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Improving the production of highly custom single-stranded DNA sequences for
applications in DNA nanotechnology through the development of novel
bacteriophage-based DNA-production methods
by

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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AND

UNIVERSITY OF CALIFORNIA, BERKELEY

Abstract

DNA origami is a powerful tool for the production of self-assembled nanostructures and has shown promising applications in a wide range of fields. These structures are typically assembled through a reaction between one long single-stranded DNA (ssDNA) scaffold and many short oligonucleotide staples. In our efforts towards developing applications of DNA origami, we determined that two important properties for scaffold production include the ability to customize its size and sequence, and the ability to produce scaffolds cost effectively at scale. Several *in vitro* methods exist for producing custom scaffolds. However, because they rely on enzymatic processes, these methods are unreliable at large scale. On the other hand, scalable production of scaffolds based on the M13 bacteriophage genome has been demonstrated. However, the M13 genome will only accommodate insertions up to 2.5 kilobases (kb) reliably, limiting customizability. Likewise, phagemid vectors allow for the production of phage-based scaffolds with larger custom regions, but they still require 2.9 kb of fixed sequence. To overcome these issues, we developed a modified expression system using a novel phagemid, which we call pScaf. pScaf includes a standard M13 origin of replication (M13 ori) as well as a modified M13 ori which behaves as a terminator of ssDNA synthesis, allowing for packaging and export of the intervening sequence as a custom phage particle, while reducing the fixed-sequence region to only 381 bases.

Here, I discuss the steps taken to develop this novel phagemid vector. I also describe the resulting custom scaffolds which we produced using this vector and demonstrate the usefulness of these scaffolds for applications in DNA origami.

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I. DNA Nanotechnology

History and Assembly Methods

The field of DNA nanotechnology was born with the publication of “Nucleic Acid Junctions and Lattices”¹ in 1982 by Nadrian Seeman in the *Journal of Theoretical Biology*. As the story goes, Seeman was inspired by the M.C. Escher engraving titled *Depth*, where a grid of fish, each with four symmetrical fins protruding out of them, are aligned perfectly in a three-dimensional lattice. Seeman theorized that partially complementary oligonucleotides could be designed and synthesized, such that they could assemble into two- and three-dimensional networks. Using this framework, Seeman subsequently published the assembly of a cube structure² formed by the hybridization of ten oligonucleotides of size 80 bases or shorter. This was also followed by the design and assembly of a truncated octahedron³ composed of eight 120-mers which form into hexagons, six 80-mers which form into squares, and 24 40-50-base linkers which assemble them into the final structure. Seeman’s work showed that Watson-Crick base-paired DNA wasn’t just limited to linear helical structures, but could be used to construct other unique complexes, paving the way for DNA nanotechnology as we know it today.

After Seeman’s groundbreaking work, the next major contribution to the field was the publication of the Shih octahedron⁴ by William Shih and co-authors in 2004. This work is credited as the first application of what we now refer to as single-stranded Origami

(ssOrigami), where a long strand of ssDNA is folded onto itself into a target shape with the help of few shorter strands (or none at all). In this case, a 1.7-kb ssDNA was folded with the help of five 40-mer oligonucleotides into a three-dimensional octahedron. This work served as a promising proof-of-concept for the assembly of complex nanoscale shapes from DNA. However, due to the self-folding nature of the long scaffold, significant control is needed over the sequence, and production of custom scaffolds was challenging.

Just two years later, in 2006, Paul Rothemund⁵ published a new assembly method that brought DNA origami closer to the mainstream. Rothemund's scaffolded DNA assembly method utilizes a long multi-kilobase scaffold strand that is folded into a target shape with the help of many short oligonucleotides, called 'staples'. Unlike Shih's method, the scaffold never pairs with itself, only binding with staples that act as intermediates to bridge different parts of the scaffold. As a result, the scaffold sequence is less important, making custom scaffolds less necessary. As a result, Rothemund opted to use the readily available and easily producible M13 bacteriophage ssDNA genome as his scaffold strand. As a result, many different shapes could be built from the same scaffold sequence, simply by varying the pool of staple strands. In this work, Rothemund applied this method to the production of six two-dimensional DNA origami shapes.

Subsequently, in 2009, Shawn Douglas and colleagues in Shih's lab expanded this framework for the assembly of three-dimensional scaffolded DNA origami

nanostructures⁶. Meanwhile, Douglas's release of DNA origami design software, cadnano⁷, vastly streamlined the process, providing a graphical interface that allows users to create DNA origami designs and export the staple sequences necessary for folding the desired structure.

In 2016, in work led by Stefan Niekamp, our lab published a method for scaffolded DNA origami that uses reusable sets of staples⁸. Whereas in traditional DNA origami a fixed-sequence scaffold is folded by a unique set of staples into the prescribed shape, in the reusable staple method, a fixed pool of staples is used to fold different shapes from varying scaffold sequences.

Another popular approach that has its roots in Seeman's original work is the use of DNA 'tiles'. In 1998, Erik Winfree, in collaboration with Seeman, published a strategy for the production of tiles that are made up of four or five oligonucleotides with four specific sticky-ends⁹. These tiles could then be arranged such that the sticky ends align in a precise manner, forming a two-dimensional DNA crystal. In 2011, Liu and colleagues in Seeman's lab developed additional DNA tiles, which either have parallel domains or orthogonal domains, allowing for assembly into different types of two-dimensional arrays¹⁰. In this case, however, rather than being made up of a small number of oligonucleotides, each tile was folded from an M13mp18 scaffold with 201 oligonucleotide staples to create a larger monomer unit. Unpaired sticky ends on these tiles then pair with other tiles to create the desired array.

In 2011, Zhao and co-authors also published a method for assembly of DNA origami tiles into more complex superstructures by using pre-formed scaffold frames¹¹. In this method, the M13 scaffold is again used to generate tiles of varying shapes. However, rather than using sticky ends and oligonucleotide staples to assemble the tiles to one another, an additional DNA origami structure is folded to serve as a frame where each of the individual tiles can dock, allowing for precise positioning of the tiles.

While the previously discussed tile-based methods rely on the assembly of pre-formed structures, Wei and colleagues in Peng Yin's lab published a related method where each subunit is a single oligonucleotide¹². In this single-stranded tile (SST) method, each oligonucleotide is a 42-mer divided into four domains. Each domain is designed to be complementary to exactly one domain on another adjacent oligonucleotide. The mixture of all of these oligonucleotides results in the self-assembly of a two-dimensional structure. The end result is similar to the structures that can be built using scaffolded DNA origami, but this method only uses staples instead of a scaffold-staple combination. However, the reported assembly yields for SST is much lower than for DNA origami.

In a follow-up to the SSTs, Ke and colleagues published a strategy for the staple-only self-assembly of three-dimensional nanostructures using DNA 'bricks'¹³. In this method, each monomer is a 32-base oligonucleotide with four domains. Complementary domains on adjacent bricks assemble such that they form a 90 degree angle. This allows for assembly of the staples in three-dimensional space, resulting in a pre-

determined structure. Like its SST predecessor, the DNA brick method also resulted in low reported yields compared to scaffolded DNA origami.

A recent method that presents a novel alternative to traditional origami assembly methods was published by Florian Praetorius in Henrik Dietz's lab in 2017¹⁴. This method creates self-assembled structures using a combination of DNA and proteins, in a method that could be classified as double-stranded DNA origami. Here, the single-stranded scaffold is replaced by a double-stranded template, and the staple oligonucleotides are replaced by DNA-binding transcription activator-like (TAL) effector proteins. TAL proteins can be designed to bind to specific DNA sequences. Therefore, they can be used to induce user-specific folding of the dsDNA template into a specified conformation.

DNA Nanotechnology Applications

Since the first DNA origami structures were folded, researchers have been developing applications for the technology. By its nature, DNA origami allows for the assembly of structures of specific shape and size. Additionally, because the DNA sequence is known at every position of the structure, designers have the ability to precisely position functional elements, such as proteins, along the structure. Some of the most-publicized applications of DNA nanotechnology have been for therapeutic applications.

In 2012, Shawn Douglas and Ido Bachelet, along with George Church, reported a logic-gated DNA origami nanorobot, which was shown to selectively deliver a protein payload to cancer cells *in vitro*¹⁵. This design was a clamshell structure, which houses the protein therapeutic on the inside of the device. This clamshell is held shut by a pair of aptamer locks, which are released in the presence of specific cell-surface receptors. The aptamer locks are released only when both receptors are present, creating an AND gate. Satisfaction of AND gate conditions releases the locks, allowing the spring-loaded clamshell to open up, releasing the protein payload to the cell surface. In 2014, Bachelet expanded upon this work to create OR, XOR, NAND, NOT, CNOT, and half-adder gates¹⁶. Furthermore, this year, Li and co-authors demonstrated aptamer-based logic-gating behavior by a DNA nanorobot *in vivo* using a mouse model¹⁷.

Additionally, there were two other reports of DNA origami drug delivery systems in 2012. Zhao and co-authors in Bjorn Hogberg's lab, as well as Jiang and collaborators, published doxorubicin-loaded DNA origami nanostructures as cancer therapeutics. While doxorubicin is a commonly used cancer drug, it has significant drawbacks. First, free doxorubicin, like many other chemotherapeutics, lacks specificity for cancer cells, resulting in adverse side effects. Additionally, cancer cells are able to quickly adapt to many therapeutics, developing resistance to them. Researchers believe that DNA origami carriers can mitigate these concerns. Doxorubicin is an anthracycline, which inhibits DNA synthesis in cells by intercalating between base pairs. Because of this ability, it can also be loaded in between the bases of DNA nanostructures. Jiang and co-authors used a doxorubicin-loaded DNA nanostructure to deliver the therapeutic to

doxorubicin-resistant cells *in vitro*, reversing the drug-resistance phenotype and resulting in cell death¹⁸. Meanwhile, Zhao and colleagues demonstrated that the origami-delivered doxorubicin exhibited increased cytotoxicity and residence time in the cell compared to the free drug¹⁹. They also showed how alterations in the design of the nanostructure, specifically by modulating the global twist, could be used to tune the drug-loading efficiency and release rate. In 2014, Zhang and colleagues followed up on Jiang's earlier work and demonstrated the anti-tumor efficacy of the same doxorubicin-loaded particles *in vivo*²⁰. These studies show great promise for DNA origami in therapeutics. However, the main factor holding it back is the difficulty of producing large amounts of the nanostructures in a cost-effective manner. As we develop new techniques to bring assembly costs down, DNA nanotechnology can become a much more powerful therapeutic tool.

Among the many other applications for DNA nanotechnology, another interesting one is in electronics. In 2010, Maune and co-authors developed a method for a DNA origami 'nanobreadboard', which allows for the two-dimensional arrangement of single-walled carbon nanotubes with nanometer precision²¹. Using this method, the authors were able to build a nanostructure with transistor-like behavior. In 2011, Liu and colleagues described a method for plating of branched DNA origami nanostructures with silver and gold, providing a method for the production of DNA-templated nanocircuits²². More recently, in 2016, Teschome and co-authors published a method for creating electrical contacts between DNA-origami-based metallic nanowires, allowing for propagation of an electric signal through a DNA-based circuit²³.

II. Traditional ssDNA Scaffold Production

M13 Bacteriophage

M13 is a filamentous bacteriophage with an ssDNA genome encapsulated by a coat of proteins. This genome is primarily composed of genes encoding 11 essential phage proteins responsible for its structure, replication, packaging, and export. These genes are very efficiently packed onto the genome with some overlapping reading frames. As a result, the potential for making modifications to the genome is limited. Another important feature of the genome is the 508-bp intergenic region, which includes the signals for initiation and replication of the genome, as well as a packaging signal sequence. A key property of M13 is that it is non-lytic, meaning that it does not lyse its host bacterium during export. In fact, the protein coat is assembled around the genome as it is being secreted from the bacterium. As a result, infected bacteria are able to continue to grow and divide while the continuously replicated phage particles are exported out of it. Additionally, because the viral coat is not pre-formed, it is able to adapt to the genome it is encapsulating, meaning that larger or smaller genomes can also be encapsulated, with the size of the phage particle correlating to the size of its genome²⁴.

M13 begins its replication process by infecting a host cell. It is only able to infect bacteria that have an F pilus. The phage initiates replication by making contact between its minor coat protein pIII and the pilus. This causes pIII to interact with the host TolQ, TolR, and TolA proteins, allowing for the removal of the pIII proteins and insertion of the

M13 DNA into the bacterium. Once the plus-strand ssDNA has entered the bacterium, it uses the host's machinery to convert into a double-stranded circular replicative form (RF). This RF contains genes that allow for the expression of all of the viral proteins necessary for propagation of the phage. The gene II protein, pII, initiates replication of the viral DNA by nicking the plus-strand of the RF at a specific point in the M13 origin of replication. DNA polymerase from the host is then able to add nucleotides to the free 3' end of the strand using the opposite strand as a template, creating a copy of the plus-strand. Once the entire genome has been replicated, pII returns to nick and re-circularize the replicate. Initially, these copies are converted into the dsDNA RF such that they can continue to express proteins and replicate further. However, once a threshold concentration of RF has been reached, conversion to the RF stops. This is regulated by the ssDNA-binding protein, pV, which binds and sequesters the plus-strand just after synthesis. Therefore, it is unable to be converted to the RF and thus remains as ssDNA. From this point on, all replicated DNA follows this pathway, allowing it to be packaged and exported as phage. This ssDNA, still bound to pV, migrates to assembly sites in the host's membrane. Next, the membrane-associated coat proteins, pVII and pIX, interact with the packaging sequence in the complexed ssDNA's intergenic region. Meanwhile, pI, pIV, and pXI create a pore in the membrane for the ssDNA to pass through. As the ssDNA is secreted, it sheds its pV coat and replaces it with major coat protein, pVII. Finally, the ssDNA is capped by minor coat proteins pVI and pIII, completing the phage structure. Because the phage is non-lytic, the host bacteria survive and can continue to generate more phage particles²⁴. As a result, bacteriophage is a very efficient vehicle for amplifying large amounts of ssDNA.

The field of DNA origami took off with the publication of Paul Rothemund's scaffolded assembly method published in 2006. In these studies, Rothemund demonstrated a proof-of-concept by folding the 7249-base M13mp18 ssDNA genome into a series of nanoscale shapes⁵. M13mp18 is a variant of M13 that has been engineered to be more suitable for cloning and modification. It is a derivative of the first modified M13, M13mp1, created by Joachim Messing at the Max-Planck Institute (the source of the "mp" in the phage's name). M13mp1 differed from wild-type M13 only in that it contained lac sequences, added within the phage's intergenic region, allowing for blue-white screening of inserts placed into the M13mp1 vector. Messing and colleagues further improved upon this by incorporating a series of different multiple cloning sites (MCS) into the phage vector until arriving at the M13mp18 vector commonly used today (M13mp19, which contains the same MCS as M13mp18 but in reverse order, is also commonly used)²⁴.

This MCS in M13mp18 allows for the insertion of small fragments into the M13 genome. As a result, DNA nanotechnologists who wanted to use sequences other than M13mp18 (also referred to as p7249 due to its size in bases) could increase the size of the scaffold by introducing additional fragments. This led to the development of other commonly used DNA origami scaffolds, such as p7308, p7560 and p8064. However, the problem lies in its lack of significant customizability. Because the phage genome has a significant number of essential genes and regulatory elements, all of these scaffolds must be ~7kb or longer and include these fixed sequences. At the upper limit,

the phage is often intolerant of large inserts, making cloning difficult. Therefore, in practice, users are limited to scaffolds in the 7-10kb range, with a significant majority of that sequence being fixed, non-custom sequence. Still, there are significant advantages to using these phage-based scaffolds. These sequences are still phage genomes, so they can be easily introduced to bacteria, either by transformation of the ssDNA or dsDNA form, or by infection of the phage particle. Once this genome has been introduced to the bacteria, it can then be easily propagated, recovered as phage particles, and then processed to recover the pure ssDNA.

Production and Recovery of M13-Based ssDNA

The production and recovery of this phage-based ssDNA is a fairly simple process. First, *E. coli* are transformed or infected with the desired M13 variant. These bacteria are then allowed to grow and divide, typically in shake flasks, as they produce more M13 following the standard M13 life cycle. Because the replicated M13 is exported out of the bacteria, bacteria and phage can be separated via centrifugation, where the larger bacteria produce a pellet at the bottom of the tube or bottle, while the much smaller phage remain in the supernatant. This supernatant may then be further clarified via filtration, followed by concentration using PEG and salt. This concentrated phage can then be stored for future infection, or it can be lysed to recover its ssDNA. A common lysis method is to add a buffer containing detergent and base to the phage. The solution is then neutralized and centrifuged. The resulting supernatant contains the phage ssDNA. This ssDNA can then be precipitated out with the addition of ethanol. An additional centrifugation step results in a pellet of ssDNA, which can then be dried and

resuspended in buffer. Not only is this a short and simple process carried out with standard lab equipment, but it can also be scaled up to larger volumes. A more detailed account of this method is included in the protocols section at the end of this document.

Some of our early efforts in the lab were focused on transitioning the production of phage ssDNA from milliliter-scale shake flasks to liter-scale bioreactors. Bioreactors are advantageous because not only do they allow for an increased volume of production, but they provide specific control over key parameters, such as the pH, temperature, and dissolved oxygen concentration, and can allow for controlled feeding of nutrients such as glucose. I assisted a colleague, John Garbutt, in developing growth conditions, bioreactor controls, and a recovery process for producing phage ssDNA at the liter scale. After optimization of these conditions, we are able to establish a fed-batch process wherein we could produce an average of 220 mg of phage ssDNA per 1-liter culture, compared to our yields of 10 mg ssDNA per 1-liter culture at the flask scale.

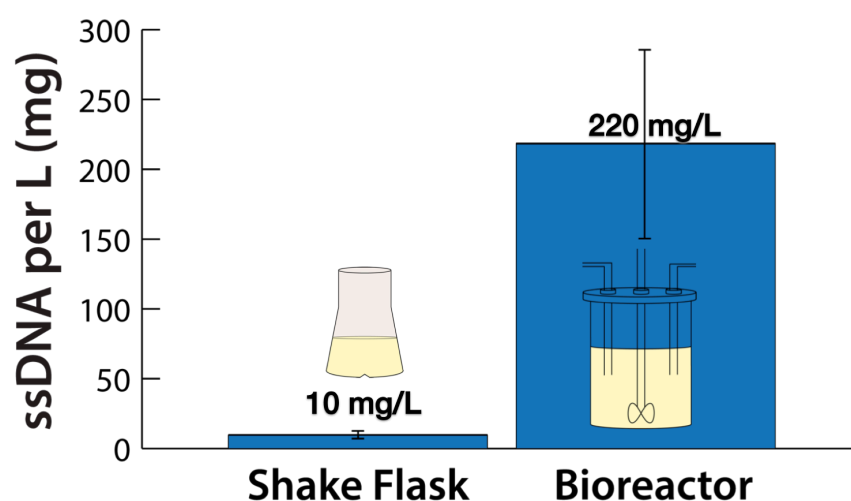


Figure 1: Shake Flask vs. Bioreactor ssDNA Yields

Meanwhile, Kick and colleagues in the Dietz Lab developed and published their own process for production of M13 ssDNA in bioreactors²⁵. In contrast to our complex medium, they used a defined medium. Similar to our process, they grew their cultures at 37°C and pH 6.7 and provided a controlled glucose feed upon depletion of the initial batch glucose. Using this process Kick and colleagues were able to report M13 ssDNA yields ranging from 370 mg to 410 mg per 1-liter culture. This established that phage ssDNA can be cultivated at increased scales in reactors, and has the potential for production at even greater scales by increasing the volume of the culture.

III. Custom-Sequence ssDNA Production

While many in the DNA nanotechnology field still use the standard phage-based scaffolds, there are many applications that could benefit from customizable scaffolds. One simple case is greater control over size. Some applications require fewer than 7000 bases and using standard M13 scaffolds requires a large amount of unused DNA. Alternatively, many are interested in building larger structures. New methods need to be established to create larger scaffolds than the standard M13 vectors allow for. Additionally, if multiple scaffolds with orthogonal scaffolds are developed, they could be assembled simultaneously to build one-pot super-assemblies, helping increase the size limit for DNA origami. Custom sequences may also allow for sequence-guided folding. For example, precise control over the scaffold sequence can allow one to design various domains that fold independently before coming together into the final structure. This could be instrumental in the production of complex shapes that are prone to falling into kinetic traps during the folding process, if it is not controlled more precisely. Custom scaffolds also open the door for new advancements in ssOrigami, where no or few staples are used to fold a single scaffold or multiple scaffolds into a shape. Because staples are the cost-limiting step for scalable DNA origami production, a paradigm shift towards ssOrigami could be a significant step towards demonstrating the industrial viability of DNA nanotechnology.

There are several existing methods for the production of custom ssDNA sequences for DNA nanotechnology. However, each of these methods has significant problems that prevent its broad use. These methods can be divided into two categories. *In vitro*

methods rely on enzymatic reactions to generate ssDNA, which allows for the production of highly customizable scaffolds, but comes at the expense of costly and difficult mass-production. Meanwhile, phage-based methods are much easier and cost-effective to scale up, as has been demonstrated previously, but existing methods lack the requisite customizability for the production of scaffolds in a wide range of sizes and sequences.

In Vitro and Enzymatic Methods

Assymetric polymerase chain reaction (aPCR) is a method in which PCR is performed with an excess of sense primer relative to antisense primer. This allow for preferential production of the sense strand. In 2016, Veneziano and co-authors used this strategy to generate a series of origami scaffolds ranging from 721 to 3356 bases²⁶. These scaffolds were then used to fold a series of DNA origami polyhedra. In 2017, they also published a pre-print describing the application of the same strategy to the production of a 15-kb scaffold²⁷.

A similar strategy to aPCR is to amplify a sequence using traditional symmetric PCR, but then use an exonuclease to remove the undesired strand, leaving a single-stranded product. This is achieved by using one 5' phosphorylated primer and a 5' Cy3-tagged primer during the amplification. Lambda exonuclease is then added to digest the 5' phosphorylated sacrificial strand, retaining the Cy3-tagged strand. Zhang and colleagues used this method in 2012 to generate a 26kb scaffold in order to fold a large

rectangular origami structure²⁸. Despite the customizability of these PCR-based methods, there is no discussion of product yields by the authors.

Additionally, exonuclease-based methods are not limited to sequences generated via PCR. In 2009, Douglas and colleagues generated an ssDNA scaffold from a pre-existing plasmid which they are able to propagate in bacteria⁶. This propagated and recovered plasmid was then nicked with a restriction endonuclease that only cuts one of the two strands, generating a free 5' end for T7 exonuclease to digest. This results in complete digestion of the nicked strand, allowing the intact strand to be used as an ssDNA scaffold. In this case, the authors generated the scaffold from a pre-existing plasmid, one which contains the gene for eGFP. However, this strategy could be used to generate a partially custom scaffold by cloning a user-defined sequence into the plasmid at some position. It may, however, not be entirely custom as the plasmid is likely to still include a plasmid origin of replication and an antibiotic-resistance gene. This is similar to the method employed by Erkelenz and co-authors in 2014²⁹. In their study, a sequence-of-interest is PCR-amplified such that one of the primers introduces a nicking site. This amplified sequence is then cloned into a host vector and propagated in bacteria. Similar to the strategy employed by Douglas, the propagated plasmid is then nicked and exonuclease-digested to produce a custom scaffold. The authors used this strategy to generate 5 scaffolds ranging in size from 1847 to 5438 bases. However, because the final product contains non-custom sequences originally on the plasmid, these products are only between seven and 68% custom.

An alternative strategy to exonuclease-based digestion of the undesired strand is to separate the sense and antisense strands after PCR by binding one to a bead or column. For example, Pound and co-authors performed PCR using one standard primer and one biotinylated primer³⁰. The amplified product was then bound to streptavidin-coated magnetic beads via the biotinylated strand. NaOH was then added to denature the DNA, freeing one strand, while keeping the other strand bound to the magnetic beads. The beads may then be removed, leaving behind a solution containing the ssDNA. Using this method, they generated four different scaffolds ranging in size from 756 bases to 4808 bases. They then used these scaffolds to generate thin, branched DNA origami nanostructures. While this method allows for the production of highly custom scaffolds, the authors only discuss yields for one of their products, stating that they recovered 1.1 µg of ssDNA from 4.3 µg of amplified product.

A recent method developed by Krieg and Shih, similar to the affinity-based method, uses polymers to separate the two strands of the PCR product³¹. Here, one strand of a dsDNA amplicon is labeled with an acrylamide moiety. The acrylamide-tagged DNA may be polymerized with the addition of tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) resulting in a polymer mesh containing the dsDNA. This DNA-polymer hybrid can be purified via precipitation under native conditions. Subsequently, the pure DNA-polymer can be precipitated under denaturing conditions, pelleting the polymer-bound strand, while leaving the free strand in solution. The free solution may then be purified via precipitation with isopropanol. This method was used to create ssDNA scaffolds of size 1650, 3315, and 7301 base pairs. The method is

highly customizable, as it simply requires amplification of a template sequence prior to strand-separation. However, size scalability is a concern, as the authors report a decreasing yield with increasing scaffold size. This is in addition to concerns of mass scalability due to the method's reliance on PCR amplification.

Rolling circle amplification (RCA) is an *in vitro* version of a replication method commonly found in biology. For example, M13 bacteriophage uses a similar process to replicate its genome within the host bacteria. *In vitro*, a primer is added to a circular template, while a DNA polymerase is used to generate copies of the template. In 2013, Ducani and other members of the Shih Lab combined this with a novel 'monoclonal stoichiometric' (MOSIC) method for enzymatic generation of short ssDNAs³². Specifically, they generated a plasmid containing a series of 14- to 378-base sequences separated by hairpins. Single-stranded copies of this plasmid can be generated using RCA, and subsequently an enzyme may be used to cleave the ssDNA plasmid at each hairpin, resulting in a pool of oligonucleotides. This is mainly intended for the production of staple oligonucleotides. However, the authors also produced a 378-base sequence, which they successfully used as a custom-sequence scaffold.

Another strategy is to simply start with a dsDNA source and use heat to separate the two strands, resulting in two scaffolds that can be simultaneously used for origami folding. This method was employed by Yang and colleagues in 2012 to generate three pairs of scaffolds of size 2027, 2322, and 4349³³. They also performed the technique on non-custom 7249-base sequence obtained from a commercial vendor. This method

provides some customizability. However, because the separated strands must be complementary, half of the bases in the final mixture are constrained, and therefore not user-definable. Additionally, because of the complementarity of the strands, additional design constraints must be employed when designing the DNA origami.

Existing Phage-Based Methods

A move towards more scalable production of custom scaffolds can be accomplished by returning towards phage as a source for ssDNA-production. One strategy proposed by Said and colleagues in 2013 requires selective digests of an existing phage-based scaffold³⁴. Specifically, they produce a single 704-base scaffold by first annealing a pair of 20-base oligonucleotides at EcoRI and BglII sites on wild-type M13 ssDNA. This creates double-stranded regions that can be recognized and digested by the restriction enzymes. They then perform the digest and gel-purify the desired product. Finally, another oligonucleotide is added to bridge the two free ends of the linear scaffold, while a ligase is added to circularize the DNA. The authors called this 704-base scaffold the M1.3 as it is approximately 10% of the size of wild-type M13. This method allows one to produce scaffolds of arbitrary length, given that the final size is less than approximately 7kb and that restriction sites exist or can be incorporated at the desired sites. It, however, does not allow for customization of sequence, as one is limited to a subset of the existing phage sequence. Additionally, what the method gains in scalability by being phage-based, it loses through its reliance on enzymatic reactions and gel-based purification.

On the opposite end of the spectrum, in 2014, Marchi and co-authors attempted to produce an extremely large 52kb scaffold by creating a hybrid of M13 and Lambda phages³⁵. To do so, they introduced an M13 origin of replication into the lambda genome and produced lambda phage containing this modification. The phage were then used to infect *E. coli*. Here, the M13 origin of replication allows for the production of the entire lambda genome in a single-stranded form, which can then be packaged and exported as M13 phage. The DNA can then be recovered from the phage using standard methods. While notable for producing the largest scaffold to-date, this method lacks customizability, as it is only able to produce scaffolds based off of the lambda genome. However, this method is very similar to phagemid-based methods, which provide slightly more customizability.

A phagemid is a plasmid that contains an M13 or f1 phage origin of replication, in addition to a standard plasmid origin of replication and antibiotic-resistance gene. The dual origins allow for replication of the full plasmid in both double-stranded and single-stranded forms, though the latter is only possible in the presence of specific phage proteins. In 2012, Zadegan and colleagues created a 1983-base phagemid which they transformed into *E. coli*³⁶. The bacteria was cultured in the presence of helper phage M13KO7, which is a mutant of M13 which has a modified packaging signal, allowing it to infect the bacteria and provide the necessary phage proteins to the phagemid, while being less likely to be packaged itself. In theory, this leads to the selective packaging of the phagemid over the helper phage, but in practice, the end result is not binary, with a

mixture of both helper and phagemid phage being exported. One strategy to avoid this helper phage contamination is to use a helper plasmid, first proposed by Chasteen and colleagues in 2006 as a strategy for eliminating the need for helper phage in phage display³⁷. This, however, can be applied to phagemids as well. The helper plasmid contains genes for all of the phage proteins, but does not include a phage origin. Therefore, the helper plasmid sequence is never generated as ssDNA nor is it exported as phage. In 2015, Brown and co-authors generated a 2404-base phagemid-based scaffold similar to Zadegan, but replaced the helper phage with a helper plasmid, allowing for production of the phagemid phage without other phage contaminants³⁸. Both of these attempts allow for the production of smaller-than-typical scaffolds, providing some size customization. However, they are completely lacking in sequence customization, as the phagemid is essentially just an M13 origin placed into a standard pUC plasmid (the researchers in the two papers removed different non-essential parts of the plasmid backbone to obtain their respective phagemids). Phagemids, however, are capable of providing some customizability. Commercial phagemids, such as pBluescript, contain an MCS, allowing for the insertion of a custom sequence which can be incorporated into the final ssDNA product. For example, Shih did this in 2004 in order to generate the scaffold for his Shih Octahedron⁴. To do so, he cloned a 1.7kb insert into the pBluescript vector, transformed the resulting plasmid into *E. coli*, infected with helper phage and recovered the resulting phage ssDNA. However, the final product contained more than just his 1.7-kb insert. Because the phagemid allows for the replication and packaging of the entire plasmid, an additional 3kb of non-custom sequence are also produced, resulting in just 36% custom sequence in the case of the

octahedron scaffold. Shih got around this by using restriction digest followed by gel purification to excise the unwanted sequence. However, these steps are not easily scalable. Therefore, we sought to improve the customizability of phagemids in another way: by eliminating the replication of the unwanted sequence within the bacterium, such that the recovered phage ssDNA requires no additional processing.

pScaf Terminating Phagemid Vector

The ability to clone a custom sequence into a plasmid prior to phage production in *E. coli* is very important, as is the simplicity of recovering the ssDNA from the bacteriophage. In order to retain the advantages of phage-based ssDNA production while maximizing customizability, we set out to develop an improved phagemid vector. Phagemids take our understanding of bacteriophage biology, specifically the role of the M13 origin as a trigger for ssDNA synthesis, to engineer a plasmid encoded with this same behavior. Taking a cue from other areas of molecular biology, we envisioned the development of an M13 terminator motif, which could signal the stoppage of DNA synthesis. We hypothesized that if such a terminator were placed within a phagemid downstream of the M13 origin, this could allow for specific synthesis and packaging of the intervening sequence in the form of bacteriophage.

To begin our pursuit of an M13 terminator, we began an extensive search of the literature pertaining to bacteriophage biology, and specifically DNA replication in bacteriophage. Significant to our goals was a discovery published by Dotto et al. in 1982³⁹. In this work, researchers determined that the M13 origin simultaneously acts as

both an initiator and terminator of ssDNA synthesis, and that these initiation and termination behaviors are linked through overlapping regions of the origin. In wild-type bacteriophage, this behavior makes sense. The genome is circular, so the DNA synthesis begins at the M13 origin and ends when the origin is reached once again. Dotto's work went further, however, to show that if a plasmid is engineered to contain two identical M13 origins, two ssDNA products are formed – one product beginning at the first M13 origin and ending at the second, and a second product beginning at the second M13 origin and ending at the first. Furthermore, they discovered that the resulting product is circular, suggesting that even when DNA synthesis is initiated and terminated from two different origins, the resulting product is able to re-circularize. Furthermore, Dotto and colleagues showed that if the origin is truncated at specific points, its initiation and/or termination capabilities can be partially blocked. Therefore, if a plasmid contains a wild-type origin and a truncated origin, it may be able to produce a single DNA product bounded by the origin and the terminator. This provided a good starting point for our goals.

Looking for additional examples of phage DNA synthesis terminator, we came across a 1992 paper by Specthrie and colleagues⁴⁰. In this work, the researchers set out to create the smallest bacteriophage particle possible. To do so, they chose to develop a plasmid featuring back-to-back M13 origins and terminators, such that entire phage genome is composed only of these two features. In order to obtain the termination-only behavior on the second origin, similarly to Dotto and colleagues, they performed a truncation 29 bases from the end of the origin. Thus, they refer to this terminator as

M13ori Δ 29. In addition to inclusion of the terminator, they further minimized the size of the origin by removing stem-loops that they deemed to be unnecessary. Using this strategy, they were able to produce and recover bacteriophage containing only a 221-base genome. They did, however, report a significant number of slightly larger phage being produced as well, suggesting that termination may be inconsistent, resulting in the occasional production of phage containing the entire plasmid's DNA.

In our first attempt to develop a terminating phagemid system, we set out to directly apply the microphage plasmid into a phagemid vector. To do so, we generated a pUC plasmid containing the phage elements from Specthrie and colleagues with a single change: we placed an MCS between the origin and terminator, such that custom sequences could be placed within the vector. To evaluate this strategy, we inserted a 2476-base sequence from the kringle fluorescent protein expression plasmid into the MCS and attempted to produce the custom-sequence microphage. Because the microphagemid provides no phage proteins, bacteria containing the microphagemid were grown in the presence of helper phage. In doing so, we were able to recover the microphage containing only the kringle insert, unlike the traditional phagemid which yielded a larger phage containing extraneous elements from the plasmid. As a proof-of-concept, we folded the kringle scaffold into origami, showing that we are able to produce and fold custom-sequence scaffolds. However, we found that this microphagemid was limited in its use. For example, for some insert sequences, we observed no phage production. The following gels depict a series of products we attempted to recover using this vector. The first gel depicts a successful kringle-based

scaffold produced using the μ phage vector and four unsuccessful products based on GFP, GRFP and dynein (μ Dyn-3C contains a 3kb insert and μ Dyn-6 contains a 6kb insert). The subsequent gel shows a successful 974-base scaffold whose insert is derived from a the pUC-18 plasmid and another successful kringle product. It also shows six different attempts at producing the GFP-based scaffold from six different colonies, none of which were successful.

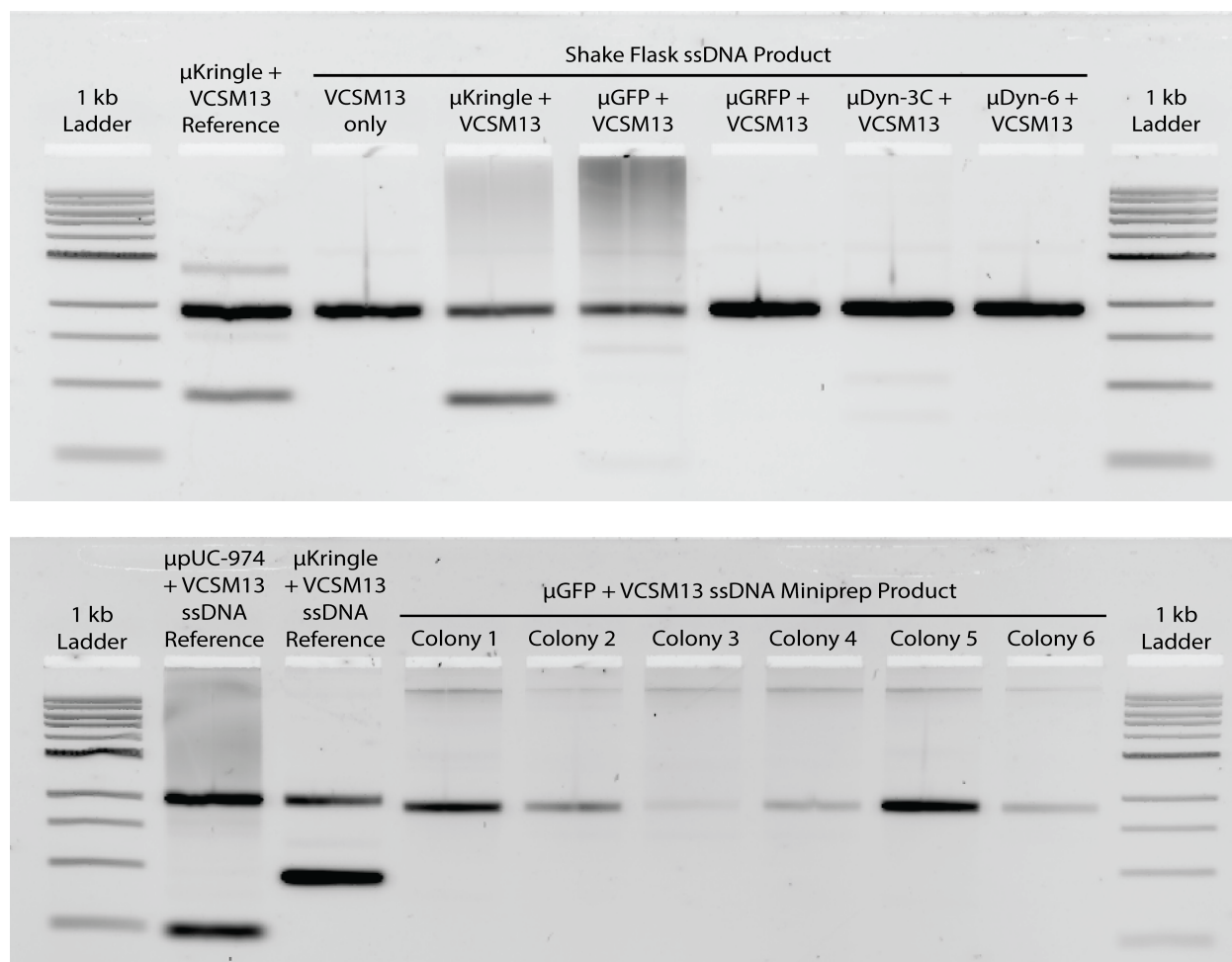


Figure 2: Scaffold Production from μ Phage Vector

Additionally, when we attempted to eliminate helper phage by combining the microphagemid with a helper plasmid, we found it to be incompatible in this system, producing an unknown phage of the incorrect size. Finally, even when we were able to produce the correct product, we often observed an additional, larger product, suggesting that the initiation behavior had not been completely eliminated.

To address these issues, we began making modifications to the origin and terminator. The first change we made was to replace the minimal sequences from the Specthrie paper with full-size origins and terminators by reintroducing the regions which those authors had deemed unnecessary. Thus, we replaced our μ ori and μ ori Δ 29 with ori and ori Δ 29. This change alone proved fruitful, as we were now able to produce phage containing inserts which appeared to be incompatible with the previous version. For example, the following gel depicts the same GFP-based insert placed into the μ phagemid vector and a vector that contains a full origin and Δ 29 terminator. The addition of the full-sized origin appears to allow for the production of the desired scaffold product. It does, however, still have the issue of spurious initiation as an additional band is observed between the desired product and the helper phage band.

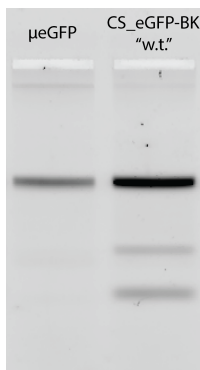


Figure 3: Comparison of μ Phage Origin and Full-Size Origin on ssDNA Product

Additionally, we were now able to successfully use the helper plasmid to produce our custom phage without contamination by helper plasmid, as will be demonstrated later. The only issue left to address was the spurious initiation. The first question we decided to ask was, “assuming truncation is the best method for terminating ssDNA synthesis, is $\Delta 29$ indeed the ideal truncation site?” To answer this question, we designed a series of mutant terminators that are truncated between sites 20 and 38. We generated a library of phagemid vectors containing each of these truncation variants by producing PCR fragments that started at the beginning of the origin and ended at the desired termination site, and replaced the original terminator in the vector by cloning the fragment between BamHI and EcoRI sites. What we observed was that between $\Delta 20$ and $\Delta 28$, wild-type behavior was maintained – that is to say, both the unmodified and modified M13 origins behaved as both initiator and terminator, resulting in the production of two distinct ssDNA products, similar to the results obtained by Dotto when exploring phagemids with two M13 origins. However, additional truncations, particularly $\Delta 29$ and $\Delta 30$, appeared to reduce the modified origin’s initiation capabilities. The result with $\Delta 29$ was expected, as it agrees with the results from Sepcthrie, as well as our own earlier findings. It was notable, however, to observe that the $\Delta 30$ truncation provided a similar results, but with less off-target product. However, this was not completely consistent. For example, for one particular insert derived from the eGFP coding sequence, ssDNA production from the $\Delta 30$ mutant in the presence of helper phage, resulted in little-to-no off-target background, while production of the same ssDNA from the same plasmid in a helper-plasmid-bearing strain resulted in significant off-target

production. Finally for truncations at $\Delta 31$ and above, off-target production became more significant again, with $\Delta 36$ and $\Delta 38$ producing an even larger product consistent with the complete phagemid, suggesting that in these cases, only the wild-type origin was functioning, with the truncated M13 origin behaving as neither initiator nor terminator. These results are partially summarized in the following gel.

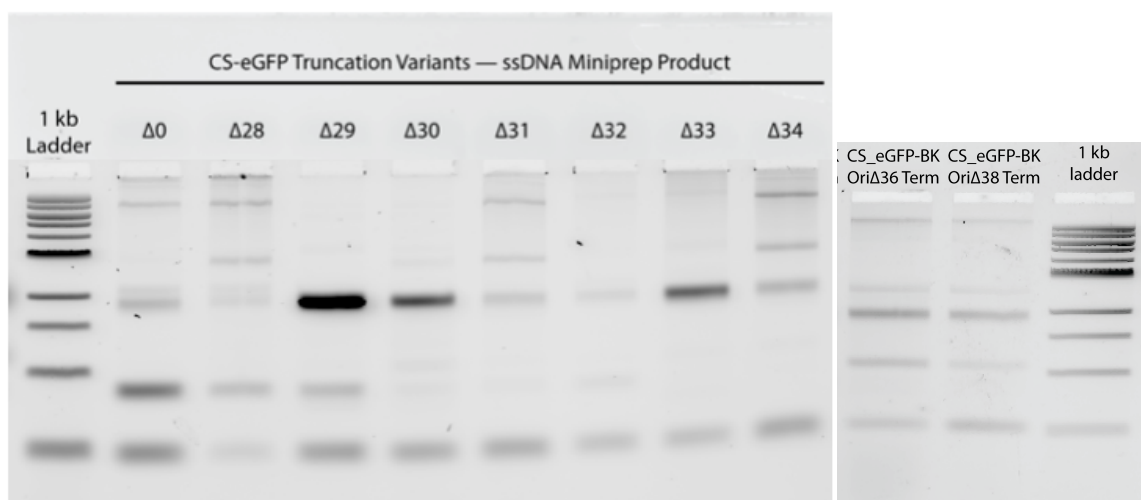


Figure 4: Comparison of Terminator Truncation Position on ssDNA Production

These results, however, were also inconsistent. For example, while the $\Delta 31$ - $\Delta 34$ truncations appear to produce clean bands in the experiment from which the above gel was obtained, there was an another experiment where no $\Delta 30$ product was produced but the $\Delta 31$ - $\Delta 34$ products contained significant spurious initiation impurities. Nevertheless, we used the $\Delta 30$ -truncation terminator in a phagemid vector to produce a series of scaffolds. In the following gel, we depict a recreation of the Shih octahedron scaffold and a custom scaffold derived from the kringle gene. We also attempted to fold each

into a representative shape – the octahedron scaffold into the original octahedron, and the kringle scaffold into a nanotube.

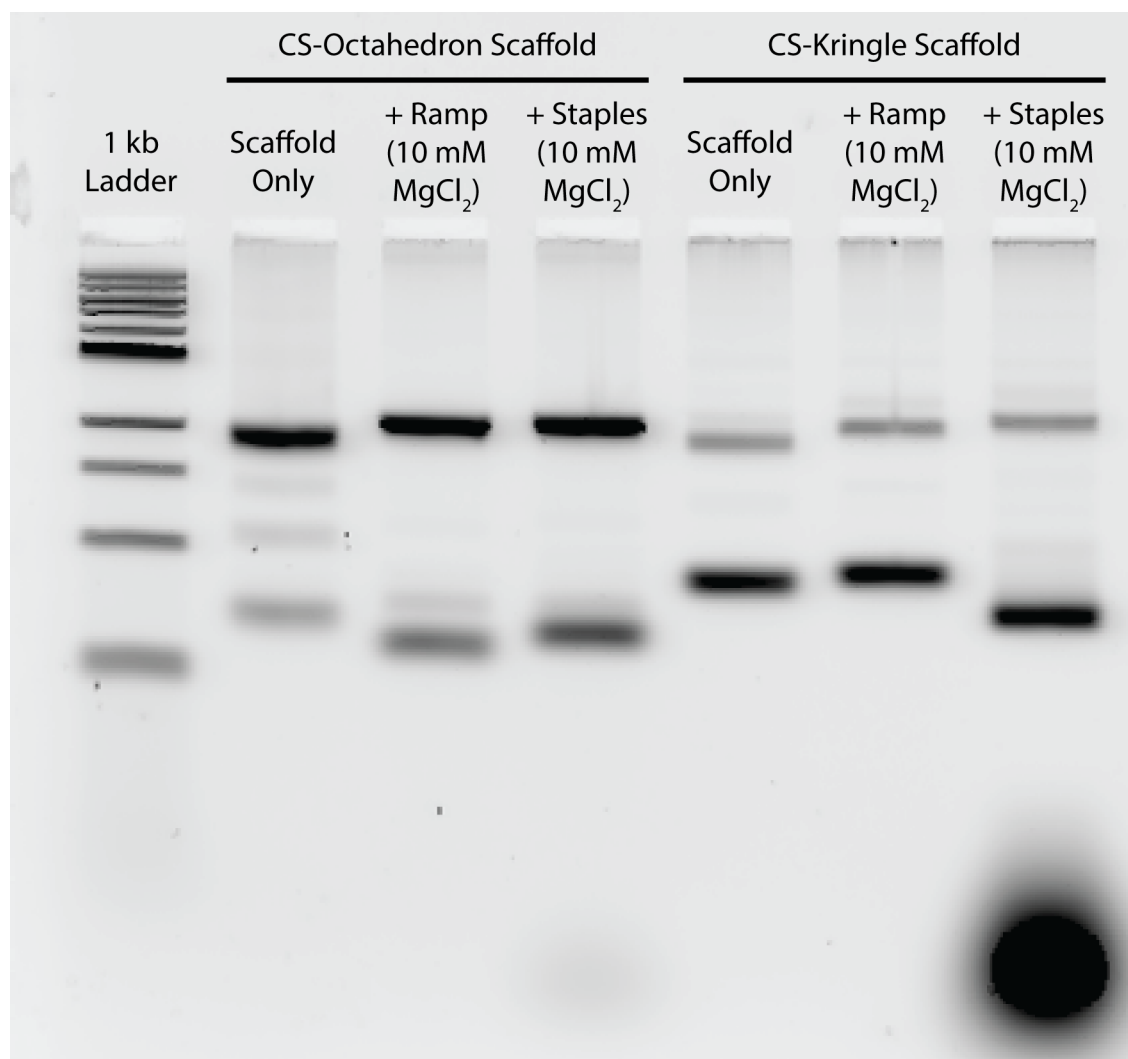


Figure 5: Production and folding of Octahedron and Kringle Scaffolds via $\Delta 30$ Termination Phagemid

However, scaffold production results were also inconsistent for different inserts. For example, the following gel depicts a series of 9 different inserts placed into the vector containing a $\Delta 30$ -truncation terminator. Over the course of multiple phage preps, for example, the 1512.1 product would have impurities that overshadowed the desired product, while the other products would appear much purer.

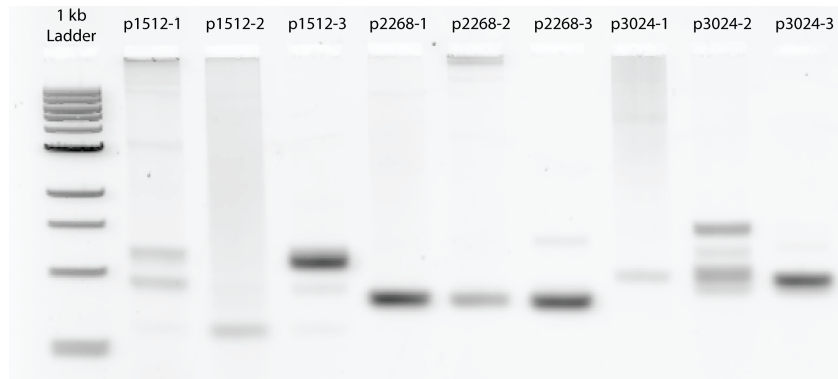


Figure 6: Production of ssDNA Scaffolds using $\Delta 30$ Terminator and Helper Plasmid

Interestingly, however, these differences were less pronounced when helper phage was used instead of helper plasmid, as shown below.

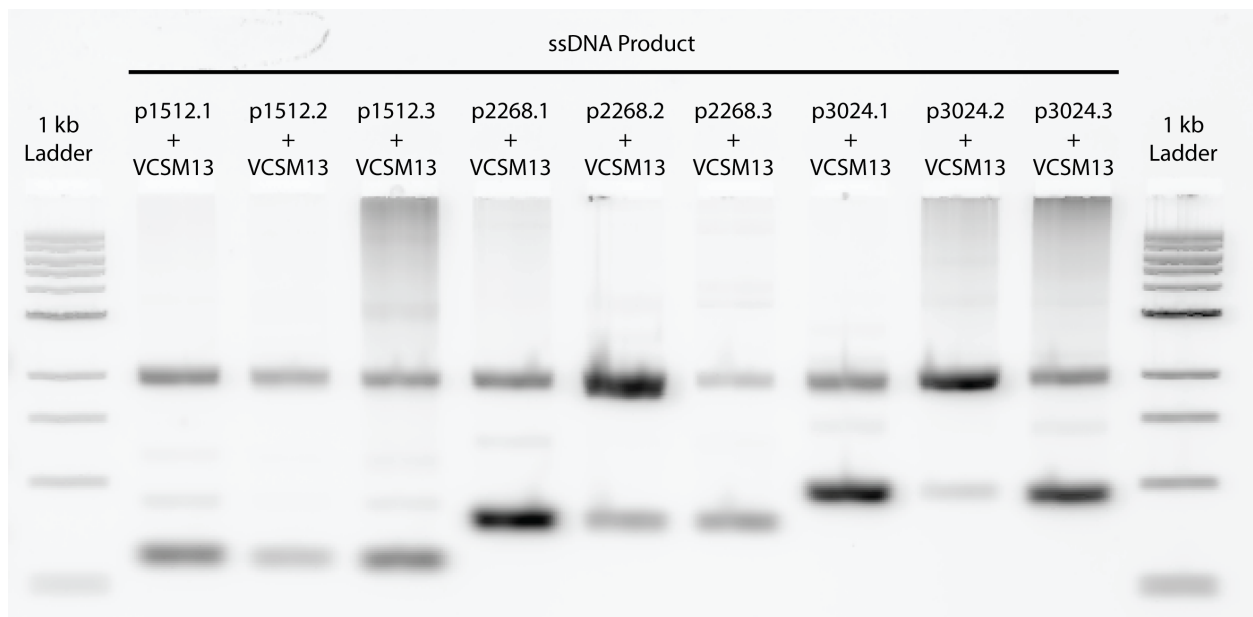


Figure 7: Production of ssDNA Scaffolds using $\Delta 30$ Terminator and Helper Phage

Therefore, we knew that we needed to find a more consistent way to achieve the desired behavior. In order to better understand the mechanism between truncation and

ssDNA production, we mapped the position of these truncations onto an annotated M13 ori sequence.

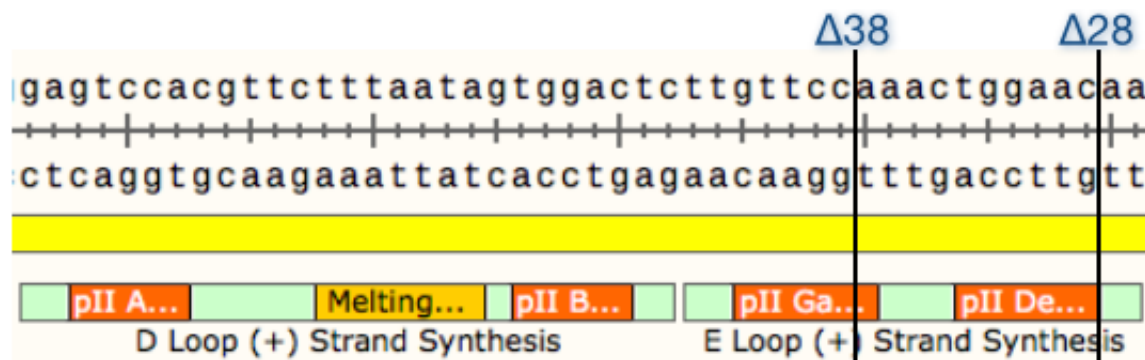


Figure 8: Annotated pII Binding Sites

Specifically, we annotated the M13 origin's pII binding sites. These are four positions on the origin, where M13 phage protein pII binds, causing the DNA to bend into a hairpin, at which point pII is able to nick one of the DNA strands, allowing for ssDNA synthesis⁴¹. ssDNA synthesis also ends by recircularization at this nick site. Therefore, it is unsurprising that modifications in this region may affect initiation and termination capabilities of the M13 origin. What we observed in our overlay is that all truncations at $\Delta 28$ and below are downstream of these binding sites, which may explain why they have no effect on ssDNA production. However, $\Delta 29$ and $\Delta 30$, which appear to partially eliminate the initiation capability, both truncate the pII-delta binding site. Finally upstream truncations such as $\Delta 38$ not only eliminate all of the pII-delta site, but also partially truncate the pII-gamma site, which may explain why these mutations prevent both initiation and termination behavior.

From this, we hypothesized that the pII binding sites are necessary for termination behavior, but specific modifications to the pII-delta site may allow us to obtain a better non-initiating variant with less off-target production. We also observed that the pII-delta and –gamma sequences are reverse complements of one another, suggesting that specific sequence may be necessary for the binding, bending, and/or nicking behavior initiated by pII. To explore this, we created a series of variants with point mutations within the pII-delta binding site. Specifically, we made a series of sequential changes where the bases starting from the downstream end were replaced by thymines (unless the base was originally a thymine, in which case it as replaced by adenine). These mutations are summarized in the following schematic:

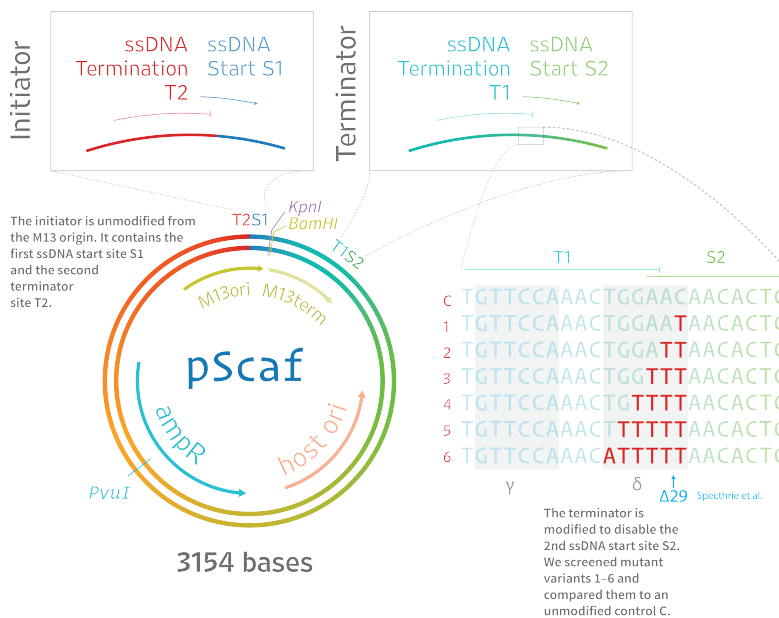


Figure 9: pScaf Design and pII Mutation Variants

These mutations essentially mutate out the pII-delta site in a stepwise manner, starting with a single-base mutation, and ending with a variant in which the entire binding site has been removed. Similar to the truncation mutants, these variants were created by first generating PCR products that were composed of the entire M13 origin with the desired substitution mutations. These mutant fragments were then cloned in at BamHI and EcoRI, replacing the original terminator. We then used each of these mutant terminators to produce phage and recovered the ssDNA from the phage. We then compared the resulting ssDNA products via gel electrophoresis.

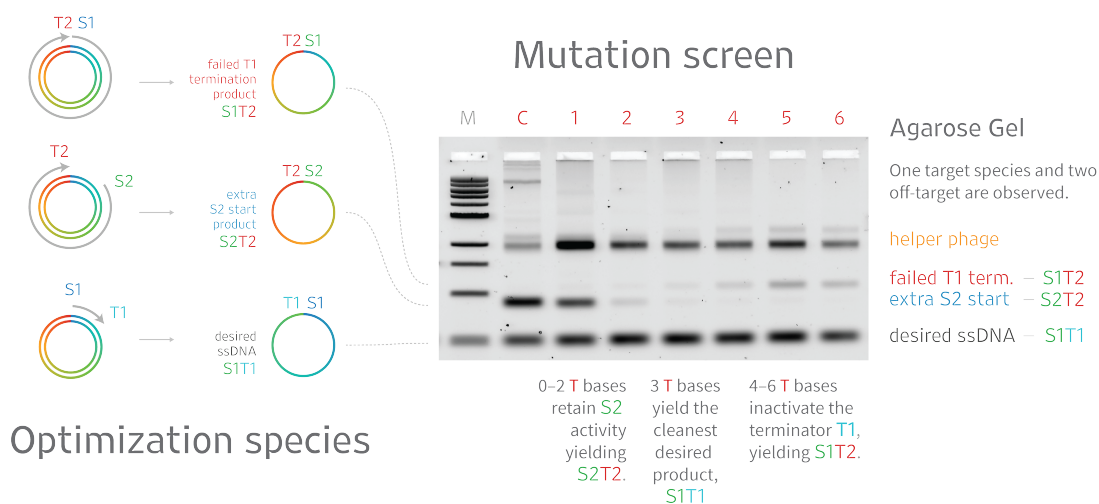


Figure 10: Gel of ssDNA Products from pII Mutation Variants with eGFP Insert

Among the different mutation variants, we observed 3 types of ssDNA product (not including the helper phage ssDNA). In the case of the wild-type, we observe two distinct species of ssDNA, with both M13 ori behaving as initiator and terminator, as we had observed previously. A single-base mutation in the pII-delta site yields a similar result. However, we observe that with subsequent mutations, the spurious initiation product,

which is caused by the modified ori behaving as initiator, begins to disappear – with the product almost completely eliminated in the T3 mutation. We then observe that subsequent mutations result in a larger product, similar to what we had seen with the $\Delta 36$ and $\Delta 38$ mutations earlier. This product is the result of the mutated origin failing to terminate on occasion, causing it to produce ssDNA representing the entire plasmid. Nevertheless, the key takeaway from these experiments was that the pII-delta-T3 mutation appears to successfully create an M13 terminator with minimal leak. The resulting product is a custom-sequence ssDNA which is primarily made up of the user-defined sequence, save for a 381-base region carried forward from the trailing end of the wild-type origin and the leading end of the mutated origin. These ends appear to circularize at the position of the pII nick site, just as a single-origin phage would.

In the delta mutation screen above, all products were produced using an eGFP-based insert – the same one that did not produce any product in the μ Phage vector. We additionally performed the same experiment using another insert containing an approximately 2kb insert from dynein. For this experiment, we cloned the insert into each of the mutation variants at BamHI and KpnI, replacing the eGFP insert. We then produced phage, recovered the ssDNA product, and ran the results on a gel. We also attempted to fold each of the scaffolds into a nanotube in order to study the effect of impurities on folding.



Figure 11: Gel of ssDNA Products from pII Mutation Variants with p2268.2 Insert

In the above gel, we observe that the T2 and T3 mutations appear to produce the cleanest scaffolds, while the T3 mutation appears to produce a cleaner origami product than the T2 mutant. Regarding the origami folding, there are a couple of important notes to point out. First, the helper phage contaminant is not removed prior to folding, yet it appears to have no effect on the folding of the origami, as the sequences are orthogonal and do not interfere with one another. Second, even the scaffold products that have significant impurities appear to fold into the correct structure, although the final product contains impurities as well.

In order to ensure that our pII-delta-T3 mutant could consistently produce the correct custom-sequence ssDNA with minimal off-target products, we generated a terminating phagemid containing this modified origin. This phagemid, which we call pScaf, contains a wild-type origin and a pII-delta-T3 mutant terminating origin, separated by BamHI and

KpnI sites, allowing for the placement of inserts of varying size and sequence between the initiation and termination blocks. We first, cloned a series of inserts that would produce final ssDNA products of 1512, 2268, and 3024 bases. We cloned each of these by PCR amplifying the desired sequence off of an existing plasmid, while incorporating BamHI and KpnI sites on opposite ends via the primers. We could then insert the PCR fragment into the pScaf vector via restriction digest and ligation.

Additionally, we observed that for longer inserts, cloning was less efficient. For example, the following gel depicts the undigested and digested miniprep products of a transformed single-insert ligation attempting to produce 6kb and 7kb scaffolds.

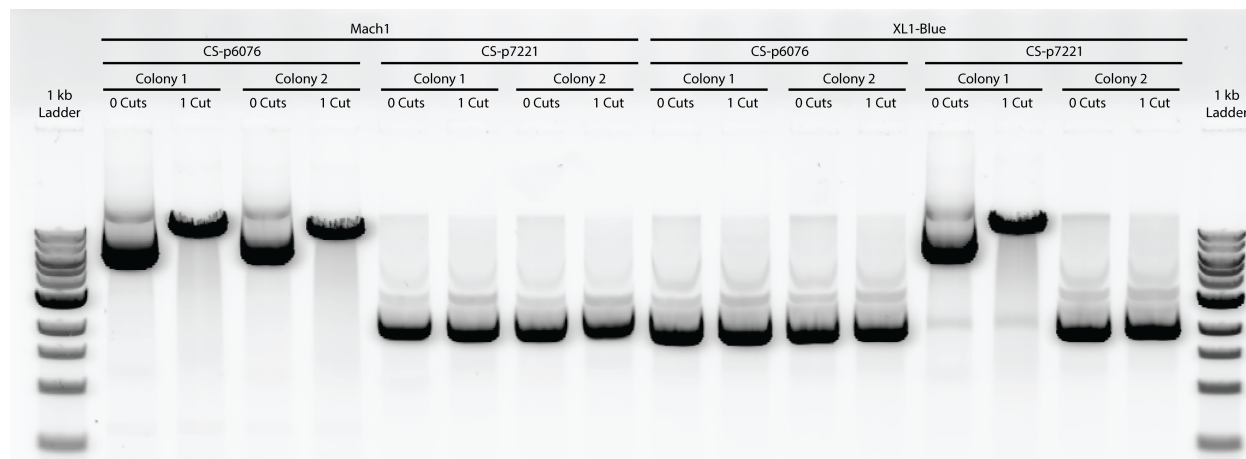


Figure 12: Miniprep Products from Attempted Single-Step Cloning of 6kb and 7kb Scaffolds

While successful clones could be obtained, only three of the eight screened colonies contained the correct product. This is actually relatively high efficiency compared to some of our other attempts, where many colonies would contain an undesired recombination product, meaning that often dozens of colonies would need to be screened to find a successfully cloned plasmid. While this was not impossible, we sought to improve the efficiency of inserting longer sequences into the pScaf vector.

Specifically, we chose to make two changes to the cloning strategy. First, we decided to break down large sequences into multiple smaller inserts that could be incorporated sequentially. Second, we chose to utilize the PvuI and BsaI sites in the antibiotic resistance gene for cloning, such that unsuccessful ligation products – i.e. those in which the ampicillin-resistance gene is not properly restored – can be easily selected against. We are able to complete this cloning, partially summarized below, using a multi-step process. First, we create our initial part vectors by PCR amplifying regions of interest, typically in the sub-3kb range with BamHI sites on one side and adjacent KpnI/BglII sites on the other side. We can then clone this sequence into the pScaf vector at BamHI and KpnI, while introducing a BglII site on the upstream end of the insert in the process. We can use this process to create a library of “part vectors”. The schematic below shows part vectors pScaf-A and pScaf-B. In addition to the BamHI and BglII sites flanking each insert, the part vectors contain PvuI sites within the ampicillin-resistance gene (they also contain BsaI in the event that PvuI cannot be used due to its presence in an insert sequence). We can then assemble these part vectors into larger vectors by performing selective digests and ligations. For example, as shown below, if pScaf-A is digested with PvuI and BamHI, while pScaf-B is digested with PvuI and BglII, the recovered fragments containing the inserts may be ligated to create pScaf-AB. This takes advantage of the fact that BamHI and BglII have compatible sticky ends, allowing the sites cut with these enzymes to ligate together, while producing a sequence at the ligation site that is no longer recognized by either enzyme – that is to say, the restriction site is destroyed upon ligation. This results in a new vector, pScaf-AB which contains the inserts from pScaf-A and pScaf-B, while maintaining the

ampicillin-gene-PvuI, the upstream BglII, and the downstream BamHI. This allows for the addition of subsequent inserts to the vector using the same process, allowing for the production of increasingly large custom-sequence inserts. Additionally, because the cloning requires the splitting of the antibiotic-resistance gene, this allows for facile selection of the correctly ligated product, greatly increasing the cloning efficiency compared to our previous strategies. Additionally, it should be noted that insertion order of the custom inserts can be altered by modifying the restriction enzymes used on each part. For example, if we cut pScaf-A with BglII instead of BamHI, and pScaf-B with BamHI instead of BglII, the resulting ligation product would be pScaf-BA, where the “B insert” would appear upstream of the “A insert”.

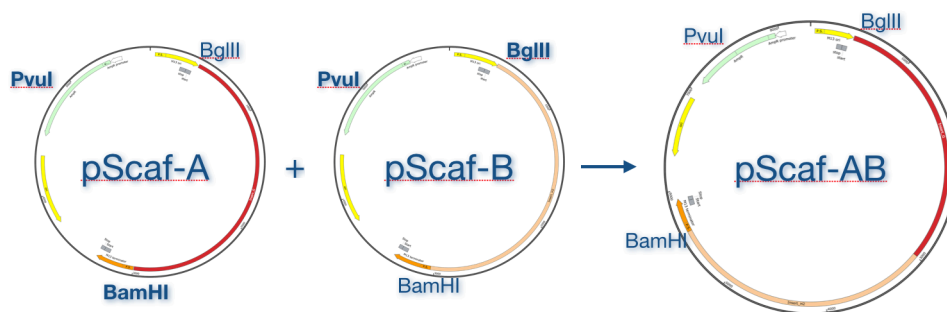


Figure 13: Stepwise Cloning Strategy

We were able to use this cloning strategy to create a series of custom pScaf vectors that would produce ssDNA sequences of size 5544, 7560, 8064, and 10080. Additionally, we are able to easily build larger vectors by assembling any number of existing pScaf vector variants using the cloning strategy outlined above. Each of our custom pScaf constructs was transformed into *E. coli*, where the phage was produced.

We then recovered the custom ssDNA from the phage. At this point, we had also transitioned to using helper plasmid in place of helper phage, resulting in more pure products. The gel below depicts some of the scaffolds produced from our pScaf constructs generated with either one, two, three, or four inserts.

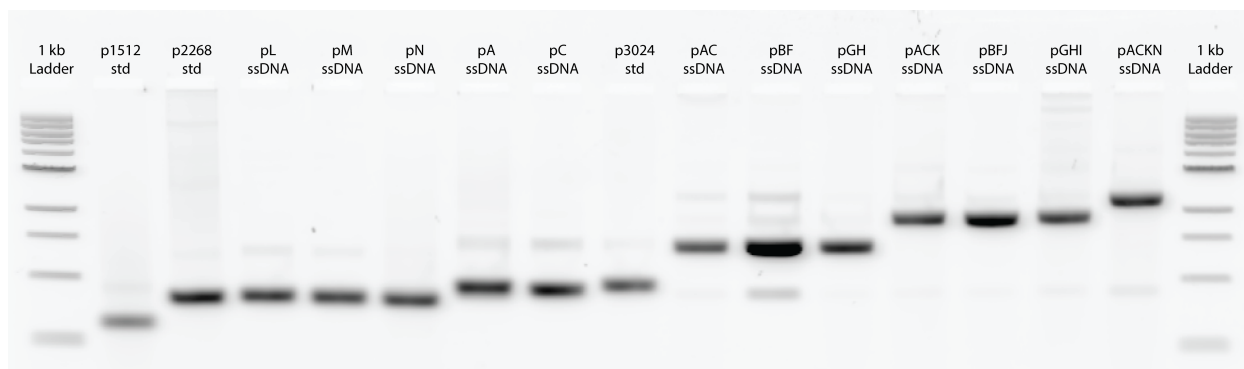


Figure 14: Sampling of Custom ssDNA Scaffolds Produced from Various pScaf Constructs

In the above gel, the two-insert constructs pAC, pBF, and pGH produce scaffolds of size 5509. In order to produce a scaffold of a size that is more useful for typical origami geometries, we also set out to build a set of 5544-base scaffolds. Like the p5509 scaffolds, these also use two parts, one of which is equivalent to the ones used in p5509. For example, pH'C uses the same part vector C used in pAC, while H' is the part vector H used in pGH, but modified to be 35 bases longer. These are summarized in the following gel.

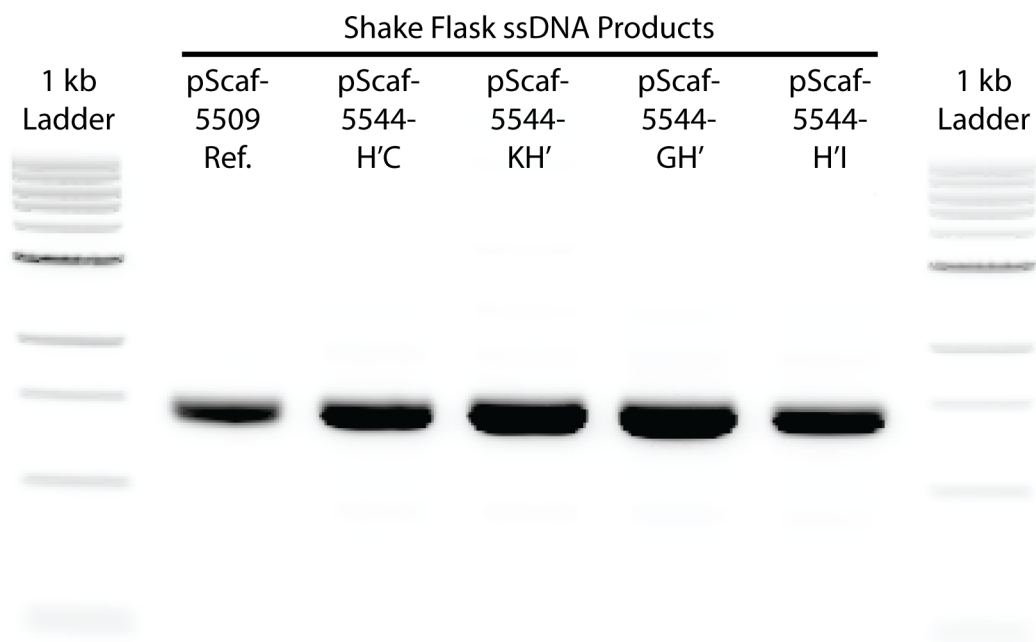


Figure 15: 5544-Base Scaffolds Produced from pScaf Constructs

Another gel compares the three different 8064-base scaffolds produced using the stepwise assembly method: 8064-ACK, -BFJ, and -GHI. These are placed alongside a commercially purchased non-custom 8064-base scaffold derived from standard M13.

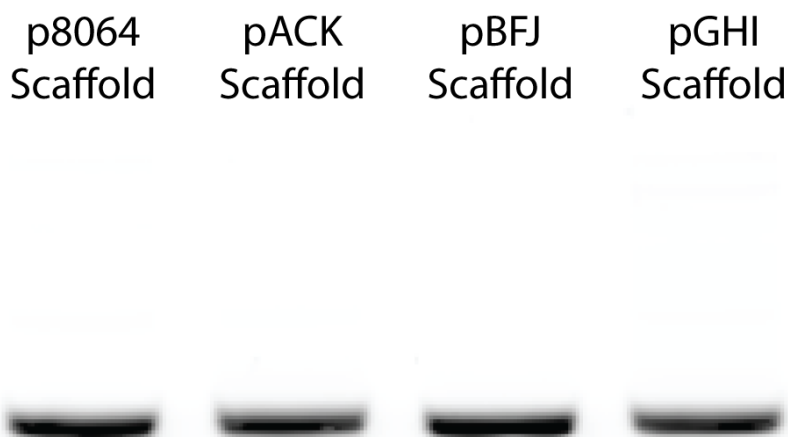


Figure 16: Comparison of Custom p8064 Scaffolds to Commerical p8064

Additionally, we attempted to produce a series of 10080-base scaffolds using a four-insert assembly method. Six of these attempts are depicted in the gel below, alongside the 8064-base constructs that were used as precursors prior to the final insertion. Interestingly, only one of the p10080-base scaffolds, pACKN, produced the correct product.

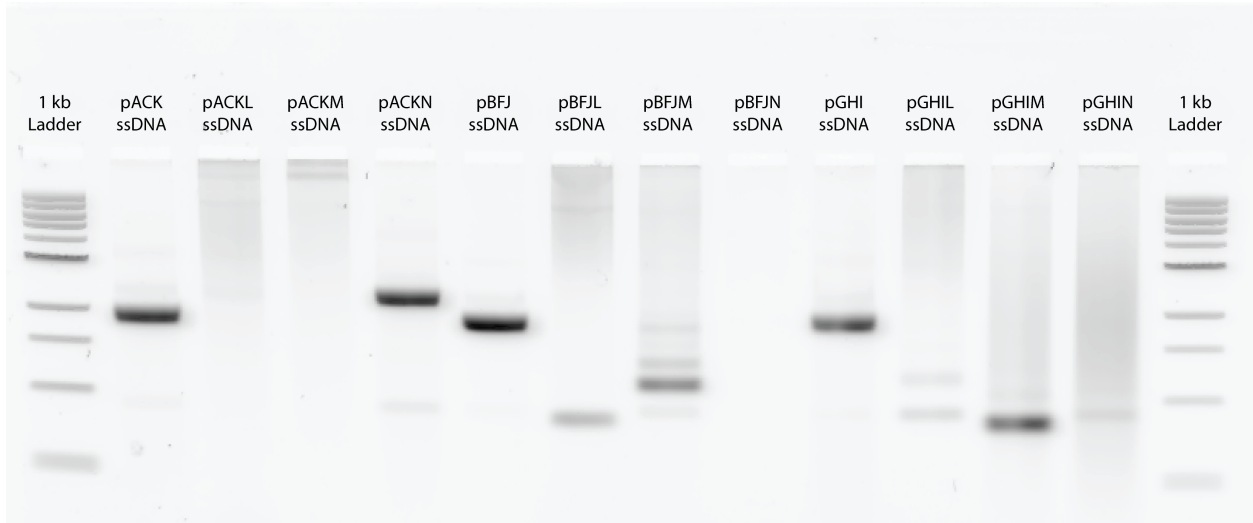


Figure 17: 10080-Base Scaffolds Produced from pScaf Constructs

Interestingly, however, when we attempt to produce these scaffolds with helper phage, we obtain wildly different results.

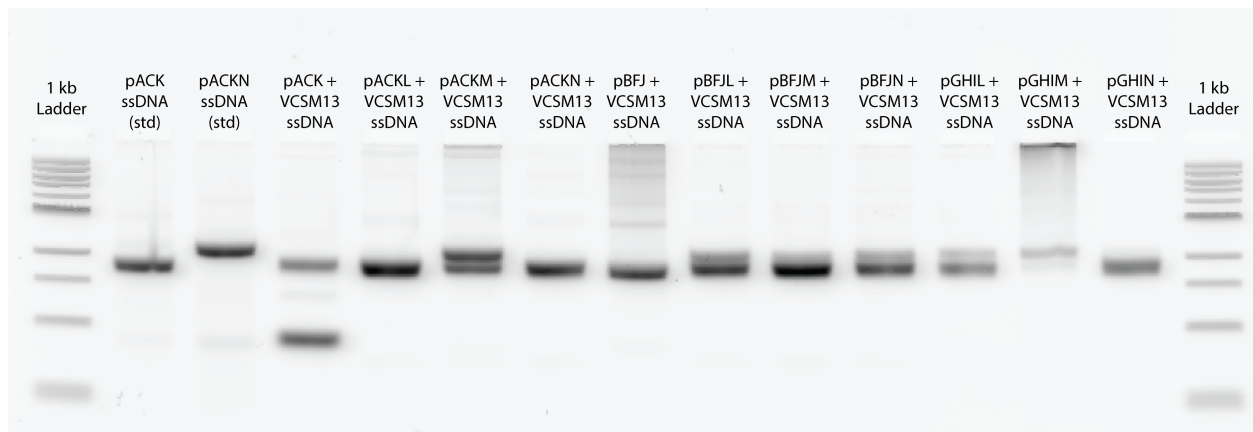


Figure 18: 10080-Base Scaffolds Produced from pScaf Constructs with Helper Phage

pACKN, while was successful in helper plasmid, does not produce a product with helper phage, while other constructs, such as pACKM, now do. It is not clear if this is a sequence-related issue or simply colony-dependent. Nevertheless, we proceeded with pACKN as our preferred p10080 construct.

All of the part vectors and pScaf constructs produced are summarized in the following figure, along with summarized results of the attempted ssDNA production (if applicable).

Round 1					
Part	Scaffold Length	Successful Cloning?	ssDNA Recovery Attempted?	ssDNA Recovered? (helper plasmid)	
A	2954	✓	✓	✓	
B	2954	✓			
C	2954	✓	✓	✓	
D	2954	✗			
E	2954	✗			
F	2954	✓			
G	2954	✓			
H	2954	✓			
H'	2989	✓			
I	2954	✓			
J	2954	✓			
K	2954	✓			
L	2415	✓	✓	✓	
M	2415	✓	✓	✓	
N	2415	✓	✓	✓	

Round 2					
Part1	Part2	Scaffold Length	Successful Cloning?	ssDNA Recovery Attempted?	ssDNA Recovered? (helper plasmid)
A	C	5509	✓	✓	✓
B	F	5509	✓	✓	✓
G	H	5509	✓	✓	✓
H'	C	5544	✓	✓	✓
H'	I	5544	✓	✓	✓
G	H'	5544	✓	✓	✓
K	H'	5544	✓	✓	✓

Round 3							
Part1	Part2	Part3	Scaffold Length	Successful Cloning?	ssDNA Recovery Attempted?	ssDNA Recovered? (helper plasmid)	ssDNA Recovered? (helper phage)
A	C	K	8064	✓	✓	✓	
B	F	J	8064	✓	✓	✓	✓
G	H	I	8064	✓	✓	✓	

Round 4								
Part1	Part2	Part3	Part4	Scaffold Length	Successful Cloning?	ssDNA Recovery Attempted?	ssDNA Recovered? (helper plasmid)	ssDNA Recovered? (helper phage)
A	C	K	M	10080	✓	✓	✗	✓
A	C	K	N	10080	✓	✓	✓	✗
A	C	K	L	10080	✓	✓	✗	✗
B	F	J	N	10080	✓	✓	✗	✓
B	F	J	L	10080	✓	✓	✗	✓
B	F	J	M	10080	✓	✓	✗	✓
G	H	I	L	10080	✓	✓	✗	✓
G	H	I	M	10080	✓	✓	✗	✓
G	H	I	N	10080	✓	✓	✗	✗

Figure 19: Summary of pScaf Part Vectors and Constructs

The gel below also highlights a subset of these custom sequence scaffolds that we subsequently used for origami studies. The 8064-base scaffold depicted here is from our pScaf-8064-BFJ, which is one of five different custom 8064-base scaffolds we have produced, while the p5544 scaffold depicted here is from pScaf-5544-GH'.

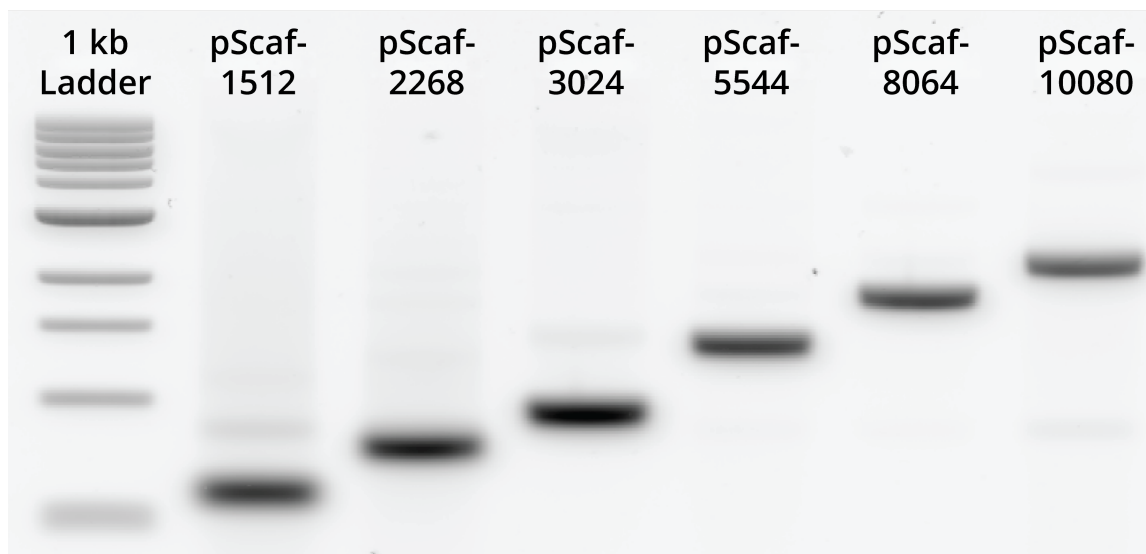


Figure 20: Gel of Custom ssDNA Scaffolds Used for Origami Studies

Furthermore, we sought to show that these custom scaffolds could be used to fold DNA origami in the same manner as traditional non-custom scaffolds. To do so, we designed a series of DNA origami shape that could be folded from the scaffolds. Initially, we folded a set of six-helix bundle nanotubes of increasing size from the 1512-, 2268-, and 3024-base sequences. We attempted to determine the proper folding conditions for screening properties such as magnesium concentration and folding ramp. An origami gel-shift assay, depicting shifts between the pure scaffold strand and the folded origami structure resulting from mixing the scaffold strand with oligonucleotide staples, which was used to perform a partial magnesium screen for a particular folding ramp, is shown below.

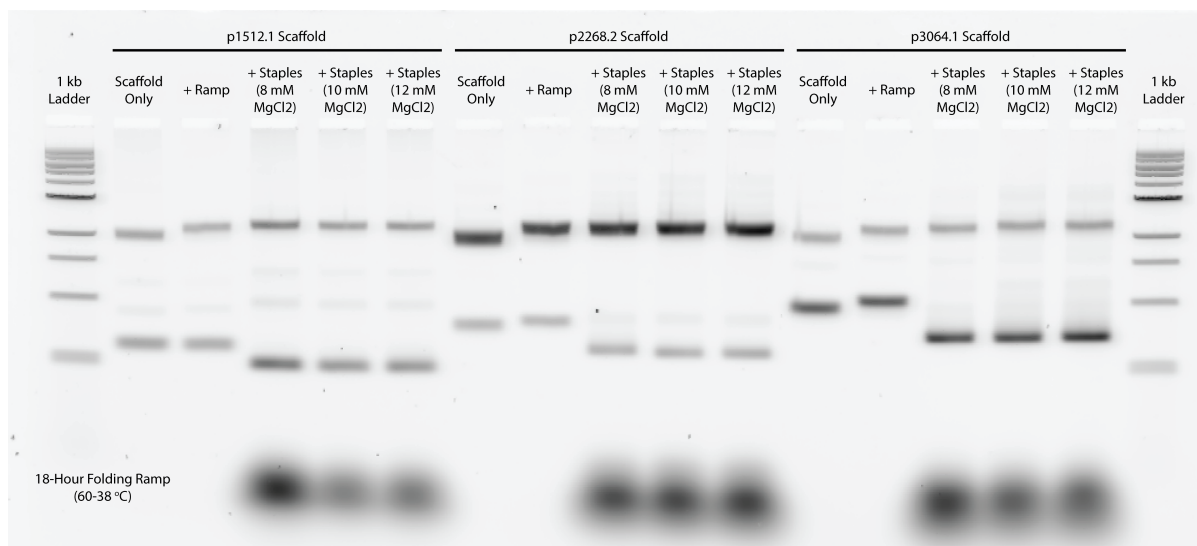


Figure 21: Magnesium Screen for p1512, p2268, and p3024 Nanotube Origami

Furthermore, in order to demonstrate the orthogonality of the scaffold sequences, we showed that we could fold three different nanotubes from three different scaffolds in a single-pot reaction. Additionally, we showed that these separate nanotubes could be induced to fold into a multimeric nanotube consisting of all three scaffolds in a single reaction. These mixed scaffold and origami conditions, along with the original monomeric origami products are summarized in the following gel.

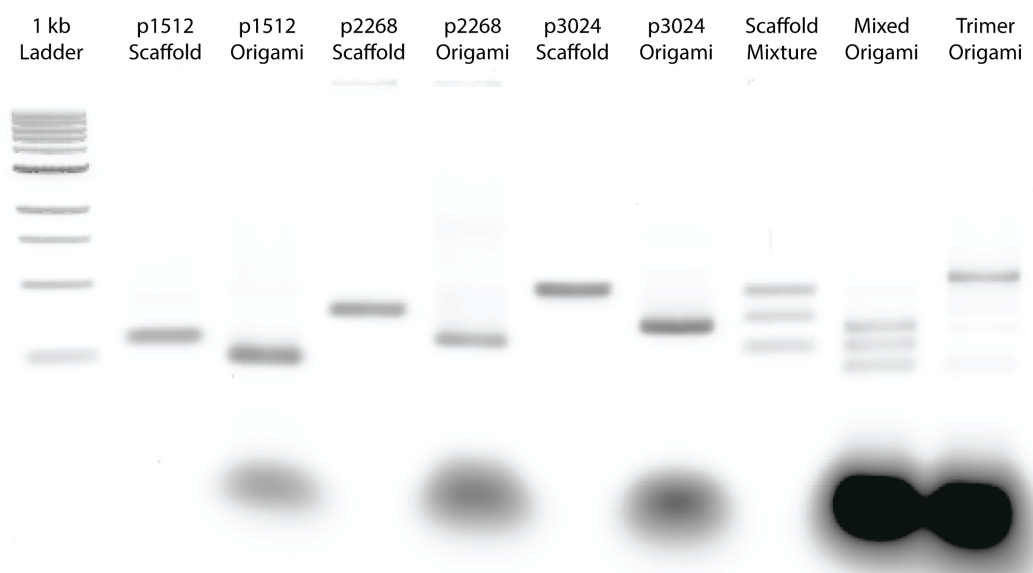


Figure 22: Gel of Nanotube Origami Structures

In the above gels, we observe characteristic shifts as origami staple sets are added to each scaffold. The down-shifted product represents the folded origami, while the dark and fuzzy bands at the bottom represent excess staples. However, obtaining the trimer origami was not a simple endeavor. We first looked at two different methods for end-to-end attachment, labeled “Trimer v1” and “Trimer v2” in the following gel. We also explored the effect of leaving the non-assembling interfaces free of staples (“uncapped”) or with poly-T overhangs that would minimize unwanted assembly (“capped”). We observed that capping the origami yielded better trimers, while both attachment methods provided similar results (when the non-assembling ends were capped).

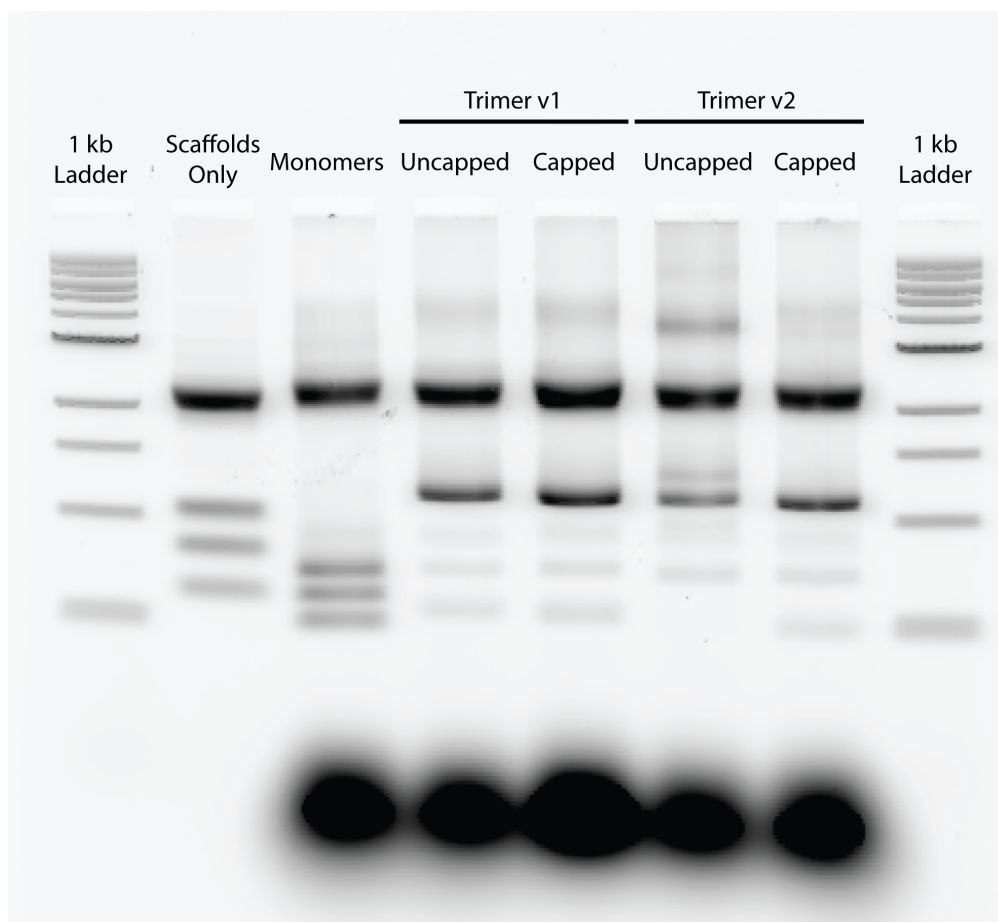


Figure 23: Evaluation of Assembly Methods for Trimeric Superassemblies

While the trimer appeared to form robustly under many stoichiometries, monomer or dimer bands would often remain present if the scaffold concentrations were not tuned properly. Therefore, we had to screen several different relative concentrations until the nearly perfect trimer was achieved. The following gel summarizes a subset of these conditions.

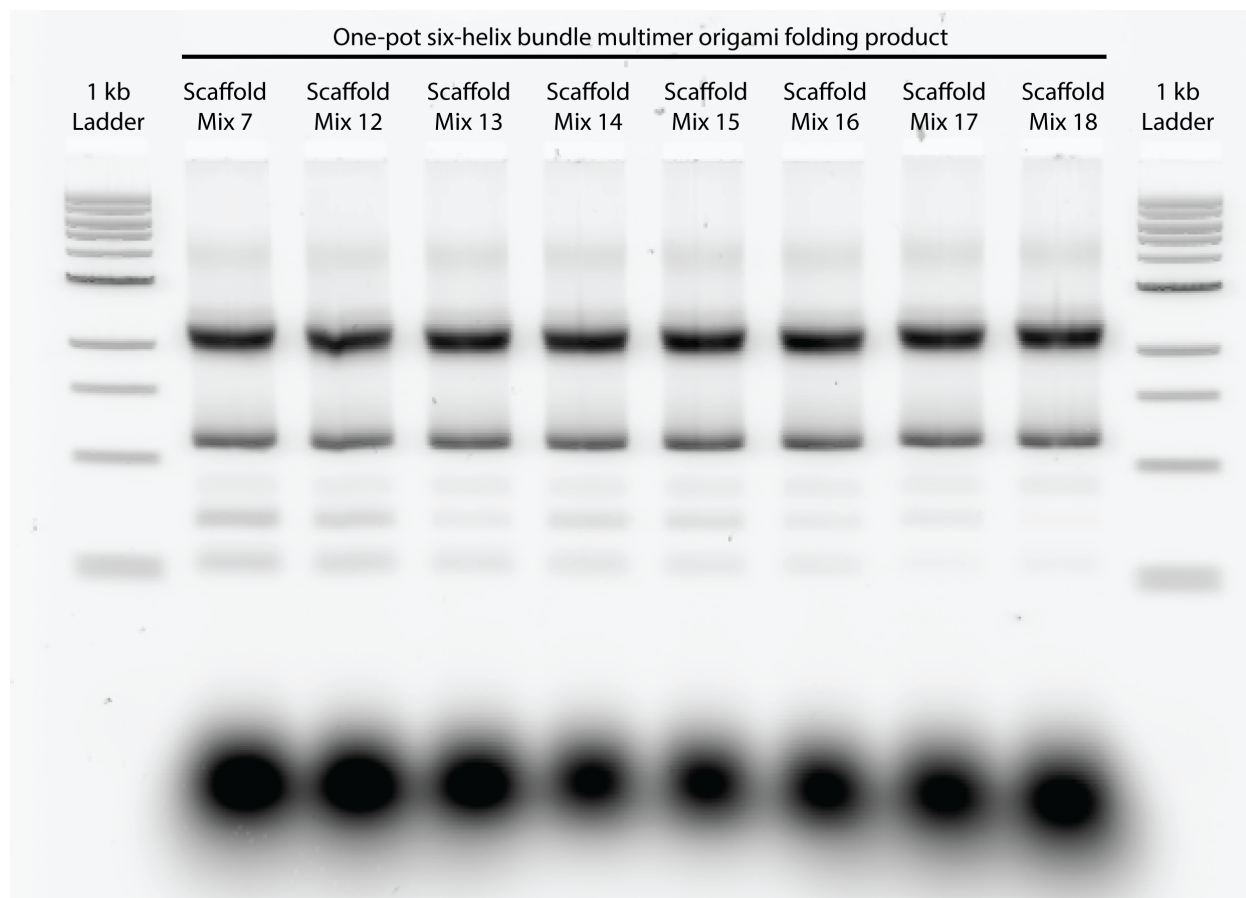


Figure 24: Effect of Scaffold Stoichiometry on Trimeric Superassembly Formation

We were further able to characterize these nanotubes by performing transmission electron microscopy (TEM) of the individual monomers and the trimer. In the micrographs below, class-averaged particles are displayed for the 1512- and 2268-base nanotubes. Meanwhile, due to the floppy nature of the longer nanotubes, we were unable to obtain class averages of the 3024-base and trimeric structures. Therefore, we

display representative images of those. Finally, we were able to observe that the measured size of each nanotube agrees with the expected size based on our original designs.

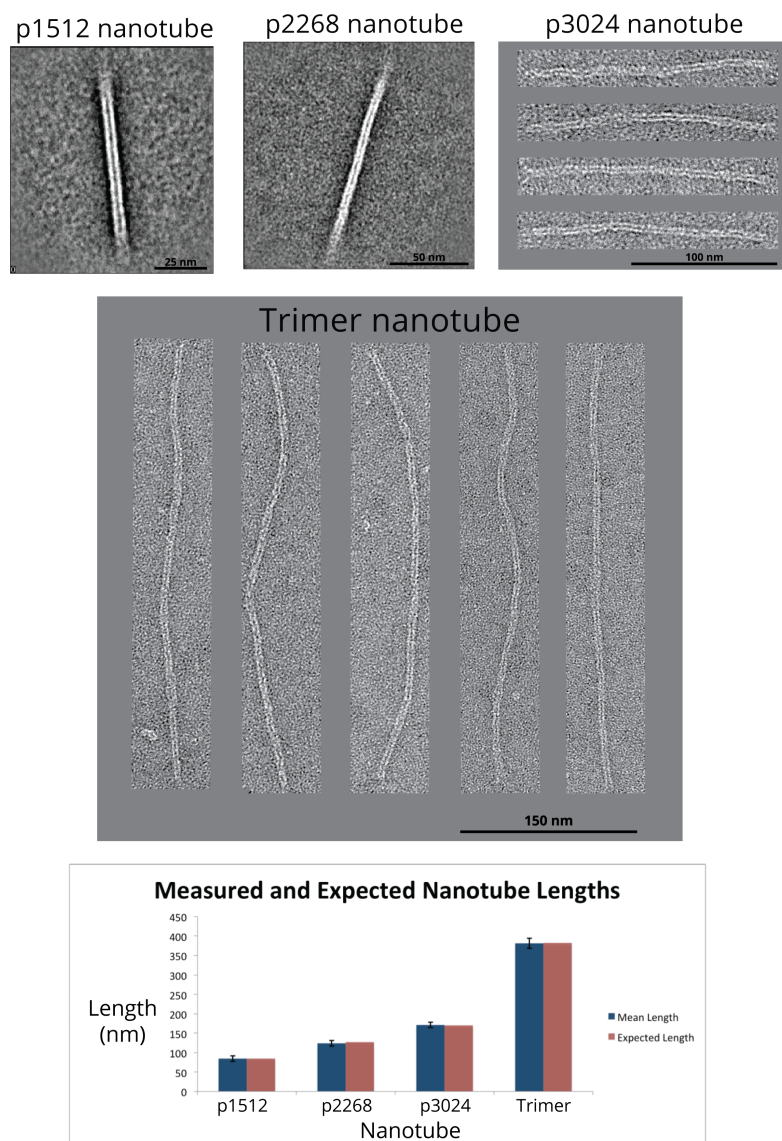


Figure 25: TE Micrographs and Size Analysis for Origami Nanotubes

As a side note, when we first began producing the p1512, p2268, and p3024 scaffolds, we had been using helper phage as the source of phage proteins. For that reason, many of the above gels (except those that were updated and re-run later with products

produced with helper plasmid) contain the additional helper phage band. When we first began making the transition from helper phage to helper plasmid, we were surprised to see that the helper plasmid system appears to completely fail when cultures are grown at 37°C. Meanwhile, at 30°C, it appears to behave better, as summarized in the following gel.

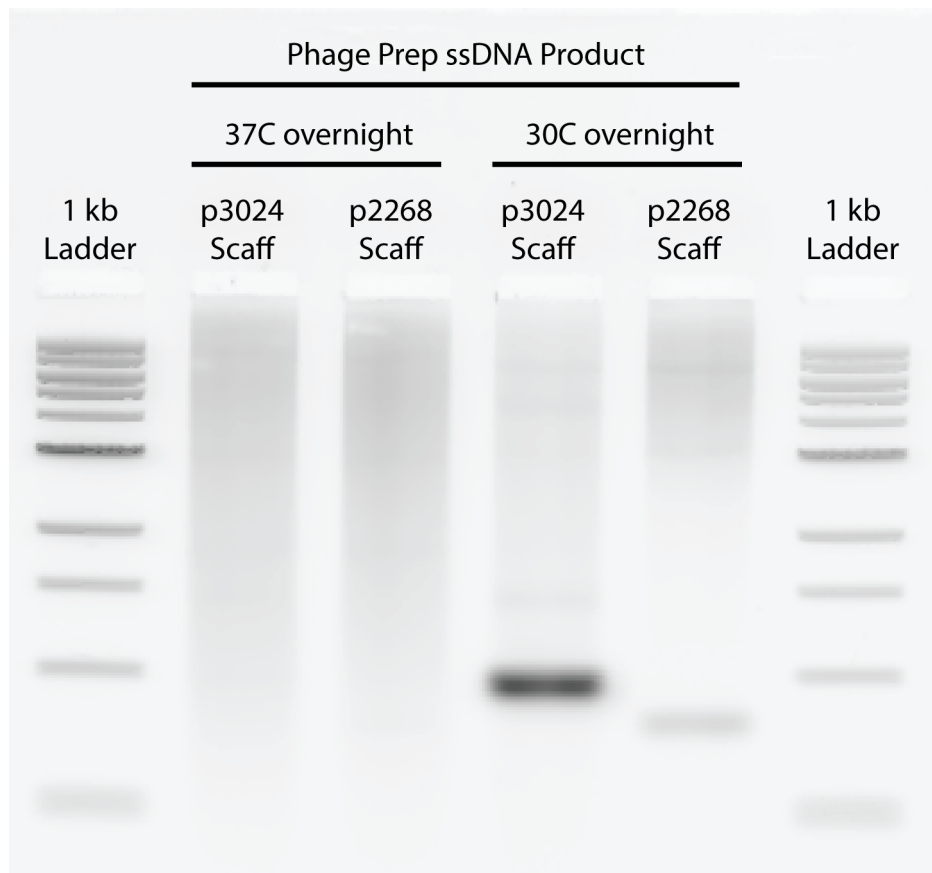


Figure 26: Effect of Temperature on Scaffold Production with Helper Plasmid

Additionally, we designed a series of brick-like origami structures, which we folded from our 1512-, 2268-, 3024-, 5544-, 7560-, 8064-, and 10080-base custom scaffolds. The three smallest bricks each contain the same number of helices, but are stretched out further to accommodate for the increase in scaffold bases. Meanwhile, the 5544-base scaffold and larger were used to build bricks with increasing numbers of helices. We

first attempted to fold the p8064-ACK and p10080-ACKN scaffolds into brick-like shapes. For these shapes, we screened magnesium concentration, as well as the presence of sodium chloride in the folding reaction. The gel below displays the results of this screen.

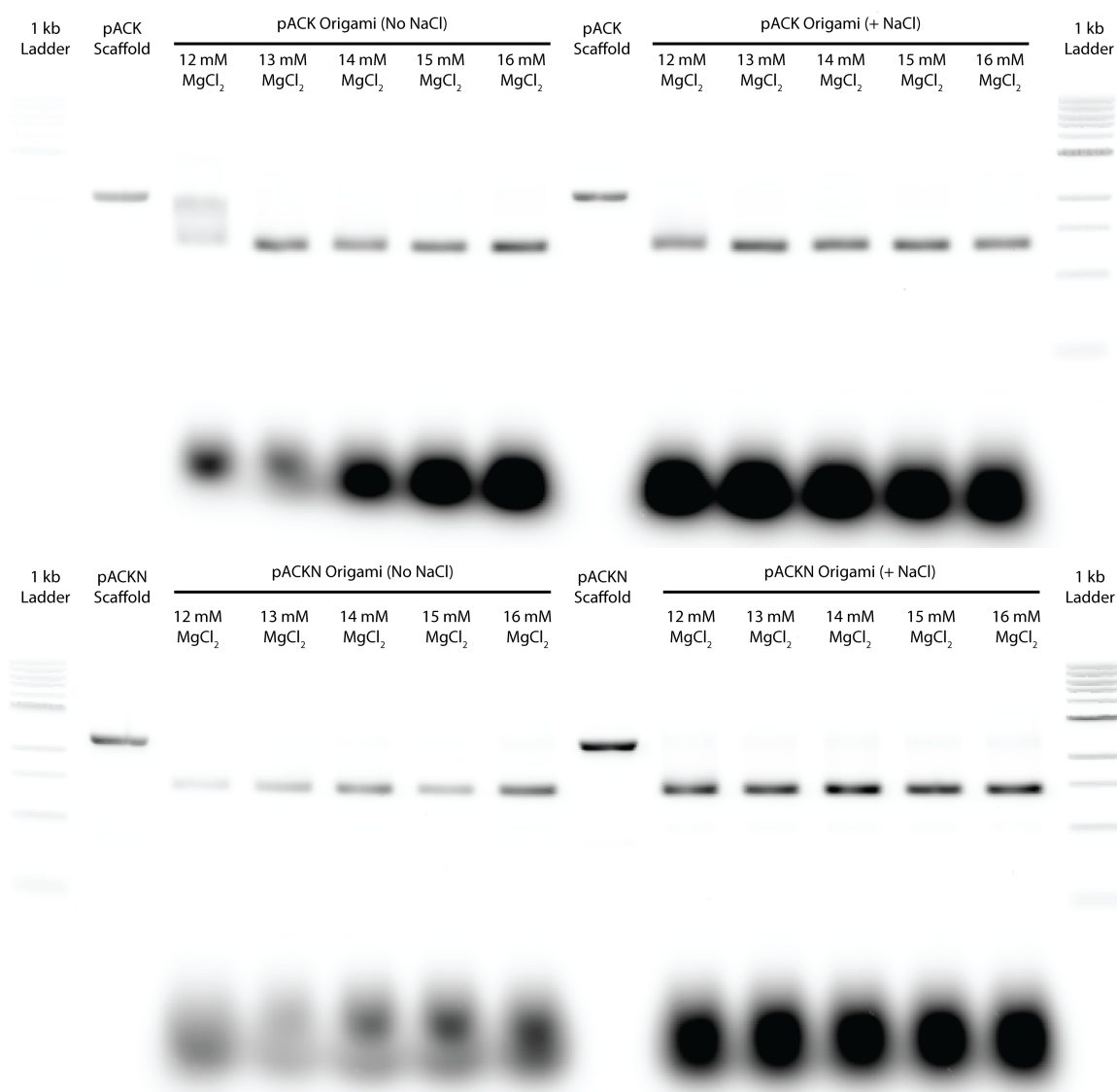


Figure 27: Magnesium and NaCl Screen for Folding Brick Origami from pACK and pACKN Scaffolds

We then performed a similar screen for two other 8064-base custom scaffolds. In this case, however, all folding reactions contained NaCl and only magnesium concentration was varied.

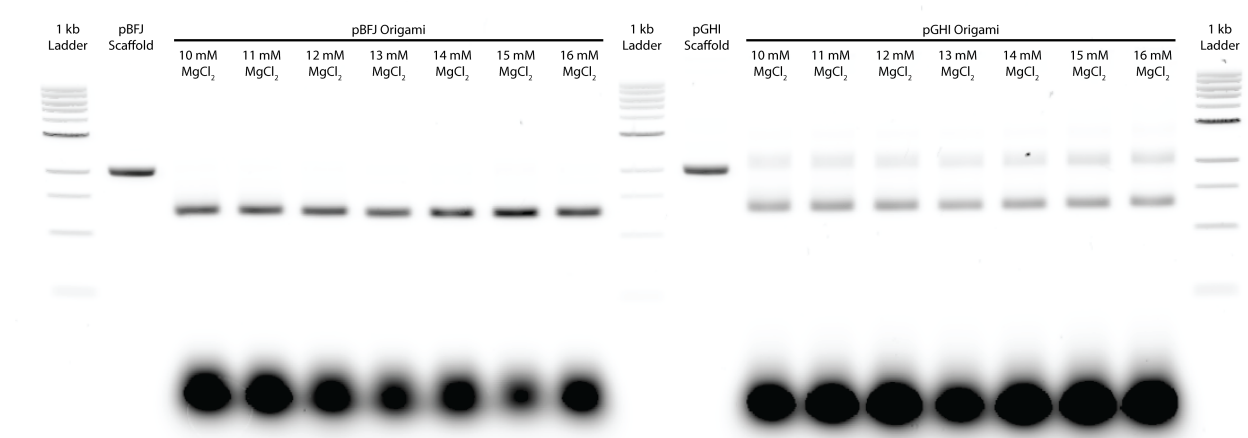


Figure 28: Magnesium Screen for Folding Brick Origami from pBFJ and pGHI Scaffolds

The gel shifts for each of these bricks folded under their optimal conditions is also summarized below.

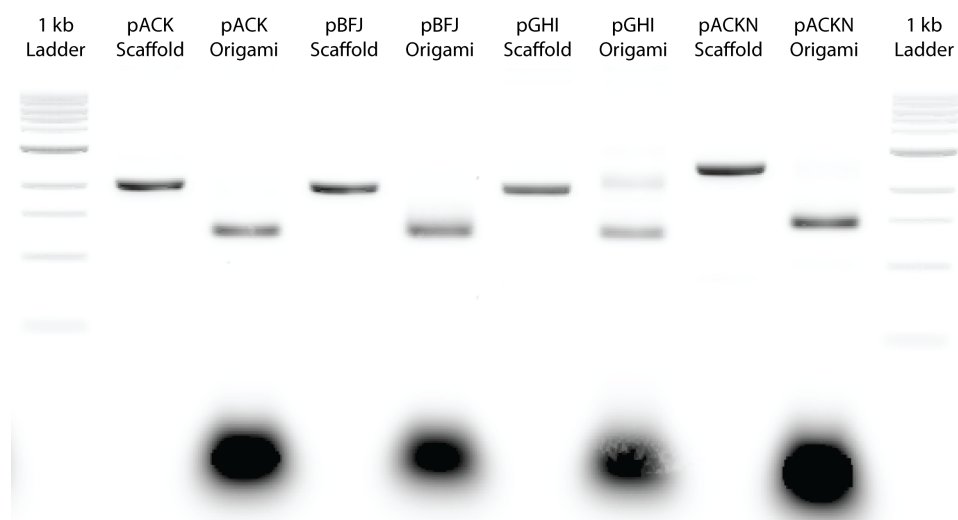


Figure 29: Gel Shift Assay for 8064- and 10080-Base Custom Scaffold Brick Origami

The agarose gel summarizing a panel of folded origami brick structures from six of our scaffolds is reproduced below. As with the similar scaffold panel above, the 8064-base custom scaffold depicted here is the pBFJ variants.

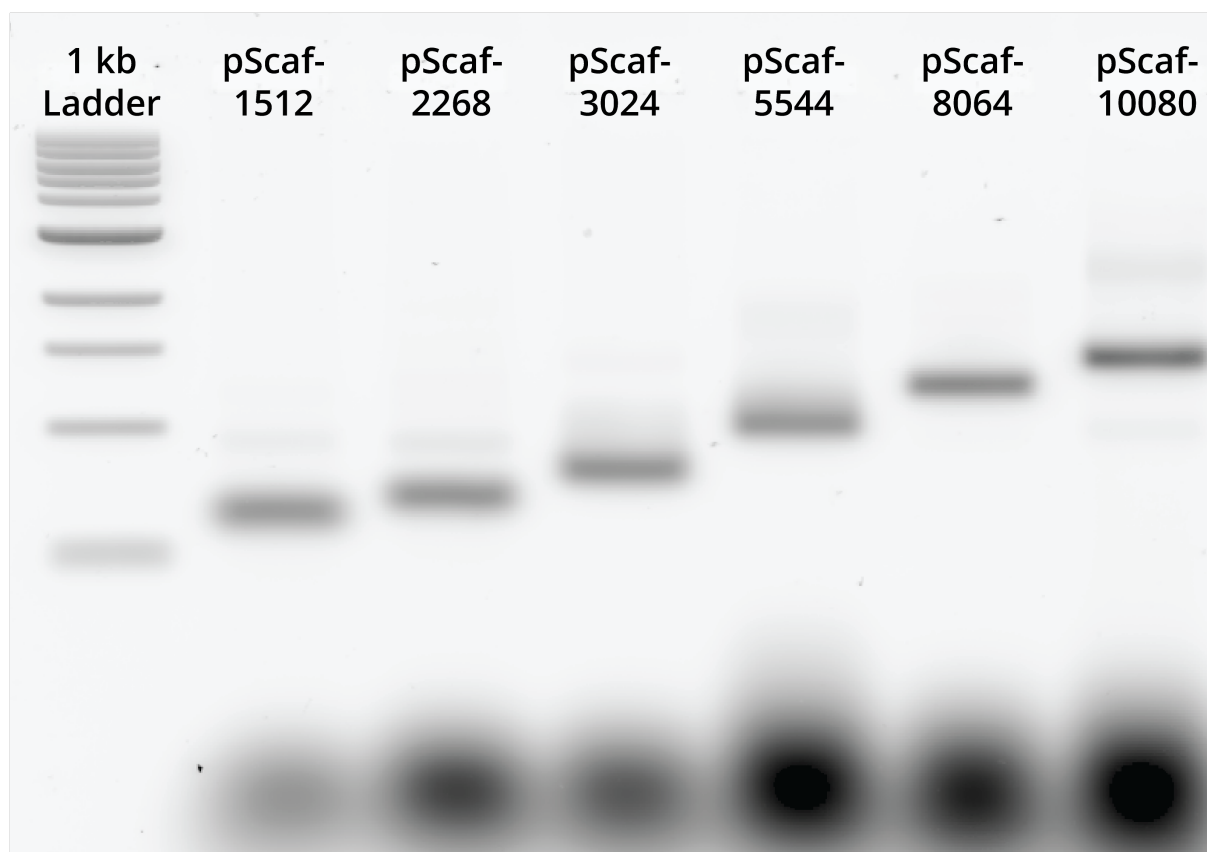


Figure 30: Gel of Brick Origami Structures

Prior to imaging, we used a method called PEG fractionation to separate folded origami from excess staples. In short, the origami reaction product is mixed with a PEG buffer to a final concentration of 7.5% PEG. The mixture is then centrifuged to pellet the origami, while keeping the staples in the supernatant. While this pellet is often invisible, careful removal of the supernatant and resuspension of the pellet generally results in highly pure origami. The results of this purification are summarized below for the p8064, and p10080 Bricks.

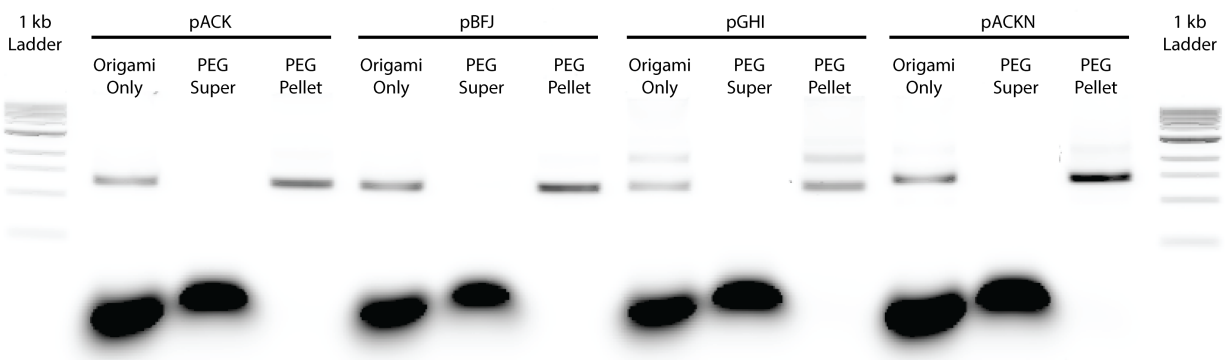


Figure 31: PEG Fractionation of p8064 and p10080 Brick Origami from Excess Staples

For the p5544 brick, we were unsure if 7.5% PEG would be sufficient. Therefore, we screened a series of concentrations, as depicted below.

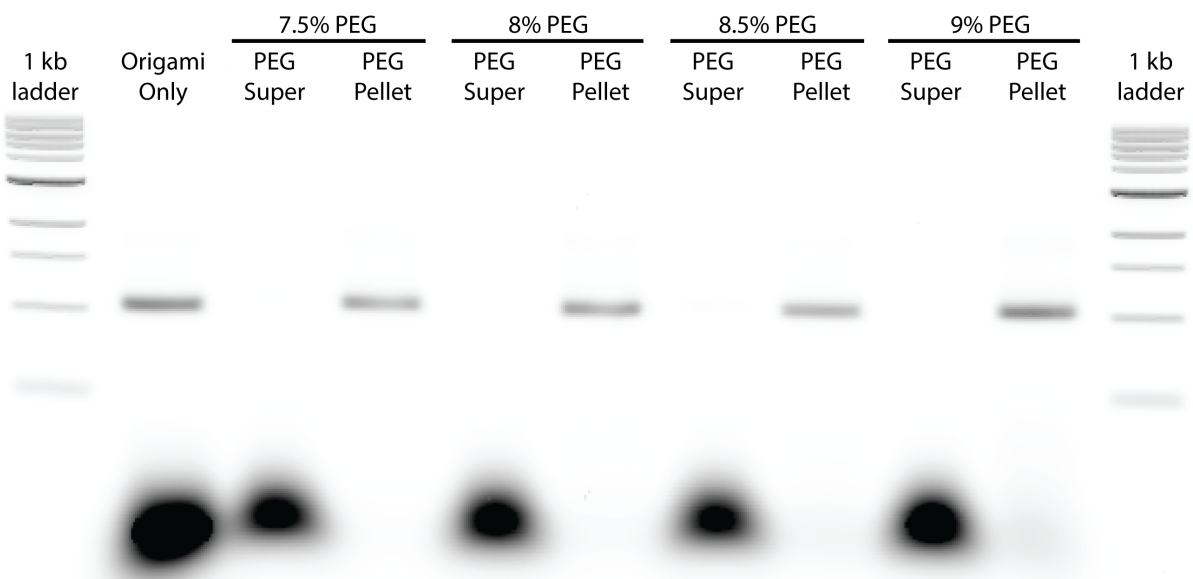


Figure 32: PEG Fractionation of p5544 Brick Origami from Excess Staples

We determined that 7.5% PEG was indeed sufficient to precipitate and purify the p5544 brick. For our p1512, p2268, p3024, and p5509 bricks, however, we were unable to find a PEG concentration that cleanly purified them. Therefore, we used gel purification to separate those structures from excess staples, as we had done for the nanotubes.

After TEM imaging, the raw micrographs can be imported into software such as EMAN2⁴², which can be used to pick individual origami particles and obtain averages of the structures. Using EMAN2, we generated average particles for 7 brick shapes, as shown below.

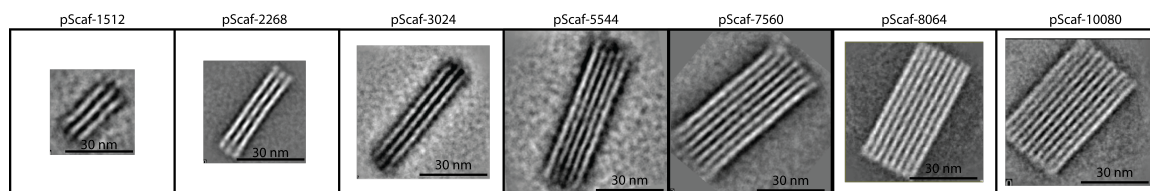


Figure 33: Class-Average TE Micrographs of Brick Nanostructures

It would also be prudent to point out a few additional scaffolds which we produced using methods other than or stepwise assembly strategy. Two pairs of additional custom p7560 and p8064 scaffolds were produced. One set was derived from regions of the lambda genome, and the other from regions of the coding region for dynein. In both cases, each pair shares the same 7560-base region, with the 8064-base product containing an additional 504 bases. These were also produced using a two-step cloning process, but using a more traditional strategy, where the inserts are simply ligated end-to-end without making use of restriction sites in the antibiotic resistance gene. The p7560-lambda scaffold was used to produce the origami brick whose EM class average is pictured above. The production of these scaffolds is summarized on the following gels

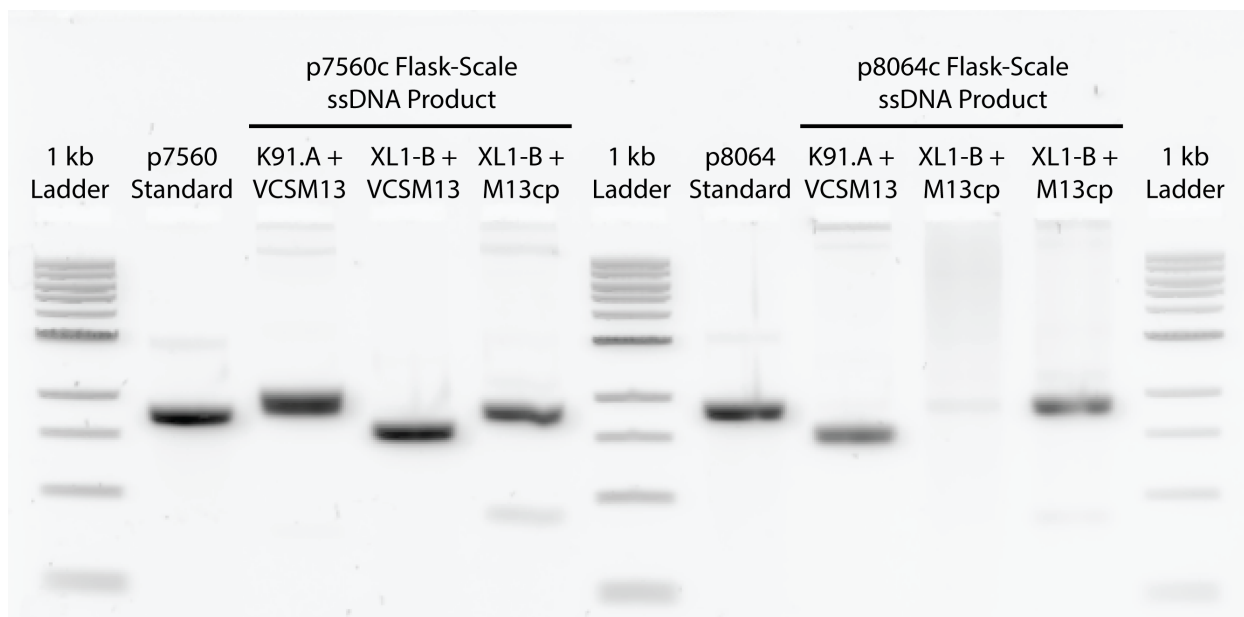


Figure 34: Custom p7560 and p8064 Scaffolds Derived from Dynein

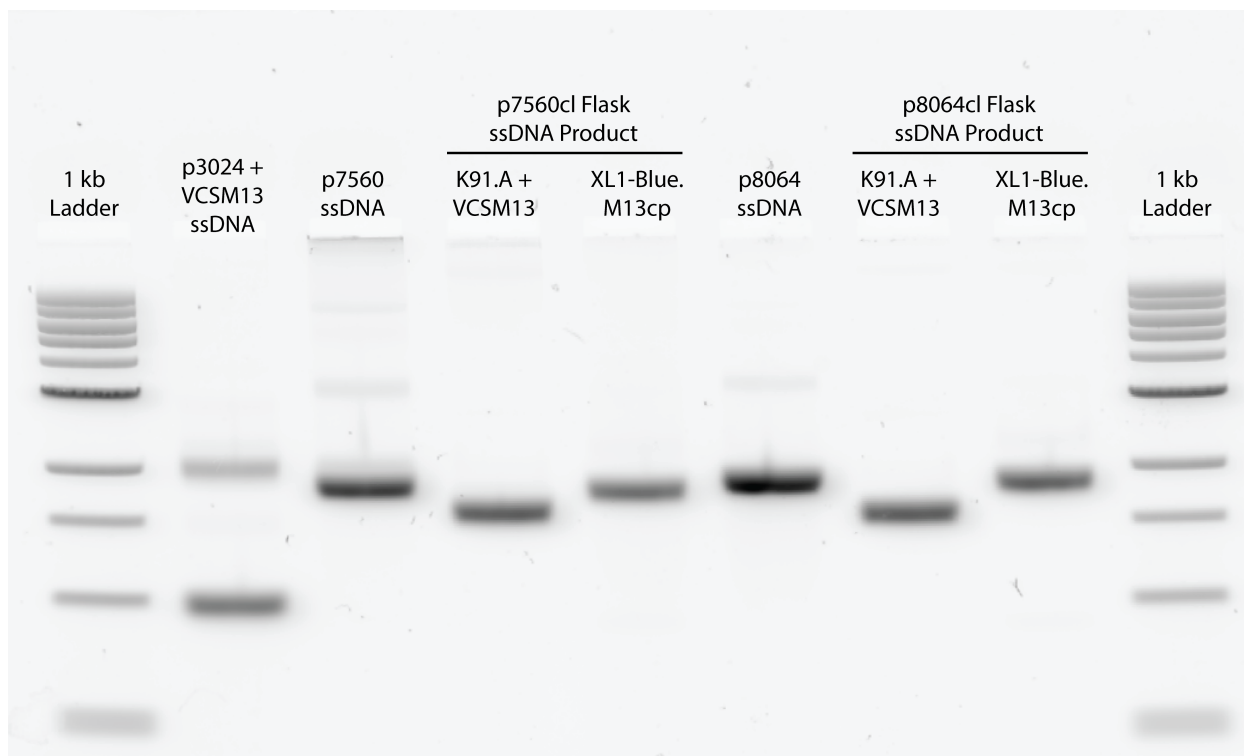


Figure 35: Custom p7560 and p8064 Scaffolds Derived from Lambda

One interesting observation with these scaffold was that while they appeared to produce the desired product in helper plasmid (M13cp), they failed to do so when produced in the presence of helper phage (VCSM13).

Additionally, prior to producing the p10080 scaffold discussed above, we produced a p10087 scaffold by making a 2023-base addition to p8064-lambda. The ssDNA product from a series of flasks is reproduced below.

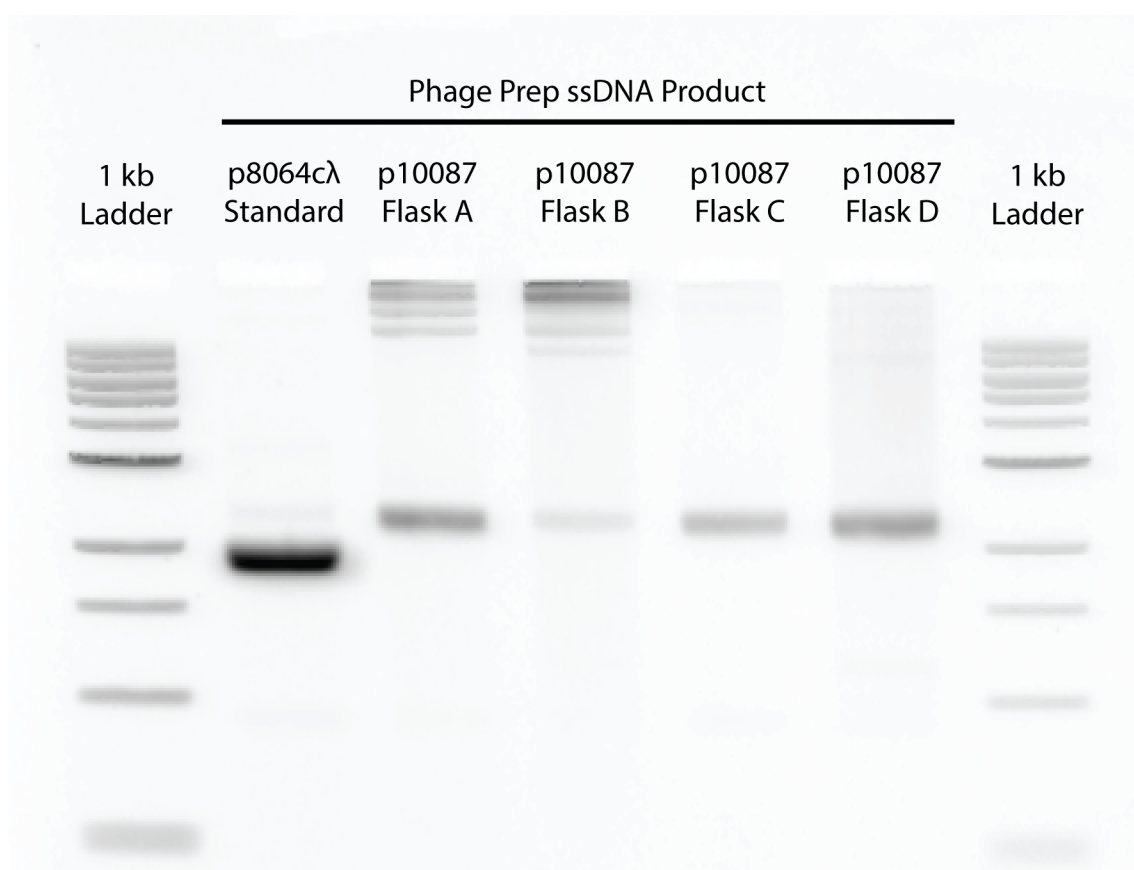


Figure 36: Custom10087-Base Scaffold

IV. Applications for Custom Scaffolds

Reusable Staples

One of the key barriers to the proliferation of DNA origami applications in areas, such as therapeutics, is the difficulty in producing DNA origami at large scale in a cost-effective manner. As I have discussed, the production of M13-based scaffolds is simple and scalable, as has been demonstrated by our work in bioreactors and work published by the Dietz Lab. Staple synthesis, however, is the cost-limiting step. It has been reported that the cost to produce enough staples to fold 1 gram of traditional DNA origami is \$100,000, while the cost to produce enough scaffold is much lower. As a result, methods that use a fewer number of unique staples may be potentially more cost-effective to produce. For example, this could include Niekamp's work in our lab where a reusable pool of staples is used to fold different custom scaffolds into different shapes⁸. With our pScaf vector, we now have a strategy to easily produce scaffolds of user-defined size and sequence. This can be coupled with a pool of reusable staples to prototype many different structures without having to synthesize a unique pool of staples for each.

ssOrigami

Similarly, custom scaffolds enable researchers to develop more ssOrigami designs, like the Shih Octahedron⁴, that rely on far fewer staples than scaffolded DNA origami to fold into a target shape. The complete elimination of staples from the assembly reaction would go a long way in allowing for scalable mass-production of DNA nanostructures. In a very recent paper from Han and Qi and collaborators in the labs of Hao Yan and Peng Yin, a method is designed for the design of scaffolds that will self-fold into a user-defined structure⁴³. To validate these methods, the authors generated scaffolds using non-scalable methods such as PCR, exonuclease digest, endonuclease digest, and gel-purification. However, scalable methods such as the pScaf vector may now be applied to generate these self-folding scaffolds.

Additionally, members of our lab are currently developing an updated version of cadnano. Among its many new features, this version now allows for the design of self-complementary scaffolds. This new design tool will enable researchers to more easily design scaffolds that could be used for ssOrigami. Our pScaf vector could then be used to generate the scaffolds designed *in silico* using cadnano.

Hierarchical Assembly

Additionally, custom scaffolds can allow for the assembly of larger structures than one can typically build traditional M13 scaffolds. Because traditional M13 scaffolds are

primarily composed of the same sequence, superstructures must be built by assembling individual monomers separately, and then performing a subsequent hierarchical assembly step to build larger structures. However, with custom scaffolds, one has the ability to utilize scaffolds that are orthogonal in sequence. As a result, as we have shown, multiple scaffolds can be simultaneously folded into different shapes in a single one-pot reaction. Meanwhile, these different shapes can also be induced to assemble into superstructures in the same one-pot reaction. As a result, custom scaffolds enable the one-pot hierarchical assembly of large DNA nanostructures. In order to further expand these capabilities, we must now look for the assembly of larger scaffolds and more complex shape. This will allow us to demonstrate the reliability of this strategy for a wider range of applications.

V. Further Study for Custom Scaffold Expression

Exploration of Sequence Constraints

As we expand the capabilities of the pScaf vector for production of custom ssDNA scaffolds, it is important to understand if there are any significant size or sequence constraints which would need to be designed around. For example, ssOrigami scaffolds inherently have a greater amount of secondary structure than traditional scaffolds due to the need for self-complementation. It is important to determine if this can increase the difficulty of cloning into the vector in the first place or if it may affect phage ssDNA yield. If such scaffolds appear problematic to clone, these issues may be mitigated by breaking the sequence up into multiple non-problematic parts which are then assembled sequentially. Alternatively, if phage production is the limiting step, one may be able to produce multiple sub-scaffolds which may still be able to fold together into the same target shape as a single complete scaffold.

Exploration of Size Limitations

Additionally, it is important to explore the size limits of custom scaffolds. On the lower end, we have already established a lower limit of 381 bases, which may be produced from an insert-free pScaf vector. On the upper end, the largest scaffold we have produced is approximately 10kb. The previously reported Lambda/M13 hybrid³⁵,

however, packages an approximately 50-kb sequence into a phage particle, suggesting that sequences at that scale are technically feasible. An important follow-up is to attempt to build larger pScaf constructs with inserts ranging from 10kb to 50kb and beyond. It is likely that yield will decrease with increasing size, as a greater number of phage proteins will be necessary to encapsulate the DNA. However, modified growth conditions may be able to mitigate the yield issue. Alternatively, if production of large scaffolds appears to be problematic, hierarchical assembly of nanostructures folded from smaller scaffolds can still be used to generate large structures, as discussed previously.

Production of Custom Scaffolds at Large Scale

Finally, an important consideration is the ability to produce custom scaffolds via the pScaf vector in bioreactors. We have previously demonstrated the ability to produce M13 ssDNA on the order of 100s of milligrams per 1-L culture in bioreactors. We have also applied this to the production of traditional phagemid scaffolds, with or without helper phage, reproducing the original Shih Octahedron scaffold in a pBluescript phagemid vector. The next step is to determine whether this bioreactor-based production protocol may be applied to the production of the modified-phagemid pScaf vector.

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VII. Origami Designs, Sequences, and Micrographs

pScaf-1512 Nanotube

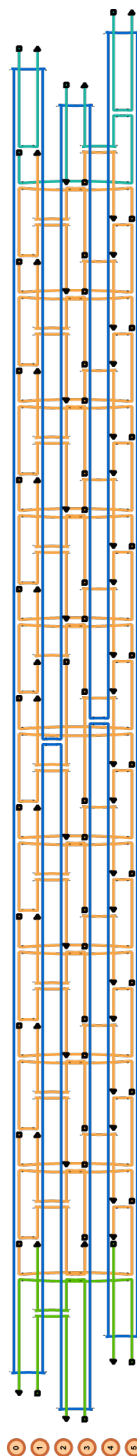


Figure 37: cadnano Design for pScaf-1512 Nanotube

pScaf-1512.1 Scaffold Sequence

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Staple Sequences for pScaf-1512.1 Scaffold

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3[77]	1[97]	GTAAATGTAAGATCGATCTCATAAAAGGTTAGCGC	#f6a444
3[217]	4[210]	ATGATGTTCTTGGATAAGGC	#f6a444
5[168]	2[154]	GACGGGGAAAATTTGTGACCACTGGCATACTTCAGCTCTGGT	#f6a444
3[203]	1[223]	TTTGTGTTGTCAGCCAATACCTTCTCGCAAAACATT	#f6a444
3[196]	4[189]	CCATTCTGTGGATAAGTAATG	#f6a444
3[182]	1[202]	AGTTAACTTTGATAATTAACCTTTGTTTCATATGAT	#f6a444
5[105]	2[91]	TGGGGTCATTCTTCACCTTTATTTGGCTTTAGGTAAATGTAA	#f6a444
5[231]	2[217]	GGGCGCTTACAAATAAAATTTGAACACCTCTTCTTTAAATC	#f6a444
5[42]	3[34]	GGCGAAACCTGGAAATATAGGTTGCGTCTTCGGCGCCTGAGCTCTTTA	#f6a444
3[238]	4[231]	TTATAGTCGCCGCTACAGGGC	#f6a444
3[35]	1[55]	AACTTTGGATGAAGGTCTACTTACTTTTCGGCGCCT	#f6a444

3[133]	4[126]	ACGTCAGTAATGGGCTTCATC	#f6a444
3[224]	1[244]	AAACATTGTGAGAGGTTACCAATAACCGAAAGTAG	#f6a444
3[119]	1[139]	TGAATTTGTTGTCCGAAGGCGGATGAAAAAATAG	#f6a444
0[118]	4[119]	GTGAATAGAGGTGCCGTAAAGGTGTTGC	#f6a444
0[76]	4[77]	TTCTCAGCCCACTACGTGAACTATAAAAT	#f6a444
5[147]	2[133]	GGAGCCCCACCATCTAATTCATTCTTTCTTACCGTCATCTTT	#f6a444
5[210]	2[196]	AAGCGAAATTTACCGTAAGTACTGGGTATTTAATTCGATTCT	#f6a444
3[98]	1[118]	ACGCGTTTTGTTGTGTTTAAGTGGGTCATCTTTTA	#f6a444
0[97]	4[98]	GACATGCCAAATCAAGTTTTTCCCGAAA	#f6a444
5[189]	2[175]	TGGCGAGCTTCACCTTCACCGAGTCATGAAGGTATCACCTTC	#f6a444
0[223]	4[224]	AAGGTCAAGGAGCGGGCGCTAGCGTGGA	#f6a444
3[140]	1[160]	CTAATTGAACAGAACTTGTAGTTGAACATAACCTT	#f6a444
3[91]	4[84]	TCATAGTTCGGCAAAAATCCCT	#f6a444
5[84]	2[70]	CATCACCCCTGAAAATAAAGACTTCTGCTAGAACGCGTTTTG	#f6a444
0[139]	4[140]	ACCAAATCGGAACCCTAAAGTTGTTGA	#f6a444
3[112]	4[105]	TCTTTGTGCAAGGCCTGGTAC	#f6a444
3[56]	1[76]	GTTGTCCTTCGTTTTTGTCTATTTAGCTTTTCAG	#f6a444
0[181]	4[182]	GAGACAGAAAGCCGCGCAACGGTTGTCT	#f6a444
3[154]	4[147]	CCGTCTTCCAATTGGAGTATT	#f6a444
5[63]	2[49]	GCGATGGTCGTAGGGATATCAGAGCATCTTGTTGAATTTGTT	#f6a444
0[55]	4[56]	TAATCTCAACCGTCTATCAGGGAGATAG	#f6a444
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0[34]	4[35]	TTCTCGCCTCCAACGTCAAAGCCAGTTT	#f6a444
3[175]	4[168]	ATTTTGAGGTAACAAGACTGG	#f6a444
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0[202]	4[203]	GCATCACAAAGGAAGGGAAGAAGATTGA	#f6a444
5[126]	2[112]	CACTAAATTGGGACAACACCAAACCTTTGTTTCGTTGTTCTTC	#f6a444
3[161]	1[181]	CAATGTTGTGTCTAAAACCTTGGGCAGACTTGAAAA	#f6a444
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4[34]	4[11]	GGAACAAGAGTCCACTATTTTTTT	#57bb00
1[5]	2[0]	TTTTATAATCGGTTGAAGCTTTTTAGCTTCTGCTATT	#57bb00
5[11]	0[5]	TTTTTAAAGAACGTGGATAGCAGTAGTTGGAATATCTTTT	#57bb00
0[264]	1[264]	TTTTTGGAAGTGGCAATTTATGACTAAGGTTGGCCATTTT	#1cb6a2
4[271]	5[271]	TTTTACCACACCCGCGCGTACGCTGCGCGTAACTTTT	#1cb6a2
2[257]	2[238]	TTTTATTTGTGACCTAAAAT	#1cb6a2
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pScaf-1512.3 Scaffold Sequence

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3[77]	1[97]	TTGCGGCACGACTTAGATCGTGACTTGATCAATGA	#f6a444
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3[154]	4[147]	CGAGGCGCCCACTTATCGAGT	#f6a444
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3[133]	4[126]	TGATATCGATTGTCCATGTCTG	#f6a444
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3[238]	4[231]	GATGCCTCGCCGCTACAGGGC	#f6a444
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5[11]	0[5]	TTTTAAAGAACGTGGACTGTTTCAGCACCCACTGCTTTT	#57bb00
4[34]	4[11]	GGAACAAGAGTCCACTATTTTTT	#57bb00
1[5]	2[0]	TTTTAAAGCCTTCTCGGGAGCCTGTTGTCATCCAATTTT	#57bb00
0[244]	3[257]	CATAGTAGGCAAGTG TAGCGCTTAATGTGAAGTTGTTTTT	#1cb6a2
4[271]	5[271]	TTTTACCACACCCGCCGCTCACGCTGCGCGTAACTTTT	#1cb6a2
2[257]	2[238]	TTTTTTGGGCTGAACAGTGA	#1cb6a2
0[264]	1[264]	TTTTCTGCCTCTGTGGAATCACGTAGGTGGCCAACTTTT	#1cb6a2

Electron Micrographs for pScaf-1512 Nanotube

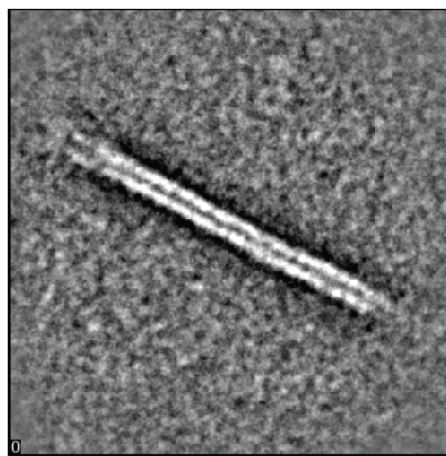
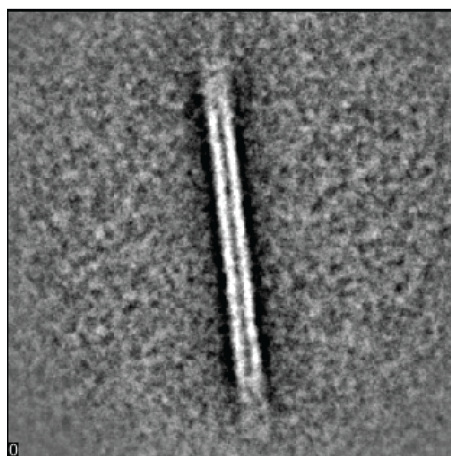
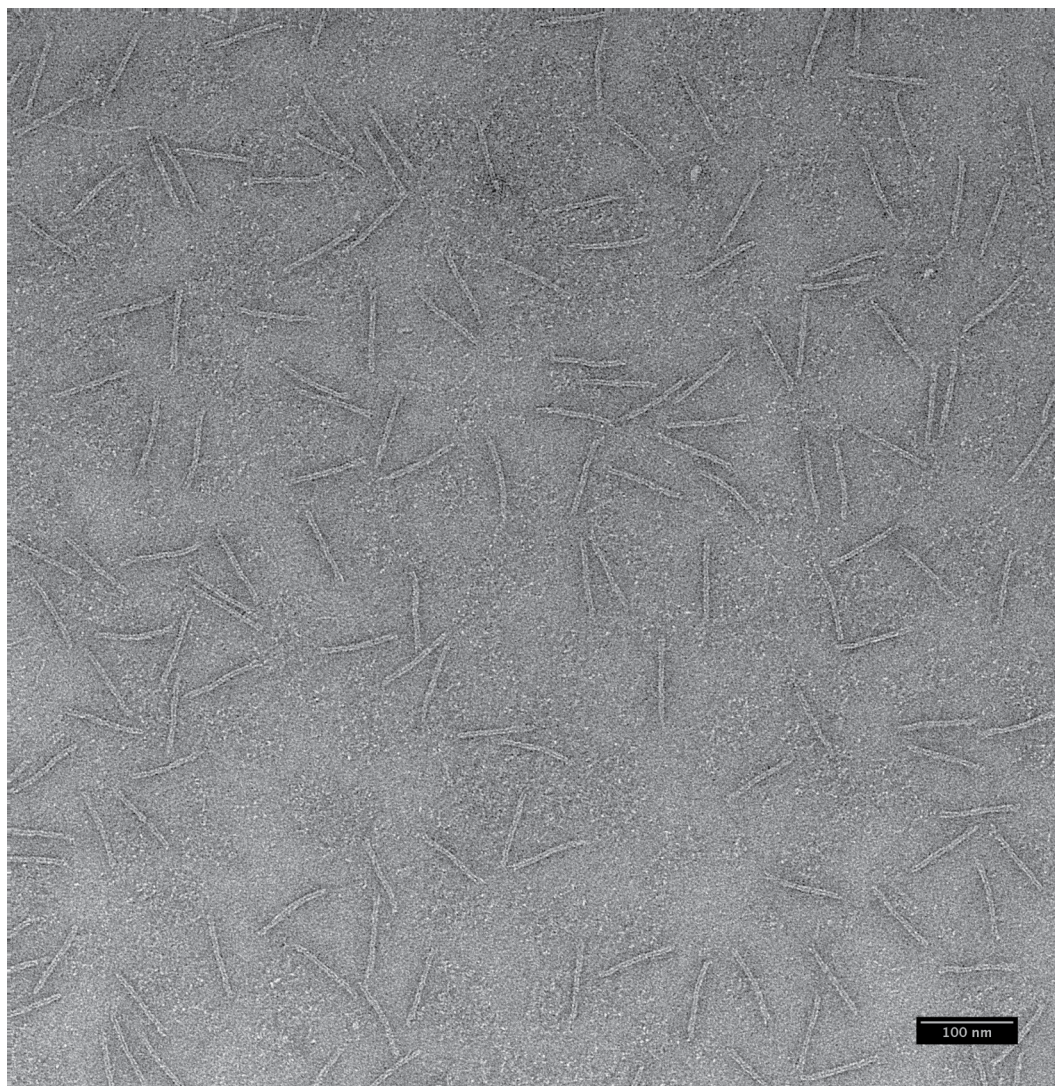


Figure 38: Electron Micrograph and Class Averages for pScaf-1512 Nanotube

pScaf-2268 Nanotube

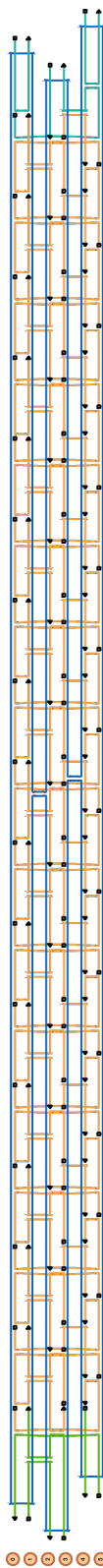


Figure 39: cadnano Design for pScaf-2268 Nanotube

pScaf-2268.2 Scaffold Sequence

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Staple Sequences for pScaf-2268.2 Scaffold

Start	End	Sequence	Color
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3[182]	1[202]	GAGTCGTGACCAACCATCGATTTGGGTTGACAGGC	#f6a444
0[34]	4[35]	AGATTGGCTCCAACGTCAAAGCCAGTTT	#f6a444
3[91]	4[84]	TTCATGGTCGGCAAAATCCCT	#f6a444
0[118]	4[119]	ATCCACCGAGGTGCCGTAAAGCTAACTC	#f6a444
5[168]	2[154]	GACGGGGATGAACTCAGCCCATCGTGGCTTGTCCGAAACACT	#f6a444
3[77]	1[97]	GCGTTCTGCCATGCGGCTGTTGCTCAACCAGAAGT	#f6a444
3[175]	4[168]	CGCGACAGGTAATCAGGATCA	#f6a444
3[70]	4[63]	GTCTCTACAAAAGAATAGCCC	#f6a444
5[105]	2[91]	TGGGGTCAGAAGGTCCCTCCTGCCCTCTCAGGAAACAGCAAG	#f6a444
5[315]	2[301]	GCTATGGAAGACGATAAGCCAAGAGCTAGCTCTGTTCTCTCT	#f6a444
5[231]	2[217]	GGGCGCTAACTCCGCATCCTGTGCAATGTTCTCTCTCTCCGA	#f6a444
3[119]	1[139]	GCGGCCCATACTCATCCCGACGGGACCCGAAGACC	#f6a444
0[307]	4[308]	TCCTTCTGGGCGCGTGGATCCCAACGCC	#f6a444
0[97]	4[98]	CCCAATGCAAATCAAGTTTTTCCCGAAA	#f6a444
0[202]	4[203]	TCAAAGTAAAGGAAGGGAAGAGCTGTGT	#f6a444
3[350]	1[370]	AATCACCACTGGATTGGACATCAGCGCAACAAGA	#f6a444
3[203]	1[223]	TGCACCTGGTTGTCCCTGAGGTGGTGCCAAGGACG	#f6a444
5[252]	2[238]	TCACGCTTCTCATCTCAGGTGGTCTGCTGGTTGGACCAAATT	#f6a444
3[112]	4[105]	GACGCTTGAGGCATGCGGTAC	#f6a444
5[189]	2[175]	TGGCGAGTCGATCGCGAGATCTCCAGAAAAAGCAGAGAAGCA	#f6a444
0[181]	4[182]	TGGAGCAAAAGCCGGCGAACGTTGCGGT	#f6a444
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3[196]	4[189]	ATGAACTTTGAACGATGTGCC	#f6a444
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3[56]	1[76]	TCGACAGCCCAAGCTTCTCTGCCAGAAGTTGGCCT	#f6a444
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5[273]	2[259]	CACCCGCCACGCTCATCACAGACGGAGGAGGCGCAACTACAT	#f6a444
3[364]	4[357]	CTCAAGAATTCTGATCATTAT	#f6a444
3[259]	4[252]	CCCAAAGGGCCAAGCTGACTT	#f6a444

3[224]	1[244]	TGCCTGTCTTGGAGCTACGAGGGAGTTTGTCCATG	#f6a444
3[133]	4[126]	CCCGATGCTCAAGAATCAGGT	#f6a444
0[244]	4[245]	ATTTCCCGGCAAGTGTAGCGGGGCTATC	#f6a444
0[160]	4[161]	GTGCTCACCGATTAGAGCTTATGGATT	#f6a444
3[308]	1[328]	TTACACCTATCCTCCGCACATTTTGAAGCAATTCT	#f6a444
5[210]	2[196]	AAGCGAACTATTACTGCAGGCTCTTTGAGCCGGTTACAATAT	#f6a444
5[84]	2[70]	CATCACCACCGCCAAGGATCACTGCCCTGGAAGATCAGTGGA	#f6a444
3[217]	4[210]	TCCTGGCAGGAATGGTGACTG	#f6a444
5[63]	2[49]	GCGATGGTGTCTTTTATTATCCAGAAGTAAATTTGTGCGCTC	#f6a444
0[349]	4[350]	TACAGGGTGGGTTTGAAGGAATTCTCCA	#f6a444
3[301]	4[294]	CCAAACTGAAAGATCAGGAGA	#f6a444
5[147]	2[133]	GGAGCCCACCTGTGGAAGAACTCTGCATCTGAATTGTTCCA	#f6a444
0[328]	4[329]	ATTCCAGCCCTCTTGCCTGCCCAGGAA	#f6a444
3[245]	1[265]	TGAACACCTTGCAATCCTCAAGGAGAACGCCATCT	#f6a444
3[322]	4[315]	CTCCAGTCTCCAGGAGCTCGC	#f6a444
3[343]	4[336]	ACTTATGGGAGCCGATCCCTC	#f6a444
0[139]	4[140]	CTGAACCTCGGAACCCTAAAGCTTGTTT	#f6a444
5[357]	2[343]	GTCTCTCGCAGCATTCTGGATTGTTCAACCTGAACAAATCTC	#f6a444
3[98]	1[118]	GTTGGATTCTCAACCTCCGCGGCCTGGCAATCCA	#f6a444
3[329]	1[349]	CAAGCCTCAAGATCTAAGAAATAGCGTCATTGACG	#f6a444
0[286]	4[287]	AACATTGCGCGCTTAATGCGCATGTTAC	#f6a444
5[126]	2[112]	CACTAAAAGAACTCCAACCTGAGTGGAACATTCCTGAACGC	#f6a444
0[223]	4[224]	GGCCGCTAGGAGCGGGCGCTAATGCGCA	#f6a444
0[265]	4[266]	CGCATGAGCGCGTAACCACCAGGGCCAG	#f6a444
0[76]	4[77]	CCAATTCCTCCACTACGTGAACTATAAAT	#f6a444
3[35]	1[55]	ATCAACTGTCCGAGCTGGAAGTACACTGCTCTCAT	#f6a444
5[11]	0[5]	TTTTTAAAGAACGTGGATCCAGTCAGTTCATCAATCTTTT	#57bb00
4[34]	4[11]	GGAACAAGAGTCCACTATTTTTT	#57bb00
1[5]	2[0]	TTTTACTGGCAGCCTTCTCCAGATCAAGATCTCAGTT	#57bb00
3[0]	1[34]	TTTTGACTGCTCCTGGAAGGCTGCTCCTTCGGCCAAAGAGCAAAGTGATCC	#57bb00
2[383]	2[364]	TTTTTGCGCACATATGAATC	#1cb6a2
4[397]	5[397]	TTTTAGGAGACACTCGAAATCTCAAGAGGCTGTACATTTT	#1cb6a2
0[390]	1[390]	TTTTCAGCGTGAGCCTGCCAAGTCCATCTTTCCCTATTTT	#1cb6a2
0[370]	3[383]	CAAAGCTCACTCCCACTCAATCGAGCCCAGAATCTCATTTT	#1cb6a2

Electron Micrographs for pScaf-2268 Nanotube

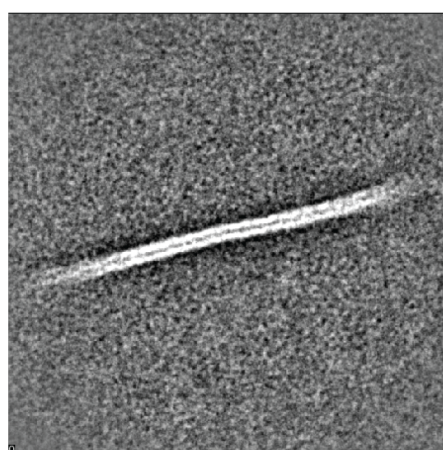
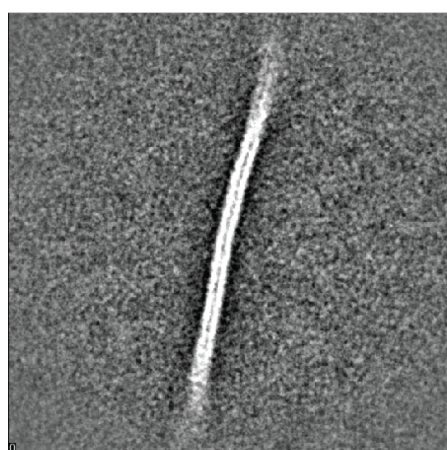
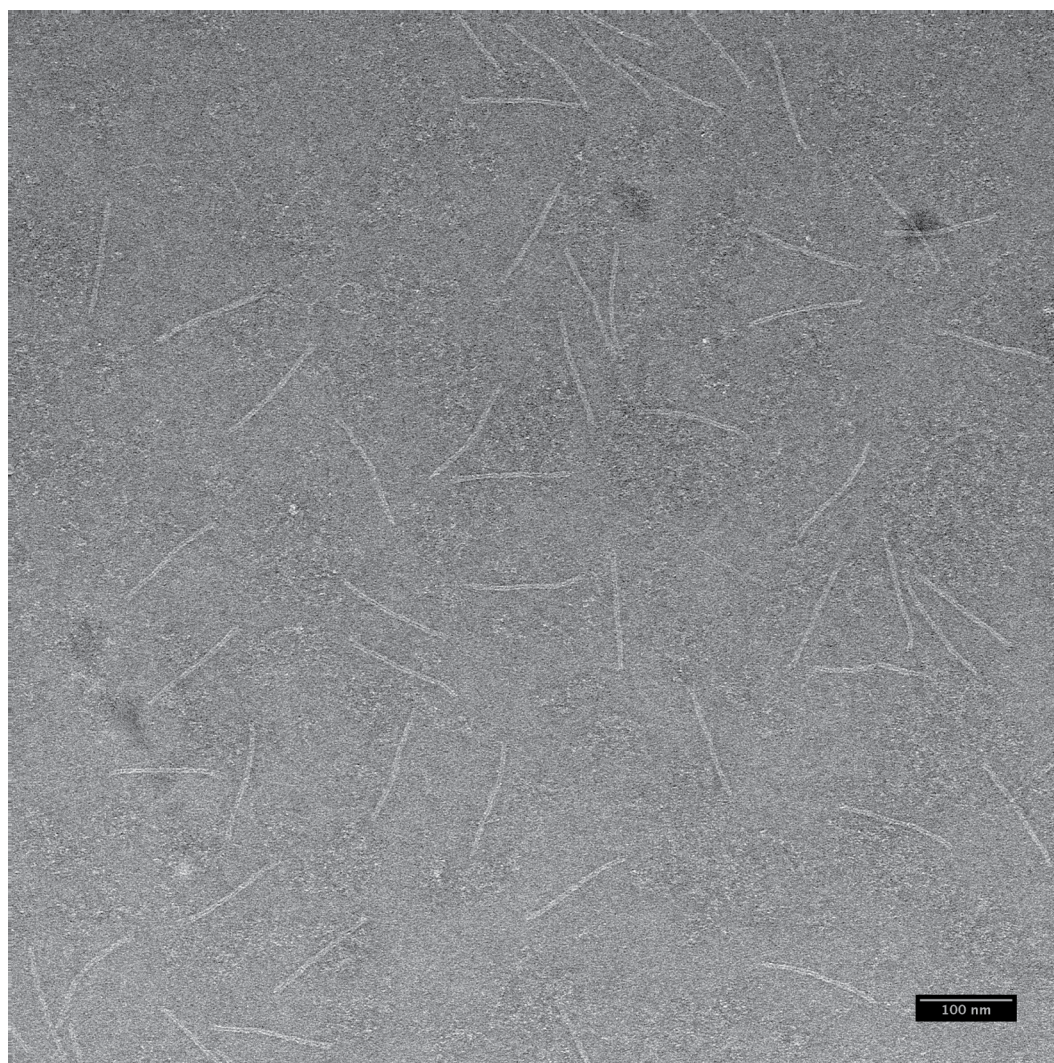


Figure 40: Electron Micrograph and Class Averages for pScaf-2268 Nanotube

pScaf-3024 Nanotube

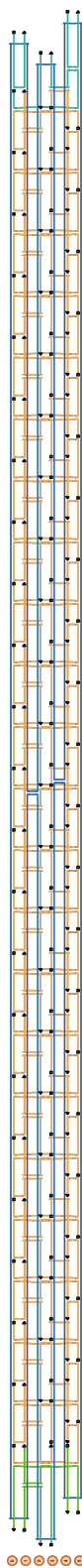


Figure 41: cadnano Design for pScaf-3024 Nanotube

pScaf-3024.1 Scaffold Sequence

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GGATTCCAAGAAATGGCCAATGAAGATTGGATCACCTTTCGCACTAAGACCTACTT
GTTTGAGGAGTTTCTGATGAATTGGCACGACCGCCTCAGGAAAGTGGAGGAGCAT
TCTGTGATGACTGTCAAGCTCCAATCTGAGGTGGACAAATATAAGATTGTTATCCCT
ATCCTGAAGTACGTCCGCGGAGAACACCTGTCACCCGATCACTGGCTGGATCTGT
TCCGCTTGCTGGGTCTGCCTCGCGGCACATCTCTGGAGAAACTGCTGTTTCGGTGA
CCTGCTGAGAGTTGCCGATACCATCGTGGCCAAGGCTGCTGACCTGAAAGATCTG
AACTCACGCGCCCAGGGTGAAGTGACCATCCGCGAAGCACTCAGGGAAGTGGATT
TGTGGGGCGTGGGTGCTGTGTTTCACTGATCGACTATGAGGACTCCCAGAGCCG
CACCATGAAGCTGATCAAGGATTGGAAGGACATCGTCAACCAGGTGGGCGACAAT
AGATGCCTCCTGCAGTCCTTGAAGGACTCACCATACTATAAAGGCTTTGAAGACAA
GGTCAGCATCTGGGAAAGGAAACTCGCCGAAGTGGACGAATATTTGCAGAACCTC
AACCATATTCAGAGAAAGTGGGTTTACCTCGAACCAATCTTTGGTCGCGGAGCCCT
GCCCAAAGAGCAGACCAGATTCAACAGGGTGGATGAAGATTTCCGCAGCATCATG
ACAGATATCAAGAAGGACAATCGCGTCACAACCTTGACTACCCACGCAGGCATT
GCAACTCACTGCTGACCATCCTGGACCAATTGCAGAGATGCCAGCGCAGCCTCAA
CGAGTTCCTGGAGGAGAAGCGCAGCGCCTTCCCTCGCTTCTACTTCATCGGAGAC
GATGACCTGCTGGAGATCTTGGGCCAGTCAACCAATCCATCCGTGATTGAGTCTCA
CCTCAAGAAGCTGTTTGCTGGTATCAACTCTGTCTGTTTCGATGAGAAGTCTAAGC
ACATTACTGCAATGAAGTCCTTGGAGGGAGAAGTTGTGCCATTCAAGAATAAGGTG
CCCTTGTCGAATAACGTGCAACCTGGCTGAACGATCTGGCCCTGGAGATGAAGA
AGACCCTGGAGCAGCTGCTGAAGGAGTGCCTGACAACCGGACGCAGCTCTCAGG
GAGCTGTGGACCCTTCTCTGTTCCCATCACAGATCCTGTGCTTGGCCGAACAGATC
AAGTTTACCGAAGATGTGGAGAACGCAATTAAGATCACTCCCTGCACCAGATTGA
GACACAGCTGGTGAACAAATTGGAGCAGTATACTAACATCGACACATCTTCCGAGG
ACCCAGGTAACACAGAGTCCGGTATTCTGGAGCTGAAACTGAAAGCACTGATTCTC
GACATTATCCATAACATCGACGTGGTCAAGCAGCTGAACCAATCCAAGTGACACAC
CACCGAAGATTGGGCCTGGAAGAAGCAGTTGAGGTTCTACATGAAGTCCGACCAC
ACCTGTTGCGTTCAGATGGTTGACAGCGAGTTCCAGTACACCTATGAGTACCAAGG
AAATGCCAGCAAGCTCGTTTACACTCCACTCACTGACAAGTGTTACCTCACCTTGA
CACAGGCTATGAAGATGGGCCTGGGAGGCAACCCATACGGTCCAGCTGGCACTG
GTAAGACAGAGAGCGTTAAGGCACTCGGAGGTCTGCTGGGCAGGCAGGTCCTCG
TGTTCAACTGTGATGAAGGAATCGACGTTAAGTCCATGGGAAGAATCTTTGTTGGC
CTCGTTAAGTGTGGAGCTTGGGGTTGCTTCGACGAGTTCAACAGGCTGGAGGAAT
CTGTGCTGAGCGCCGTCTCTATGCAGATCCAGACCATCCAGGACGCATTGAAGAA
CCACAGGACCGTCTGCGAGCTGTTGGGTAAAGGAAGTGGAGGTGAACTCCAACCTCC
GGAATCTTCATCACAATGAATCCCGCAGGTAAAGGATATGGAGGAAGACAGAACT
CCCAGACAACCTGAAGCAGCTGTTCCGCCAGTGGCTATGTCCCATCCAGACAAT
GAGCTGATCGCCGAAGTCATCCTCTATTCCGAGGGATTCAAAGATGCTAAAGTTCT
CTCCAGAAAGCTCGTGGCCATCTTCAATCTGTCAAGAGAACTCCTGACACCTCAGC
AGCATTACGACTGGGGTCTGAGAGCCCTCAAGACCGTCCTGAGAGGTTTCAGGAAA
TCTCCTCAGGCAGCTGAACAAGAGCGGTACAACACAGAATGCAATGAGAGCCAC
ATTGTCGTCCAGGCTCTGAGGCTGAATACCATGTCAAAGTTCACATTACAGACTG

CACAAGATTTGACGCTCTGATTAAAGATGTGTTCCCTGGTATTGAACTCAAAGAAGT
 GGAGTATGACGAGCTGAGCGCCGCTTTGAAGCAGGTGTTTGAGGAGGCTAACTAT
 GAGATTATCCCTAATCAGATCAAGAAAGCATTGGAAGTGTATGAACAGCTGTGTCA
 GAGGATGGGAGTGGTGATTGTGGGCCCATCAGGCGCAGGTAAGAGCACTCTCTG
 GAGAATGCTGAGAGCAGCACTGTGCAAGACTGGAAAGGTGGTGAAGCAATACACC
 ATGAATCCCGGATCCACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGGTGTGG
 TGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTT
 CGCTTTCTTCCCTTCTCTTCTCGCCACGTTGCGCGGCTTTCCCCGTCAAGCTCTAA
 ATCGGGGGCTCCCTTTAGGGTTCGATTAGTGCTTTACGGCACCTCGACCCCAA
 AAAGTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTT
 TCGCCCTTTGACGTTGGAGTCCACGTTCTTT

Staple Sequences for pScaf-3024.1 Scaffold

Start	End	Sequence	Color
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5[420]	2[406]	CACTCCCATGTTAGTATACTGGTGTGGTCTCTGTTGAACTCGT	#f6a444
3[35]	1[55]	GTCACCTCACCTGTCTGGGAGGAAGGCGCTGCGC	#f6a444
5[273]	2[259]	CACCCGCGCTCCCTGAGAGCTTATGGGTGTCTTACCAGTGC	#f6a444
3[224]	1[244]	ATAGGGATAACAATACTTTCTCTTTGGGCAGGGCT	#f6a444
3[238]	4[231]	CTTATATCACAGAATGCTCCT	#f6a444
3[385]	4[378]	TGGGCGGGACGACAATGTGGC	#f6a444
3[427]	4[420]	TCCTCCATGTGAATGTGAACT	#f6a444
0[55]	4[56]	GTGAGACAACCGTCTATCAGGGAGATAG	#f6a444
3[91]	4[84]	GATGGTATCGGCAAAATCCCT	#f6a444
3[49]	4[42]	GGCGCGTGGTTGAGTGTGTT	#f6a444
5[462]	2[448]	TTTCTTGATACCGGACTCTGTGCCAATCTGGATCTGCATAG	#f6a444
3[364]	4[357]	GTCTGGATGCATTCTGTGTTG	#f6a444
5[42]	3[34]	GGCGAAATGAATCACGGATGGAAGCGAGGTCTCATAGTCGAGCGGATG	#f6a444
0[307]	4[308]	CTGTGATGGGCGCGTGGATCCTCAGGAC	#f6a444
3[490]	4[483]	CACTTCCCACTTCTTTGAGTT	#f6a444
0[433]	4[434]	TGTGTCGATCCTCTGACACAGTGCAGTC	#f6a444
5[126]	2[112]	CACTAAAAAGGACTTCATTGCACTGAGTGGACTGCAGGAGGC	#f6a444
3[301]	4[294]	GGAGAGAGGTCTTGAGGGCTC	#f6a444
3[259]	4[252]	TTGGAGTTGTCAGGAGCTTGA	#f6a444
3[196]	4[189]	CAGGTGTCATCAGAACTCCT	#f6a444
3[434]	1[454]	TATCCTTTACCTGCAGACGGCACTGCTTCTTCCAG	#f6a444
3[280]	4[273]	GAAGATGCCAGTCGTAATGCT	#f6a444
3[203]	1[223]	TCTCCGCGGACGTAGGTTCTGCCCTGTTGAATCTG	#f6a444
3[77]	1[97]	GCAGCCTTGCCACTGACGATGCTGGCATCTCTGC	#f6a444
0[286]	4[287]	GTCCACACGCGCTTAATGCGCTCAGACC	#f6a444
5[399]	2[385]	GCCTGATTTGTTACCAGCTGTCAACCAACCCAAGCTCCAC	#f6a444
3[329]	1[349]	TCGGAATAGAGGATCGATTCTGTAAACGAGCTTG	#f6a444

3[371]	1[391]	TGGGACATAGCCACACTTAACACTGGAACCTCGCTG	#f6a444
3[308]	1[328]	ACTTTAGCATCTTTACACGAGAACACTTGTCAGTG	#f6a444
5[357]	2[343]	CAGTGCTTTAATTGCGTTCTCCTGGCATCATGGACTTAACGT	#f6a444
5[252]	2[238]	TCACGCTGTTGTACGCACTCCCGCGACTTCGAGGTAAACCC	#f6a444
3[343]	4[336]	GACTTCGCTTGTTTCAGCTGCC	#f6a444
5[84]	2[70]	CATCACCCAGACAGAGTTGATAGGCTGCGTCCTTCCAATCCT	#f6a444
3[448]	4[441]	GGGATTCAGCGTCAAATCTTG	#f6a444
3[245]	1[265]	TTGTCCACCTCAGACAATTGGCAAAGGCTGGACCG	#f6a444
0[412]	4[413]	CTCCAATGGGCCCAATCACTTGACAT	#f6a444
3[322]	4[315]	GAATCCCATTTCTGAACCTC	#f6a444
3[133]	4[126]	CTCCAGATTCTTGGAATCCCT	#f6a444
3[217]	4[210]	CTTCAGGCCTGAGGCGGTCGT	#f6a444
5[483]	2[469]	AATCTCAGCTTTAGTTTCAGACTTGACTTCAATGCGTCCT	#f6a444
3[406]	4[399]	GTTGTCTGGTATTTCAGCCTCA	#f6a444
0[244]	4[245]	CTTCAGCGGCAAGTGTAGCGGCAGTCAT	#f6a444
5[210]	2[196]	AAGCGAAATCTCCAGGGCCAGTCATCCACAAATATTCGTCCA	#f6a444
3[392]	1[412]	AACAGCTGCTTCAGCGAAGCATCTGAACGCAACAG	#f6a444
5[294]	2[280]	CGCTACAGGGAACAGAGAAGGATCTTCATCCGAGTGCCTTAA	#f6a444
5[189]	2[175]	TGGCGAGAGCCAGGTTTCGACGTCATGAGAGTTTCCTTTCCC	#f6a444
3[98]	1[118]	TCGGCAACTCTCAGATCTATTCCAGGATGGTCAGC	#f6a444
3[469]	4[462]	TCCGGAGAGGGAACACATCTT	#f6a444
5[105]	2[91]	TGGGGTCTGCTTAGACTTCTCAATTGGTGTGCGCCACCTGGT	#f6a444
0[223]	4[224]	CTTCTTCAGGAGCGGGCGCTACCACTTT	#f6a444
0[202]	4[203]	ATCGTTCAAAGGAAGGGAAGAGCCAATT	#f6a444
3[266]	1[286]	TCTCTTGACAGATTCGCTCTCTGCCTCCCAGGCCC	#f6a444
3[476]	1[496]	TTGGAGTTCACCTCTGTGGTTTTTGGTTCAGCTGC	#f6a444
3[287]	1[307]	GCCACGAGCTTTCTGCAGACCTAGCCTGTGTCAAG	#f6a444
0[328]	4[329]	CTGTTCGATGGTGTATTGCTTTGAGGAG	#f6a444
5[315]	2[301]	GGGATTCGCCAAGCACAGGATGTGAGGTGACCTGCCTGCCCA	#f6a444
5[441]	2[427]	CTGTTCAAGGTCCTCGGAAGAAACCTCAGCTCAGCACAGATT	#f6a444
0[454]	4[455]	GTTACCTTACAGTTCCAATGCTAATCAG	#f6a444
0[349]	4[350]	CACATCTTTTCCAGTCTTGCATACCGCT	#f6a444
0[370]	4[371]	GTGATCTGCTCTCAGCATTCTTCTCATT	#f6a444
3[182]	1[202]	CAGTGATCGGGTGAGTTCGGCTGCTGCGGAAATCT	#f6a444
3[119]	1[139]	CCGAACAGCAGTTTCTTCAATGCGAATGCCTGCG	#f6a444
0[118]	4[119]	AGTAATGGAGGTGCCGTAAAGGCTGGAA	#f6a444
3[70]	4[63]	CAGGTCACAAAAGAATAGCCC	#f6a444
3[56]	1[76]	GAGTTCAGATCTTTTGATCAGCCAGGAACTCGTTG	#f6a444
0[97]	4[98]	ATCGAAACAAATCAAGTTTTTCCCGAAA	#f6a444
0[76]	4[77]	ACCAGCACCCACTACGTGAACTATAAAT	#f6a444
0[181]	4[182]	GTTATTGAAAGCCGGCGAACGCAAACAA	#f6a444

5[231]	2[217]	GGGCGCTAGCTGCTCCAGGGTGTCTGCTCTGAATATGGTTGA	#f6a444
3[154]	4[147]	CAGACCCGATCCAATCTTCAT	#f6a444
3[175]	4[168]	ATCCAGCGTAGGTCTTAGTGC	#f6a444
3[413]	1[433]	GGGAGTTTCTGTCTCCTCCAGCGGACTTCATGTAG	#f6a444
5[147]	2[133]	GGAGCCCAATGGCACAACCTTCTGGGTAGATAGTATGGTGAGT	#f6a444
0[34]	4[35]	ATTGGTTCTCCAACGTCAAAGCCAGTTT	#f6a444
0[160]	4[161]	ATTCTTGCCGATTTAGAGCTTGAAAGGT	#f6a444
5[63]	2[49]	GCGATGGAACAGCTTCTTGAGTTCTCCTCTTCATGGTGCGGC	#f6a444
5[378]	2[364]	CCAGAGAATCTGGTGAGGGATAGGTGTGAGGCCAACAAAGA	#f6a444
0[265]	4[266]	GCGTCCGGCGCGTAACCACCAGCTGAGG	#f6a444
5[168]	2[154]	GACGGGGGACAAGGGCACCTTCGATTGTGACCTTGTCTTCAA	#f6a444
0[139]	4[140]	TCCCTCCTCGGAACCTAAAGTGGCCAT	#f6a444
3[350]	1[370]	GCGATCAGCTCATTTTCTTCCTTCCTTGGTACTCA	#f6a444
3[112]	4[105]	CAGGTCACTCTTCGTAGGTAC	#f6a444
0[391]	4[392]	TGTCTCAGTGCTCTTACCTGCGAGCCTG	#f6a444
3[140]	1[160]	GATGTGCCGCGAGGAGCCTTTTCAAGGTTGTGACG	#f6a444
3[161]	1[181]	AGCAAGCGGAACAGAGATGCTCCTTCTTGATATCT	#f6a444
3[455]	1[475]	ATTGTGATGAAGATGGATGGTCTTCGGTGGTGTGC	#f6a444
0[475]	4[476]	CTCCAGAATCTGATTAGGGATCAATACC	#f6a444
1[5]	2[0]	TTTTGCAGGTCATCGTGAACACAGCACCCACGCCCTT	#57bb00
4[34]	4[11]	GGAACAAGAGTCCACTATTTTTTT	#57bb00
3[0]	1[34]	TTTTACAAATCCAGTTCCTGAGTGCTTCTCAGTGTCTCCGATGAAGTAG	#57bb00
5[11]	0[5]	TTTTTAAAGAACGTGGAGACTGGCCCAAGATCTCCATTTT	#57bb00
0[516]	1[516]	TTTTTATGGATAATGTCGAGTTGACCACGTCGATGTTTTT	#1cb6a2
0[496]	3[509]	AATCAGTTAGTTAGCCTCCTCCATACTCTTACCCAACTTTT	#1cb6a2
4[523]	5[523]	TTTTGCGGCGCTCAGCTCGTAAACACCTGCTTCAAATTTT	#1cb6a2
2[509]	2[490]	TTTTAGCTCGCAGACGGTCC	#1cb6a2

Electron Micrographs for pScaf-3024 Nanotube

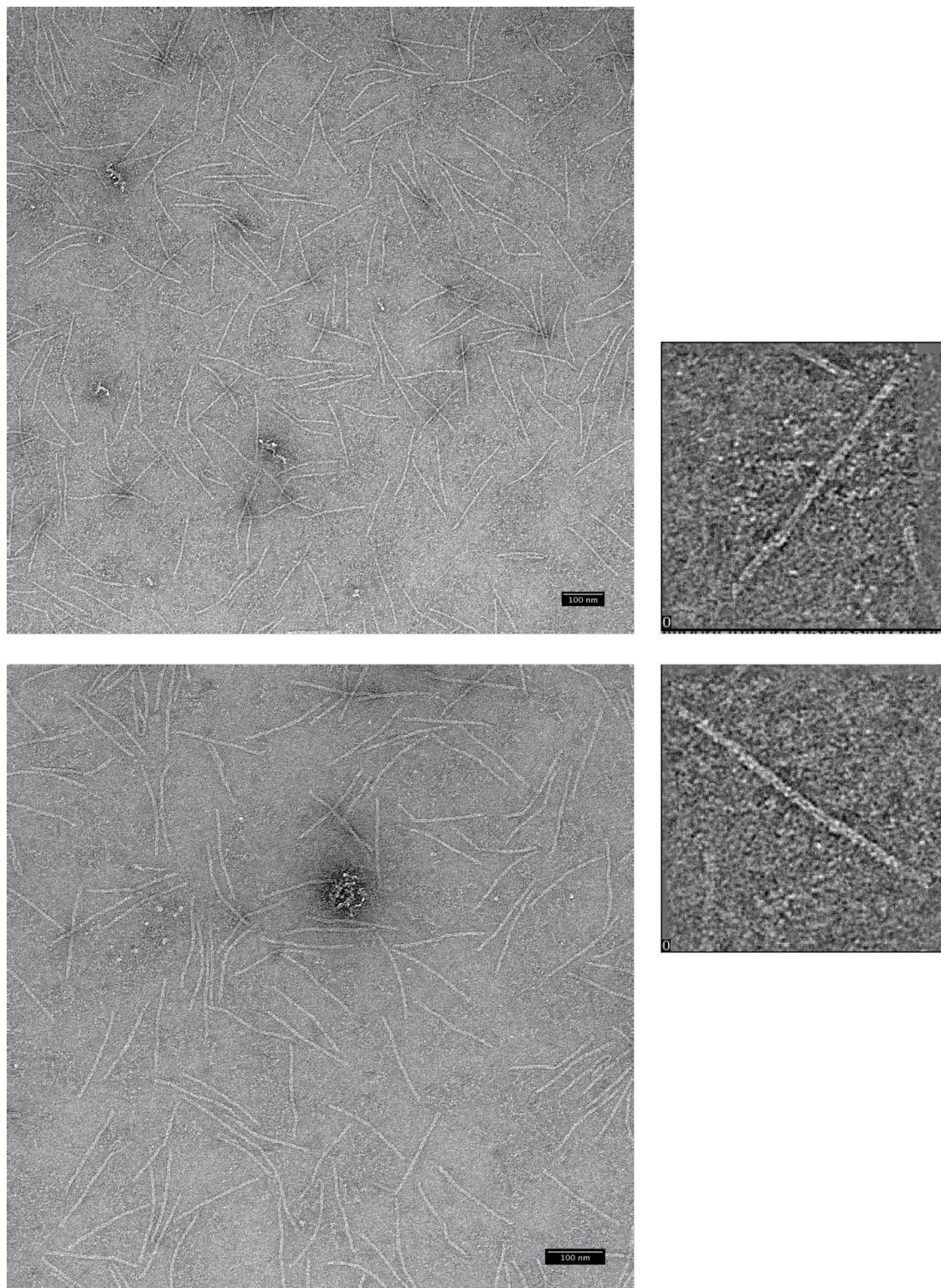


Figure 42: Electron Micrographs for pScaf-3024 Nanotube

pScaf-1512 Brick

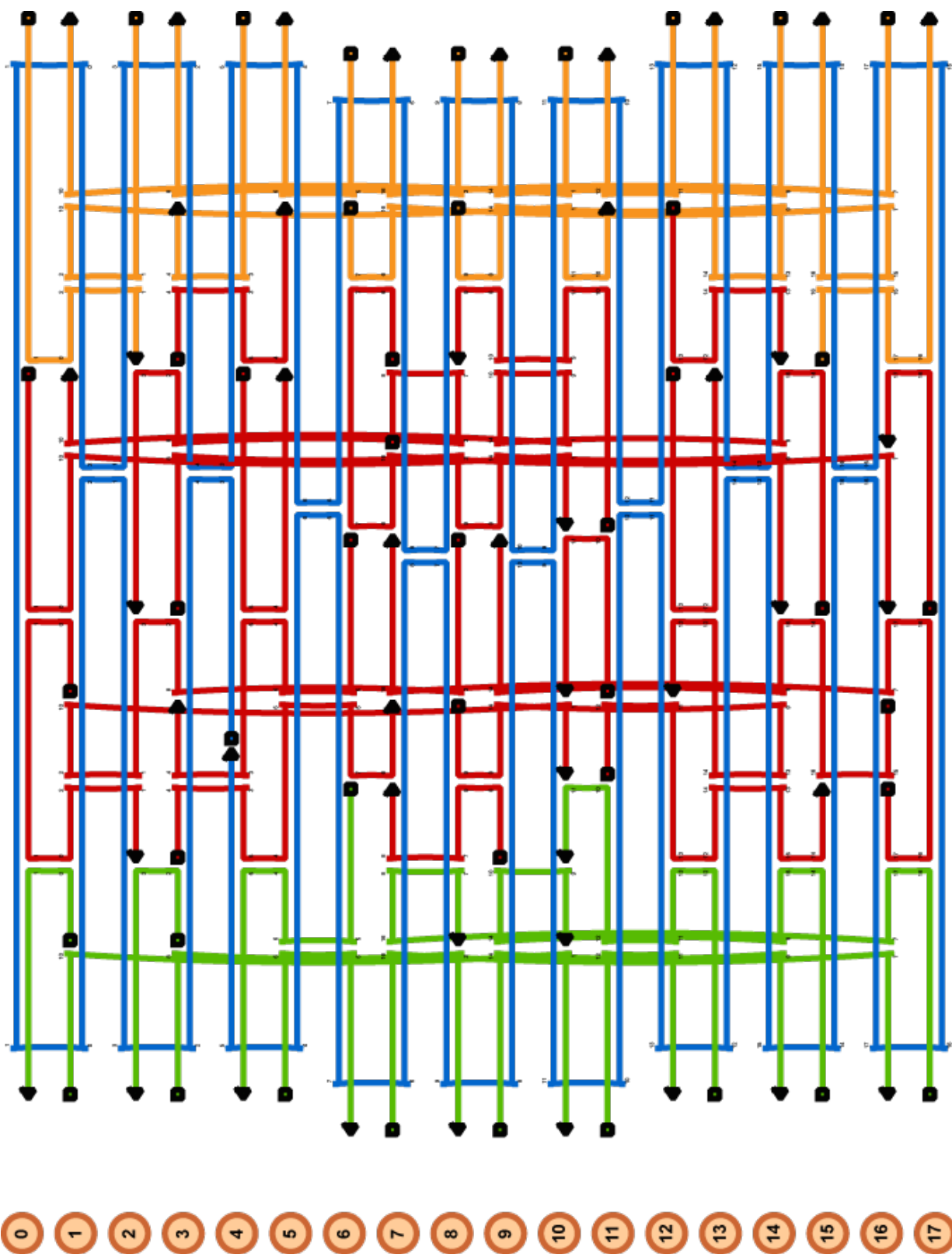


Figure 43: cadnano Design for pScaf-1512 Brick

pScaf-1512.3 Scaffold Sequence

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TTTTGATTTATAAGGGATTTTGCCGATTTTCGGGGTACCAACTGCTGGGCCATATCG
ACATGGACACCAGAGAATGGAGCGACGGCGTGCTCACAACTCCGCCAGACAAGT
CGTGCGCGAACCTCAAGACGTCAGCTCTTGATCATCTGCGATGGTGATATTGAC
CCTGAGTGGATCGAGTCCCTGAATTCCGTGTTGGATGACAACAGGCTCCTCACAAT
GCCTTCTGGTGAGAGAATCCAGTTCGGTCCTAACGTGAACTTCGTGTTTCGAGACAC
ACGATCTCAGCTGTGCTAGCCCAGCTACTATCTCCCGCATGGGAATGATCTTCTTG
TCCGACGAGGAGACAGATTTGAACTCATTGATCAAGTCTTGGCTCAGAAACCAGCC
TGCAGAATATAGGAATAACCTGGAGAACTGGATCGGTGATTACTTCGAGAAGGCTT
TGCAGTGGGTGCTGAAACAGAACGACTATGTCGTCGAAACCAGCCTGGTTCGGTAC
AGTTATGAACGGACTCTCCCATCTGCACGGATGCAGAGATCACGATGAGTTTATCA
TCAATTTGATCCGCGGACTGGGAGGTAACCTGAATATGAAATCTCGCCTGGAGTTC
ACTAAAGAAGTGTTCCACTGGGCTAGGGAGTCACCACCTGACTTCCACAAACCTAT
GGACACCTACTATGATTCCACAAGAGGCAGGTTGGCCACCTACGTGCTGAAGAAG
CCTGAGGACCTCACCGCTGACGACTTCTCCAACGGACTGACTCTGCCCCGTGATCC
AGACTCCAGACATGCAGCGCGGACTCGATTACTTTAAGCCCTGGCTCAGCTCCGA
TACCAAGCAACCTTTTCACTCTCGTGGGACCAGAGGGATGTGGTAAAGGAATGCTC
CTGAGGTATGCATTCTCCAGCTCCGCTCAACCCAAATTGCCACTGTTCACTGTTC
AGCCCAAACAACCTTCAAGGCATCTCCTCCAGAAGCTCAGCCAGACCTGTATGGTTA
TCAGCACCAACACCGGCAGAGTTTACCGCCCAAAGGATTGTGAGCGCCTCGTGTT
GTACCTCAAAGATATCAATCTGCCCAAACCTCGATAAGTGGGGCACTTCCACCCTCG
TGGCATTCTCCAACAGGTGCTGACCTACCAGGGCTTCTACGACGGGATCCACGC
GCCCTGTAGCGGCGCATTAAGCGCGGGCGGGTGTGGTGGTTACGCGCAGCGTGAC
CGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCTTTC
TCGCCACGTTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGG
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GTCCACGTTCTTT

Staple Sequences for pScaf-1512.3 Scaffold

Start	End	Sequence	Color
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8[118]	13[134]	ACGACATAACTGTATGCCTCTGGAAGTCAGGTGGTGACTCTTTT	#f7931e
6[118]	15[134]	CTATATTACCGATCAGAGTCATCAGGCTTCTTCAGCACGTTTTT	#f7931e
12[134]	11[131]	TTTTCTAGCCAGTGAGTCCGCGGTTTT	#f7931e
6[131]	5[134]	TTTTAGGTTATTCCTGTCTCCTCGTTTTT	#f7931e
10[131]	1[134]	TTTTATCAAATTGCTGTTGTCATCCTTTT	#f7931e
14[134]	9[131]	TTTTAGGTGGCCAACCCCGACAGGTTTT	#f7931e
4[134]	3[118]	TTTTCGGACAAGAAGATCATTCTGAGATC	#f7931e
16[134]	7[131]	TTTTTGGATCACGGGCCAGTTCTCCTTTT	#f7931e
8[131]	3[134]	TTTTCTGGTTTCGGTGTGTCTCGAATTTT	#f7931e
2[134]	11[118]	TTTTCACGAAGTTCACGTTAGGAGAGGAGCATGATAAACCTCCC	#f7931e

0[104]	10[91]	CTCAGGGTCAATATCACCATCTCCATTCTCTGGCACTGCATC	#cc0000
8[76]	12[77]	AGAAAGGCACGCTGAGGTCTGCCTTTGGGCGGTAACCCACTT	#cc0000
7[98]	2[84]	TTCTCGACTGTTTCTGGGCTAGGATTCTCTTGTCATGTCTGA	#cc0000
16[69]	7[90]	GCATTCCAAGGTTGCTTGGTATCGGAGCTGCATACCGGGGAAAGCCGTG	#cc0000
16[76]	9[90]	CTCAGGATGAACAGTGGCAATACCATAACCGCGTAACCACCTG	#cc0000
3[105]	5[118]	GCACAGCCATGCGGCAATGAGTTCAAAT	#cc0000
11[77]	10[77]	AGAAATGCCACGAGCGACACCCGCCGCG	#cc0000
15[84]	1[104]	TTGGGTTGAGCGGTCAGCGGTTTCATAGTGAGAGTCGTGATCTCCAGAAG	#cc0000
9[63]	7[69]	TAGCGGTAAGGGAAGATTTAG	#cc0000
12[118]	14[105]	GAACACTTCTTTAGGGTTTGTGTGGAA	#cc0000
3[63]	7[76]	GAATAGCGAACGTGTCAGGGCGATGGCCTTGGGGTAGCTTGA	#cc0000
1[77]	2[63]	CCGTCGCGCAGATGATCCAAGAGCTGACAGTTTGTGTGGTACC	#cc0000
8[90]	10[70]	ACGCGAACGTGGCGAGGGTTGTATGGCCCAGCAGTGAGCACGCTTAATG	#cc0000
3[84]	14[84]	AGTGTTGTTCCAGCAGCACCCCAGATGGAGTTGGTGCTGATA	#cc0000
17[84]	16[98]	TGAGCCAGGGCTTAAAGTAATGGAGAAG	#cc0000
11[70]	15[69]	CTGTTGGATCGAGTTTGGGCATCACAATGCTGAGCTGAACAG	#cc0000
7[105]	16[84]	AGTAATCCTGCAGGCTGGTTTCTGAGACCAAAGCCTCGAGCTGGGAGAA	#cc0000
12[104]	13[104]	TGAACTCCAGGCTGGAAGTGCACCTCTGCCGGTGGTGTCCATA	#cc0000
6[90]	3[76]	CCAAATCAAGTTTTCTACTACGAGTCCACTATTAACCGAGAT	#cc0000
11[91]	8[105]	GGGAGATTTTCATATTCAAGTTACTCATCCGTTTCATAGTCGTT	#cc0000
4[104]	5[104]	GAGATAGTAGTTTGGAAACAAGTGAACCATCCCAAGACTTGAT	#cc0000
6[69]	4[43]	CGAGGTGCCGTAAACCGTCTAGACTCCAACGTCAAAGTTTT	#57bb00
9[40]	14[43]	TTTTGCTAGGGCGCTGGGAGATGCCTTTT	#57bb00
1[56]	0[43]	CTGGCGGGTCTTGAGGTTTCGCGCTTTT	#57bb00
3[43]	8[40]	TTTTCCCTTATAAAAAGGAGCGGGCTTTT	#57bb00
11[40]	12[43]	TTTTTCGTAGAAGCCCATCTTTGAGTTTT	#57bb00
15[43]	10[56]	TTTTTTGAAGTTGTTTGGGCTTCTGGAGCAAGTGACAGGGC	#57bb00
7[40]	16[43]	TTTAAACCCTAAAGGGCATCCCTCTTTT	#57bb00
17[43]	8[56]	TTTTGGTCCCACGAGAATGATTTACCAAGCCCCGAAAGCG	#57bb00
1[43]	10[40]	TTTACGACTTGTGCGTGGATCCCGTTTT	#57bb00
13[43]	10[63]	TTTTGTACAACACGAGGCGCGATTGATTGGTAGGTCAGCACCGCCGCT	#57bb00
5[43]	6[40]	TTTTGGCGAAAAAGCACTAAATCGGTTTT	#57bb00
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Electron Micrographs for pScaf-1512 Brick

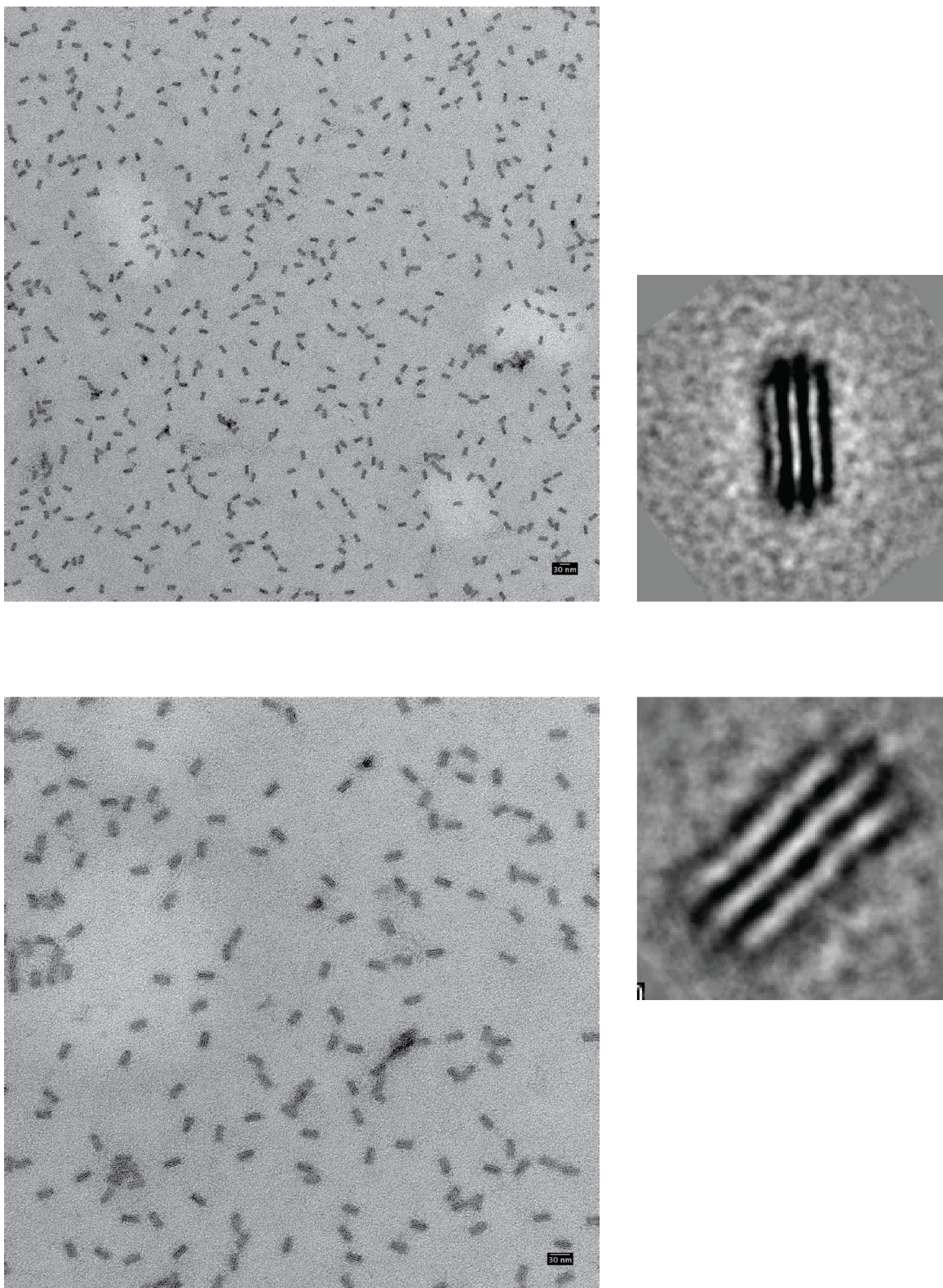


Figure 44: Electron Micrographs and Class Averages for pScaf-1512 Brick

pScaf-2268 Brick

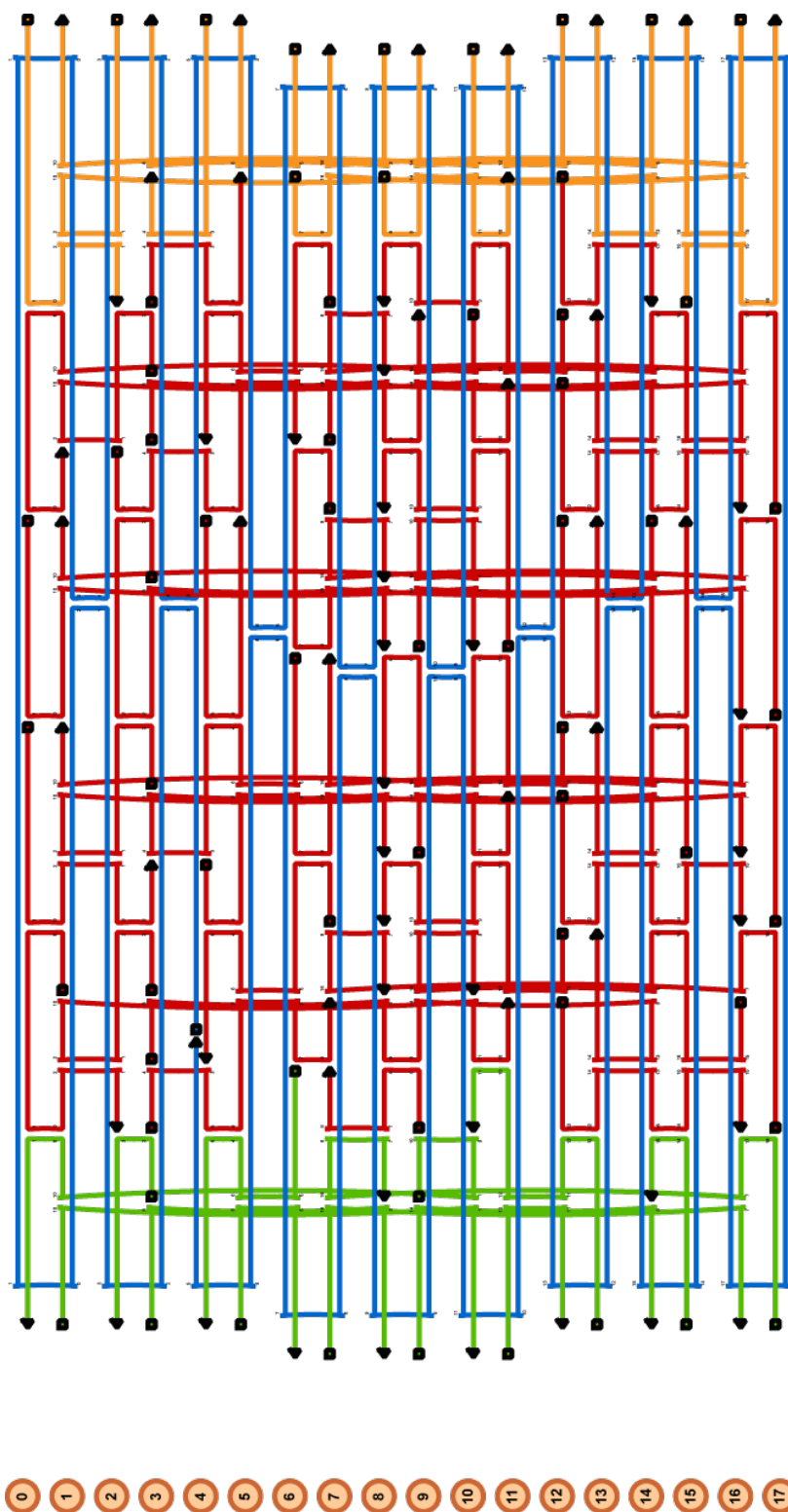


Figure 45: cadnano Design for pScaf-2268 Brick

pScaf-2268.2 Scaffold Sequence

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CTGATTCTTGAGAAACAAGGCGGCTCCACACTGAATCCATTGATCCTGATTACCAC
CGCAAGGCACATCGATGTTGGTCACGACTCTGTGCGCTCAGCGGATGTGTACAC
TGGCAAAGAAATGCACTCATCGGGTGAGTATGGGCCGCAAGCGTCTTGAGGAATC
CAACCCATGAAGCATGGCAGAACGCTAGAGACGCTTGGGCTGTGAGCTGATTCT
CGGACAGTTGATTACCGTCTGAAGGAGCAGCCTTCCAGGAGCAGTCCTGAGATCTT
GATCTGGAGTTTGGCCTCTTGCACTCTACCCTTCCAGGAGGCGACAAATTTGAGAG
AATCCACTGATCTTCCAACAGCCCTTGCTGTTTCTGCGGAGGGGCGTTGAGGAAT
GTGTCGGGATGGAACAATTCAGAGAGGTCCAGTGTTTCGGACAAGAGGGCCTGCT
TCTCTGCTTTATCGACCCAATTCTGGATGGCCAGTGCTCTAGCCACGAGGCCTCTG
AGATATTGCAGAGGGTCTTCGGGTCCCTCCCACTTGATTGCCAGGCCAGAGGGC
ACTTCTGGTTGAGCAGGGCAGAGGCCAACTTCTGGACTTCTGATGAGAGCAGTGT
AGTGCCGCGGATCACTTTGCTCAAGGCTGCCAGTGATTGATGAACTGACTGGACC
AATCTGATGGCGTTGAACTGCTCCAAGATAATAAAGGACAGAATTGGTGATCCTTG
GCGGTCATTGGGAGGAGGGACCTTCTGGTGGATCAGGTTGGAGTTCTGGTTGAGT
TTCTTCCACAGGTTGAGCACTGGGCTGAGTTCATTGCTCCAGATCTCGCGATCGAA
CTTTGAGCCTGCAGTAATAGAGCGGCCCAGGATGCGGAGTTGGGAAATCACCTGA
GATGAGATCATGCGCTGTGATGAGCGTGCAATGTTGGCAGGCAGACCGAAGAAGG
ATGGCTTATCGTCTTCTGGAATCTTCTCAATGACAGCCCTGTAATCCAGAATGCTG
CAGCTTTGTGGCAGGCTCACGCTGTAGGGAAAGATGGACTTCTTGTTGCGCTGATT
GAACACGTCAATGACGCTAGAGTTGAAGAATTGCTTCAAATAGCTCTGCAGGACGC
GCAAGTCGAAATAGTTATCGATGCGGCCCTCCGTAGATGGCGTTCTCCAGCAGACC
ATGGACAAACTCCCATTGCACGTCCTTGGCACCATCAAAGAGCCTGTCAATGATAT
TGTAACCGGCCCTCAGGTCGGAGAGAGAGAACTCGTAGAATTTGGTCCAACCTTG
AGGAATGTAGTTGCGCCTCTCTTGGCATGCGGCGTGAAACCAGGCCAGAGAGAAC
AGAGCATGTGCGCGGTGAGTGTTATCTTTCTTAGAGATTTGTTGAGGTGTCCAAGA
TTCATATGTGCGCATGAGATTCTTCTTGAGTCCAGGTGGTGATTCATAAGTGATCTT
GAGGCTTGACTGGAGGAGGATAGGTGTAAAGTTTGGGTGCACTTCGGCTGTCAAC
CAGAGCCTGAAAGTGTCTTTGGGTGCAAGGTGTTGAGTTCCTTCTCCAAGACAGG
CAGCCAGGAGACAACCAGGTGCAAGTTCTTCAAACACAGCCAGTCACCATTCTT
GCGCATTCTTGAGCATTGATAGCCAAGTCAGCTTGGCCCTGGCCCATTTGCAA
CCTGGTGGTAACATTCTCCTGATCTTTCGGCGTTGGCGAGCTCCTGGAGTTCCTG
GGAGGGATCGGCTCCTGGAGAAATAATGATCAGAATGGGCTCGATTTGAGTGTC
TCCTTGACAGCCTCTTGAGATTGAGTGGGAGTGGAGAGACTTCCTTCAAACCCAA
GGTCTTGACAGGCAAAGAGGGCCATAGCGGATCCACGCGCCCTGTAGCGGCGCAT
TAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCG
CCCTAGCGCCCGCTCCTTTGCTTTCTTCCCTTCTTCTCGCCACGTTGCGCCGGC
TTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTT
ACGGCACCTCGACCCCAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCA
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Staple Sequences for pScaf-2268.2 Scaffold

Start	End	Sequence	Color
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15[43]	14[56]	TTTTCAAGCCTCAAGATCAACCTATC	#57bb00
3[43]	8[40]	TTTAGGTCTAACCAGGGCGCGTGGTTTT	#57bb00
9[40]	14[43]	TTTTATCCGCTATGGCCTCCTCCAGTTTT	#57bb00
17[43]	8[56]	TTTTTCACGCCGCATGCCAATCTCTCTGCGAGAAGCCGCTA	#57bb00
6[69]	4[43]	CCCGATTAGAGCTCCGTCTAGACTCCAACGTCAAAGTTTT	#57bb00
1[43]	10[40]	TTTTATACTCACCTTCTCCAGGAGCTTTT	#57bb00
5[43]	6[40]	TTTTGGCGAAAAATGACGGGGAAAGTTTT	#57bb00
9[56]	0[43]	CCTCTTTTCATTATCGATGAGAAGACGCTTGCGGCCCTTTT	#57bb00
3[56]	2[43]	TCGAGGCTTGTTTCTCAAGAATCTTTT	#57bb00
7[40]	16[43]	TTTTCCGGCGAACGTGGGCCTGGTTTTTT	#57bb00
11[40]	12[43]	TTTTCGATCCCTCCCAGTGACTGGCTTTT	#57bb00
12[104]	1[104]	GGCCAAGTTACCACCAGGTTGAATCTCAAGAGGCTCGACAGA	#cc0000
15[91]	8[98]	GAATCTCATGCGCATTTCAAGACTCCCACTCAACCAGGTAGCGGTCACG	#cc0000
3[63]	7[76]	ATGCGGTGAACGTGTCAGGGCGATGGCCGGGAGCCGAAGAAA	#cc0000
10[146]	1[132]	GCGAGATACTGCAATCGACGGTAATCAACTGTCCGTCCTGGA	#cc0000
4[90]	16[91]	GAACAAGTGAACCATCACCCAGCACTAACGCTAGGTAAGAAA	#cc0000
7[133]	9[146]	CACTGGCGAACGCCCTTGCGCGTCTGCGCGCAACCCTGATC	#cc0000
3[77]	11[76]	AATCGGCTGGATTCAAGTGTGGCTTTGCCCAGCCCTCGCCAA	#cc0000
7[84]	4[70]	GAGCGGGATCGGAACCCCTAAACACTACGAGTCCACTATTAAA	#cc0000
12[125]	13[125]	GCACGCTCATCAATGGGCCAGGAACACCTTGACGTCGAGCCT	#cc0000
16[76]	10[77]	CGCACATATCACCACTGGACCGAAGTGGGGTTTGTGCAAAT	#cc0000
1[77]	2[63]	AGTGTACGCTTCATGGGTTGGATTCTCTGCATTTAGCCGCC	#cc0000
4[125]	5[125]	GAAGCAGGCATAGGGTTGAGTTTTTTGGGCTCTGGCCTGGC	#cc0000
9[63]	7[69]	GCCTGCATTAATGCAGGAAGG	#cc0000
17[63]	8[77]	GAGAGGCGCAACTACATTCCTCTCACCGGCGAAAGCACACCC	#cc0000
7[147]	4[133]	AGCAAAGGTCCAGAAGTTGGCGGACCCGGAATTGGGTCGATA	#cc0000
3[147]	5[160]	ATTGTTCCCATCCAAAGACCCTCTGCAA	#cc0000
3[119]	8[112]	TCTTGCTCTCTGAAAATCGATGTGCCTGAATAGCCCGAGCCCGCCATC	#cc0000
3[133]	13[146]	CTGGACCTATTATCACTCCAAAAGAAGTAGCTGCAGCATTCT	#cc0000
9[112]	15[125]	TGTGGAAAGACCCAAAGACACCATATGAATCTTTCGATAACT	#cc0000
2[132]	6[133]	AGTGGATCGAAACAAAGCAGAAATCCAAGTGGGAGCTCTGCC	#cc0000
12[146]	8[147]	TTCGGTCTTCCCAACTCCGCATTGATCCACCAGACCAATTC	#cc0000
3[140]	11[139]	TCTCTGAGCTGTTGGAAGATCAGGGTAGCTGGAGCAGGTGAT	#cc0000
17[126]	8[140]	AGGGCCGGTTACAATATCATTTGCTGGAAGCCTTGTGTCCTT	#cc0000
7[126]	16[105]	CATCAATCTGCTCAACCAGAAGTGCCGTTGGTCCACAGGACACCTGAAC	#cc0000
11[112]	8[126]	CACAGCGCATGATCTCATCTCAATGAACAACCAGATTGGAGC	#cc0000
12[139]	16[126]	TGCCTGCCAACATTGCCACAACCATCTTATTTTGAATCTACG	#cc0000

12[83]	8[84]	CAAATGCCGCCGAAAGATCAGGAGACACAAGGAAGTAACCAC	#cc0000
0[125]	10[112]	AGAATCAGCTCGACAGCCCAAGTCGTGACCAACTTAGTGCTC	#cc0000
9[91]	13[104]	TCTCTCCCTCTGGTTTGGAGAAGGAACT	#cc0000
12[76]	16[63]	TCAAGGAATGCGCAGCACCTGACTTTACCTTATGAGCTCTGT	#cc0000
3[70]	13[83]	ACCCCGAGCCGCGCAGACCTTCACCCAAGTTGTCTCCTGGCT	#cc0000
12[160]	14[147]	ATAAGCCATCCTTCGGATTACTCAATCA	#cc0000
17[105]	8[119]	GAGTTCTCTCTCTCCGACCTGGAGGCCGGTCAGTTAGTTCAA	#cc0000
12[97]	16[84]	CTGACTTGGCTATCGCCTGTCTGACAGCTCAAGAAGATAACA	#cc0000
6[111]	8[91]	CGAGGTGCCGTAAAAATCAAGGTTGTTCCAGTTTGCTTATAACTGCGCG	#cc0000
3[98]	11[97]	ATCAAAATGCGGTGGTAATCACTGAGCGGTACAAGGAGAATG	#cc0000
0[104]	3[90]	GCGTCTCTAGCGTTCTGCCATACATCCGGGATCAAAAAATCC	#cc0000
14[125]	1[125]	TCCCTACGAAACTGTCAGCCCTGTGCGC	#cc0000
17[84]	7[111]	CAAGGTTGGACCAAATTCTACAAATCTCGCGCTGGCAAGTAT	#cc0000
6[173]	5[176]	TTTTACTGCTCTCTATCTCAGAGGCTTTT	#f7931e
2[176]	11[160]	TTTTACGCCCTCCGCCAGGAAACAACCTCCACTCAAAGTCCTGGG	#f7931e
15[147]	17[176]	AGAGCTACATGGTCGACAGGCTCTTTGATGGTGCCAAGGATTTT	#f7931e
14[176]	9[173]	TTTTTCTAGCGTCATTTCTCCCAATTTT	#f7931e
8[173]	3[176]	TTTTTGACCGCCAACACATTCCTGATTTT	#f7931e
12[176]	11[173]	TTTTTTCCAGAAGACGCCGCTCTATTTTT	#f7931e
10[173]	1[176]	TTTTTACTGCAGGGATCAAGATCTCTTTT	#f7931e
0[176]	2[147]	TTTTAGGACTGCTCCTGGAAGGCTGCTCCTGAGGCCAAGCAAGG	#f7931e
16[176]	7[173]	TTTTCGTGCAATGGGACGGCACTACTTTT	#f7931e
6[160]	15[176]	ATCAGAATGATCCGTTTTGTCTTTGAAGCAATTCTTCAACTTTT	#f7931e
8[160]	13[176]	AGGATCAAGGTCCCGACGTGTAGGGCTGTCATTGAGAAGATTTT	#f7931e
4[176]	3[160]	TTTTCTCGTGGCTAGAGCACTGGCATCCCCG	#f7931e

Electron Micrographs for pScaf-2268 Brick

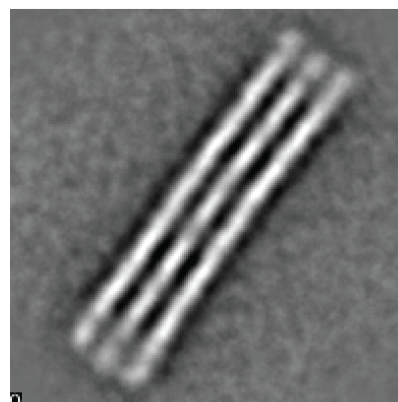
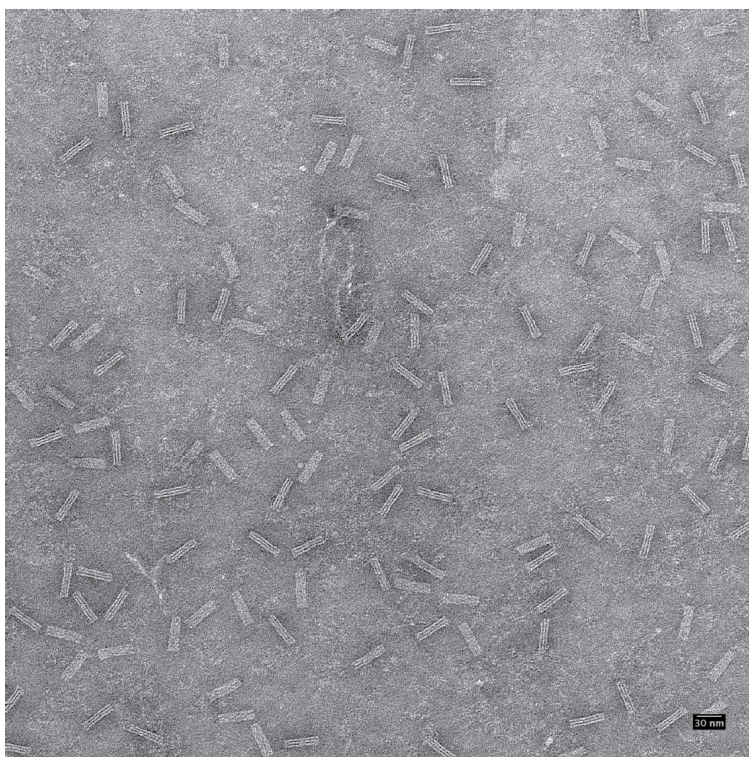
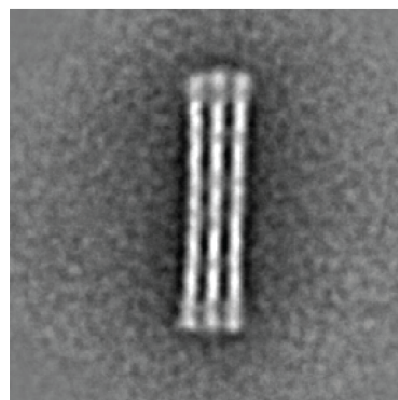
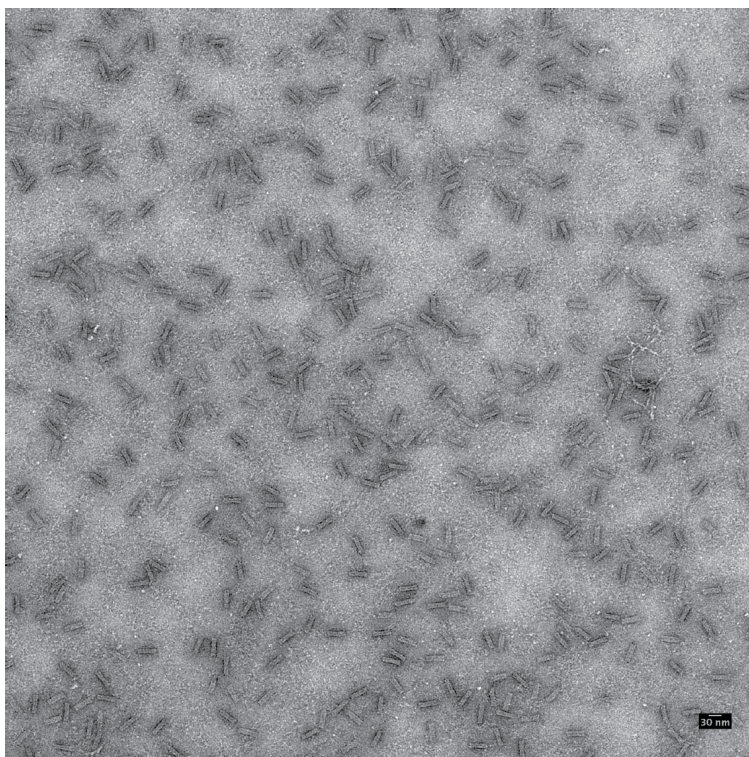


Figure 46: Electron Micrographs and Class Averages for p2268-Brick

pScaf-3024 Brick

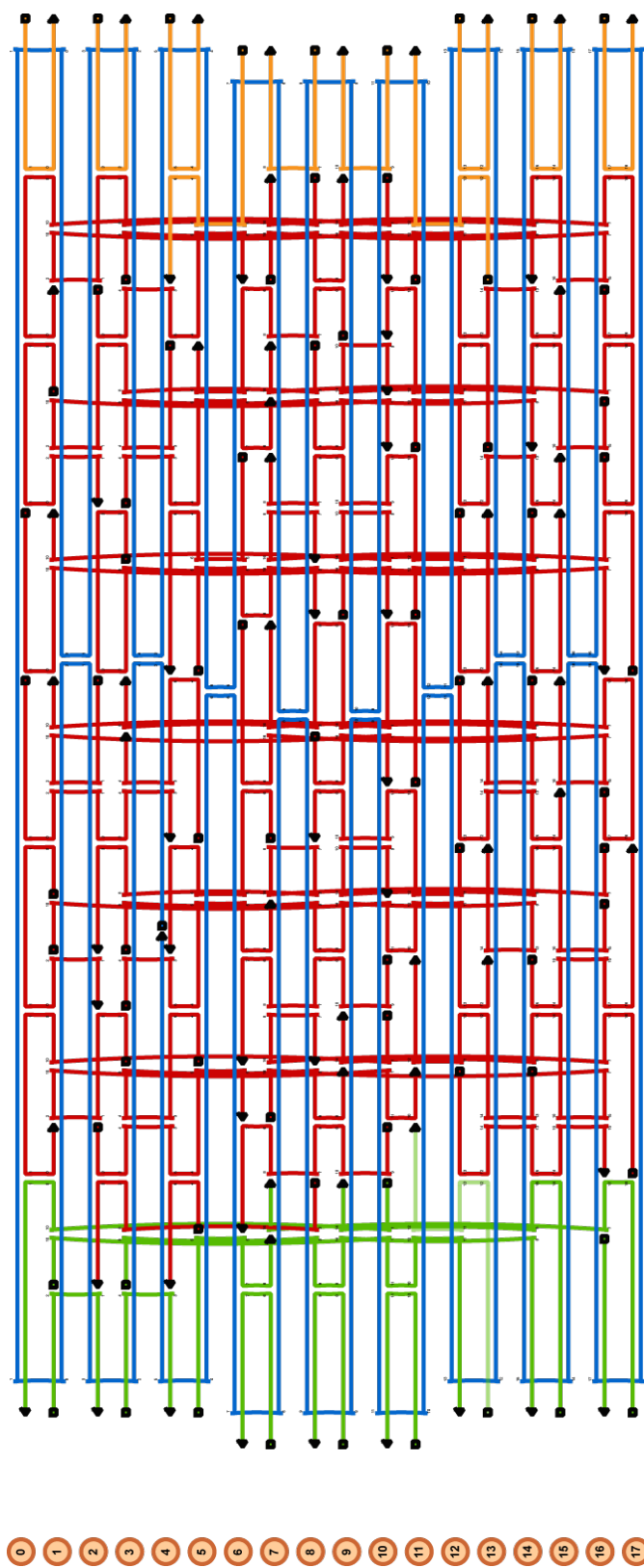


Figure 47: cadnano Design for pScaf-3024 Brick

pScaf-3024.1 Scaffold Sequence

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GGATTCCAAGAAATGGCCAATGAAGATTGGATCACCTTTCGCACTAAGACCTACTT
GTTTGAGGAGTTTCTGATGAATTGGCACGACCGCCTCAGGAAAGTGGAGGAGCAT
TCTGTGATGACTGTCAAGCTCCAATCTGAGGTGGACAAATATAAGATTGTTATCCCT
ATCCTGAAGTACGTCCGCGGAGAACACCTGTCACCCGATCACTGGCTGGATCTGT
TCCGCTTGCTGGGTCTGCCTCGCGGCACATCTCTGGAGAAACTGCTGTTTCGGTGA
CCTGCTGAGAGTTGCCGATACCATCGTGGCCAAGGCTGCTGACCTGAAAGATCTG
AACTCACGCGCCCAGGGTGAAGTGACCATCCGCGAAGCACTCAGGGAAGTGGATT
TGTGGGGCGTGGGTGCTGTGTTCACTGATCGACTATGAGGACTCCCAGAGCCC
CACCATGAAGCTGATCAAGGATTGGAAGGACATCGTCAACCAGGTGGGCGACAAT
AGATGCCTCCTGCAGTCCTTGAAGGACTCACCATACTATAAAGGCTTTGAAGACAA
GGTCAGCATCTGGGAAAGGAAACTCGCCGAAGTGGACGAATATTTGCAGAACCTC
AACCATATTCAGAGAAAGTGGGTTTACCTCGAACCAATCTTTGGTCGCGGAGCCCT
GCCCAAAGAGCAGACCAGATTCAACAGGGTGGATGAAGATTTCCGCAGCATCATG
ACAGATATCAAGAAGGACAATCGCGTCACAACCTTGACTACCCACGCAGGCATT
GCAACTCACTGCTGACCATCCTGGACCAATTGCAGAGATGCCAGCGCAGCCTCAA
CGAGTTCCTGGAGGAGAAGCGCAGCGCCTTCCCTCGCTTCTACTTCATCGGAGAC
GATGACCTGCTGGAGATCTTGGGCCAGTCAACCAATCCATCCGTGATTCAGTCTCA
CCTCAAGAAGCTGTTTGCTGGTATCAACTCTGTCTGTTTCGATGAGAAGTCTAAGC
ACATTACTGCAATGAAGTCCTTGGAGGGAGAAGTTGTGCCATTCAAGAATAAGGTG
CCCTTGTCGAATAACGTGCAAACCTGGCTGAACGATCTGGCCCTGGAGATGAAGA
AGACCCTGGAGCAGCTGCTGAAGGAGTGCCTGACAACCGGACGCAGCTCTCAGG
GAGCTGTGGACCCTTCTCTGTTCCCATCACAGATCCTGTGCTTGGCCGAACAGATC
AAGTTTACCGAAGATGTGGAGAACGCAATTAAGATCACTCCCTGCACCAGATTGA
GACACAGCTGGTGAACAAATTGGAGCAGTATACTAACATCGACACATCTTCCGAGG
ACCCAGGTAACACAGAGTCCGGTATTCTGGAGCTGAAACTGAAAGCACTGATTCTC
GACATTATCCATAACATCGACGTGGTCAAGCAGCTGAACCAATCCAAGTGACACAC
CACCGAAGATTGGGCCTGGAAGAAGCAGTTGAGGTTCTACATGAAGTCCGACCAC
ACCTGTTGCGTTCAGATGGTTGACAGCGAGTTCCAGTACACCTATGAGTACCAAGG
AAATGCCAGCAAGCTCGTTTACACTCCACTCACTGACAAGTGTTACCTCACCTTGA
CACAGGCTATGAAGATGGGCCTGGGAGGCAACCCATACGGTCCAGCTGGCACTG
GTAAGACAGAGAGCGTTAAGGCACTCGGAGGTCTGCTGGGCAGGCAGGTCCTCG
TGTTCAACTGTGATGAAGGAATCGACGTTAAGTCCATGGGAAGAATCTTTGTTGGC
CTCGTTAAGTGTGGAGCTTGGGGTTGCTTCGACGAGTTCAACAGGCTGGAGGAAT
CTGTGCTGAGCGCCGTCTCTATGCAGATCCAGACCATCCAGGACGCATTGAAGAA
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CCCAGACAACCTGAAGCAGCTGTTCCGCCAGTGGCTATGTCCCATCCAGACAAT
GAGCTGATCGCCGAAGTCATCCTCTATTCCGAGGGATTCAAAGATGCTAAAGTTCT
CTCCAGAAAGCTCGTGGCCATCTTCAATCTGTCAAGAGAACTCCTGACACCTCAGC
AGCATTACGACTGGGGTCTGAGAGCCCTCAAGACCGTCCTGAGAGGTTTCAGGAAA
TCTCCTCAGGCAGCTGAACAAGAGCGGTACAACACAGAATGCAATGAGAGCCAC
ATTGTCGTCCAGGCTCTGAGGCTGAATACCATGTCAAAGTTCACATTACAGACTG

CACAAGATTTGACGCTCTGATTAAAGATGTGTTCCCTGGTATTGAACTCAAAGAAGT
 GGAGTATGACGAGCTGAGCGCCGCTTTGAAGCAGGTGTTTGAGGAGGCTAACTAT
 GAGATTATCCCTAATCAGATCAAGAAAGCATTGGAAGTGTATGAACAGCTGTGTCA
 GAGGATGGGAGTGGTGATTGTGGGCCCATCAGGCGCAGGTAAGAGCACTCTCTG
 GAGAATGCTGAGAGCAGCACTGTGCAAGACTGGAAAGGTGGTGAAGCAATACACC
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 TGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTT
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 ATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAA
 AAAGTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTT
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Staple Sequences for pScaf-3024.1 Scaffold

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16[187]	17[187]	TTTTCTGTGTCAAGGTGAGAGGCCCATCTTCATAGTTTT	#f7931e
13[154]	11[183]	CCTGGGTCCTCGGATGTTCAACAGCTCCCTGAGAGCTGCGTTTT	#f7931e
10[183]	9[183]	TTTTCCGGTTGTCAATGTGCTTAGACTTTT	#f7931e
6[183]	4[154]	TTTTTGAGGCTGCGTGGCATCTCCCTGTTGACCAAAGATTGGT	#f7931e
8[183]	7[183]	TTTTTTCTCATCGAAACCAGGAACTCGTTTTT	#f7931e
0[187]	1[187]	TTTGTATGGTCACTTCACCCCCCTGAGTGCTTCGCGTTTT	#f7931e
12[187]	13[187]	TTTTGTATACTGCTCCAATTAGATGTGTGATGTTATTTT	#f7931e
4[187]	5[187]	TTTTTTGGGCAGGGCTCCGCGAATCTGGTCTGCTCTTTTT	#f7931e
14[187]	15[187]	TTTCCAGGCCCAATCTTCGACCTCAACTGCTTCTTTTTT	#f7931e
16[132]	7[146]	TGCTGGCTCTGTCTTACCAGTGCCAGCTAGTGTAAGCGAG	#cc0000
9[112]	15[125]	AGGGCACGGATAATGTCGATCATGTGTACTGGAAGTCTGCTGT	#cc0000
16[83]	2[70]	CATAGAGGGATTACACAGTGAATCAAACCAATTCATCAGAA	#cc0000
17[42]	8[56]	CCCATGGACTTAACGTCGATTAGATTCCGCTACAGCTCCAGA	#cc0000
12[83]	8[84]	TTCCTGATTGCATTCTGTGTTGCTCAGTCCTCAAAGTCTTG	#cc0000
14[55]	11[69]	GGTTGTCTTTCTGGAGAGAACTCTGAGGGACGACAATGTGG	#cc0000
8[97]	13[104]	CCTTCCACACCTGTGTCTGGAGGATGACTTCGGC	#cc0000
3[70]	13[83]	CCTTATACTGCTCTTCTCATACTGGGCGTCTTTGAATCCCTC	#cc0000
12[104]	15[90]	CGTTCAGCTGCCTGAGGAGATGGAATAGATGGGACAGACGGT	#cc0000
5[35]	4[28]	AAATCAAAAAACCGTCTATCA	#cc0000
3[126]	7[139]	TTCCTTTTGAATATGATATCTGTCATGACAGCAGTGAAGTAG	#cc0000
0[125]	10[112]	ACGATGGTATCGGCAACTCTCCCCTCTGGGAGTCCTCGTTCA	#cc0000
3[56]	11[55]	AAATCGGAAACAAGTAGGTCTAACAATCATACCAGAGAGCCT	#cc0000
16[139]	10[140]	ACGAGCTCTGAACGCAACAGGTTAGCTTTCTCCCAGGGTC	#cc0000
10[167]	1[153]	GCACTCCTCCAGTTTGGGCGCGTGAGTTCAGATCTCCACGCC	#cc0000
1[70]	13[69]	CTCAGATTACTCCACTCTCATACCTCTCAGGACGGTTTAGCA	#cc0000
3[154]	14[154]	CAAAGCCAGTTGATACTTCATGCACTTG	#cc0000

14[125]	1[125]	ATGTTATCTTATTCGGCCAGATCATAGT	#cc0000
10[48]	6[35]	GGAACACAATGCTTCTTACCTGCGCTTAGAAAGGAGCGGGCG	#cc0000
9[147]	5[146]	TCCAAGGACCAGCAGGAAGGCTGGTCCAGGATGGTTGCTGCG	#cc0000
2[153]	6[154]	CCTGGTTTTGTCTTTGAGGTGAAATCTTCATCCACTGCAAT	#cc0000
7[84]	4[70]	TGGTGTAGGGAAAGCCGCGACGGAACCAGTCCACTATTAAA	#cc0000
7[154]	9[167]	GCTGCGCTTGTCAGGGACTTCATGTAGAGTGGTGTTCAGTA	#cc0000
1[77]	2[63]	TGGAGCTTTCTCCAGAGATGTGCCGCGATGTCCACACTCCTC	#cc0000
17[105]	8[119]	CCTCCGAGTGCCTTAACGCTCATTTCTTCATCGTTGAATCA	#cc0000
2[104]	10[91]	CACCTTCCTGAGGCCATCACAAAAGCGG	#cc0000
8[41]	2[28]	GCGCCTGGAACCTAAAGGTGATCCAAT	#cc0000
5[105]	16[105]	TGTGACGCGATTGTTGCGTGGCAGCAGGTGGTACTCATAGGC	#cc0000
16[153]	7[167]	TGAGTGGGGACCGTATGGGTTGCCTCCCCTAACACTTCTCCT	#cc0000
4[146]	14[133]	AAACCCACTTTCTCCCCAGATTCTTGAGGCACAACGCTTGAC	#cc0000
12[55]	16[42]	GGCTCTCAGACCCCCACGAGCTGGGAGTCGGAGTTTGTTGAA	#cc0000
2[48]	6[49]	TAGTGCGTCGTAGGAGGGCGAGTTTTTTGGGGTCGAGAAAGC	#cc0000
12[125]	7[132]	TAATTGCCGGCCAAGCACAGGTCTCCAGTTGAATGGTGAGACCTCCGAT	#cc0000
7[49]	9[62]	ATGCGCCTCCAGCCGGAGTTCACCTCCATGCTTCACTGATTA	#cc0000
5[56]	9[55]	AGGTGCCGACTCCAACGTCAATACCCCGAGTGCTTCTTGAT	#cc0000
6[111]	15[104]	GTAGTCAAGTAGAGCTTGACGTTGCTTCATGGTCTCCTGTGGTTCTTCA	#cc0000
8[146]	10[133]	AACAGCTGCTGACCGACGATGTCCTTCCTGTGAACCTTCTTCA	#cc0000
16[76]	10[77]	ACGGCGCACCCAACAGCTCGCATAGCCAGTTAGCCCTCGTCA	#cc0000
10[62]	1[48]	GAGTTCATTATATTGGCAGACCCAGCAAGCGGAAGTGGGAT	#cc0000
3[63]	7[76]	CAAAATCGAACGTGGTAAAGCACTAAATACGTGGCGGATCCG	#cc0000
11[91]	1[104]	GTACCGCTCTTGGTAAACTTGCCAGGTTTCGATCGAATGCT	#cc0000
8[167]	10[154]	CAGACAGTTTATAGTCTATTGTGCGCCACCACAAATTCAGCA	#cc0000
1[140]	2[126]	ACAGCACTTCAGGTCAGCAGCCTTGCCCGATCAGAATCCTT	#cc0000
5[84]	3[97]	CTAAAGGGAGCCCCGATTGTGTTGTTCCAGTTTGCCCGAGA	#cc0000
3[119]	8[112]	GGCGAGTGATCAGCTTCATGGTGCAGTTCGATTCGATTCAGTTCCGGATGG	#cc0000
16[90]	7[111]	GGATCTGAGGACCTGCCTGCCAGCAGAGTCTGGACGCCCAAGATCTC	#cc0000
6[132]	4[105]	GAGTTGCGAATGCCCTTCTTGTTGAGGTTCTGCAAATAGT	#cc0000
14[69]	17[83]	GAACAGCCTTCCTTTCAGCACCTTCATCACAGTTGAACACG	#cc0000
13[133]	10[147]	AGAATACCGACTCTCTGGTGATGGGAACAGAGAAGCTGCTC	#cc0000
0[104]	4[84]	AGCAGGTCACCGAACAGCAGTTGACAGTGGTCGTGAGAATAGGAACAAG	#cc0000
11[133]	15[132]	ATCTGTGCAGGGAGTGATCTTCAGCTCCCACGTCGCAACCAT	#cc0000
14[104]	3[104]	AGCTCATCTCGTTATTGGACAATTGGTTGACTGCATAGGGTT	#cc0000
10[69]	6[56]	CTTCTTTGGGATAACAGCATTGGCGCGTGAGAAAGGAAGGGA	#cc0000
11[112]	13[125]	ATCTGTTGTTCTCCACATCTTGAGAATCAGTGCTTTCAGTTT	#cc0000
11[154]	15[153]	GGGTCCACAGCTGTGTCTCAATGTGTTAGATTTGGTGTGGTC	#cc0000
9[8]	8[8]	TTTTATCTCTGACACAGCTCACAATCACCCTCCCTTTT	#57bb00
3[28]	14[12]	CCTGCTGATGGGCCGTTTCATATTCCTCCATATCCTTTACCTTTT	#57bb00
10[41]	0[12]	ATCTTTATTACAGGAAGATCCAGCCAGTGATCGGGTGACAGTTTT	#57bb00

11[8]	10[8]	TTTTGTCTGTGAATGTGAACCGTCAAATCTTGTGCATTTT	#57bb00
7[8]	6[8]	TTTTGTCACGCTGCGCGTAAGCTGGCAAGTGTAGCGTTTT	#57bb00
16[34]	16[12]	AAGCAACCCCAAGCTCCACTTTT	#57bb00
3[12]	4[12]	TTTTTTTCTTGGAATCGGGCGATGGCCCTTTT	#57bb00
15[12]	9[41]	TTTTTGC GGGATT CATTGTGATGAAGATTCTTCTGTCCAGTTCC	#57bb00
1[28]	12[12]	GACGTACATCAGAGTTTGACAATGCTGCTGAGGTGTCAGGTTTT	#57bb00
5[12]	7[34]	TTTTACTACGTGAACCATCACCCCTAGGGCCCACCAC	#57bb00
17[12]	7[41]	TTTTACTTAACGAGGCCAACAAGATTCTTCTCGTCGACCCGCC	#57bb00
13[12]	11[48]	TTTTAGTTCTCTTGACAGATTGAAGATGGCAGTCGTATGGTATTCAGCCTC	#57bb00
1[12]	2[12]	TTTTGTGTTCTCCGCGCTTCATTGGCCATTTT	#57bb00

Electron Micrographs for pScaf-3024 Brick

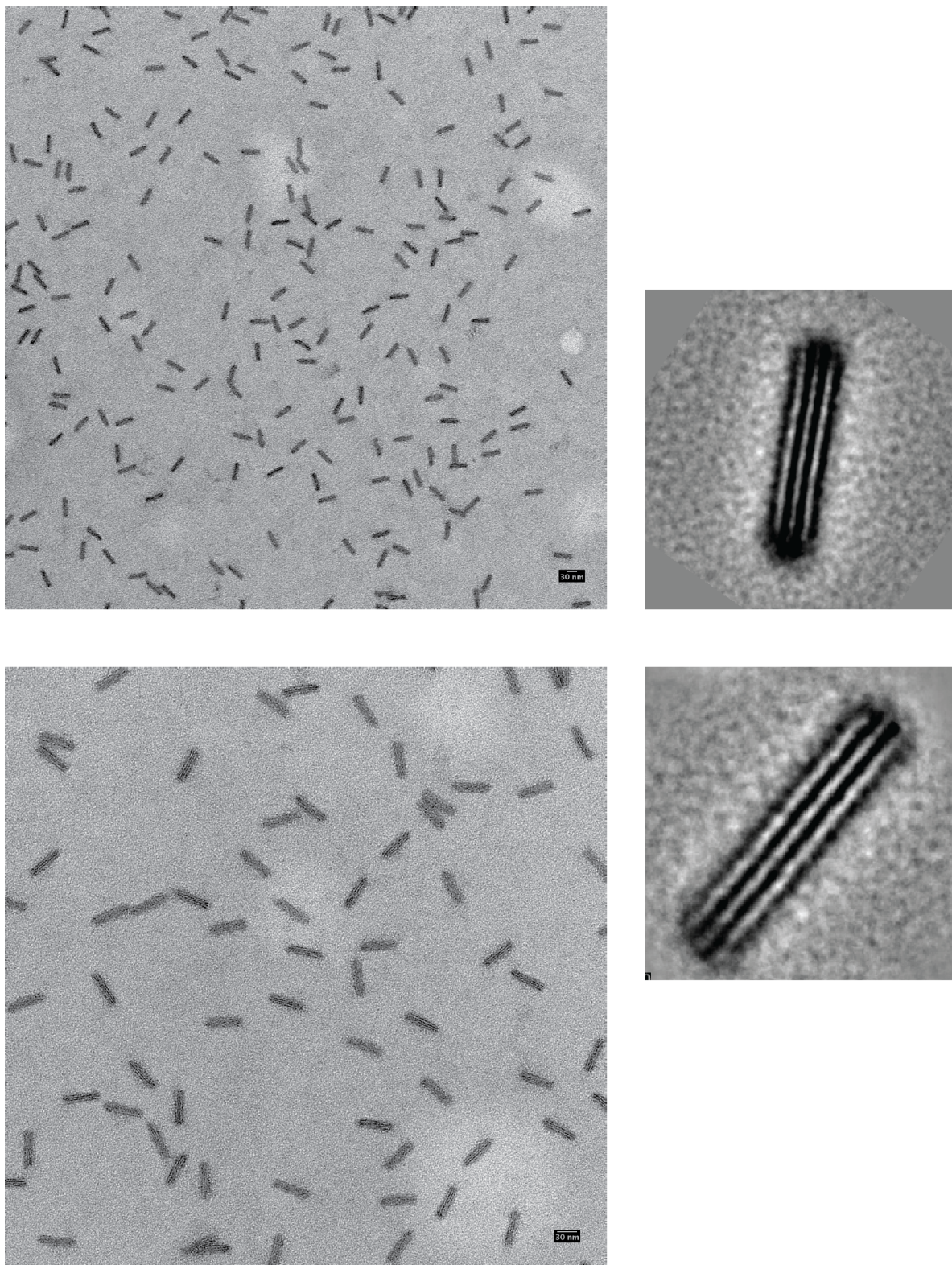


Figure 48: Electron Micrographs and Class Averages for pScaf-3024 Brick

pScaf-5509 Brick

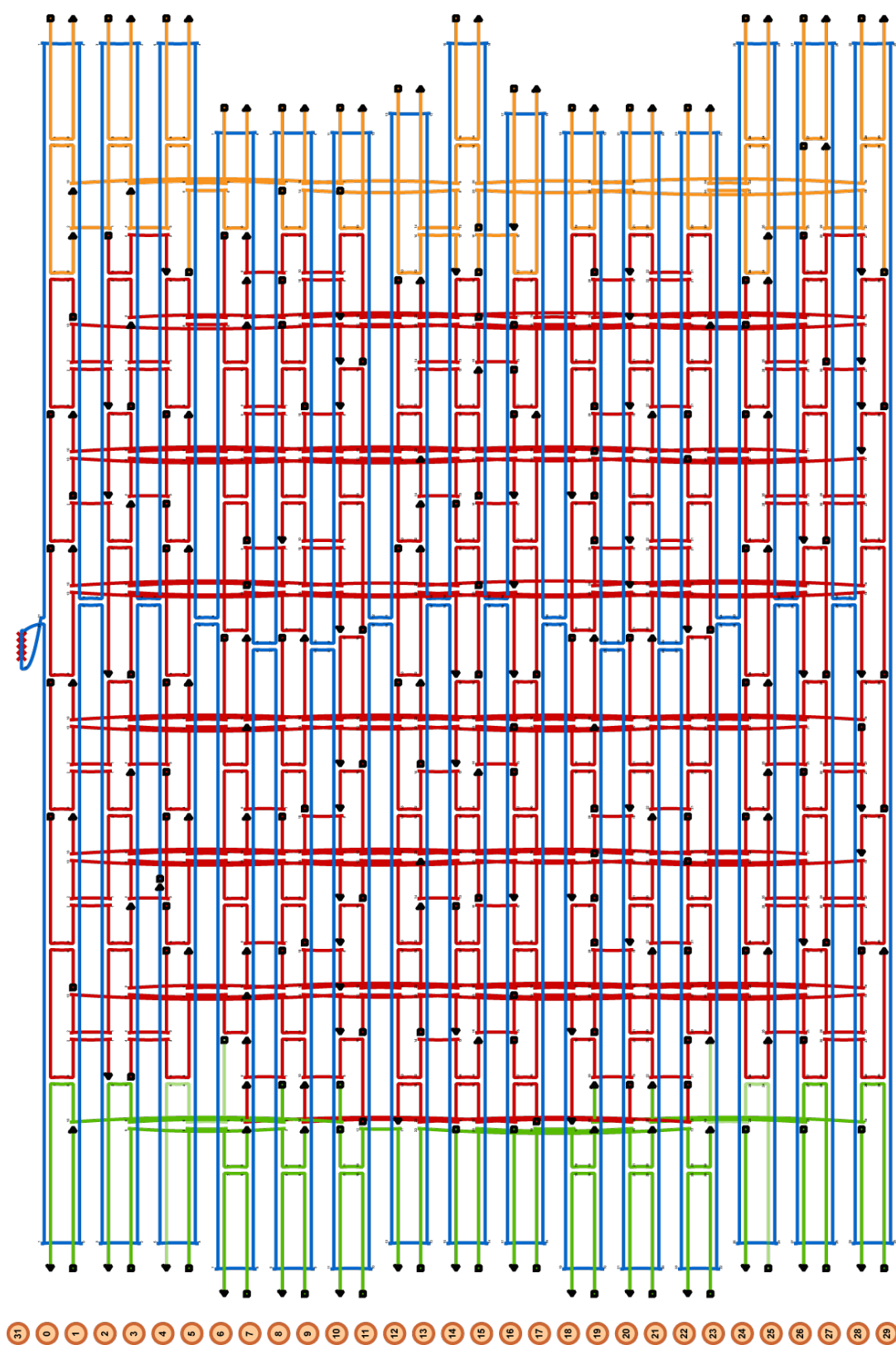


Figure 49: cadnano Design for pScaf-5509 Brick

pScaf-5509 Scaffold Sequence

AATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGCTATTC
TTTTGATTTATAAGGGATTTTGCCGATTTTCGGGGTACCTTTTTTAGATCTCTGTTACA
CACTGTTCCGGGATGCTTATCATCTGGCGGGGATTGAGATGCCGGACTTTTCATCGT
GAGGATGACTGGTGGCGTAACGGCCAGAATCTCTATCTGGATAATCTGGAGGCGA
CGGGGCTGTATCAGGTGCCGTTGTCAGCGGCACAGCCGGGCGATGTGCTGCTGT
GCTGTTTTGGTTCATCAGTGCCGAATCACGCCGCAATTTACTGCGGCGACGGCGA
GCTGCTGCACCATATTCCTGAACAACGAGAGAGGTACACCGACAAA
TGGCAGCGACGCACACACTCCCTCTGGCGTCACCGGGCATGGCGCGCATCTGCC
TTTACGGGGATTTACAACGATTTGGTCGCCGCATCGACCTTCGTGTGAAAACGGG
GGCTGAAGCCATCCGGGCACTGGCCACACAGCTCCCGGCGTTTCGTCAGAACT
GAGCGACGGCTGGTATCAGGTACGGATTGCCGGGCGGGACGTCAGCACGTCCGG
GTTAACGGCGCAGTTACATGAGACTCTGCCTGATGGCGCTGTAATTCATATTGTT
CCAGAGTCGCCGGGGCCAAAGTCAGGTGGCGTATTCCAGATTGTCCTGGGGGCTG
CCGCCATTGCCGGATCATTCTTTACCGCCGGAGCCACCCTTGACAGCATGGGGGGC
AGCCATTGGGGCCCGGTGGTATGACCGGCATCCTGTTTTCTCTCGGTGCCAGTATG
GTGCTCGGTGGTGTGGCGCAGATGCTGGCACCGAAAGCCAGAACTCCCCGTATA
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TGCCCAGGGCAATGTTCTGCCTGTTCTGTACGGGGAAATGCGCGTGGGGTCACGC
GTGGTTTCTCAGGAGATCAGCACGGCAGACGAAGGGGACGGTGGTCAGGTTGTG
GTGATTGGTCGCTGATGCAAAATGTTTTATGTGAAACCGCCTGCGGGCGGTTTTGT
CATTTATGGAGCGTGAGGAATGGGTAAAGGAAGCAGTAAGGGGCATACCCCGCGC
GAAGCGAAGGACAACCTGAAGTCCACGCAGTTGCTGAGTGTGATCGATGCCATCA
GCGAAGGGCCGATTGAAGGTCCGGTGGATGGCTTAAAAAGCGTGCTGCTGAACA
GTACGCCGGTGCTGGACACTGAGGGGAATACCAACATATCCGGTGTACGGTGGT
GTTCCGGGCTGGTGAGCAGGAGCAGACTCCGCCGGAGGGATTTGAATCCTCCGG
CTCCGAGACGGTGCTGGGTACGGAAGTGAAATATGACACGCCGATCACCCGCACC
ATTACGTCTGCAACATCGACCGTCTGCGCTTTACCTTCGGTGTACAGGCACTGGT
GGAAACCACCTCAAAGGGTGACAGGAATCCGTCGGAAGTCCGCCTGCTGGTTCAG
ATACAACGTAACGGTGGCTGGGTGACGGAAAAAGACATCACCATTAAGGGCAAAA
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TAATATCCGGATGCGCAGGATGACGCCGGACAGCACCACAGACCAGCTGCAGAAC
AAAACGCTCTGGTCGTCATACTGAAATCATCGATGTGAAACAGTGCTACCCGAA
CACGGCACTGGTCGGCGTGACAGGTGGACTCGGAGCAGTTCGGCAGCCAGCAGGT
GAGCCGTAATTATCATCTGCGCGGGCGTATTCTGCAGGTGCCGTGAACTATAAC
CCGCAGACGCGGCAATACAGCGGTATCTGGGACGGAACGTTTAAACCGGCATACA
GCAACAACATGGCCTGGTGTCTGTGGGATATGCTGACCCATCCGCGCTACGGCAT
GGGGAAACGTCTTGGTGCGGCGGATGTGGATAAATGGGCGCTGTATGTCATCGG
CCAGTACTGCGACCAGTCAGTGCCGGACGGCTTTGGCGGCACGGAGCCGCGCAT
CACCTGTAATGCGTACCTGACCACACAGCGTAAGGCGTGGGATGTGCTCAGCGAT
TTCTGCTCGGCGATGCGCTGTATGCCGGTATGGAACGGGCAGACGCTGACGTTCC
TGCAGGACCGACCGTCGGATAAGACGTGGACCTATAACCGCAGTAATGTGGTGAT
GCCGGATGATGGCGCGCCGTTCCGCTACAGCTTCAGCGCCCTGAAGGACCGCCA

TAATGCCGTTGAGGTGAACTGGATTGACCCGAACAACGGCTGGGAGACGGCGACA
GAGCTTGTTGAAGATACGCAGGCCATTGCCCGTTACGGTCGTAATGTTACGAAGAT
GGATGCCTTTGGCTGTACCAGCCGGGGGCAGGCACACCGCGCCGGGCTGTGGCT
GATTAACAGAACTGCTGGAAACGCAGACCGTGGATTTCAGCGTCGGCGCAGAA
GGGCTTCGCCATGTACCGGGCGATGTTATTGAAATCTGCGATGATGACTATGCCG
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CGCTCGACCGTGAAATCACGGATCTTGATCACCAGTATGGTGGCCAGCGGCTATG
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CATTACCTATGAAATGTGAGGACGCTATGCCTGTACCAAATCCTACAATGCCGGTG
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CCGGCGAACTGACCGCTGAGTCCTATGACGACAGCTATCTCGATGATGAAGATGC
AGACTGGACTGCGACCGGGCAGGGGCAGAAATCTGCCGGAGATACCAGCTTCAC
GCTGGCGTGGATGCCCGGAGAGCAGGGGCAGCAGGCGCTGCTGGCGTGGTTTAA
TGAAGGCGATACCCGTGCCTATAAAATCCGCTTCCCGAACGGCACGGTCGATGTG
TTCCGTGGCTGGGTCAGCAGTATCGGTAAGGCGGTGACGGCGAAGGAAGTGATC
ACCCGCACGGTGAAAGTCACCAATGTGGGACGTCCGTCGATGGCAGAAGATCGCA
GCACGGTAACAGCGGCAACCGGCATGACCGTGACGCCTGCCAGCACCTCGGTGG
TGAAAGGGCAGAGCACACGCTGACCGTGGCCTTCCAGCCGGAGGGCGTAACCG
ACAAGAGCTTTCGTGCGGTGTCTGCGGATAAAACAAAAGCCACCGTGTGCGTCA
TGGTATGACCATCACCGTGAACGGCGTTGCTGCAGGCAAGGTCAACATTCCGGTT
GTATCCGGTAATGGTGAGTTTGTCTGCGGTTGCAGAAATTACCGTCACCGCCAGTTA
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GAAACGGCAGGCAGAACAGGCGGAGTCAGACAGCAACCGGAAGTTTACTGTGGA
AGACGCCATCAGAACCGGCGCGTTTTCTGGTGGCGATGTCCCTGTGGCATAACCAT
CCGCAGAAGACGCAGATGCCGTCCATGAATGAAGCCGTTAAACAGATTGAGCAGG
AAGTGCTTACCACCTGGCCACGGAGGCAATTTCTCATGCTGAAAACGTGGTGTA
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GACGCCGGGGCCCGCAGAGCCTGTTTCTGCGGGAAAGTGTTTCGACGGTGAGCTGA
GTTTTGCCCTGAACTGGCGCGTGAGATGGGGCGACCCGACTGGCGTGCCATGC
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TTATTTTCATGATGTTCTGCTGGATATGCACTTTTCCGGGCTGACGTACACCGTGCT
CAGCCTGTTTTTCAGCGATCCGGATATGCATCCGCTGGATTTCACTCTGCTGAACC
GGCGCGAGGCTGACGAAGAGCCTGAAGATGATGTGCTGATGCAGAAAGCGGCAG
GGCTTGCCGGAGGTGTCCGCTTTGGCCCGGACGGGAATGAAGTTATCCCCGCTTC
CCCGGATGTGGCGGACATGACGGAGGATGACGTAATGCTGATGACAGTATCAGAA
GGGATCGCAGGAGGAGTCCGGTATGGCTGAACCGGTAGGCGATCTGGTCGTTGA
TTTGAGTCTGGATGCGGCCAGATTTGACGAGCAGATGGCCAGAGTCAGGCGTCAT
TTTTCTGGTACGGAAAGTGATGCGAAAAAACAGCGGCAGTCGTTGAACAGTCGCT
GAGCCGACAGGCGCTGGCTGCACAGAAAGCGGGGATTTCCGTCGGGCAGTATAA
AGCCGCCATGCGTATGCTGCCTGCACAGTTCACCGACGTGGCCACGCAGCTTGCA
GGCGGGCAAAGTCCGTGGCTGATCCTGCTGCAACAGGGGGGGCAGGTGAAGGAC
TCCTTCGGCGGGATGATCCCATGTTCAAGGGGGCTTGCCGGTGCGATCACCTGC
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GCGGGGGTAAGCGGTGAGGCTCAGATTGCGTCCATCAGCCAGAGTGTGGCGCGT
TTCTCCTCTGCATCCGGCGTGGAGGTGGACAAGGTCGCTGAAGCCTTCGGGAAGC
TGACCACAGACCCGACGTCGGGGCTGACGGCGATGGCTCGCCAGTTCCATAACG
TGTCGGCGGAGCAGATTGCGTATGTTGCTCAGTTGCAGCGTTCCGGCGATGAAGC
CGGGGCATTGCAGGCGGCGAACGAGGCCGCAACGAAAGGGTTTGATGACCAGAC
GGATCCACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGGTGTGGTGGTTACGC
GCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTT
CCCTTCCTTTCTCGCCACGTTCCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGG
CTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGA
TTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTTCGCCCTT
TGACGTTGGAGTCCACGTTCTTT

Staple Sequences for pScaf-5509 Scaffold

Start	End	Sequence	Color
17[12]	19[34]	TTTTCGGGCCAAAGCGGACACCTCAGAACACCAGTCG	#57bb00
28[34]	28[12]	CTTCATTAAACCACGCCAGTTTT	#57bb00
9[8]	8[8]	TTTTCTGTGGTCAGCTTCCCAGCCCCGACGTCGGGTTTTT	#57bb00
15[12]	21[34]	TTTTCGTACCAGAAAAATGACGCGGGCATTGGTACAC	#57bb00
29[12]	19[41]	TTTTCAGCGCCTGCTGCCCCTGCTCTCCGGGTATCGCGCATACT	#57bb00
23[8]	22[8]	TTTTTCCGGTTGCTGTCTGATCTTCCACAGTAAACTTTTT	#57bb00
7[8]	6[8]	TTTTCACCCGCCGCGCTTAAGTGCAGTAAACCACATTTT	#57bb00
19[8]	18[8]	TTTTTACTGTAAAAGCGGTGTCATGAAAATAATGGGTTTT	#57bb00
13[12]	23[34]	TTTTTCACCTGCCCCCCTGTTGGATGGCGCTCCGCC	#57bb00
3[12]	9[34]	TTTTCCCGCCAGATGATAAGCATCGCCGTCGAAGGCT	#57bb00
21[8]	20[8]	TTTTTACATACCAGACAGCCATTCACCACAAACTCATTTT	#57bb00
10[34]	12[12]	TGCCCCGAGTCCCAGTCCCAGCGCAAGGAGTCCTTTTT	#57bb00
11[8]	10[8]	TTTTCATACGATCTGCCGTCTCTGGACAGGACCAGTTTT	#57bb00
8[41]	2[12]	GAGCCATCCCGGAACACGATGAAAGTCCGGCATCTCAATCTTTT	#57bb00
5[12]	7[34]	TTTTACTACGTGAACCATCACCCGGTCACGTGCGCCG	#57bb00
25[12]	23[48]	TTTTCCGGATAACAACCGGAATGTTGACCTTCTGCAACTGTTCTGCCTGCCG	#57bb00
14[34]	14[12]	TTTTTTTCGCATCACTTTCTTTT	#57bb00
27[12]	21[41]	TTTTTGCCGGTTGCCGCTGTTACCGTGCTGCGAGGTGCACGTTT	#57bb00
16[34]	16[12]	GGATAACTTCATTCCCGTCTTTT	#57bb00
1[12]	1[34]	TTTTATGGTGCAGCAGCTCGCCG	#57bb00
26[34]	26[12]	CTGGCAGGCGTCACGGTCATTTT	#57bb00
10[41]	0[12]	TGCCGCCTCGCCGCTCTCGTTTGCTCAGTTGTTTCAGGAATTTTT	#57bb00
24[34]	24[12]	CGCAGCAAACTCACCATTATTTT	#57bb00
6[48]	4[12]	CTGGCAAGTGTAGCAAATCAAAAAACCGTCTATCAGGGCGATGGCCCTTTT	#57bb00
24[160]	28[147]	ATCAGCCACAGCCCTAGTCATGTGCGAGCGGTAATGGGTGGTC	#cc0000

24[146]	20[147]	GGCGCGGGACCGTAACGGGCAGGCATTAATAGGTCCGAGCAG	#cc0000
22[41]	12[35]	CGGTTCTCAGCAGGGGGGATC	#cc0000
11[91]	15[90]	TGATACCAGCGAGGTGGCCCCTCGGTGACGCTTTCCAACGAC	#cc0000
24[83]	20[84]	ACATCGCTGCAGGGCTGACAGGTCTTCTTCCTGCTCGAACAC	#cc0000
11[49]	15[48]	AGGACCAAGCCCCCTGAACATATCAGCCAACGACTTGGCCAT	#cc0000
14[69]	18[70]	CTGTCGGTGGCCGCATCCTCCGCACATCATCTTCAGCTGAGC	#cc0000
11[112]	8[126]	GCAGCGCAGACGGTCGATGTTGGAACACCAGTGTCTCGCTTC	#cc0000
16[90]	19[97]	TACTGTCCGTCAGCCTCGCGCATCCGGACTCACGC	#cc0000
22[146]	10[147]	TGGCGGTTTTGCCCTGGTTTCGTAATGGTGCGGGTTCTGCTC	#cc0000
16[97]	25[104]	CTTCTGACAGATCGTCACCGTCAATCTGGTTACGCGTGGCTTTTGT	#cc0000
19[49]	21[62]	TGACATCTTTTATACTGCCATCGACGGAGGTGCTCTGCCTCC	#cc0000
3[147]	7[160]	CTGCCCCACACCACATTGCCCTGGGCAATCCCCTTACCGCCC	#cc0000
6[111]	5[104]	GGAAAGCCGCGCAACGATT	#cc0000
8[83]	10[70]	CAACATAAATCGGCTATCCAGATAGAGATGAACCACTGAGTG	#cc0000
8[62]	10[49]	CCGACACGATCTAACGTTACGCCACCAGTGCGGCGTTAAACG	#cc0000
24[104]	13[104]	CAAATGAAGCGTGACGGTCACCGATTATGGACGGAGGCAGC	#cc0000
1[133]	13[132]	TTAACCCCCAGCCCTGCAGACCACCACTGCCTGTATACTGCG	#cc0000
15[70]	25[83]	ATCCAGACAGAAACAGGTGGTGCCACGGGTCATACCACTGAC	#cc0000
28[62]	10[56]	AAGCGGACCGGCAAGCGGGCCTCAAATCCTCAGCGACGCCGGGGTCTGG	#cc0000
7[126]	16[105]	CCATTCCGAGAAACCACGCGTGACCCGGAAGTCTTCTCGGACTCCTCT	#cc0000
8[167]	10[154]	CACACTCGCAAGGGGCCCCGCGACTCTCCCGCCCGGCGGAG	#cc0000
0[125]	10[112]	TTGTAAATCCCCAAGGCAGATGTGCCGCTGACATCACACCGG	#cc0000
19[168]	7[167]	CTGGTCGATCCACATCCGCCGGTCCCAGTACGCCCGCAGGCG	#cc0000
20[41]	9[41]	TGTTTCAGCTGACTCGCCGCTGTCAGCGA	#cc0000
17[35]	7[41]	CCGGCAAGAAGCGGCTACAGG	#cc0000
12[125]	22[112]	CACCGAAGGTAAACCGGTCGCATACGCATGGCGGAAGCGGAA	#cc0000
22[76]	26[63]	GTTATGCAATGCGCTGACTCTCCGGATTGGTGATGTCAGCGT	#cc0000
2[174]	4[168]	TGACTTGTGGCTCCTTCGGTG	#cc0000
0[146]	3[132]	GTCGATGCGGCGACCAAATCGTGCGCCGGCCATCATCATACC	#cc0000
19[63]	7[62]	GCATGGCACGGTGTACGTGAGTGCATCAGTCATGTCTGGTCA	#cc0000
16[146]	2[133]	TACGGCTCCATAAACTTCAGGCCAATGGATATGAATTACAGC	#cc0000
22[83]	10[84]	GCGGATGGGCCACGCACCATCGGGTTGAGTTGCCCCCTTAAC	#cc0000
27[126]	28[140]	ATAGCGTCGGCATTGATAGCTGTCGTCATAGGACTCCGGCAC	#cc0000
19[140]	1[146]	CCGTGCCAAATCGCAGTGCCGGGGCGCGGGCGTACCTGCTCAGGACGTG	#cc0000
6[174]	3[160]	CAACCTGACCACCGCCATGTTCCAGCATCTGCGCCCCATGCT	#cc0000
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24[62]	20[63]	AACTGGCAGGGCGAGATGCTCCACAGGGGTGGGCCAGGCTCT	#cc0000
17[105]	16[119]	GCAGACTGACACAGACACCAGCGAACTG	#cc0000
12[104]	1[104]	CACCGCCAGGCATACGCCAGCATGCCTCACCGCTTCCCGGCT	#cc0000
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0[104]	3[90]	GCGCGCCATGCCCCGGTGACGCACAGCAGTCCAGATAAAATCC	#cc0000
24[167]	20[168]	TGTTTTAGCGTATCTTCAACAGTCAATCCGGTCGGTACCGGC	#cc0000
27[154]	13[167]	ACATAAGTCAGATCTCCGTGATATCCGACAGTTCATTTTTCC	#cc0000
8[160]	23[160]	AGCAACTCAGCACGTCCGGATTGATGTCCTCAACATGGCCT	#cc0000
7[119]	2[105]	CCTTTACGCGCGGGATGCCGGGGCAGAGTCACGGCACCTGAT	#cc0000
16[48]	18[49]	ATCCGGGGCCCTGCCGCTTTCCCCGGAA	#cc0000
16[153]	28[154]	TGATAATGGTTTAAACGTTCCACCAAGCCGTCCGCCCACAG	#cc0000
3[42]	7[55]	CAGTGTGAGGGCGAGTTTTTTGGGGTCGTAGGGCGGATCCGT	#cc0000
26[48]	29[62]	TCACCACCGATCTTGGCACGGGCATCCACGCCAGCGTGAAGC	#cc0000
4[83]	13[76]	AGTCCACTATTAACCCCGACGCAATCGGAGAAACCAGCGCGCTGCGT	#cc0000
22[62]	10[63]	ACATCGCTTGCCCGACCGGCAGCGTTTTGTTGAAAACCTCGCT	#cc0000
23[112]	20[126]	ACAAAGGCATCCATCTTCGTAGGCGCTGCATCACCATCCAC	#cc0000
14[132]	18[133]	GTTACCCCTGCACTGGCTGCGCCATGTTGTTGCTCGTAGCG	#cc0000
15[133]	25[146]	GCCGACCTGAGCACACATTACTCCGGGTTGCTGATACCGGCA	#cc0000
12[167]	7[174]	GATTCTGTGTCATATTTACATTCAAAGCCATCCATCGATGTTTAC	#cc0000
4[62]	14[49]	GACTCCAACGTCAATAACAGAGTTATGGCACCTCCACTGTTT	#cc0000
28[97]	21[111]	CCCAGCCGGGTGATCACTTCCCTTGTCGTTTAAACGGCTTCGC	#cc0000
22[139]	26[126]	CCTTCAGACATTACTGTGCCTGCCCCGCCACCGGCTGGCTG	#cc0000
1[161]	2[147]	GGCAATCAGCCCCCGTTTTTACACGAAGCTGACGTGGGAACA	#cc0000
9[147]	5[146]	TGTTTCAGGCGTGGATGACAAACGTCTGCCGTGCTGGCAGAAC	#cc0000
8[111]	10[91]	CCTTCATCGCCGGAATCAAAAACAGCCCCGTCGCCACATCGACCCCCG	#cc0000
18[111]	7[111]	TCCAGCGGATGCATCGGTTACGCGATCCCAATGCCCCGGCTT	#cc0000
19[70]	21[83]	ACGCCAGCCGTGCCATTGGTGACTTTCCTGGAAGAAGCACT	#cc0000
13[91]	25[90]	ACTGTGCCATCTGCTTCAGAATTCGGTTTTTCAGGACGACACG	#cc0000
19[147]	17[146]	GCCAAAGACGTTTCCCCTATGCGTATGCC	#cc0000
11[154]	15[153]	GATCGGCGTCACCCTTTGAGGTTAATGGATTAAACTGTTTCG	#cc0000
16[160]	25[167]	GCGCAGAGTAGCACGCATCGCCACGTCTTTTCACGCATCGCAGATTCA	#cc0000
4[104]	14[91]	GTTGTTCCAGTTTGCTTATAAACGCTGCGCTGATGAAATCCC	#cc0000
11[70]	13[69]	TCGGACAGGCAGGGTGATCGCCCTGCAA	#cc0000
9[63]	5[62]	ATGCAGATGCTCCGTCAAACCAAAGGAGCGGGCGCAGGTGCC	#cc0000
29[147]	20[161]	CAGCGGTCAAGTTCGCCGGGGCGTTATAAAGCACTGAATACAGC	#cc0000
4[69]	16[70]	GAACGTGGTAAAGCACTAAATGAAAGCGCTTTCGTTTACGTC	#cc0000
4[132]	16[133]	GCACCGACCCGTACAGAACAGATCTCCTTCACGCTCACCTGC	#cc0000
26[90]	28[84]	CCTCCGGACCGTGACGGAAC	#cc0000
0[83]	3[69]	CAGAGGGAGTGTGTGCGTCGCGCACTGATTCTGGCAAAAGGT	#cc0000
4[125]	5[125]	GAGAAAACAATAGGGTTGAGTGAGCTTGACCACGCGCATTTT	#cc0000
15[119]	1[125]	GAGTCCAATCACCATTCCCCTACCGTGATGTAAC	#cc0000
19[77]	1[83]	TCGGGTCTTTCCCGCTCAAATTGTGCAGCGCGCCACAGTGCAGAACAGC	#cc0000
29[84]	19[111]	GCCCCTGCCCGGTGCGAGTCCCTGCTGAGCCAGTTTCAGGTC	#cc0000
11[35]	7[48]	CCTGATTGCCGGAATCAGCCCCCTTGTCAACTGGCGCGCGTG	#cc0000
19[84]	7[83]	GCCCCATTGCTGAAAAACAGGGCTCTTATCAGCATGCGGCC	#cc0000

26[174]	28[168]	ATCAAGAGGCTGAAGCTCCCC	#cc0000
15[161]	10[161]	TGTTTCACTGCGCACTTTTATCCCTCC	#cc0000
19[133]	21[146]	CGCGGCTCTTTCACCTCACAATTCATAGTCAGCGTGCGGTT	#cc0000
15[105]	26[105]	G TTCAGCCATACCCGCTGTGTGCGCCATCCCGCACGAAAGCT	#cc0000
29[105]	20[119]	AGTCTGCATCTTCATCATCGAGTAGGATCATTACAGCCTTAC	#cc0000
27[63]	28[77]	CGTCCCAGTTCGGGTGGTATCTCCGGCAGATTTCTACATCGA	#cc0000
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27[105]	13[125]	TTCGCCGTCACCGGTACAGGCTTACCGCATCCGGAAGCTGTGGCCAGA	#cc0000
9[84]	5[83]	CACTCTGAACTGAGTCGTTTCGAGAAAGGAAGGGAACGGAACC	#cc0000
16[55]	25[62]	CCGCCACCTGCTCGCGGCGTCGAGAAATTGCCCTTGCAACGCCGTTTAC	#cc0000
13[49]	25[48]	ACGGACTCACCAGATTTTCATCGGTGACGGTAATTTGCCTGCA	#cc0000
4[90]	7[97]	GAACAAGCTAAAGGGAGCCCCCGTGGCGCCGCCTG	#cc0000
3[105]	14[105]	GAATAGCCCGAGGGGTATGCCGTTGGTACCGCTTTATACTGC	#cc0000
19[126]	28[105]	GGTGATGCGGATGGGTCAGCATATCCAAAGGTACGTTGCCTTACCGATA	#cc0000
24[125]	25[125]	GCTGGTACAGCCCGTTATGTTATCCGCAGACACAGCACACGA	#cc0000
22[194]	25[174]	TTTTCCCAGCCGTTGTTTCGGAGCTCTGTTTCCAGCAGTTCATAACAT	#f7931e
5[168]	7[194]	ATCCAGTGAGGAGAATCACCAATAAAACATTTTGCATTTTT	#f7931e
16[197]	16[175]	TTTTGTTATAGTTCGACGGCACC	#f7931e
26[188]	23[194]	CCACCATACTGGTGCGCCCGGTACATGGGGTCTGCTCGCCGTCTTTTT	#f7931e
20[194]	9[194]	TTTTGCGTCTGCCGTGTATGGTGGTGCCCTTCAATCTTTT	#f7931e
10[181]	11[194]	CCGGAGGTTCCGTACCCAGCACCTTTT	#f7931e
12[197]	14[168]	TTTTCTGAACCAGCAGGCGGACTTCCGACGGTCACCCCGTCATC	#f7931e
14[208]	15[208]	TTTTGTTCTGCAGCTGGTCTACGACCAGAGCGTTTTTTTTT	#f7931e
2[208]	3[208]	TTTTAGCCCCCAGGACAATCATCCGGCAATGGCGGCTTTT	#f7931e
4[208]	5[208]	TTTTTCCGTTGTCTGTATACGTTCTGCTTACCGTTATTTT	#f7931e
18[194]	27[188]	TTTTCATACAGCGCCCATTTTCAGTACTGGTCACCCTCCACAAGCCCGC	#f7931e
6[194]	3[181]	TTTTCAGCGACCAAATAGGTGGGGAGTTCTGGCTGGCGGTA	#f7931e
28[208]	29[208]	TTTTGTCTGAAAGCGGATTCGACGCGACCAAGTCAACTTTT	#f7931e
8[194]	1[181]	TTTTGGCCCTTCGAAGAATGTGGAATACGCCACCGATACCA	#f7931e
24[208]	25[208]	TTTTCGACGCTGAAATCCACCGAAGCCCTTCTGCGCTTTT	#f7931e
15[168]	17[197]	CATCGATTGCAGAAATACCGCTGTATTGCCGCGTCTGCGGTTTT	#f7931e
15[175]	21[194]	GATTTACGTTCCATCCTGCACGAACGTCATTTT	#f7931e
26[208]	27[208]	TTTTTAGTCATAGCCGCTGGATCATCGTCGCGCCGGTTTT	#f7931e
0[208]	1[208]	TTTTACGCCGGGAGCTGTGTCTCAGTTTCTGACGAATTTT	#f7931e
29[168]	19[194]	TCAGGTCTTTAACTTTTGCCAGCGTAAGGGCCGATGATTTT	#f7931e
10[194]	1[174]	TTTTGTCTCGGAGGCCGTCGGGCCAGTGCCCGGATGGCTTCCGTACCT	#f7931e
8[181]	13[197]	CTGATGGCACCGGATGTCCGGAGCCACCGTTACGTTGTATTTT	#f7931e

Electron Micrographs for pScaf-5509 Brick

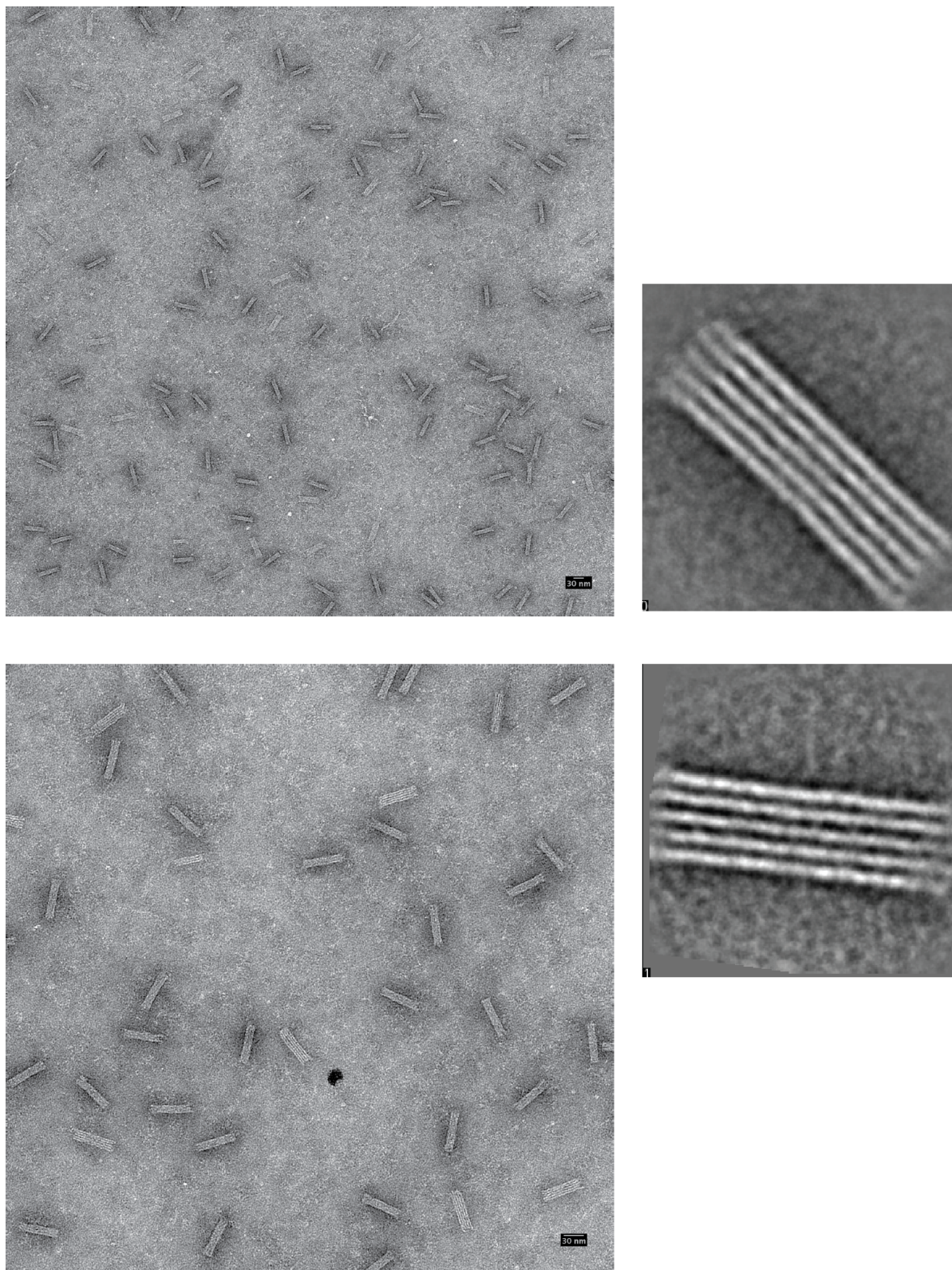


Figure 50: Electron Micrographs and Class Averages for p5509 Brick

pScaf-5544 Brick

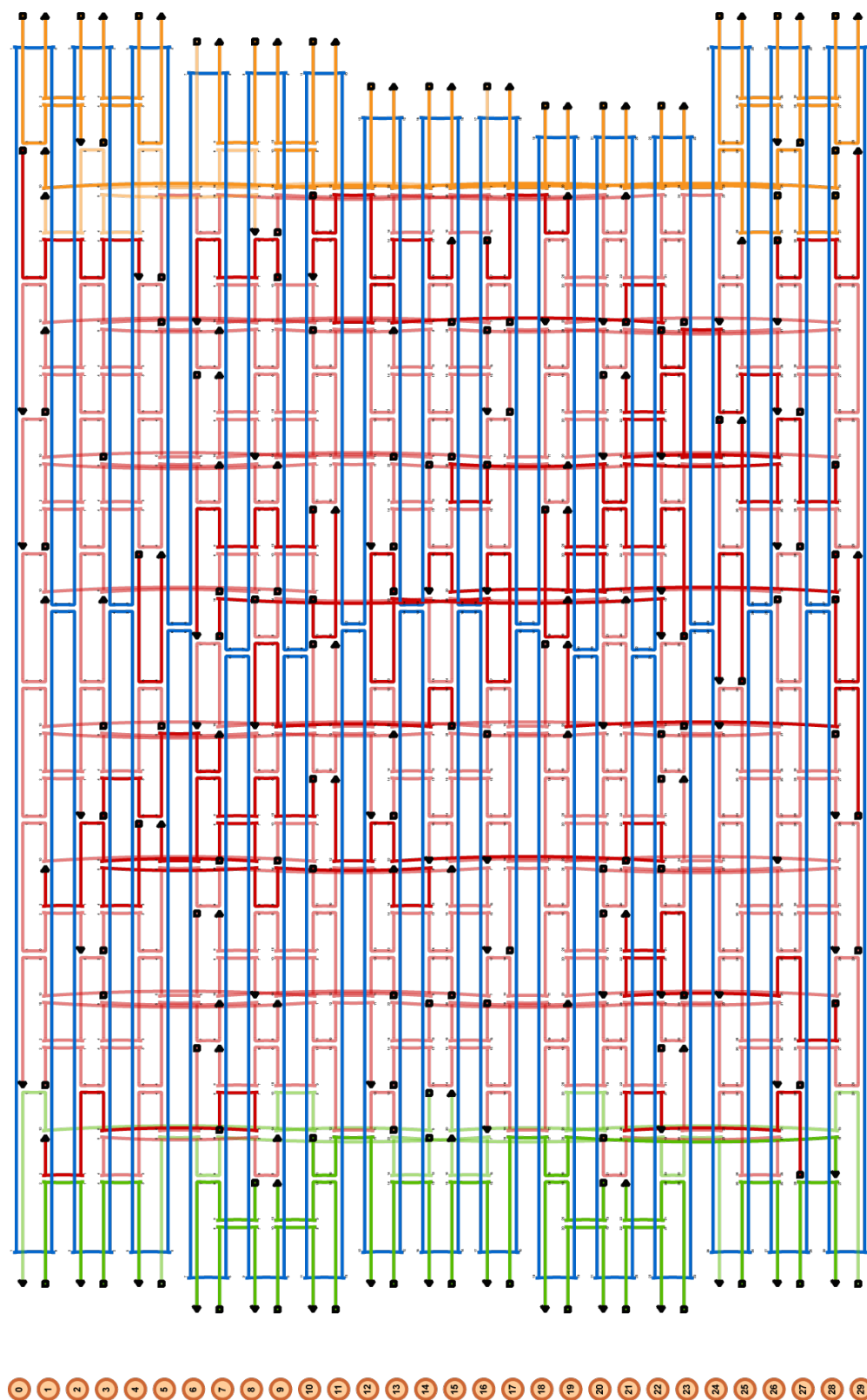


Figure 51: cadnano Design for pScaf-5544 Brick

pScaf-5544 Scaffold Sequence

AATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGCTATTC
TTTTGATTTATAAGGGATTTTGCCGATTTGCGGGTACCTTTTTTAGATCTCTGTTACA
CACTGTTCCGGGATGCTTATCATCTGGCGGGGATTGAGATGCCGGACTTTCATCGT
GAGGATGACTGGTGGCGTAACGGCCAGAATCTCTATCTGGATAATCTGGAGGCGA
CGGGGCTGTATCAGGTGCCGTTGTCAGCGGCACAGCCGGGCGATGTGCTGCTGT
GCTGTTTTGGTTCATCAGTGCCGAATCACGCCGCAATTTACTGCGGCGACGGCGA
GCTGCTGCACCATATTCCTGAACAACACTGAGCAAACGAGAGAGGTACACCGACAAA
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TTTACGGGGATTTACAACGATTTGGTCGCCGCATCGACCTTCGTGTGAAAACGGG
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GAGCGACGGCTGGTATCAGGTACGGATTGCCGGGCGGGACGTCAGCACGTCCGG
GTTAACGGCGCAGTTACATGAGACTCTGCCTGATGGCGCTGTAATTCATATTGTT
CCAGAGTCGCCGGGGCCAAGTCAGGTGGCGTATTCCAGATTGTCCTGGGGGCTG
CCGCCATTGCCGGATCATTCTTTACCGCCGGAGCCACCCTTGCAGCATGGGGGGC
AGCCATTGGGGCCGGTGGTATGACCGGCATCCTGTTTTCTCTCGGTGCCAGTATG
GTGCTCGGTGGTGTGGCGCAGATGCTGGCACCGAAAGCCAGAACTCCCCGTATA
CAGACAACGGATAACGGTAAGCAGAACACCTATTTCTCCTCACTGGATAACATGGT
TGCCAGGGCAATGTTCTGCCTGTTCTGTACGGGGAAATGCGCGTGGGGTCACGC
GTGGTTTTCTCAGGAGATCAGCACGGCAGACGAAGGGGACGGTGGTCAGGTTGTG
GTGATTGGTCGCTGATGCAAAATGTTTTATGTGAAACCGCCTGCGGGCGGTTTTGT
CATTTATGGAGCGTGAGGAATGGGTAAAGGAAGCAGTAAGGGGCATACCCCGCGC
GAAGCGAAGGACAACCTGAAGTCCACGCAGTTGCTGAGTGTGATCGATGCCATCA
GCGAAGGGCCGATTGAAGGTCCGGTGGATGGCTTAAAAAGCGTGCTGCTGAACA
GTACGCCGGTGCTGGACACTGAGGGGAATACCAACATATCCGGTGTACGGTGGT
GTTCCGGGCTGGTGAGCAGGAGCAGACTCCGCCGGAGGGATTTGAATCCTCCGG
CTCCGAGACGGTGCTGGGTACGGAAGTGAAATATGACACGCCGATCACCCGCACC
ATTACGTCTGCAAACATCGACCGTCTGCGCTTTACCTTCGGTGTACAGGCACTGGT
GGAAACCACCTCAAAGGGTGACAGGAATCCGTCGGAAGTCCGCCTGCTGGTTCAG
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TAATATCCGGATGCGCAGGATGACGCCGGACAGCACACAGACCAGCTGCAGAAC
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CACGGCACTGGTCGGCGTGCAAGGTGGAAGTGGAGCAGTTCGGCAGCCAGCAGGT
GAGCCGTAATTATCATCTGCGCGGGCGTATTCTGCAGGTGCCGTGCAACTATAAC
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GCAACAACATGGCCTGGTGTCTGTGGGATATGCTGACCCATCCGCGCTACGGCAT
GGGGAAACGTCTTGGTGCGGCGGATGTGGATAAATGGGCGCTGTATGTCATCGG
CCAGTACTGCGACCAAGTCAGTGCCGGACGGCTTTGGCGGCACGGAGCCGCGCAT
CACCTGTAATGCGTACCTGACCACACAGCGTAAGGCGTGGGATGTGCTCAGCGAT
TTCTGCTCGGCGATGCGCTGTATGCCGGTATGGAACGGGCAGACGCTGACGTTG
TGCAGGACCGACCGTCGGATAAGACGTGGACCTATAACCGCAGTAATGTGGTGAT
GCCGGATGATGGCGCGCCGTTCCGCTACAGCTTCAGCGCCCTGAAGGACCGCCA
TAATGCCGTTGAGGTGAACTGGATTGACCCGAACAACGGCTGGGAGACGGCGACA
GAGCTTGTTGAAGATACGCAGGCCATTGCCCGTTACGGTCGTAATGTTACGAAGAT

GGATGCCTTTGGCTGTACCAGCCGGGGGCAGGCACACCCGCGCCGGGCTGTGGCT
GATTAACAGAACTGCTGGAAACGCAGACCGTGGATTTCAGCGTCGGCGCAGAA
GGGCTTCGCCATGTACCGGGCGATGTTATTGAAATCTGCGATGATGACTATGCCG
GTATCAGCACCGGTGGTCGTGTGCTGGCGGTGAACAGCCAGACCCGGACGCTGA
CGCTCGACCGTGAAATCACGGATCTTCCGGTGATGAGCGATATCCCGGCACTGTC
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GCGGGCTTGTGGAGTTCAGCCGATCTGACTTATGTCATTACCTATGAAATGTGAGG
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TGGTCGCGTCTGGCAAAAGTTAAAGACCTGACGCCCGGCGAACTGACCGCTGAGT
CCTATGACGACAGCTATCTCGATGATGAAGATGCAGACTGGACTGCGACCGGGCA
GGGGCAGAAATCTGCCGGAGATAACCAGCTTCACGCTGGCGTGATGCCCGGAGA
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TAAATCCGCTTCCCGAACGGCACGGTCGATGTGTTCCGTGGCTGGGTCAGCAGT
ATCGGTAAGGCGGTGACGGCGAAGGAAGTGATCACCCGCACGGTGAAAGTCACC
AATGTGGGACGTCCGTGATGGCAGAAGATCGCAGCACGGTAACAGCGGCAACC
GGCATGACCGTGACGCCTGCCAGCACCTCGGTGGTGAAAGGGCAGAGCACCAAG
CTGACCGTGGCCTTCCAGCCGGAGGGCGTAACCGACAAGAGCTTTCGTGCGGTG
TCTGCGGATAAAACAAAAGCCACCGTGTCGGTCAGTGGTATGACCATCACCGTGA
ACGGCGTTGCTGCAGGCAAGGTCAACATTCCGGTTGTATCCGGTAATGGTGAGTT
TGCTGCGGTTGCAGAAATTACCGTCACCGCCAGTTAATCCGGAGAGTCAGCGATG
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GTCAGCCCTGCAGCGCATTGAGCATCTCGCCCTGATGAAACGGCAGGCAGAACAG
GCGGAGTCAGACAGCAACCGGAAGTTTACTGTGGAAGACGCCATCAGAACCGGC
GCGTTTCTGGTGGCGATGTCCCTGTGGCATAACCATCCGCAGAAGACGCAGATGC
CGTCCATGAATGAAGCCGTTAAACAGATTGAGCAGGAAGTGCTTACCACCTGGCC
CACGGAGGCAATTTCTCATGCTGAAAACGTGGTGTACCGGCTGTCTGGTATGTATG
AGTTTGTGGTGAATAATGCCCCTGAACAGACAGAGGACGCCGGGGCCCGCAGAGC
CTGTTTCTGCGGGAAAGTGTTGACGGTGAGCTGAGTTTTTGGCCTGAACTGGCG
CGTGAGATGGGGCGACCCGACTGGCGTGCCATGCTTGCCGGGATGTCATCCACG
GAGTATGCCGACTGGCACCGCTTTTACAGTACCCATTATTTTCATGATGTTCTGCTG
GATATGCACTTTTCCGGGCTGACGTACACCGTGCTCAGCCTGTTTTTCAGCGATCC
GGATATGCATCCGCTGGATTTAGTCTGCTGAACCGGCGCGAGGCTGACGAAGAG
CCTGAAGATGATGTGCTGATGCAGAAAGCGGCAGGGCTTGCCGGAGGTGTCCGC
TTTGGCCCGGACGGGAATGAAGTTATCCCCGCTTCCCCGGATGTGGCGGACATGA
CGGAGGATGACGTAATGCTGATGACAGTATCAGAAGGGATCGCAGGAGGAGTCC
GGTATGGCTGAACCGGTAGGCGATCTGGTCGTTGATTTGAGTCTGGATGCGGCCA
GATTTGACGAGCAGATGGCCAGAGTCAGGCGTCATTTTTCTGGTACGGAAAGTGA
TGCGAAAAAACAGCGGCAGTCGTTGAACAGTCGCTGAGCCGACAGGCGCTGGC
TGCACAGAAAGCGGGGATTTCCGTGCGGCAGTATAAAGCCGCCATGCGTATGCTG
CCTGCACAGTTCACCGACGTGGCCACGCAGCTTGACGGCGGGCAAAGTCCGTGG
CTGATCCTGCTGCAACAGGGGGGGCAGGTGAAGGACTCCTTCGGCGGGGATGATC
CCCATGTTGAGGGGGCTTGCCGGTGCGATCACCTGCCGATGGTGGGGGCCACC
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ACCCTGTCCGATTTCAACAAAACGCTGGTCCTTTCCGGCAATCAGGCGGGACTGA
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TTAACCAGACCAGCGAGTCACTCAGCGCACTGGTTAAGGCGGGGGTAAGCGGTG
 AGGCTCAGATTGCGTCCATCAGCCAGAGTGTGGCGCGTTTCTCCTCTGCATCCGG
 CGTGGAGGTGGACAAGGTCGCTGAAGCCTTCGGGAAGCTGACCACAGACCCGAC
 GTCGGGGCTGACGGCGATGGCTCGCCAGTTCCATAACGTGTCGGCGGAGCAGAT
 TCGGTATGTTGCTCAGTTGCAGCGTTCCGGCGATGAAGCCGGGGCATTGCAGGCG
 GCGAACGAGGCCGCAACGAAAGGGTTTGATGACCAGACGGATCCACGCGCCCTG
 TAGCGGCGCATTAAAGCGCGGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTAC
 ACTTGCCAGCGCCCTAGCGCCCCGCTCCTTTTCGCTTTCTTCCCTTCTCTCGCCA
 CGTTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCG
 ATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTTGGGTGATGGTTCAC
 GTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCAC
 GTTCTTT

Staple Sequences for pScaf-5544 Scaffold

Start	End	Sequence	Color
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14[41]	0[11]	ACTTTTT	#57bb00
20[27]	18[7]	CCGAGAGGACCCAGCTGATCTCCTGAGAATTTTT	#57bb00
10[34]	12[11]	TACGGCTCCTGCACCCGAGGCCAGATACTGCGATTTTT	#57bb00
5[11]	15[34]	TTTTTGGAATGGCTGCGTATCTCGACGGTCCATACCTCGCTGAACCGTCT	#57bb00
		TTTTTAAAGAACGTGGACTCCAACGTCAAAGCCAGTTTTTCCCCGGCACCATC	
29[11]	15[41]	GGAGCC	#57bb00
17[11]	28[28]	TTTTTCACTCAGCAACTGCGTGGACTGCCGTGCGCATGGAACAA	#57bb00
13[11]	14[11]	TTTTTGGTGGTTTTGCCTCATATTTCACTTTTT	#57bb00
3[11]	4[11]	TTTTTCGGTGCTGATACACGACCGTAACGTTTT	#57bb00
11[7]	9[27]	TTTTTAACTGCTCCGAGTCCACACCTGCTTTCCCC	#57bb00
7[7]	8[7]	TTTTTTCAGCGTCTATCCGCCGCTTTTT	#57bb00
25[11]	26[11]	TTTTTTGTGCGTCGCTGTTATCCAGATAGTTTT	#57bb00
23[7]	21[27]	TTTTTGGACGTGCTGACGTCCACTGCGCTACCACC	#57bb00
19[7]	20[7]	TTTTTACCACGCGTAAACAGGATTTTT	#57bb00
1[11]	2[11]	TTTTTATTTATAGGTAGCACACGACCACTTTTT	#57bb00
21[7]	22[7]	TTTTTTGCCGGTCACGTTAACCCTTTTT	#57bb00
9[7]	10[7]	TTTTTACCAAGACGTGGCTGCCGTTTT	#57bb00
27[11]	28[11]	TTTTTAGATTCTGGCCGGAGTCCACTATTTTT	#57bb00
15[11]	16[11]	TTTTTCCGTACCCAGCTGGCATCGATCATTTTT	#57bb00
14[34]	24[11]	CGGCGTGCTTAATGTCATGTACGCCGGGGTGACGCCAGAGGGAGTGTTTT	#57bb00
8[27]	6[7]	TATCCACGCCCGTTCCGTCCTGCACGAACGTTTT	#57bb00
		CGGTTACTCATGCCGTTGCCTTCTCTCAGGGCCCACCAGCTGAAAATAAA	
5[161]	13[160]	AGC	#cc0000
28[118]	29[125]	CGTAAAAAAGGTACTGGGGTCGAGGTGCCGTAAAG	#cc0000
17[63]	26[77]	GTATGCCCTTACTATCAGCGCAACCATCAAAGAATCCCCGCCAGATGCATC	#cc0000

		GCC	
15[98]	8[98]	CCGTGACCCACCAGACGTTCCGTCCCCGGACAGGTGATGCGC	#cc0000
		GGTGCCACCCTGGGACCAATCACCACAAGCGCGGGGCCATCCCAGAAATGTC	
20[69]	8[56]	GCAG	#cc0000
		CAGCCACGGGCGCGGTAGCACTGTTTCACGGCACCAACCAGCTGACTGCGC	
13[56]	7[69]	TGAG	#cc0000
10[181]	15[174]	ATGAGAACACAAACCACGCCAGTCGGGTGTCGGCAGCATATCCTTCGTC	#cc0000
		ATCGGCTGGGTCTGTGCGAGAGGCATCCGCTCTGTGCGCGTCGTTATAGCGC	
1[42]	7[55]	CGAG	#cc0000
23[112]	19[132]	TCGCTCTGGACAGGACCAGCAAGCGAGGGCAAGCCGCAGGCAGAAATCC	#cc0000
		TGATGGCAGCGGATTACTCCGTGAGTTGGCGTTTTCCCCGCCTTAACCATGC	
9[175]	25[174]	AGA	#cc0000
24[146]	21[153]	GTCTGGTTGCCGTCAGTCCCGAGCGCACATCATCC	#cc0000
		CTTTTTCATGGTGCGGAGGATCTTCAATTTGTCCTTCGCTTCCCTGACCCAGGC	
13[42]	19[55]	AG	#cc0000
13[119]	22[112]	TATCCAGCCGTGGAACACTAGCTGGTCTTCTGTCACCCTCACACCATC	#cc0000
		ACTTGCCGGCAATATACGGGAGTGAGGATAAAACATTTTGCCTTCCTGTT	
22[90]	16[77]	CAGC	#cc0000
		AACATCGGAGCGTCAGCGTCCGAACCTACGCCCTGTTCGGGCGGCAGGTT	
3[56]	12[42]	ACCC	#cc0000
		GAAATCCCGGGATATCGCTCACCGCTGGATTGCCGACGACCATGTCCGGCGT	
3[98]	12[84]	CATC	#cc0000
		GCCGGAAAGGACCACCCTGATTTACCTCTTTGCCGACTGTTTCCGTACCAGA	
23[161]	18[161]	AAA	#cc0000
0[188]	4[168]	GGACTCAGCGGTCAGTTCGCCTGCCCGGATAGGCACCGCCTTGTCACGG	#cc0000
22[160]	26[147]	ATACGCCCCTGATTTGAGTGACTCGCTGCCTTGTCAACTGGC	#cc0000
18[132]	19[118]	TCAAATCTGGCCGCATCCAAATATACTG	#cc0000
28[139]	22[140]	CCACACCGGATCCGTCTGGTCGAGCCATCATGGGGCGGTGCGC	#cc0000
		GCGATGGCCCACTACGTGAACTATAAATGTTATCCGAGTTCTGCTCACCAGGT	
29[63]	14[77]	AAA	#cc0000
16[174]	19[181]	ATGTCCGGCGATCCCTTCTGAATCACTTCAACGAC	#cc0000
		CGGGGAAATCATCTAAGCTGCCGCCGAAGTTATGGCACCTCCACGCCGGAGT	
16[160]	24[161]	GCGC	#cc0000
		CCCGGTACCCCCGGCGTTGTTCCGGGTACGGCATCCACATCCAGCACGCTGCT	
3[63]	15[76]	CCT	#cc0000
16[139]	25[146]	ATTCCCGTGCCGCTGAACTGTCCCTGAACGCCGTGGAAGGCTTCAGCGA	#cc0000
		GAAGATACCGCTGTCCACCATAGGGTCACCGCTCCCCTTATAGTCATAGTCAC	
10[111]	2[84]	CGG	#cc0000
7[77]	1[76]	CACGCCTAAAGCCGAAGCCCTAAGATCCGTGATTTTCGTGCGC	#cc0000
		CACGCCACTGCTGCTGGTGACTCTGCCATGTTTTATCCGCAGACGGTGAGATT	
1[126]	7[139]	CGG	#cc0000
10[132]	6[112]	TGTTTAATGCGGATGGCTGACTTCAAATTGGTCATACCACTGACCGAGG	#cc0000

13[126]	19[139]	CAGAACATCAGCCCCAAGCCCTCCGGGCCGACCAGATCGCCTCTGCTCGCCG	#cc0000
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		CGCAT	
6[69]	9[55]	ACCACATTACTGCGTCCCAGCCTGGTACAGCCAAATTTCAATTACTGGCAGCA	#cc0000
		TAT	
23[98]	11[111]	GTGCCCCGATGGCTGGATCTGGAATACGGACGGATGTGGTGCGAGCGTTTT	#cc0000
		GTTCT	
16[55]	24[56]	ACCGGACTCAAATCCGCCACATGCTGCACACCTGATCGTTTGCTCAGTTCCCG	#cc0000
		TAA	
9[119]	1[118]	CGTCTTCCGGCTTCGGGCATCGTCTGAAAGCGGATTCGCGTAACTGGTGATC	#cc0000
		AACC	
5[98]	13[97]	TTATGGCGTTTTAATCAGCCACGACGCTGGCTCCGGGTTTAATGCCTGTGACT	#cc0000
		TCC	
26[118]	22[119]	GTTTCGGCACTGATGGTAACAGAGATCCCGCTACAGACGTCGGCGCACCGTGG	#cc0000
		CCCC	
29[84]	19[111]	CATCACCCAAATCAAGTTTTTCCCGAAAGGTGTTCTGCTTTT	#cc0000
		GTCCACGTCTTATCTCAACAAATCTTCGTAACATTCGGCATAGCCCATTATGCC	
6[48]	9[34]	GT	#cc0000
10[118]	11[132]	ATTCATGGCTTCCCGCAGAAACAGGCTC	#cc0000
		GGCGCGTCGCCGCGCACTAAATCGGAACCCTAAAGGTAACCACTGTGCACGT	
27[126]	20[140]	CGGT	#cc0000
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5[168]	21[181]	CCCC	#cc0000
		ATCCTCATGTTGTTGGCGAAAAACCGTCTATCAGGGAGATAGAACATTGGCA	
27[42]	20[56]	TCTG	#cc0000
		CATCAGGCCCCCACCACCGATACAGAAACCGTCCCCTTCGTCTTCAGGCGGC	
22[48]	16[35]	CCT	#cc0000
26[174]	29[188]	TGCTCCGCTCGTTCGCTGGCAGACGGGGAAAGCCGGCGAACG	#cc0000
		TCAGGCTCGGATCGAAACGCGCCGTGGGCTGCCCCGGGCGTCAGGTCTTTAA	
15[161]	0[147]	CTTT	#cc0000
7[112]	16[119]	GTCACACGAGGTATTCCCCTCTAAATGACAGACTCAAATCAACAAAGCG	#cc0000
		TGTTGCTGGGTTATGCCGGTAAACCCACAGGGTGGTCCCGGCCGCATCACAC	
9[77]	2[63]	GGTC	#cc0000
		TTCTGCAGAGCACGCCACAGGCTTCCTGGCTGGTATGCCAGACGCGACCACT	
15[140]	0[126]	CAAC	#cc0000
		CGTTATGAGTTCAGCCCACATCCCTGCTCTATCTGACAGTGACGGTCTGCGT	
7[119]	3[118]	TGT	#cc0000
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		CCACCTGGTGGCCACGAAGGTCGATGCGGCTCGCCAGCAGCAATAAGCAA	
22[97]	28[84]	TCCCT	#cc0000
9[168]	6[161]	CCGGTTCTGCCTGCTTAACTGCCGGAATGTTGACC	#cc0000

17[161]	1[160]	TCCTCCTCCACATCCTCCGGACGTTTCAGCCGTCACGGGTATCGCCTTCCAGAT	#cc0000
		TT	
		GGCTTTCGGTAAAGCGGCTGTAATATGGTGCAGCAGCGACCAACGCCGGA	
20[76]	23[90]	GCTGT	#cc0000
4[83]	13[76]	GCGCGGTGTGCCTGCATGGCGTCCGGCAGCCATGTGCGCAGATTGTATC	#cc0000
28[125]	14[119]	CTTAATGCCCCGACGGCATAACGCCTCCGGGGAAAAG	#cc0000
		ATCAAACGCTGCGCGGAGCCCCCGATTAGAGCTTAGTGTAGGCTCAGCCGC	
27[147]	20[161]	CTGC	#cc0000
		ACCGCCCGCAGGCGCGCTCCAAGTGTCCAGGTACGCATTACGGCGCTGAAGC	
18[111]	6[98]	TGTA	#cc0000
4[125]	5[125]	TCGACGGACTCCAGCAGTTCTGGTCCTTCACACGGTGGCTTT	#cc0000
		GCAGGCGACACCGAAGCCCGGGCGTACTTTACCCATTCTCAGTTTCACAGA	
13[84]	19[97]	AATA	#cc0000
27[28]	22[35]	TTACGCCACCAGTCCCGTCGCATGGCTGCAGAGTC	#cc0000
		TCGGCAATCCCGGAACAGTGTAACCAAAGCCCCCAGGACACAGCACCGTTAT	
28[97]	20[98]	CCGT	#cc0000
21[77]	11[90]	AATGATCCCCGGCGTGAACCACTGCGCAGATTTCAAGTGTATG	#cc0000
		CCAGGTGCTGTTTCATCTCACGCGCCAGTGGTACTGACAGGCTTCAGCACGCG	
10[160]	16[147]	GGGA	#cc0000
		AGCACCGAACACCATGTCTGTGGCGGCAACAGCACGTCGCCGCAGTAAATTT	
16[97]	24[98]	CACA	#cc0000
		ACTCTGGTGACGAAAATCGTTGTAAATCGTTCAGGGCCGCTGCATCTCAATA	
22[76]	28[63]	GCCC	#cc0000
		ACTGGCAGGCCCCACTCCAGACCATTTGTGGTGTCTATGCCCCAATCCGTACC	
20[34]	23[48]	TGA	#cc0000
3[84]	7[97]	TCTGCGCCAGCCCGTCACCTCAACGGCAGCGGAACTGTGGTC	#cc0000
		AGTTCGACATCGATTCCGGATATTAAACCGTTACGCGGTCGACGGAGTCTTTT	
10[76]	16[63]	TAA	#cc0000
28[55]	22[56]	GGTTGAGCGATGAAAGTCCGGACAACGGAGGGTGGATGAATT	#cc0000
		GTGGCCAGCCAGCGCTGACTCTGGCCATACCGGTTTAACTTCTTTTCAAGTCAA	
20[153]	8[140]	TGC	#cc0000
7[35]	1[34]	GGCATAACATACAGCGTCATCAGCTGTTACCGCCAATGACAT	#cc0000
10[90]	5[83]	CGTCTGCGTATGCCTGCCGCCTACGCTGGGCGCGCCATCATCATCCAGT	#cc0000
		TCTCCGGATTAAACTGATCACGCTGTTAACGAAAGCTCTTGTTTGCCTGGCTG	
1[147]	7[160]	ACT	#cc0000
23[56]	21[69]	CGTCGCTCAGTTTCGAACAATCTCCGGC	#cc0000
		GTGCGGGCACGCCAGCAGCGCGCGTGAACCTCAATCTGCGGGCCAAAACCTCA	
3[140]	12[126]	GCTCA	#cc0000
25[105]	24[105]	TTGCGGCGTGATCTGTGGTCAGCCGCCTGCCCGAGCCCCCGT	#cc0000
		CAGCCATACCGGACATGACGCCCTGTCGCGGTACCCCTTCGTTGCGGCCCCG	
17[147]	26[161]	ACAC	#cc0000
14[139]	26[126]	GTGTACGTCATGAACACCGCCTACGATCTAAACGTCAGCCCTGCTTCCAGCC	#cc0000

		CCG	
8[118]	21[118]	AAAGCGTGCATCTGTGTTGAGGTGGTTTACCGGATATGTTACATGGCGGGG	#cc0000
		TGAT	
6[153]	9[139]	CAGCAACGCCGTTACACCGCCCGTGCTGCGATCTTTTCACCGCTGCAGGGTT	#cc0000
		ATG	
14[55]	26[42]	AGACGTACGTACACACAGCGCTACCAGCAGGCAGATGCGCGCACCTCTCTAC	#cc0000
		AGCC	
13[140]	7[153]	AATAATGTTTCAGGGCCGGCGTCCTCTGTGTAAGCAGACATCGGAGATGCGAA	#cc0000
		CATC	
15[56]	0[42]	CCTCCGGTGTTTGCCCCACAGTGCAGAAACAAGCCACCTTTACCGGCATTGT	#cc0000
		AGG	
3[189]	5[209]	CCCAGCCACCACCGGGTCAGCGTGGTGCTCTTTTT	#f7931e
12[198]	11[205]	TTTTTCGGCAAGCATGGTCATACATACCAGACAGCCTTTTT	#f7931e
		TTTTTCATTACGTCATCCGGTAATGACTCCGCTGCTGAGGGAAGCGGATTTTT	
16[198]	1[181]	CGCAGT	#f7931e
22[195]	13[198]	TTTTTGACAGGGTTGGATGACATCCTTTTT	#f7931e
		TTTTTAGCAAACCTACCATTACCGAGGCCACAGGTGCTGGCAGGCACCGATA	
6[205]	8[175]	CCTGTTC	#f7931e
14[198]	1[188]	TTTTTAGACTGAAATCCGTCTTCCTTTTCAGCCCAGTCT	#f7931e
28[181]	21[195]	CTAGGGCGCCGCCTGCAATGCCAACATACTGTTGCAGTTTTT	#f7931e
2[209]	1[209]	TTTTTATCGACCGTCATCATCGATTTTT	#f7931e
20[195]	15[198]	TTTTTCAGGATCAGCGCCGGTTCAGCTTTTT	#f7931e
26[181]	23[195]	CGCAATCGGAGAAACGCGCCACCGCTTAGTTGAAATCTTTTT	#f7931e
10[205]	9[205]	TTTTTGGTACACCACGTACAGTAACTTCTTTTT	#f7931e
4[209]	3[209]	TTTTTTGCCCTTTCACGGAACACTTTTT	#f7931e
0[209]	2[189]	TTTTTGATAGCTGTCGTCATAGCATCTTGCCGTTT	#f7931e
24[209]	26[189]	TTTTTCGCAATCTGAGCCTCACACTCTGAACTGAG	#f7931e
26[209]	25[209]	TTTTTGAACGCTGCGCTGATGGATTTTT	#f7931e
28[188]	19[195]	GCGGGCGTGCCGCTGTTTTTT	#f7931e
8[205]	7[205]	TTTTTCGGTTGCTGTCTTTCTGCAACCGCTTTTT	#f7931e
28[209]	27[209]	TTTTTGAAGAAAGCTCATCGCCGTTTTT	#f7931e
27[189]	29[209]	CCCGGCTGAAAGGATGGCGAGAAAGGAAGGTTTTT	#f7931e
18[195]	17[198]	TTTTTTTTTTTCGCTACTGTCATCAGTTTTT	#f7931e

Electron Micrographs for pScaf-5544 Brick

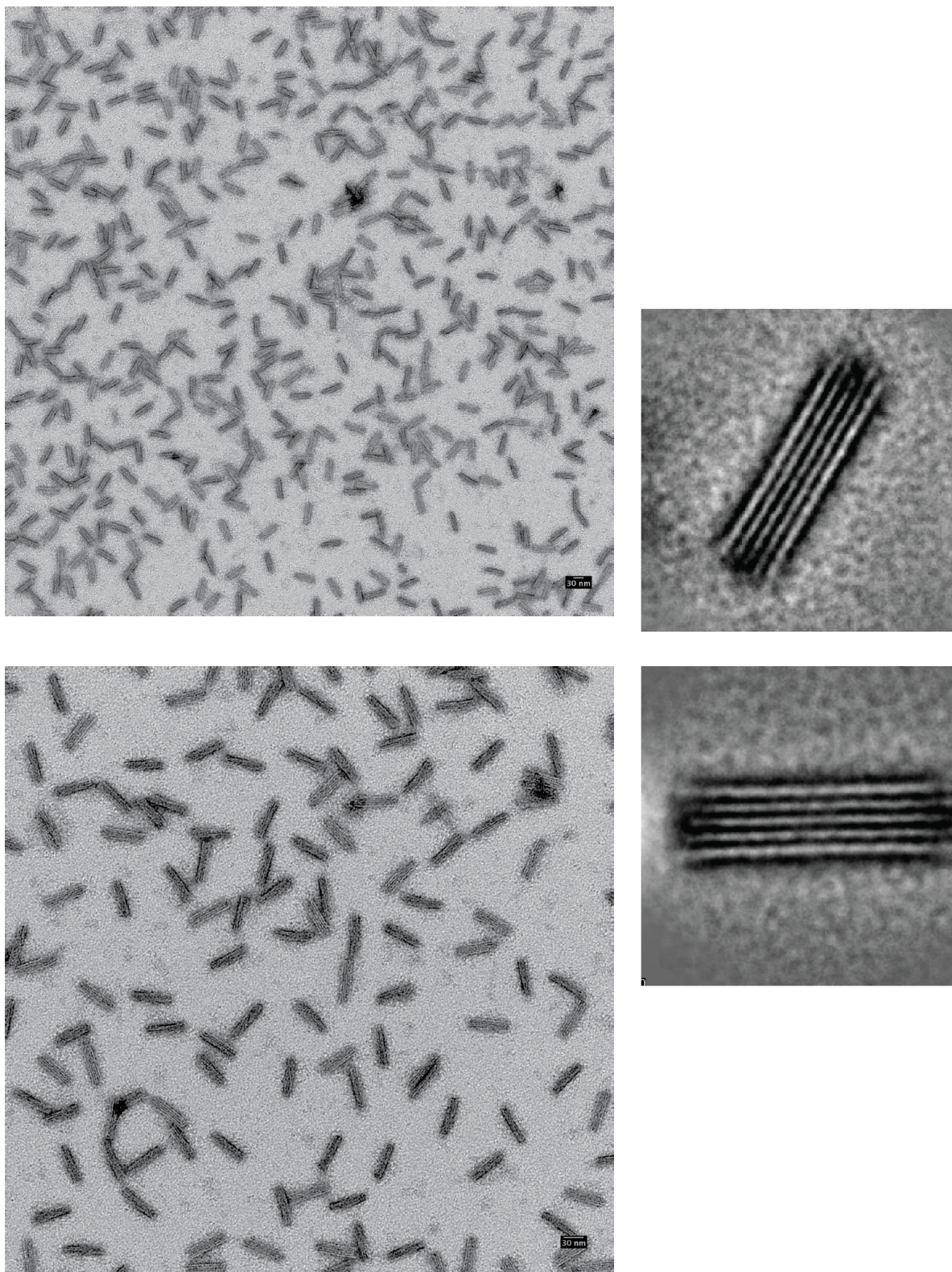


Figure 52: Electron Micrographs and Class Averages of pScaf-5544 Brick

pScaf-7560 Brick

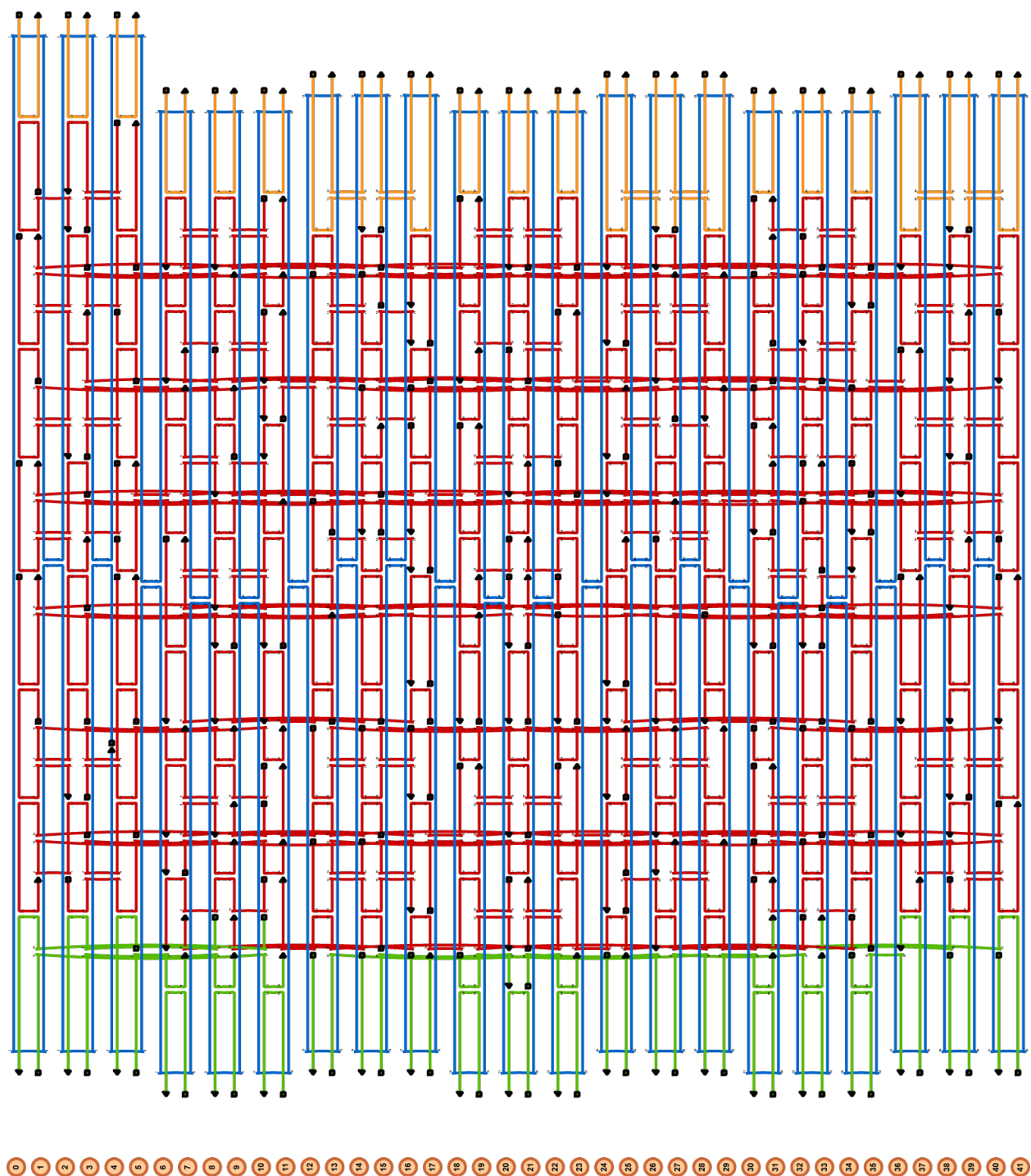


Figure 53: cadnano Design for pScaf-7560 Brick

pScaf-7560 Scaffold Sequence

AATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGCTATTC
TTTTGATTTATAAGGGATTTTGCCGATTTTCGGGGTACCGGAGGATGGCAGCGTGAT
TTCACGGTCGAGCGTCAGCGTCCGGGTCTGGCTGTTACCGCCAGCACACGACCA
CCGGTGCTGATACCGGCATAGTCATCATCGCAGATTTCAATAACATCGCCCCGGTAC
ATGGCGAAGCCCTTCTGCGCCGACGCTGAAATCCACGGTCTGCGTTTCCAGCAGT
TCTGTTTTAATCAGCCACAGCCCCGGCGCGGTGTGCCTGCCCCCGGCTGGTACAGC
CAAAGGCATCCATCTTCGTAACATTACGACCGTAACGGGCAATGGCCTGCGTATCT
TCAACAAGCTCTGTGCGCGTCTCCCAGCCGTTGTTTCGGGTCAATCCAGTTCACCTC
AACGGCATTATGGCGGTCTTCAGGGCGCTGAAGCTGTAGCGGAACGGCGCGCC
ATCATCCGGCATCACCACTACTGCGGTTATAGGTCCACGTCTTATCCGACGGTC
GGTCCTGCACGAACGTCAGCGTCTGCCCGTTCCATACCGGCATACAGCGCATCGC
CGAGCAGAAATCGCTGAGCACATCCCACGCCTTACGCTGTGTGGTCAGGTACGCA
TTACAGGTGATGCGCGGTCTCGTGCCGCCAAAGCCGTCCGGCACTGACTGGTCG
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GCCGTGTTTCGGGTAGCACTGTTTCACATCGATGATTTTCAAGTGTATGACGACCAGAG
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TGATCTGAACCAGCAGGCGGACTTCCGACGGATTCTGTACCCCTTTGAGGTGG
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CGTAATGGTGCGGGTGATCGGCGTGTGCATATTTCACTTCCGTACCCAGCACCGTCT
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CGCTGATGGCATCGATCACACTCAGCAACTGCGTGGACTTCAGGTTGTCCTTCGCT
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CCGTTTTACACGAAGGTCGATGCGGCGACCAAATCGTTGTAAATCCCCGTAAAG
GCAGATGCGCGCCATGCCCGGTGACGCCAGAGGGAGTGTGTGCGTCGCTGCCAT
TTGTCGGTGTACCTCTCTCGTTTGCTCAGTTGTTTACAGGAATATGGTGCAGCAGCTC

GCCGTCGCCGCGCAGTAAATTGCGGCGTGATTGCGCACTGATGAACCAAAACAGCAC
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CGACCAGACTCTGCATATCTTCCGCCATCCCGGTGACCATAACCGTACAGGTTAGAA
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TCCTGCCGATATCCTGCATCAGTTCACCACTGTTCAAACCTCTGCGCTGAACTCA
ACACGCAGCATACTGACCCGCGACGACCATTTTGCGCAGGTCACCTTTATCTGCC
GCCACTCATAAGGCGGCGTCCACAGAAAGGATTTCCAGCCCCCGTGCTCTTCCAG
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AGCGCACCTTTCTTACAGAAGGGACCGAAGCCACATCCATACCGGGTTTTCACTTTC
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TGTCTGAATTTTCATCACGGGCACCTTGCGGGCCATGTCATACACCGCCTTCAGAG
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CGACATAACCGCCCGGTGGCATAGCCGCGCATCAGCCGGTAAAGATTCCCCACGC
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CGCTGGCTCATATTTGCCGCCGGTTCCCGTAAATCCTCCGGTTGCAAAATGGAATT
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CAGCCCCGCCAATGGCGCTGCCGATACTCCCGACAATCCCCACCATTCCTGCTT
AAGCAGAATTTCTGTCATCATGGACAGCACGGAACGGGTGAAGCTGCGCCAGTTC
TGCTCACTGCCGGTCAGCATCGCCGCCATATTCTGTGCAATACCATCAAAGGTCTG
CGTGGCTGCACTTTTTACCTGCGACATACTGTCCGTGGCGCTCTCTTCCCACTCAC
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CAAGCATGGCACGCCAGTCGGGTGCCCCATCTCACGCGCCAGTTTCAGGGCAA
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Staple Sequences for pScaf-7560 Scaffold

Start	End	Sequence	Color
40[197]	39[197]	TTTTGGTGCCCGTGATGAAATCCGAGCGCCTGCCGGGCTGATTTT	#f7931e
36[197]	38[168]	TTTTTGC GTGTTGAGTTCAGCGCAGAGTTTAAAATGGCAGCGTG	#f7931e
38[197]	37[197]	TTTTATGCCAACCTGAAAACGTATCGTCGCGGGTCAGTATGCTTTT	#f7931e
8[194]	9[194]	TTTTGTGACAGGAATCCGTGCAAACACCTCAAAGGTTTT	#f7931e
2[208]	3[208]	TTTTCGGCACGGAGCCGCGCTGCCGGACGGCTTTGGTTTT	#f7931e
20[194]	21[194]	TTTTGCGCATCTGCCTTTACCGTCACCGGGCATGGCTTTT	#f7931e
26[197]	25[197]	TTTTCCGGAAGACTGGCTGCAGGGTTCCGCTGTGTGCCGCATTTTT	#f7931e
16[197]	15[197]	TTTTACCGGCATCCTGTTTTCTCTGTTCTGCCTGTTCTGTACTTTT	#f7931e
22[194]	23[194]	TTTTAGCGGCACAGCCGGGCATCAGGTGCCGTTGTCTTTT	#f7931e
24[197]	26[168]	TTTTCTCACCGGGCGGCGCTTTGAGCACGGTTCATAACAGAAAT	#f7931e
39[168]	41[197]	TTCTCAGAGACACAAGGCGGTGTATGACATGGCCCGCAAGTTTT	#f7931e
27[168]	29[197]	GAGCCGGAATCCACGGCAACTTTGGCGGCTTCCTTTCCATTTT	#f7931e
4[208]	5[208]	TTTTAGACGCGGCAATACAGTCGAACTATAACCCGCTTTT	#f7931e
28[197]	27[197]	TTTTTTAACAACCTTTTCGCAGTAAGGCTATTTCCGTATGTCGTTTT	#f7931e
18[194]	19[194]	TTTTCGCAGTTACATGAGACACGTCCGGGTAAACGGTTTT	#f7931e
14[197]	13[197]	TTTTGGGGAAATGCGCGTG GGGTGGGTAAAGGAAGCAGTAAGTTTT	#f7931e
15[168]	17[197]	AGGGCAATCGGTGCGGGCAGCCATTGGGGCCGGTGGTATGTTTT	#f7931e
12[197]	14[168]	TTTTGGGCATACCCCGCGCGAAGCGAAGGAGAGGAATCACGCGT	#f7931e
6[194]	7[194]	TTTTACCACAGACCAGCTGCGATGACGCCGGACAGCTTTT	#f7931e
10[194]	11[194]	TTTTGTTCCGGGCTGGTGAGCCGGTGTACGGTGGTTTTT	#f7931e
0[208]	1[208]	TTTTGCGCCGTTCCGCTACAGATGCCGGATGATGGCTTTT	#f7931e
30[194]	31[194]	TTTTCATGCTGGCCAACACCTTTTTCCGGGACGTATTTTT	#f7931e
32[194]	33[194]	TTTTAAGGTTTACGCCCCGTTAACGGTGGTCCGGCGTTTTT	#f7931e
34[194]	35[194]	TTTTGTGAGCCGGTCACCTGGAGCAGAACGAAAAAGTTTT	#f7931e
30[139]	38[140]	TAGCGGTCCTCCTTGGTGGACCCTTCTGTAAGAAAGAGGCCA	#cc0000
30[132]	20[119]	CCGGCTGTCGCGGAAGGATAAGTGCGCCGTCAGAAGGGCTGA	#cc0000
25[63]	29[76]	TGCGGTGCATCTGGGAAAGAACCGAAGACTGCAGGCGGATTA	#cc0000
32[76]	36[77]	GGAGGAGGCGCCTGGCTTAAGTGACCGGCAGTGAGGATGGCA	#cc0000
32[55]	36[56]	CCAGCTGCGACAAGGATTGTCCACAGAATATGGCGCTGGAGT	#cc0000
32[41]	26[35]	AGGAGAAAAGGCCCTTTGTTA	#cc0000
20[111]	19[97]	CGGGCACGCTCCCGATTCCAGATAGTCCGTGGCTGAACCCTG	#cc0000
18[69]	8[56]	CAGGTGAAGGACTCTGCCTGCTCGTTGAGCTACAGAGCCGTT	#cc0000
2[48]	6[49]	GCTGGCGCCTCCGGAGGGCGAGTTTTTTGGGGTCGAGAAAGC	#cc0000
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1[77]	2[63]	TCGGCGCTTGCCCGTTACGGTCGTAATGCGTGATTATCAGC	#cc0000
25[147]	29[160]	GGCTGGTTGGCGCTCCCTGCGTCAGCGATTGTAAGTTCCGCA	#cc0000
10[69]	6[56]	TGTTTCGATGTGGTGTGAATGAGGCGCGTGAGAAAGGAAGGGA	#cc0000
33[77]	26[77]	AACGCGCTGGCGCACAGTTTCGCGGTACTCAGCAGAAAGAAAG	#cc0000
10[153]	6[140]	GGATTTGCTTTACCGATAACAACGCGCCCCATACTGAAATC	#cc0000
1[175]	2[168]	CGCAGTAATGTGGTGCTTCAGCGCCCTGAAGGACCCTATAACGTACCTG	#cc0000
8[146]	10[133]	CGTAACGGTGGATAGATGTGCTCAGCGATTCTGTGCCGGCTCC	#cc0000
40[153]	31[167]	GCCTGTTAGATAGGTCCGGCTGCTCTGAGATGCGTAACGGAT	#cc0000
21[35]	11[48]	CAGCGAGAGCCTTCAAGAGCCTGCATCCTGTCATCCACGGAG	#cc0000
14[55]	24[42]	ACGTAATGATGTGCGACAAGGGTGTGCGTGACCAGACCCGCC	#cc0000
12[76]	16[63]	CACCGTGCTCAGCCGAAAGCGCATGACGCTGGATGGCGGCAG	#cc0000
30[48]	20[35]	CGGGCGATCGTCAGGAACAAGGCTGCAGACCTCGCATGCTGG	#cc0000
13[77]	10[77]	TTGCCGGTGACGTACCCATTATTTTCATGAGCCTGTTTCTGC	#cc0000
32[125]	21[125]	GCAGGAGAAGCACGTCTGCCCTGAGCAA	#cc0000
31[147]	19[146]	TCCACGCGACGAGTGCGGTTATGAGCGGTTCTGGCGGTATCA	#cc0000
15[77]	8[77]	ATTTGACCCGGATGCCCCTGAACAGACAAGAAGACGCAGATG	#cc0000
35[161]	31[174]	ATTTTTTCTGTAATGCAGGGGTGCGCGGTTCTGGAGGCGCTG	#cc0000
10[48]	6[35]	TTGCCCTGCTGTCTTTGAGCAGCGCTTAGAAAGGAGCGGGCG	#cc0000
21[91]	32[91]	CGTCCATCAGAGGCTGCGTATCTCAACAGGACAAAGGAGAGT	#cc0000
15[35]	9[41]	GGTAGGCACAGTATTGTACCG	#cc0000
22[125]	10[126]	TTTACTGATGTTTTAGCGAAAAGCGTGCTGCTGAGAGACGG	#cc0000
0[104]	10[91]	TTGTTGAAGATACGCAGGCCAAGAAGGGCTTCGCCGCCCGCA	#cc0000
23[77]	22[77]	CTCAGTTGCAGCGTTGGCGCGTTTCTCC	#cc0000
16[139]	25[139]	ATGCTGGCACCTATTCGTGTGAGGTACAAGCCACCTGATTTG	#cc0000
40[104]	21[104]	AAATATGAGTTGAGGCGCAGCAATGCGCATTAAAGGCACACCA	#cc0000
25[42]	29[55]	GGCATTCTGATTATGTCTTGACGAACGGCACTGAAAGACGG	#cc0000
32[160]	36[161]	AACTTCGTATGCAGCTGTCCAAGGTGACCTGCGCGAACAGG	#cc0000
18[174]	8[161]	TCTGCCTGATGGCGGCATGGGCAGTATGATCCGGACGCCTGC	#cc0000
20[48]	7[41]	AGATCGTTGGCGGTGGGATGATCCCCATATAAAGCGACAGGCACCCGCC	#cc0000
35[112]	39[111]	AGCGTGGATGCACCCGTGCGGCGGCTGGCGGGGTTCTGAACCC	#cc0000
11[133]	15[132]	ACAGTACGTGATCGATGCCATATGTGAAACGGTGGAGCAGAA	#cc0000
4[188]	2[175]	CGGTATCTGGGACGCTGCGACCAAGTCAGATCACCTGTAATGC	#cc0000
3[161]	11[160]	GTCATCGACCACACAGCGTAAGATAAGAGCCGGAGCTGAGGG	#cc0000
30[160]	38[161]	TCGCGGTGACGAGATGGTGTGTTGGTGATGGCTAACGCTTT	#cc0000
30[174]	20[161]	TGCACCTGGACCTAATAACGTTGACACAGGCGGGATACAACG	#cc0000
20[104]	1[104]	TGGCCCGAGTCAGGAATGAAGCCACGCCATGACGGATGTACC	#cc0000
9[91]	20[91]	GAGGACGTTATCCCCGCTTCCGAGCAGATGGCCAGATTTCAA	#cc0000
30[55]	38[56]	GTGTCTGCATGCCGAGGAGGCATTACAGGCCGCTGCTGGTGGG	#cc0000
6[111]	16[84]	CGGCACTGGTAGAGCTTGACGTGTGGCATGGTACGGAAAGTG	#cc0000

7[49]	26[49]	ATGCGCCACAGTCGTCGTTGATGACGGCGCGCACTGCAAGGA	#cc0000
4[153]	16[154]	GCAACAAAGCCGTAATTATCATGGTCGTGTTAATGTGCTCG	#cc0000
3[168]	5[188]	GCCAGTAGAACGTTGGGCGTATTCTGCAGGTGCCG	#cc0000
31[91]	41[104]	TTGTGGAAGCCAGCGGGGATTAGGCGTCCGGGACGTTTGAGC	#cc0000
39[63]	40[77]	GGCGAAAGTTTGTGCGATGGCAGACAGCCGGTCGCGTTCACC	#cc0000
19[77]	18[77]	TATCAGGGCAACTCATCTGCTGCAACA	#cc0000
4[104]	5[104]	GTTGTTCCAGTTTGAACAAGCTAAAGGGAGCCCCGATTC	#cc0000
22[104]	13[97]	TGCTGGCGCCCGGATCGATATGACTTTTCCGGGCAGGTGTCCGCTTTG	#cc0000
12[160]	16[147]	AAGTCCACGCAGTTGTTTTGTTTCAGCACCAGTGGAGTGGTGT	#cc0000
21[77]	20[77]	TGAGGCTCAGATTGCAAAACGCTGGTCC	#cc0000
5[140]	9[139]	GCAGGTGCATGGCCTGGTGTGCGCGGATGTGGCTGATCGACC	#cc0000
17[105]	27[118]	TCCTGGGGGCTGCCGTGGCGTGCGTTTCAGCGGAGTCGTGGT	#cc0000
30[69]	20[56]	AGTCCAGCGTGAAGGAAAATCGGCGCAGGGTGCGCGCGGGAC	#cc0000
31[112]	33[125]	GCGAACTGCTGGAAGGTATGGATGTGGCTTTCTGGAACCTGT	#cc0000
22[153]	18[140]	GGTTCATATGGCAGCGCCGCAGGTACGGTCATATTGTTCCCA	#cc0000
35[77]	24[77]	GCAGAAAAAGACCTACAGCAGCAACGGGTGTCGCGAACGA	#cc0000
15[161]	1[167]	GTTGCCCGGTTTCTTACAGGCAGACTCCCGTGGAC	#cc0000
33[161]	24[161]	AGTCTGGCGACAGTGGGACGATGTGACG	#cc0000
25[84]	34[105]	TCCTGATACCGCGCAATTCGCGGGCGGCTGAAGCAGTCGGCCCACCAGT	#cc0000
31[140]	26[140]	TGTAAGTGAACAGTATATTTCCGGTCCAC	#cc0000
3[126]	7[139]	TTGGTGCTGTGGGACAGTTCGGCAGCCAATCGATGGGGTAAC	#cc0000
9[126]	5[125]	TGCAACCGGTGACGTGGTGAATGAAACAGTGCTACCTCGGAG	#cc0000
34[139]	38[126]	GGAGCGGGACCTGACCGGCAGGAAACACGACGCCGGGAGTCG	#cc0000
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14[76]	24[63]	TGGCGGAGCAGGGCTCTGCATTATGTTGGGCCGCAACGAAAG	#cc0000
35[35]	31[48]	GTATGGCGATAATCCCGGGGGCACTTGGTCTGCACGGAGAAG	#cc0000
3[77]	11[76]	AATCAAAGATGACTATGCCGGTTCAGCGGGGAAAGTACAGTA	#cc0000
17[140]	7[146]	ACCGCCGGGCGCAGCTGCCGC	#cc0000
33[140]	23[153]	GGGATGGATTTCAGGCCGTGGTGTTCGGTCTCTATCTGGATA	#cc0000
20[146]	10[140]	TCGACCTTTCTCCTGGCAGACGTCTGCGAATCCTC	#cc0000
11[91]	7[111]	GATGTTCTGCTGCGGTGGATGGTGAAATGATACCCATTAAGCCTCGCA	#cc0000
0[125]	3[111]	GCTGGGAGACGGCGACAGAGCGGCGGTATATGCGCGATACGG	#cc0000
35[56]	31[69]	GCTGAATAACGTCAGGCCGCCATCAGGAGCGGCGCTGAGAAA	#cc0000
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33[105]	30[91]	CGCTGACGCCGCTGCAGTGCAGACCAGCCAAGGATTATGAAG	#cc0000
4[125]	14[112]	TATGCTGACCCATCCATGGGGACATCACCGCACCAGTGGTGA	#cc0000
26[111]	30[112]	GTGAGTGAGCGGCTTCGTGCAGTCCGATATCACGATGAATAT	#cc0000
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34[41]	21[48]	CTGACTGCTGCGCGGCCTGAAGCCAGTTCATAACTCGCTGATCACTCA	#cc0000
21[56]	11[69]	GGTTAAGGGAGGTGTGATGCATGTTTTTACTGGCACCGCTTT	#cc0000
28[97]	19[104]	GATACCGAAGCGTCCTGATGGCGGCGGCGAAAAACGCAGAGCTCACACA	#cc0000
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27[133]	37[146]	GGGAAAGTACGCCGGGTCAACCGGTACTCCTTATGAGTGGCG	#cc0000
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25[49]	37[48]	AAATCCACGAAAAGCGCTGGCGAGTGGGAAGAGAGGGTATTG	#cc0000
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1[140]	2[126]	AGGACCGACTGGATTGACCCGAACAACGGCTGACGTTTCTGC	#cc0000
18[132]	8[119]	CGGGGCCAAGTCAGGCCATTGAGCCAGAGCCTCGGGAAAAAG	#cc0000
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35[119]	31[132]	TGCTCTGGGAAATCTTTTGAACGGTATATCCGGAGTGAGTG	#cc0000
5[77]	9[76]	CGGAACCAGTCCACTATTAACCTTATACCGTCCAAATAATG	#cc0000
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32[167]	26[161]	TGCGGTGCTCCGGTGCAGGGT	#cc0000
13[112]	25[111]	ATGCAAACGGCGACAGGATGAATTGAGATGCCGGAGCGCCGA	#cc0000
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0[167]	3[153]	GCCATAATGCCGTTGAGGTGAACCGTCGGGCGTGGAATGGGC	#cc0000
25[126]	29[139]	TGCAGAGCCGGTGGCCGGAGGCGGCGATATGCAGCAAATGCC	#cc0000
22[97]	21[111]	CAGAGTGTCGGCGATGAATTCATCGTGCGGCGAGCTATTCT	#cc0000

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30[76]	38[77]	CCGAAACATCAGCCGTGGTGATTCCATTTTGCAACAAATTCT	#cc0000
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34[125]	24[119]	CTGAATGCTGCAGGGGCGGGG	#cc0000
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21[28]	26[12]	ACCAGACACGATGAAGCGCGGGCGCGTTTT	#57bb00
9[8]	8[8]	TTTTAATTTCTCATGCTGAACCTGGCCACGGAGGCTTTT	#57bb00
17[12]	17[34]	TTTTGGGATTTCCGTCGGGCAGT	#57bb00
19[8]	18[8]	TTTTTCACCCTGCCGATGGTGGGCTTGCCGGTGCGATTTT	#57bb00
1[12]	11[34]	TTTTCGCGCCGGGCTGTGGCTGAGCGCGTGCGCGGA	#57bb00
23[8]	22[8]	TTTTGTGCGGGGCTGACGGCGTGACCACAGACCCGACTTTT	#57bb00
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27[12]	33[34]	TTTTTTACTGGGATGATCGTGAAATCCCTGACGCCAG	#57bb00
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5[35]	4[12]	AAATCAAAAAACCGTCTATCAGGGCGATGGCCCTTTT	#57bb00
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40[34]	40[12]	GCGTGGGGAATCTTTACCGTTTT	#57bb00
8[41]	2[12]	GGAAGTGCTGCCATGTGAACAGCCAGACCCGGACGCTGACTTTT	#57bb00
16[34]	16[12]	GCTGGCTGCACAGAAAGCGTTTT	#57bb00
12[34]	12[12]	GCTGGATTTCACTGCTGTTTT	#57bb00
31[8]	30[8]	TTTTGCTGACGCTTCAGGCAGTGCTCATGCTGCCCTTTTT	#57bb00
41[12]	31[41]	TTTTGCTGATGCGCGGCTATGCCACCGGCGCGGATTGGGACGCT	#57bb00
11[8]	10[8]	TTTTCTGGCGTGCCATGCTTAGATGGGGCGACCCGATTTT	#57bb00
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Electron Micrographs for pScaf-7560 Brick

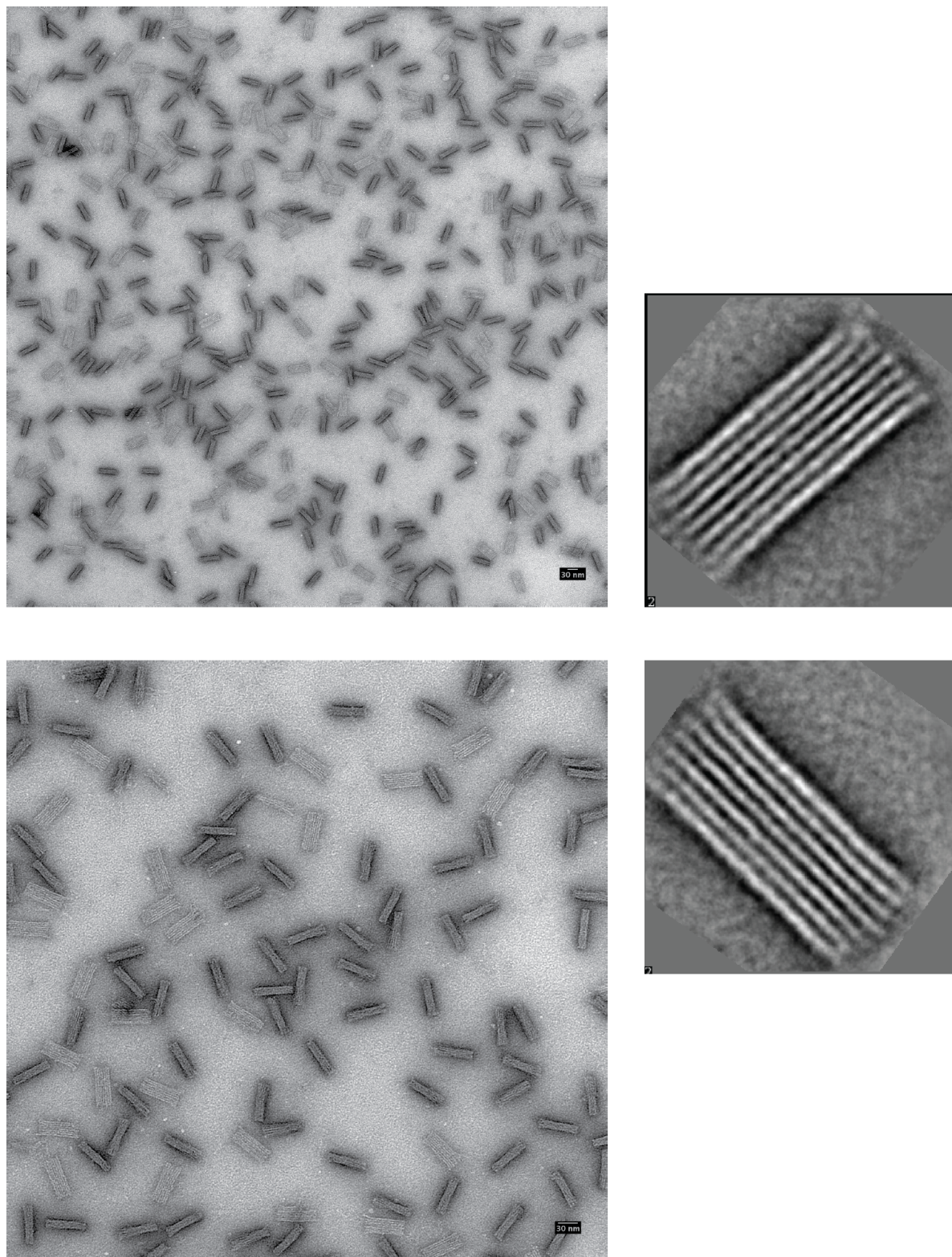


Figure 54: Electron Micrographs and Class Averages for pScaf-7560 Brick

pScaf-8064 Brick

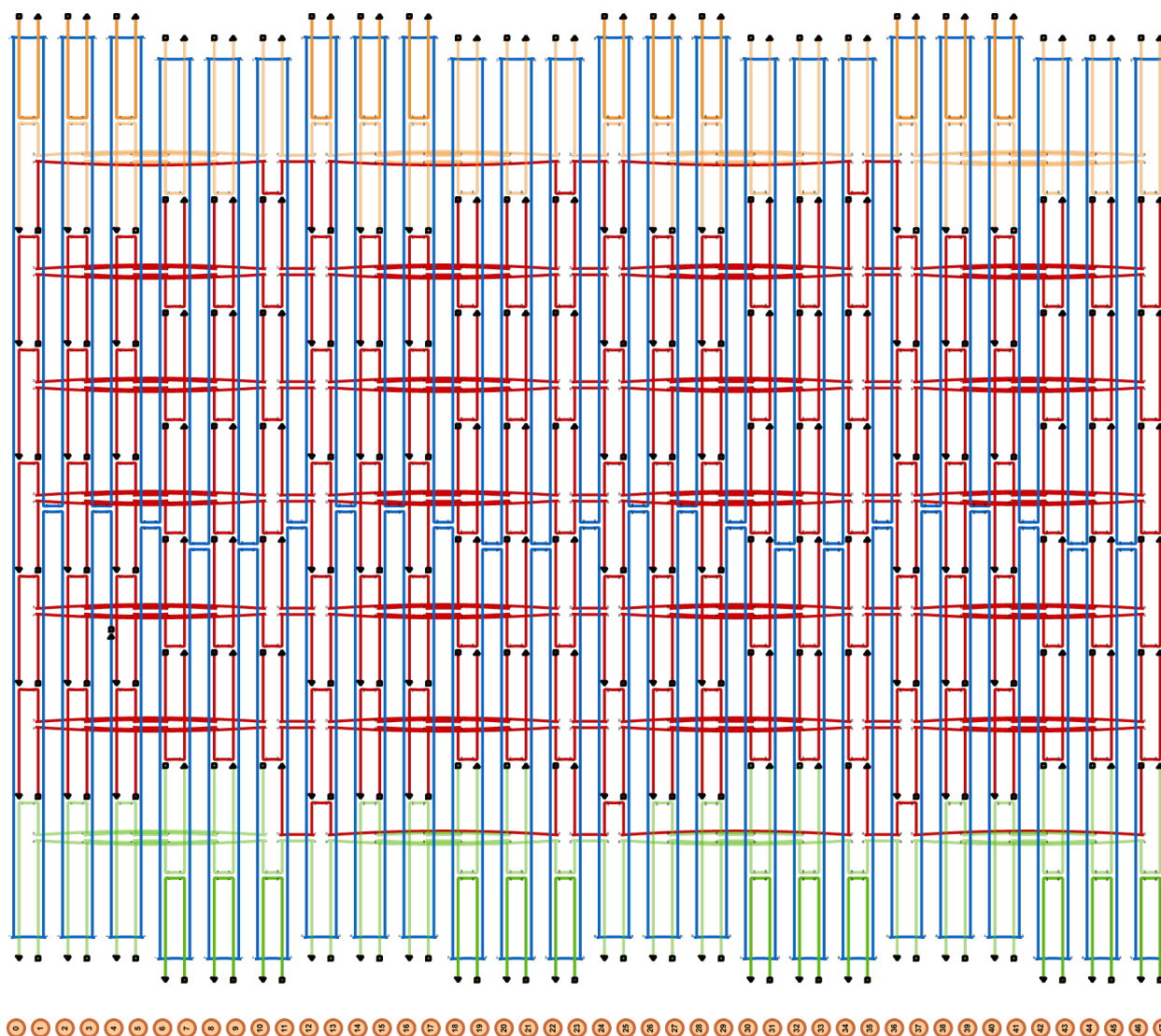


Figure 55: cadnano Design for pScaf-8064 Brick

pScaf-8064-ACK Scaffold Sequence

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11[183]	12[167]	TTTTCGCCTTTCTGAGGTTGTTTTGCATTT	#dda539
43[183]	44[183]	TTTAACTATGTCCACTCTGAATGCTCTTTT	#dda539
5[167]	6[183]	CCCAAGGACCACCCGCTCAGGACTGATTTT	#dda539
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21[183]	22[183]	TTTTCACCTATCCTCCCAAGAGAGGCGCTTTT	#dda539
27[187]	28[187]	TTTTGGTCTGCCTTCGCAAACCTTTT	#dda539
23[183]	25[161]	TTTAACTACATTCCCTCAAGGTTTCATCGATTTTGAAG	#dda539
31[183]	32[183]	TTTTGATATCCGTGAGCTGGTTATCTGCTTTT	#dda539
7[183]	8[183]	TTTTGAGGCCAGCTCCCTGATCGAGTCTTTTT	#dda539
37[174]	36[187]	CAAAAACCCTCAGAGAGAGGCTGTTTT	#dda539
47[183]	46[160]	TTTTTTTACAACCTTTTTAAGTCCTTTTATGATGCGT	#dda539
3[187]	4[187]	TTTTATCCCAGCTCCTCTGTTGTTTT	#dda539
1[187]	2[187]	TTTTGACATGTCTTCCTCATCGTTTT	#dda539
13[187]	14[187]	TTTTCCAGAATTGGTACCAGACTTTT	#dda539
45[183]	46[183]	TTTTCAGTAAATAGTAGAGAATTTAACATTTT	#dda539
5[187]	2[174]	TTTTCGCAGAGATTTGGTGGTCTGTACCACAGGCTGCCCTT	#dda539
35[183]	36[167]	TTTTTAAAGCTGAAAATGATGCTATTTTAC	#dda539
19[183]	20[183]	TTTTGGGCCAAGCTGAACCCAAACTTTATTTT	#dda539
25[174]	24[187]	AGAGCTAAACTATTTGACTTGCTTTT	#dda539
39[187]	40[187]	TTTTTGGGTTTCTAATAAGCAGTTTT	#dda539
29[187]	26[174]	TTTTTGTCACGCTAAACCCAATCACCCGCCAACATAAGCCA	#dda539
15[187]	16[187]	TTTTTCTGTGTTTGCAAGACCTTTT	#dda539
9[183]	10[183]	TTTTCTGAATCAGACTGTTCTCCCTGGCTTTT	#dda539
17[167]	18[183]	CTCTCCAACCAGGTTGCAATGGGCCATTTT	#dda539
25[187]	26[187]	TTTTGCGTCCTGCTCCTTCTTCTTTT	#dda539
13[174]	12[187]	TTCCAGGCGTCAGAGGTATGCATTTT	#dda539
31[76]	25[63]	CGATGTGGCCTCGCAGCAAGTTTTGGGTCGAATTCTATCCCTAGTGTCGT GGATCA	#800000
43[62]	38[48]	TGAAGTAATATTTTATCTGATAGGTGTCTCTGGAAATTATTCTAAAACTCT GTTTCG	#800000
2[133]	17[140]	TCGAGAACGCACTGCTTGAAGTCAGGGCAGAAACCAGATAGACAAGAG G	#800000

		AACCGGATCATGGATTCAACCTAACACGATGTGAATATTATCTGATTTC	
44[126]	37[112]	CCATAT	#800000
14[42]	1[49]	TGAGCTGATCGCCGCCACCTCTTCCACCCCCGGTCTGACCGGCAAGTG	#800000
		TCGCCTTGCGTTTTGCATTGGCTGTCAGCCGGATGTATGCCGCATTAGG	
20[70]	4[69]	TCCACT	#800000
30[35]	33[42]	GCACGACCGCTGGCCCCGGATGTTCTGTCGTGTGTGG	#800000
38[35]	43[28]	TATCGTCGCAACTCTCCTGCTAGATGAAGAGCAAG	#800000
7[48]	10[48]	ATCAGAAGACTCCTTGCTGTTCACTGTA	#800000
34[161]	39[161]	CGAAGGACTGTCATGCAATCCCAACATT	#800000
37[160]	32[160]	AGGAACCAAACCGAGGGCTGTCGATTAT	#800000
		CGGAAGATTAAAACGATGCACAAATTTGAATCGTATGTGTATGATGGT	
44[119]	39[105]	ATTCCAG	#800000
42[35]	45[42]	TGATCCCTCCGTGGAACCGCTGGCATTCTTTATGT	#800000
		ACTTCTCGTGGAAGTATAGTTCTATAATAACACGGTGTTATCGTTTTCTA	
43[153]	46[139]	TCATA	#800000
10[28]	7[35]	AACCTGCCGCGTAAAATCGGAACCCTAAAATCAAGCTTTCAGTTTATAA	#800000
4[42]	1[42]	TTGTTCTCACCCAAGGGAGCTAGCGGT	#800000
		CCCTTGATTGTCCAGTTTTCGTCGAAACACTGTTCTAACTTGTGCACCGG	
30[56]	35[56]	CACTC	#800000
46[28]	33[28]	GAGCCATCGCGTTATTTACAACACCCGC	#800000
0[42]	10[62]	GCGTGATCCGGACCGCGAACGGCGCTGTACTCCA	#800000
19[48]	23[35]	GCAGAGGGCTGATATTCTGCAAGACGTGAGTGGGCTTTTCTG	#800000
		GATTAAGGAGCGTGCAGCAATGTCACCGTAATCATCGATGCAATGATGT	
30[140]	35[140]	TGCCGCT	#800000
46[105]	37[84]	TGAAATGTGTGGCTAGATAGTAAATATATTTCCCTTGCTGAATGCCATT	#800000
23[111]	13[84]	TATCATTGACAGGCTCTTGCCTGAACAGAGTTGCATCAGTC	#800000
		GATTCCATTGATCATCCAGGATCCAGATGTACAGAGGTTAACAACATGA	
3[139]	8[153]	ACATCC	#800000
		TGAGCAACGATGGGCTCCGGCGTGGATAAAAGCAGGACATGCAACAAG	
38[105]	25[112]	A	#800000
23[118]	3[112]	GTTACAAATAACACCAAAGCCTTCATGGTGCAAATAATCTCTAACCAGC	#800000
27[167]	40[167]	TTGCACGCTCCGCTGCAATCGTGAGCAC	#800000
35[111]	25[84]	TCTGTCAGTCAGTGCGTGAACCTGGCGATAGCGTTAGTCATG	#800000
		GGGATGCCTACCGCAGATCCCCTGGGCCCCTGCCAAGTCCATGACGTGC	
29[118]	24[118]	AATGGGA	#800000
		ATCCGTAGGGTTTCTTTGTCAAACGCCTCGGCGCAGTTCAGTGAAAGCCG	
31[55]	25[42]	GCCAGT	#800000
11[48]	13[35]	TGTGAATCTTCATATTACGCGGCTGATT	#800000
17[160]	14[153]	CTCCCACTCAATCTTTCAGTCACCTACCAACCTT	#800000
38[63]	35[70]	GGATGTTATATAGTAGCACCGATAACAATGCGCAT	#800000
13[153]	11[154]	ACCTTCAGCTGATGTTGCCCCAAAGGG	#800000
26[77]	39[77]	CGGTTGATTTTTTTTGTTCGTCCTGGACATACTGACTCCCATCACTAG	#800000

		CCAAC	
42[56]	46[69]	TCGTGTAAAAAATAAAAGATTACCGACACTCCTTGCGCGTAG	#800000
		TTAGCGCGTACACGTGAACCCTAAACAATGAGTTGACAGGGTCCCCGGT	
43[104]	45[105]	CGGAAAA	#800000
9[160]	4[167]	CTTCCGCTGGAGAATGGCTGAGGCGTGGAGCCAGT	#800000
		CATGGTCTGCTGGACTCTCTCCTCTGTTGGTGGTCATCCTGGCTCTGAACT	
24[126]	8[139]	ACTTC	#800000
		CTTCATTCTGAGAACACAGAATCTGCCGTGTTGCGTCACCGCTCAAGAAG	
10[126]	26[125]	CAGCAT	#800000
38[147]	36[160]	AGTAATACAGAAGTCGCTGAACGGGATT	#800000
35[160]	26[160]	CTGCGTGAAATACACAATTCTATTCCAG	#800000
		CAGAGGAGCTTCAAGCACCGATGCCCAGCGATGACAGTGATCGCTGGAG	
11[118]	7[119]	AGAAGAC	#800000
24[140]	44[146]	GAACGCCTAGCGTCCTGCGCTTACCGTGTGCCTAAAAATTTTATTAGC	#800000
5[104]	4[104]	GCCTGTTCTGAACTCCACGCACTCCAACGTCACTGCCCT	#800000
		TAGTTCAGAATAAAACAATTCATGTGATAATGTTTGAGGGAAAACCACTA	
47[69]	32[55]	TTTGGT	#800000
1[153]	0[167]	CGGCCTGTCACCTACTTGTCCGCTGCAC	#800000
37[41]	45[28]	TGTTTTCTGGTAAACAAATATTAGGAAT	#800000
		TGATGATTTTGCTGCGCTGCATTGAGGACCGGCGGCTGAGCACAAGACG	
17[76]	12[76]	ACGCAGC	#800000
44[154]	30[160]	GATTTGTTTCTGCGCGTCTGTACCGTTGGCGCTTT	#800000
10[154]	2[167]	AATAATAAGTCTGCTGCAATCCCGCGTT	#800000
		CTGACAGACCTGAAAGACTTCTCTCCGAGTTCGAGAGAAGCGCGACAAA	
31[97]	34[111]	AATTACT	#800000
1[167]	14[167]	TGGACCATGTACAGAAAGCATGCTCTGC	#800000
		CAGACACATATTAAGGACGTTAATATCTTTTATGTCGTTATGGGCTGCAC	
36[140]	40[139]	GGTTTG	#800000
		CGTCGTGACTCGAATCCAGGAGCTCGCCATGGTGAAACCCAACCTGGAC	
16[119]	22[132]	GCACATG	#800000
		CGTCCGGATTCTCTGGCGGCGGGCCGCCCCTGGATGCCGTTGACTTGTG	
9[90]	21[77]	TCCGCAT	#800000
		TTACCCGCAAGCCAATCTTGGGCTGCCTAGACCGGCACTGGTCGTGCTGA	
26[84]	14[97]	GTCATC	#800000
		CTTCACTTGCAGCAACCAGCTTCTTTCCATGACGTGTGATTTTCTGGATTG	
31[139]	25[126]	TTCAA	#800000
3[167]	16[167]	TACTGAGCAAGGACTGGCCCTGGCCCTC	#800000
		ATATCAGGAAGCACCCGTAATCAAACGACTAATCAGATGTTGTAGCACCA	
30[77]	42[90]	TTTCTA	#800000
44[28]	31[28]	AGTGCGGAATTACTTAATAATACTGTGG	#800000
21[160]	16[160]	AAGCCTCGACAGCCTGTGGCGCAGCTAT	#800000
37[132]	21[133]	TGCAGATCTCCCCCGACTGGGGTCGTCGATGTTGATTGACGTACAGGG	#800000

		ATCACCA	
		AATTCAACATAAGGTGATGGGGAAAATTAACAAGTTACCCTTTCAAACT	
32[98]	40[97]	ATTTAA	#800000
		ACAGAGAGCTGTAAGGAGCGGGGAAAGCCGGCGAACCGTCTAAAAAAA	
11[76]	8[90]	GCCCACGA	#800000
20[28]	7[28]	TTTTGCTTCAGTGGGCGGTGGCCGACCC	#800000
13[167]	26[167]	AGAACGACGCATGCTCCAGTCAAGACGA	#800000
7[160]	2[153]	CACCATCTTTCACACCCACGCTATGTGGGATGGATAGACTCAGTTATCT	#800000
		GAAGTCTGTGACAGAGATCCCTCATTGAGAGAACTATTGCAATAATCTGC	
39[97]	27[84]	AACGGA	#800000
21[62]	41[56]	GCTGAAGCGCCGTCGCCAACCGCCGTAATCCCCAAGTTGACTGAACATA	#800000
		TGAGTCACCTGCCGTGCCGCGATTTTCTTGAGGCCACCGCATCGTGGCGC	
27[55]	15[42]	ACTTTT	#800000
15[167]	28[167]	GCCGCTTGAAGTGCCTTGGCTTACTCCG	#800000
		GCGGTTGGTGCTGTTTGTGGTGGTAGTGGTTGCTGTTTCAGCAATACCA	
23[90]	35[77]	ACTGTA	#800000
3[34]	6[48]	TTTTTTGGATAGGGTTGAGTGATCGGCAAGCCTTCATAAGCA	#800000
		AGACACTACAGCATTGAGCCCCGATTTGCAGCATCTGGAGATAAGATCA	
20[133]	4[132]	CAAGACC	#800000
		TGCGGGAGTGTCCGTATTAATCGATGCTTTTTGAACTGCTGCTCGACAGC	
43[146]	38[132]	AGCGGA	#800000
18[133]	16[132]	AACGCCGCTGTACAAGGAGACCAGGTGC	#800000
		GGCCAAAAGGAGGACAGCTTGGATCATTATTTTCATGATGCGTCGTTTTCG	
8[98]	16[97]	CCGCGT	#800000
		CCCAACTCGGTCAGGCTGGTTGGTGCTAGTTCGCAATATTGTTAATATTG	
27[125]	43[126]	GAATAA	#800000
44[161]	42[160]	GTAGTGTAGCCCTGACGGGGAATGAATC	#800000
		GTGTGAGAAGAGAGGGGAGACACTGCCTGTTCAAGGCAGGATTATCTCTG	
15[132]	1[133]	TTGACCT	#800000
		GGGCATGTAAATGCACGAGGGTGTTTTGTTGATGATGGAGGGAAAGTG	
36[56]	40[55]	GCCTGGTG	#800000
34[70]	21[70]	CCTGTAACAAAATCCATTCTTCCTCAAG	#800000
		ATCGAGCCCATTCTTTCCAGCCATCTTGAGTTGCCGACTCCCGGTGTACG	
17[118]	12[118]	AATACA	#800000
22[35]	27[35]	GCGTTTCCGTTTTGCGCCGTGCAGGCTG	#800000
29[160]	26[153]	AATCAACAGGCGCCAGCTATTGAGCGCATGAGAAG	#800000
32[35]	41[35]	CGTGTTACACTGGCATGAAGGGCATTCA	#800000
		CGGCCAGTACAAAACTGTCCCTGTTGGCACTGGCACGATGCACCGTGG	
24[42]	8[55]	GAAATCT	#800000
		CTCTCTCTGAGTCTTTCAATCCCTGATCAGCTCCATATCTGCTCGGAAGGG	
9[111]	1[84]	AAGAA	#800000
15[125]	31[126]	CAGGAATACCTTGCCTGGCTGCATGGTTCGTTGGTGGACGCTGTGGCAT	#800000

		GCCGGGC	
		GATGGCCTTGACGGGCGCTAGACAAGAGATGGAATGCTCAGGAGCGAA	
3[69]	1[77]	A	#800000
		TTTCTGATCGTTTCGCACGACCGGCACTCCTGACGCACTCCCCCAAGAC	
16[77]	29[77]	GCGAC	#800000
41[160]	39[147]	TTTTTTTCATGGTGCAGTAAACCGCCAC	#800000
37[167]	45[154]	AACTGGAGTTTCTTATGAATTATCAAAG	#800000
		CAAGAGTGCTGTGGTACAACATTCAGGCGGAATGCGGCGGAGGGACGC	
9[146]	29[140]	T	#800000
46[42]	47[28]	ACCTTCCACAGTCTAAATCTTTTCGCAC	#800000
37[139]	19[133]	TGAGCAGGTATAAAATAACGCGATGTATATCTCAGCATTGTAGCAAGGA	#800000
		GTAAGGCCAACGTTAGCGTTTCAATCTGGTCTGACGATGTATACGCAGTA	
44[77]	40[76]	TAAC TG	#800000
9[62]	29[56]	CACCACCAGCCTGTCTCTGCGTCCCGCATTGCGTCGAAAGGCAGGCTGA	#800000
9[69]	22[62]	CCGTGTTCATCCATAAATCATTACGCGTATCAGTTGCTGATGTTGCACT	#800000
38[112]	18[118]	TACACACCCAGCGGGCGGAACCGCATCCAGAAATTGTTTGACAGGAAC	#800000
		TCAGGGCATTAAAGAACGTGGCACTGAGTAATCTGTATACCTAACGGTTC	
3[76]	7[77]	CGGCGG	#800000
45[125]	30[118]	TATTTGTAACCCATGCTGACACTGCCCATTCCTTTATGGTCAAAAGTCA	#800000
		GGCTGGACTCTCAAGAGAAGACTCAGTTGGCCGCAGCCTTTAGAGAAGT	
3[132]	10[146]	ACCTCTC	#800000
		AGCGCGATCCAAC TATAAGGGCGAAAAACGTGGCGAGAAATGGAAGTC	
9[97]	3[98]	TCAACTTC	#800000
		AGATCCTTAAGCTGATTCATTTAACGAGGAGTTTCGGAAC TCAAGACTCG	
16[112]	2[111]	AAGGTC	#800000
7[97]	8[111]	GGATAAGGCGCACTAGGAATCCCAACCCCTCCAACCTCGAAG	#800000
		GTTGTCTTGTCCAGGAGCCGATCCCTCCAGAACTTAGAAGGAATGCGCA	
19[97]	22[111]	AAGAAAG	#800000
		GCCGTCCGCTTCGGTCGACTACCACCAGACGCTGCGAAGAACACATTGG	
4[112]	9[126]	GGAATCT	#800000
		TATGCTCAAATCTTCATACAGTGCTTAATAATTGCATGTATAGATCACCTT	
43[90]	39[63]	GTCAC	#800000
		AGAGATTCATTACCTCAGCGTGTCGGTTGCACATCATGCAGCAGAGTTGT	
32[126]	46[125]	CTTGAT	#800000
33[104]	44[104]	AATGCCAATTATTGAAACGAAGCCAACG	#800000
25[153]	14[160]	TCAACTCATCTACGGAGGCCGGGACCAATTCACGCATGGGATGAAGATC	#800000
		TCGTCGAGATTACTGAGGAGCCAGCTGACACTCGCGACCAGGAGGGACC	
0[84]	15[98]	GCTAAGA	#800000
16[35]	18[48]	AGGCAGACTCGTGCGCCGCCCTGGCAGA	#800000
24[35]	33[35]	GCAATCGTCATTACCTGTGTGCATGAG	#800000
10[35]	15[35]	AGGATGAACGTCTGCGCTTCCTCCGCT	#800000
32[28]	19[28]	GAGCCCCGCGGCATGATGGCCTTCGCT	#800000

		GTCAGAGTGGCAACCCTCCCAAAGAGGGTTTCTCTAGATGTATATAAGCA	
1[111]	14[125]	TCGATC	#800000
		CGACCTGAGGGCCGGTTTGTCTCAGCGCAGATGCGTACAAAGGCGCTTC	
23[132]	39[119]	TTTTCTT	#800000
		TTGACGGAATACTGAAAGAATACGGCTGCCTTTAAGCGCTGCCTGCCAG	
37[48]	21[49]	CTATATC	#800000
24[84]	34[104]	TTCCCCTGAGATGGTGCCAAGCTTTCCACGCGGC	#800000
34[42]	36[41]	GCTGGTTTACCGGACAATGAGGTGAACC	#800000
26[112]	15[112]	CAAAGCTGAATCTCACTGAACTTCCTAG	#800000
		GTCTTGGGCACCTGGCAGGCTAAGCAGCTTGGCCCGATGGATGTATTCA	
20[98]	28[97]	AGAACCT	#800000
		GCCGGGCATCCTGCAGCACATAGCAGAAAATCTCTCATATGAGCGTGAG	
12[84]	27[98]	GCTCTAT	#800000
		CTGGTGCGCAGCCACCACCGCCAACTCAGTATTGCCTCCTGCTTTAGCAG	
36[84]	44[97]	CATTAT	#800000
		TCGCAATCTGGGTATGGCGTTTGCCTACCGCGACACGTGCTGAGCCCAA	
24[63]	8[76]	CCCCAC	#800000
		CTGTCATTGATCTCGAGCAGAATCTGGGTTTTGAATTATTCCGTATGTCAG	
26[140]	42[153]	GTGCG	#800000
		CCGGATGATGCAAGTATCGTTTTTATATATGTGAGACGTTGTGACGTTTTT	
36[77]	46[76]	TGCAT	#800000
		CGCCCGGCTTCACATCCTCCGTCATGGCACCTCCGACAAGGGTCCCCTTTC	
10[77]	22[90]	TCGAC	#800000
		CCTCATCCAAGATCCACTCCAGAATAAGCCGACCACTGGCGTCTCTCTGC	
9[153]	21[140]	TTATGA	#800000
		TGAACCAAGTTTGAACAAGAATCTAAACAACGACGGACGCTTAACACT	
3[55]	8[69]	CATATTC	#800000
20[35]	29[35]	CTGCGATCGCTCCGACGGGATGCCCCTG	#800000
24[56]	44[62]	GCGGTGTTTCCCGACTGTGGAATCCTTAGAACGCGTGTCAGGAAATACA	#800000
		CCTCACACATTCTTAGCCAGATGCCTGTGCCAGAATCACCGGCGCCGCCG	
37[76]	18[76]	GAAGCT	#800000
2[49]	17[56]	CCCCGATTAGAGCCACTACGTTACTGCGTGTTACGTTGTGACCTCTTT	#800000
27[160]	18[160]	CTCATCACTGATATCCAAATGTTACC	#800000
		AAAGATCAGGAGAATGCTCAATCTGGTTAAGATCAGCCTGGTATTCTACG	
18[140]	23[140]	AGTTCT	#800000
34[28]	21[28]	GCAATACGCGTATTTATTTTCCGAATT	#800000
20[49]	5[42]	CCGCACTGCTGCGGGCTGATTCTGGCTAATATCAAAAAGGTACCCCGAA	#800000
22[28]	8[34]	GCAGCAATCTTCGCCGAGGAGCTTTCAGTGCCGT	#800000
		GCCGCCGTTGCCGAGCTGTTTGCCTCTGCGCGACAGCTGAGCCCGGTCA	
18[56]	23[56]	GTGCCGG	#800000
2[12]	1[12]	TTTTCGTAAAGCACTACCACCACACCCGTTTT	#45d511
40[12]	39[12]	TTTTGGTGAAGCACGAGCAATGCCCTCGTTTT	#45d511

22[21]	23[8]	TGGCGTTCATCCATTACCACCTTTTTT	#45d511
34[21]	35[8]	TAAATAAGGCTGCCCTGACTGTCTTTT	#45d511
12[12]	11[28]	TTTTCTGTGCCACCACGGACGCGCACGGTG	#45d511
0[12]	1[35]	TTTTCCGCGCTTAATGCGCCGCTACAGGGCCACGCTG	#45d511
24[12]	23[28]	TTTTTGACGCGTCGATAACATCTTTCGTTT	#45d511
30[8]	33[21]	TTTTCATCCTGTTCTGAGAGTGTGCGTGAGCATGGCCCCCT	#45d511
6[28]	5[12]	CTGCCGGAAATCCCTTATAAATCAAATTTT	#45d511
34[8]	33[8]	TTTTCGTTTTCGAGTTGAACCGTTTT	#45d511
20[8]	19[8]	TTTTGCCGCTTCTCAGATGACCTTTT	#45d511
30[28]	29[12]	CAATACACATCAGGACGTAGCCAGACTTTT	#45d511
42[28]	41[12]	CTGCTGAGAGCAATTGAGGCAGCGTTTTTTT	#45d511
22[8]	21[8]	TTTAAACCGCCTTCGTATTGCCTTTT	#45d511
8[8]	7[8]	TTTTCTGTACCGGGAATCCGTCTTTT	#45d511
26[12]	25[12]	TTTTAATACGGTAATTCAGTGCGTCAGGTTTT	#45d511
14[12]	13[12]	TTTTCCGCTTCTGCGCGTCTGCCGTACTTTT	#45d511
4[12]	3[12]	TTTTAGAATAGCCCGAGGGTCGAGGTGCTTTT	#45d511
16[12]	15[12]	TTTTGCTTTTCGTGGCCCCTTTGCTGATGTTTT	#45d511
32[8]	31[8]	TTTTCTCAGACTGACTTAATTCTTTT	#45d511
18[28]	17[12]	CGTTTTTTTGAAGTGGCGGCCTCTGACTTTT	#45d511
38[12]	37[12]	TTTTTAATTAAGTGAACCCGCAGCGTTATTTT	#45d511
46[21]	47[8]	GCAACCTTTGATCGAATATTTCTTTTT	#45d511
42[8]	45[21]	TTTTATGAATAAAGAACAATCGCATGGGTAACAAGTTTTCA	#45d511
36[12]	35[28]	TTTTACGGTGATGGTGTGTCCGTTGACTGT	#45d511
6[8]	9[21]	TTTTAGTACGCCAGTCGCCACAACGATTTTTCCTGCTGAAT	#45d511
10[8]	9[8]	TTTTATGGTGGAAGGTGCAGTTTTTTT	#45d511
10[21]	11[8]	AACCGTCATGGTCCCGGCGTGCGTTTT	#45d511
28[12]	27[12]	TTTTGGAACGATATCTCATTTTCCGCAATTTT	#45d511
46[8]	45[8]	TTTTTTAAAAATGCTTAATAGTTTTT	#45d511
44[8]	43[8]	TTTTATTGGTTGCAGCGACAAATTTT	#45d511
18[8]	21[21]	TTTTGGCATTCTGCTCGGAGCTGTTTGCCGCACTTTTGCA	#45d511

Electron Micrographs for pScaf-8064-ACK Brick

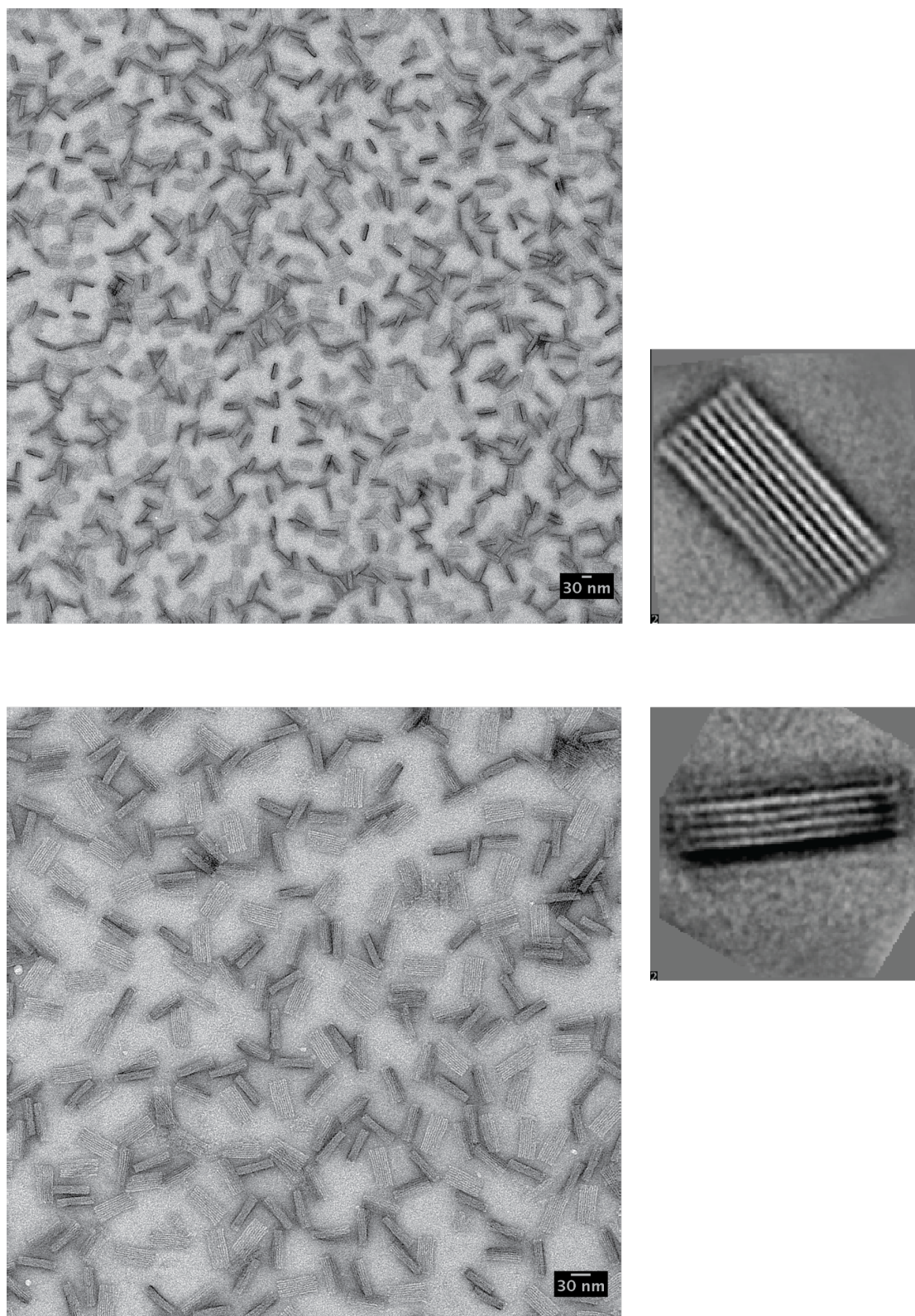


Figure 56: Electron Micrographs and Class Averages for pScaf-8064-ACK Brick

pScaf-8064-BFJ Scaffold Sequence

AATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCTATCTCGGGCTATTC
TTTTGATTTATAAGGGATTTTGCCGATTTTCGGGGTACCTTTTTTAGATCTCATTATCA
TCGCTTTTAAAACGGTTCGACCTTCTAATCCTATCTGACCATTATAATTTTTTAGAAT
GGTTTCATAAGAAAGCTCTGAATCAACGGACTGCGATAATAAGTGGTGGTATCCAG
AATTTGTCACCTTCAAGTAAAAACACCTCACGAGTTAAACACCTAAGTTCTCACCGA
ATGTCTCAATATCCGGACGGATAATATTTATTGCTTCTCTTGACCGTAGGACTTTCC
ACATGCAGGATTTTGAACCTCTTGCACTACTACTGGGGAATGAGTTGCAATTATT
GCTACACCATTTGCGTGCATCGAGTAAGTCGCTTAATGTTTCGTAAAAAAGCAGAGAG
CAAAGGTGGATGCAGATGAACCTCTGGTTCATCGAATAAACTAATGACTTTTCGC
CAACGACATCTACTAATCTTGTGATAGTAAATAAAACAATTGCATGTCCAGAGCTCA
TTCGAAGCAGATATTTCTGGATATTGTCATAAAACAATTTAGTGAATTTATCATCGTC
CACTTGAATCTGTGGTTCATTACGTCTTAACCTCTTCATATTTAGAAATGAGGCTGAT
GAGTTCCATATTTGAAAAGTTTTCATCACTACTTAGTTTTTTGATAGCTTCAAGCCAG
AGTTGTCTTTTTCTATCTACTCTCATAACAACCAATAAATGCTGAAATGAATTCTAAGC
GGAGATCGCCTAGTGATTTTAACTATTGCTGGCAGCATTCTTGAGTCCAATATAAA
AGTATTGTGTACCTTTTGCTGGGTCAGGTTGTTCTTTAGGAGGAGTAAAAGGATCA
AATGCACTAAACGAACTGAAACAAGCGATCGAAAATATCCCTTTGGGATTCTTGA
CTCGATAAGTCTATTATTTTCAGAGAAAAAATATTCATTGTTTTCTGGGTTGGTGATT
GCACCAATCATTCCATTCAAATTGTTGTTTTACCACACCCATTCCGCCCGATAAAA
GCATGAATGTTTCGTGCTGGGCATAGAATTAACCGTCACCTCAAAAGGTATAGTTAA
ATCACTGAATCCGGGAGCACTTTTTCTATTAAATGAAAAGTGGAATCTGACAATTC
TGGCAAACCATTTAACACACGTGCGAACTGTCCATGAATTTCTGAAAGAGTTACCC
CTCTAAGTAATGAGGTGTTAAGGACGCTTTCATTTTCAATGTCGGCTAATCGATTG
GCCATACTACTAAATCCTGAATAGCTTTAAGAAGGTTATGTTTAAAACCATCGCTTA
ATTTGCTGAGATTAACATAGTAGTCAATGCTTTCACCTAAGGAAAAAACATTTTCA
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Staple Sequences for pScaf-8064-BFJ Scaffold

Start	End	Sequence	Color
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33[183]	34[183]	TTTTGGCTGTCCAAGCCAAATATGCTGATTTT	#dda539
41[167]	42[183]	TCATCTTCTTCGATAACCAGAAGACCTTTT	#dda539
11[183]	12[167]	TTTTGGCGGGCGGTTTAAGACCGGACCATC	#dda539
43[183]	44[183]	TTTTTGCCCGTGCCATGATCTTCGCCAGTTTT	#dda539
5[167]	6[183]	CCATACCTGCCGTTGTAACCGGTCATTTTT	#dda539
41[187]	38[174]	TTTTTCAGTAATGTAGATGGCAGGCCTGAATGGTTGGTCT	#dda539
1[174]	0[187]	GCATCAAGTTAACAAGTTCATCATTTTT	#dda539
17[187]	14[174]	TTTTGGTGCGGTACTTTTGCAGTCACACTGGCCTAAAGTA	#dda539
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21[183]	22[183]	TTTTCAGGATACCCGTGTGGCCTGAACGTTTT	#dda539
27[187]	28[187]	TTTTTGCCCTCCATGCGTTGATTTTT	#dda539
23[183]	25[161]	TTTTAACAGTTCACCGTTAAAGGCCCGATGCCGAAA	#dda539
31[183]	32[183]	TTTTTGTAATCCCTCGTATAACCATTTTTTT	#dda539
7[183]	8[183]	TTTTCCCCGAGTACGGCCGCATCCGTCATTTT	#dda539
37[174]	36[187]	CCACAAAATCCTGCTGGTTGACTTTTT	#dda539
47[183]	46[160]	TTTLAGTAGTGCGGTTTGATTTCCACGGAGTTCTGA	#dda539
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13[187]	14[187]	TTTTGAGCCTATCCGCCACGATTTT	#dda539
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43[62]	38[48]	CGGTCAAAGTTAACGCTGGCTCACGTTACCGGACCACGTCCGACTC TGCTCCGGTT	#800000
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38[35]	43[28]	TGCAGCAGGCGGTCTGATCTGGCGGCGATGTTTTT	#800000
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29[118]	24[118]	AACTCTTTCCA	#800000
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31[55]	25[42]	GATATGTTG	#800000
11[48]	13[35]	TGGACATGCAATTGTGAAGAGTGAAAAC	#800000
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43[104]	45[105]	GCCACCACG	#800000
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26[84]	14[97]	GTATGAGCG	#800000
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43[146]	38[132]	TACTGACGA	#800000
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		TCGATGCTCCGCTTAACAACCACACCTCTTAAATGGATACTAAAAAA	
9[62]	29[56]	TG	#800000
		GACTTACCATCTGCATATCTGCTTCGAAACCACAGTATCAAACCTGA	
9[69]	22[62]	AA	#800000
		CCATGCCTCACAACCGATAAACGGCCCCGTGATGACTGTAAAAGAA	
38[112]	18[118]	TAA	#800000
		TCAGGGCATTAAAGAACGTGGTTTAAAAAGATAGGATTAGAAGGG	
3[76]	7[77]	AGACATTCGGT	#800000
		TTCAGGGATCGCCTCTCTTTGAATAGCGGGTTAGTGTGTCAGTGAAGA	
45[125]	30[118]	TAT	#800000
3[132]	10[146]	TTTCTCCCGTCCATTCCAGCATGCATCCAGTGCATCACGCATCTGCA	#800000

		GCTTGATTG	
		GGCCATGCTGTCGTTCAAGGGCGAAAAACGTGGCGAGAAAACACC	
9[97]	3[98]	TTCTTGAGCCA	#800000
		ACTGCTCGCGATGCATGCCGTAATGCCCCGCCACATGGAGCATTA	
16[112]	2[111]	CTGGCGCAAA	#800000
7[97]	8[111]	TAAGTGC GGCGTGTCCATATCGAGCAGACGTTTCATCATCATC	#800000
		CGAGTCTTTCTCCTGACTGTTCGCGCCACACATTCAAAAACCGGCA	
19[97]	22[111]	GTATGCGCT	#800000
		CTGTCCGGGCGGTTCGAGTCACCACCATCGGATCGACACCGGGCGT	
4[112]	9[126]	CGCGCATAGG	#800000
		GCTGTTGGCGATCCGACGCATTTGCTTGGCGGAGTTTGGCCGCAGG	
43[90]	39[63]	CGGTCTTCAC	#800000
		AGAACAGTATATCCACGTCTTCCCCATCTCGCTTACCGAATTGCTG	
32[126]	46[125]	CGCGTAATT	#800000
33[104]	44[104]	ACAAACCCATCCAGGATGTGCTAATGAA	#800000
		CGACGATCGGGCTTCTCCTCCGTGCATAACTGGTCCAGGCATTTTC	
25[153]	14[160]	GC	#800000
		AGTCTCTTTGGTCATTCGCCCTATATGCCGGGAGACATTAATGGC	
0[84]	15[98]	CTCCGGCAG	#800000
16[35]	18[48]	AATAGACGCAATCAAACCTATACCTTTTG	#800000
24[35]	33[35]	AAAAATAGAGTTTAGATATAATGGTATT	#800000
10[35]	15[35]	ATTAGTTCAATAATGTTTAAAGTACACA	#800000
32[28]	19[28]	TCTTCTTTGGAAAACAGAAGCTCCACTT	#800000
		AGGCCGGTCCTGAGAGACGGGATTTACGCAACCTGTGTCACTCGGG	
1[111]	14[125]	TTCAGAAACA	#800000
		CATGGTAAACGTGCTGCCGCTGGCGTTGCGTAATGCAGAACTATTC	
23[132]	39[119]	AACCTGCGTG	#800000
		TAAGCGATATCGTGATGACAGAATTGATTATATTAGTTACTATGGAA	
37[48]	21[49]	AAGGTTTTA	#800000
24[84]	34[104]	TTACAATGTCAGTCATCCTCGATTGCGTCACAACC	#800000
34[42]	36[41]	ATATCAAAAGCAATGCTTTTTATCACAG	#800000
26[112]	15[112]	GCATTGCACCGGCAGTGAATGACGAGAC	#800000
		ACGGGTTTGCACCGGGCGTTTTATCTTCCTTCTATGAGCGAGTCGTT	
20[98]	28[97]	GGTATAATG	#800000
		TTCACTAAATGCGCACTCTTCGCGGCGGTTGCTGTCCGGCTGATCG	
12[84]	27[98]	TCTGGGAAT	#800000
		AAATAATGATCTGACCACTTACCAGCTTAGCTCATGTTAATTCTCGC	
36[84]	44[97]	AGCCCCAGG	#800000
		GTTGGAATGACTATTGATGAGTTCCTTACTCTGGCATTTCAGATTAA	
24[63]	8[76]	GCTATTATC	#800000
		GGGCTGCCATATTCCGTCTGAATTTGGTCCCAAATCGCCAATACACC	
26[140]	42[153]	GTTCGCCAG	#800000

36[77]	46[76]	TGAAATCGCGCAGTTTTTCCCGAAAAATTCGCTGACGTTTGGCAGTC	#800000
		CGGATCCCG	
10[77]	22[90]	TTTGCTCTCTGCGGGTTTTATGACAATATGATAAAAAGACAAGGTG	#800000
		AAAGCATTAT	
9[153]	21[140]	AGCGCACTCGCAGCGGCGCTCCATCGTTCCGCTTCCTTCCTGGAAAA	#800000
		GCGCGCCGC	
3[55]	8[69]	TGAACCAAGTTTGAACAAGAATCTAAATTCTAAAAAATTATTAGGT	#800000
		GTCAATAAA	
20[35]	29[35]	TTGAAAATCAGATTCTGAAGAGGCTATC	#800000
		GCAGATGATTAAGGAATTAGAACTATGAAGAAAGGCATGTCACCTG	
24[56]	44[62]	CGG	#800000
		TTGTCTGCTGGCTGCAACATAAGAGAAGGAATAACATTAATAAGTT	
37[76]	18[76]	CGCCCAGCAC	#800000
		CCCCGATTTAGAGCCACTACGAGAGAAGTTTAACTATCCCAAATTTT	
2[49]	17[56]	GA	#800000
27[160]	18[160]	GCCGTGATTGCAGGGTGTCTATGGTGG	#800000
		CCAGCTCACTCTCAGCAGCGTGGGGTATTATCCCACGCGACGGGCC	
18[140]	23[140]	ACACCTTCCC	#800000
34[28]	21[28]	GTAAAATGTGTTATTCAATACAAAGCTA	#800000
		GTCCTTATGACCCAGTCAAGACGTGAGGGAAACCAAAAGGTACCCC	
20[49]	5[42]	GAA	#800000
22[28]	8[34]	ATTAATATTTTCAAAGCAATATGCAACTATCCTGC	#800000
		GGTTAATTCTATGCACGTGTGATTACTTGCAAATTTGTTTTTCATGGT	
18[56]	23[56]	GAAAAATG	#800000
2[12]	1[12]	TTTTCGTAAAGCACTACCACCACACCCGTTTT	#45d511
40[12]	39[12]	TTTTAACAGCACACACCTGCTGTCCCCCTTTT	#45d511
22[21]	23[8]	CTTATTCGTAGGTCAAAAAGAAGTTTT	#45d511
34[21]	35[8]	GAGAAACCCAACCTTAGTATAAAATTTT	#45d511
12[12]	11[28]	TTTTTCAGCCTCATTTCTAAATATTTTTATT	#45d511
0[12]	1[35]	TTTTCCGCGCTTAATGCGCCGCTACAGGGCCACGCTG	#45d511
24[12]	23[28]	TTTTGTATATTCCTGCAGTAGAATGGTTTT	#45d511
30[8]	33[21]	TTTTCGCAGCTGACTTCTTTCAGTGATATTTTTGTGTAACA	#45d511
6[28]	5[12]	GTTGATTAAATCCCTTATAAATCAAATTTT	#45d511
34[8]	33[8]	TTTTAAGCTGAACGAGCATTAGTTTT	#45d511
20[8]	19[8]	TTTTATGGCCAAAAATAGAAAATTTT	#45d511
30[28]	29[12]	TCTTTCATATCGGCTTATTAGTACTTTTTT	#45d511
42[28]	41[12]	TGTCAGGGTCCTGGCATGCCAGCGGTTTT	#45d511
22[8]	21[8]	TTTTAAGTAAGCATTTAGTAGTTTTT	#45d511
8[8]	7[8]	TTTTTGCAAGAGGATAACCACCATTTT	#45d511
26[12]	25[12]	TTTTGCCCCGTTAAGTTGAAAGAGGCGGTTTT	#45d511
14[12]	13[12]	TTTTAAGAATGCTGCCATATGGAACCTCATTTT	#45d511
4[12]	3[12]	TTTTAGAATAGCCCGAGGGTCGAGGTGCTTTT	#45d511

16[12]	15[12]	TTTTATATTTTTCTCTATATTGGACTCTTTT	#45d511
32[8]	31[8]	TTTTCGCAAGGTGGTCAAACGATTTT	#45d511
18[28]	17[12]	GTGATTTCOAACCCAGAAAACAATGATTTT	#45d511
38[12]	37[12]	TTTTACTTGCTGCCGCATCTTCTCGGGCTTTT	#45d511
46[21]	47[8]	TCTGCCGCCGGGTGATGGCAGAATTTT	#45d511
42[8]	45[21]	TTTTAAATCCAGTCTGTGTTTCATAGATGAACACACCGGGAA	#45d511
36[12]	35[28]	TTTTATAAGTCGGACACCATGGCTATAATG	#45d511
6[8]	9[21]	TTTTCTTATTATCGCAGTCCATTCTGGTTCCAAACATTCCC	#45d511
10[8]	9[8]	TTTTGTAGATGTCCAGTAGTACTTTT	#45d511
10[21]	11[8]	GTTGGCGTACTATCACAAGATTATTTT	#45d511
28[12]	27[12]	TTTTGAAACGCTTCTTCTACAATACGATTTTTT	#45d511
46[8]	45[8]	TTTTATGGTCGATAGTTGCCAGTTTTT	#45d511
44[8]	43[8]	TTTTTAACTGGCTCCACCCCGTTTTT	#45d511
18[8]	21[21]	TTTTAGTGCTCCCGGATTCATTCATTTTCGATTATTCAGGA	#45d511

Electron Micrographs for pScaf-8064-BFJ Brick

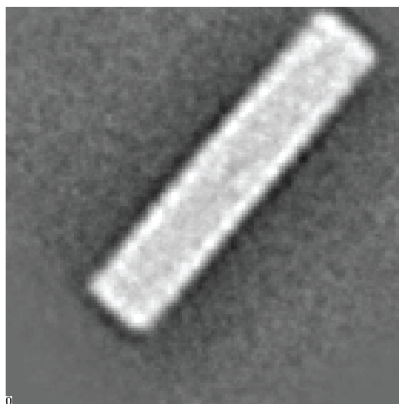
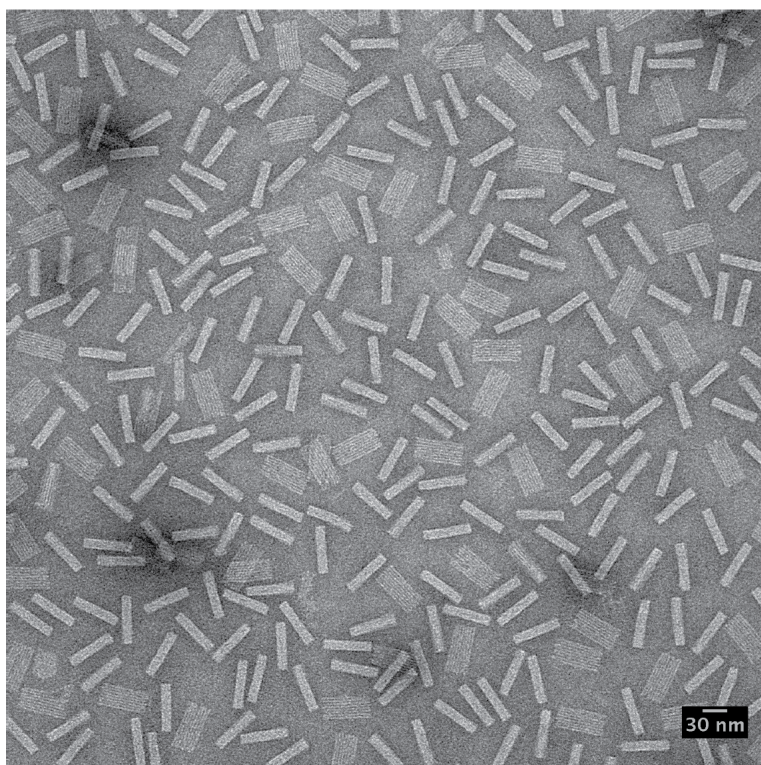
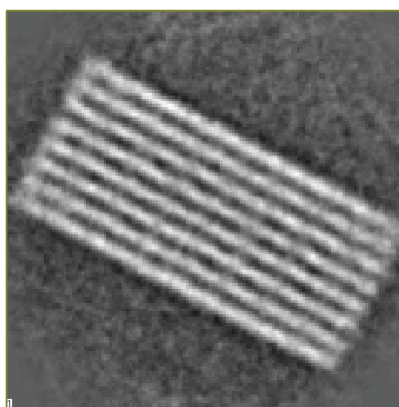
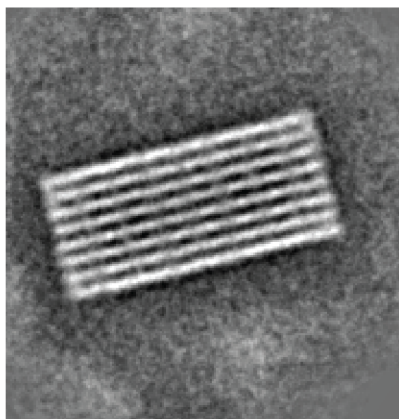
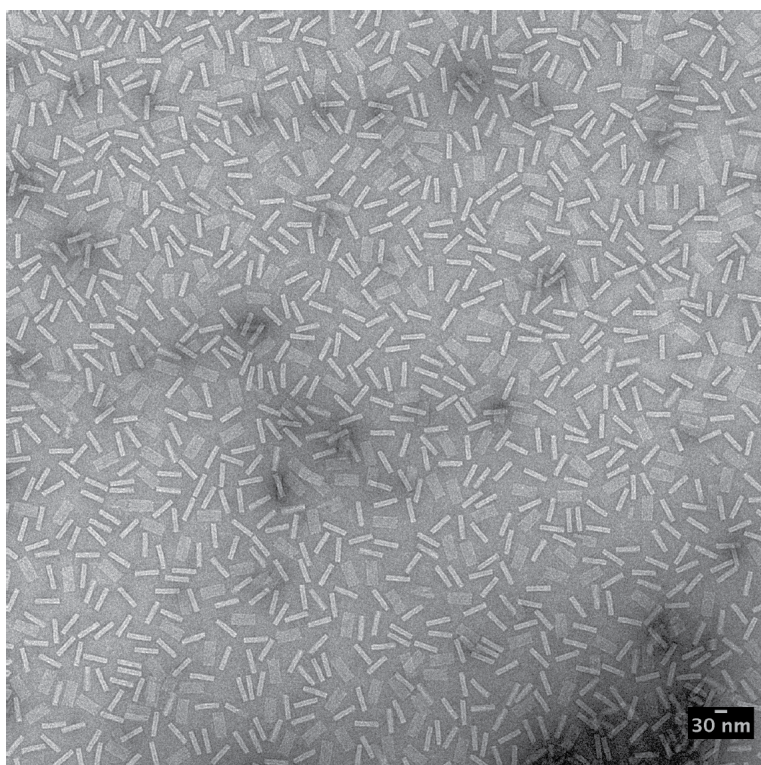


Figure 57: Electron Micrographs and Class Averages for pScaf-8064-BFJ Brick

pScaf-8064-GHI Scaffold Sequence

AATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCTATCTCGGGCTATTC
TTTTGATTTATAAGGGATTTTGCCGATTTTCGGGGTACCTTTTTTAGATCTCTGTTACA
CACTGTTCCGGGATGCTTATCATCTGGCGGGGATTGAGATGCCGGACTTTCATCGT
GAGGATGACTGGTGGCGTAACGGCCAGAATCTCTATCTGGATAATCTGGAGGCGA
CGGGGCTGTATCAGGTGCCGTTGTCAGCGGCACAGCCGGGCGATGTGCTGCTGT
GCTGTTTTGGTTCATCAGTGCCGAATCACGCCGCAATTTACTGCGGCGACGGCGA
GCTGCTGCACCATATTCCTGAACAACACTGAGCAAACGAGAGAGGTACACCGACAAA
TGGCAGCGACGCACACACTCCCTCTGGCGTCACCGGGCATGGCGCGCATCTGCC
TTTACGGGGATTTACAACGATTTGGTCGCCGCATCGACCTTCGTGTGAAAACGGG
GGCTGAAGCCATCCGGGGCACTGGCCACACAGCTCCCGGGCGTTTCGTCAGAACT
GAGCGACGGCTGGTATCAGGTACGGATTGCCGGGCGGGACGTCAGCACGTCCGG
GTTAACGGCGCAGTTACATGAGACTCTGCCTGATGGCGCTGTAATTCATATTGTT
CCAGAGTCGCCGGGGCCAAGTCAGGTGGCGTATTCCAGATTGTCCTGGGGGCTG
CCGCCATTGCCGGATCATTCTTTACCGCCGGAGCCACCCTTGACAGCATGGGGGGC
AGCCATTGGGGCCGGTGGTATGACCGGCATCCTGTTTTCTCTCGGTGCCAGTATG
GTGCTCGGTGGTGTGGCGCAGATGCTGGCACCGAAAGCCAGAACTCCCCGTATA
CAGACAACGGATAACGGTAAGCAGAACACCTATTTCTCCTCACTGGATAACATGGT
TGCCCAAGGGCAATGTTCTGCCTGTTCTGTACGGGGAAATGCGCGTGGGGTCACGC
GTGGTTTTCTCAGGAGATCAGCACGGCAGACGAAGGGGACGGTGGTCAGGTTGTG
GTGATTGGTCGCTGATGCAAAATGTTTTATGTGAAACCGCCTGCGGGCGGTTTTGT
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GAAGCGAAGGACAACCTGAAGTCCACGCAGTTGCTGAGTGTGATCGATGCCATCA
GCGAAGGGCCGATTGAAGGTCCGGTGGATGGCTTAAAAAGCGTGCTGCTGAACA
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TAATATCCGGATGCGCAGGATGACGCCGGACAGCACACAGACCAGCTGCAGAAC
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GAGCCGTAATTATCATCTGCGCGGGCGTATTCTGCAGGTGCCGTCGAACTATAAC
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GCAACAACATGGCCTGGTGTCTGTGGGATATGCTGACCCATCCGCGCTACGGCAT
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TTCTGCTCGGCGATGCGCTGTATGCCGGTATGGAACGGGCAGACGCTGACGTTG
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GCACGGTAACAGCGGCAACCGGCATGACCGTGACGCCTGCCAGCACCTCGGTGG
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ACAAGAGCTTTCGTGCGGTGTCTGCGGATAAAACAAAAGCCACCGTGTGCGTCAG
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AGACGCCATCAGAACCGGCGCGTTTCTGGTGGCGATGTCCCTGTGGCATAACCAT
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CCGGCTGTCTGGTATGTATGAGTTTGTGGTGAATAATGCCCCTGAACAGACAGAG
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GTTTTGCCCTGAAACTGGCGCGTGAGATGGGGCGACCCGACTGGCGTGCCATGC
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Staple Sequences for pScaf-8064-GHI Scaffold

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33[183]	34[183]	TTTTACCAGGCATACGAGCTGCGTGGCCTTTT	#dda539
41[167]	42[183]	AAAGCGGCCCCGGCGTCTCTGTCTGTTTTT	#dda539
11[183]	12[167]	TTTTTGATATTTTCAGGGTCATCCCCGTTA	#dda539
43[183]	44[183]	TTTTTCAGGGGCATTAGGACATCGCCACTTTT	#dda539
5[167]	6[183]	ACGCTGCTTACCAGTTTCCGGCGTACTTTT	#dda539
41[187]	38[174]	TTTTAATAATGGGTACTGTAAGCAGAAAAGCCCTCCGGGCC	#dda539
1[174]	0[187]	AAAAACACAACCTGCCGGAAAATCTTTT	#dda539
17[187]	14[174]	TTTTGACGGGCAATCAGTTCTCACCCAATTTTCATATCCGG	#dda539
29[167]	30[183]	CGCAATCGGCTGATGGACGCAATCTGTTTT	#dda539
21[183]	22[183]	TTTTATACATCCCCTTGCGCGTAGGCTTTTTT	#dda539
27[187]	28[187]	TTTTACGGGAACCGGAACGCTTTTTT	#dda539
23[183]	25[161]	TTTTCGATATCGTCGGTTCGGGTGAAACTGTCTTCGT	#dda539
31[183]	32[183]	TTTTAGCCTCACCGCTAGTTGCCCTGATTTTT	#dda539
7[183]	8[183]	TTTTCTTTGTCTCACGGTTAACGGCAGGTTTT	#dda539
37[174]	36[187]	CCGCATCCTGGCCATCTGCTCGTTTTT	#dda539
47[183]	46[160]	TTTTACTCTCCGATTAACTGGCGGTGACGTGATTCTG	#dda539
3[187]	4[187]	TTTTCTCAATATTCGCTGCCGCTTTT	#dda539
1[187]	2[187]	TTTTCGCCTCATCCCGTATCCGTTTT	#dda539
13[187]	14[187]	TTTTCCGCGCTCTCTGCGCTGATTTT	#dda539
45[183]	46[183]	TTTTCAGAAACGCGCCGGAACATCGCTGTTTT	#dda539
5[187]	2[174]	TTTTACCTTTTCAGGGATGAACAAGCATTGTTTAGTTCACG	#dda539
35[183]	36[167]	TTTTACGTCCGGTGAACGTGTCAGCCTGACT	#dda539
19[183]	20[183]	TTTTTTCGGGTAACGGGGCCACATCGCCTTTT	#dda539
25[174]	24[187]	GGCCAGCCCTGCATCTCTTCGACTTTT	#dda539
39[187]	40[187]	TTTTACCTCCGGCCATCATGAATTTT	#dda539
29[187]	26[174]	TTTTGCAACTGAGCAACATATCATCGCGGATAACTCAGAGG	#dda539

15[187]	16[187]	TTTTCCCACCTCGCGAGCGGATTTT	#dda539
9[183]	10[183]	TTTTCGGTACGCCCCGGTCCGGCATAGCTTTT	#dda539
17[167]	18[183]	ATCTTTCGCCGGTGGTCACCCAGTTTTTTT	#dda539
25[187]	26[187]	TTTTCTGAGCAATTGGAGCCGCTTTT	#dda539
13[174]	12[187]	GTCATTTTTGCATTCTGGCCAGGTTTT	#dda539
		GCATACACGCAGTACATCCGCCGCACCAAGATACCCCGCGCAGATG	
31[76]	25[63]	ATTCCGGCGT	#800000
		TTATAGGCACGGGTATCTCCCAGCGGTCAGTTCGGCGGATTCATTT	
43[62]	38[48]	CACGCGCCG	#800000
		ATGCTGCAGCGCATGCCATCGGCCATACCGATATCCGTCAGGCACA	
2[133]	17[140]	GAG	#800000
		CATGGACTGCTGTCGCGTGACAACTCACCATTACCGGATACATGAC	
44[126]	37[112]	AGTTCCTCCT	#800000
		CGAGAGAGCCCCCAATGAATTACAGCGCTACCAGCCGAAGGTGCA	
14[42]	1[49]	AGTG	#800000
		CACGCTTGCTTACCTGCCCTGCACAGCAGGCATCTAGTGTGTAACAG	
20[70]	4[69]	AGGTCCACT	#800000
30[35]	33[42]	CTCCGTGCCGCCAAGCTGAGCCACGTCGGGCGCT	#800000
38[35]	43[28]	TCATCGTTAGGTAACCTTCTTTCATTAAACCACGC	#800000
7[48]	10[48]	CTGACAAGCAGCTCTGTGTGCTTTCACA	#800000
34[161]	39[161]	GCCTGCACCAGCGCTTCCCGTGCCGCTT	#800000
37[160]	32[160]	AAATCAAACTTCAACCGGTCATCGGAC	#800000
		CTTCATTTTTTTCAGCATGAGAAAACCTCAGCACGCCAGTCGGGATATC	
44[119]	39[105]	CGCAGCAGA	#800000
42[35]	45[42]	CAGCGTGAAGCTGGATCGCCTCGCCGTCTGCGATC	#800000
		ATACATACTGCGGACTTCCACGTTCAAAGTAATTTCTGCAACCGCAG	
43[153]	46[139]	CAGGTCACA	#800000
		GCTTCAGCGCGTAAAATCGGAACCCTAAAATCAAGTGGTGCACGGC	
10[28]	7[35]	ACC	#800000
4[42]	1[42]	TTGTTCTCACCCAAGGGAGCTAGCGGT	#800000
		CGGCACTGACTGGTGCGCATCGTCTGCCGGCATTTCGCCGTCACA	
30[56]	35[56]	GCCCGGCGCG	#800000
46[28]	33[28]	GCCCTCCTGATACCGCCCGCATAGCGGA	#800000
0[42]	10[62]	GCGTGGATCCATCCAGCTCTGGGCGCTGCGATGCG	#800000
19[48]	23[35]	CACTCAGTGTCCAGGGAGTCTGACGTAAACCCTTTGAGGTGG	#800000
		AGGAGAAACGCGCCCCAGTGCTGTTGAAGCCACCGCACGGACACG	
30[140]	35[140]	CATGGCGGCTT	#800000
		GCAGGGCACCGGAATGTTGACCTTGCTATGCTCATATCACGGTCG	
46[105]	37[84]	AGC	#800000
23[111]	13[84]	ATGTTATTCTCCTCACTGCCGGACGGCTGGCTGCAAGGGTGG	#800000
		GGATTTTGAATAAACGCGCCGCTGCGGTTTTTCAGCCTGGCCCCGA	
3[139]	8[153]	CTGATCCCCC	#800000

38[105]	25[112]	GCATTACGCGATCCTGTTCAACGACTGCCCCGCTTTCCTTCACGGTCC	#800000
		GC	
		CTGCGTGCAGCGCCTGCCACAATGCAGCACCCAGATTCAACGGGTA	
23[118]	3[112]	ACG	#800000
27[167]	40[167]	CTCACCGAGGGTTGTACCCCCATATCC	#800000
35[111]	25[84]	CTGTGCAGCCAGCGCCTGTCCGGATCATCTGTTCTGCAGCTG	#800000
		AGCCCCGACGTCTGGAGATCCGGTGAGAGAGGCGACCAGATTCCCT	
29[118]	24[118]	CAACCGGATCG	#800000
		AGAAATCAGCCGTCTCCCCATGCCGTAGCCGGTTTCTCACCTCGGGT	
31[55]	25[42]	AGGGATATT	#800000
11[48]	13[35]	CGTCGCTCAGTTTCGAACAATGGACAAT	#800000
17[160]	14[153]	GTCATGGACTCCTCCTGACATATTTTCCCAGACGT	#800000
38[63]	35[70]	TGGCCACCTGGCTGCGCCGACGCTGAAAAATCAGC	#800000
13[153]	11[154]	GCAGAGACAGCCACAGCGCCTAAAAACA	#800000
		ATGACGACCTGCAGTCAGCGTCTGCCAGATTCAATCCAGTTCGTGAT	
26[77]	39[77]	CAGCATTGT	#800000
42[56]	46[69]	GGCAGATTTCTGCCGCGGATTACTGCTGCGGTTGCTCTGCCC	#800000
		TCCGTGGGCCAGGTCTACGCGCCAGTTTCAGGGCAAATTGCCAATC	
43[104]	45[105]	TGTCTGCCTG	#800000
9[160]	4[167]	CAGAGATTTTCAGCTGGTCAGGACGGTTACCCACC	#800000
		CACCGGTCATGGTGGCTCCACGAACACGCGCCGTTGACCGTGTCAT	
24[126]	8[139]	CCGTTTTATG	#800000
		GGACAAATGCTCACCGGATGTATGGCAGGCACAAACAGCCTTCTCG	
10[126]	26[125]	GAACATCTGC	#800000
38[147]	36[160]	CGGGGATCGACCAGCCAGAAAAATGACG	#800000
35[160]	26[160]	GCAGCATTTTGCCCCACGCATATCCCGG	#800000
		AATGGTGCACCTCACATCCAGCTTTATTACCATCCTCAATGGGCCTG	
11[118]	7[119]	CTGCGGTTA	#800000
		TATTTGCATGATGAGATCAGCCCAGCGAGGGGAAGTCATCTTGCGT	
24[140]	44[146]	CTT	#800000
5[104]	4[104]	TACGGGCAAATCATAAGCATCACTCCAACGTACGCTGCATG	#800000
		ATGGTCATACCACTGACCGACGGTCAGCCCAGCACGTAGTCATCCTT	
47[69]	32[55]	CATTATCCG	#800000
1[153]	0[167]	AAAAAACCAGCGCCGGTGAGATAAACGG	#800000
37[41]	45[28]	CCGGTGCGGCTGGATTCTGCCATCGACG	#800000
		CATCAGCGACCAATAGAACATGTTATCCTCATACCAATGATCCAGAG	
17[76]	12[76]	TCTCATGTA	#800000
44[154]	30[160]	TGGTTATTCTGCATAAAAGTGGCCTTAAACACTCT	#800000
10[154]	2[167]	AACGCGCGACCATCCTCTTTCGGAAGAC	#800000
		GCTCTGGACCTTCCCGAAGGCTTCAGCGCTGCCGCCTGCCGTGCAC	
31[97]	34[111]	CGGAAGGAGT	#800000
1[167]	14[167]	GCGGGGCACGCCCTTCCAAGCATCACGG	#800000

36[140]	40[139]	TTCCGTAATCGCCTCCGTTATAGTAACTTCCGGTGGCATCTCAGGC	#800000
		TCGCACGGT	
16[119]	22[132]	CGCCAGTAAGGGCCCAGCGGTGCTGACTATCCTGATTTGACGTCTC	#800000
		CAGTCGGATC	
9[90]	21[77]	GCAAAGGCAGATGCCCAATGGGTGTTCTTTTAAGCCATCCTCCCCA	#800000
		GCACCGTCTC	
26[84]	14[97]	CCAGAGCGTTTTTCAGCTTCTCCCAGCACCGAGGAGAAATAGCTGC	#800000
		CCCCATGCG	
		GACTCGCGATGCAGAACTGGCGAGCCATCCTTTCGTGTTTACGGAT	
31[139]	25[126]	TCAGCGGCGG	#800000
3[167]	16[167]	AACGCCGGGCGACGGGCAACCCAGTTGT	#800000
		CTGGCCGATGACATACCGTTCCATACCGACCCACATCATCGAGGTGC	
30[77]	42[90]	CAGTCCAGT	#800000
44[28]	31[28]	GTGATCATGACATAGTCAACGACGCCTT	#800000
21[160]	16[160]	ACCGCTTGAATACATCAGCACCGGTT	#800000
		CAGCCATTTTTTCGCATCACTTATACTGGCAGCAGTGCGACGCTTCA	
37[132]	21[133]	TGACCGCTG	#800000
		ATACGATCTGCCCCGGATGCTCGCCCCATCTCGCATCTTCAGGGTG	
32[98]	40[97]	GTCCCGGAG	#800000
		CGCCCCGAATCGTTGGAGCGGGGAAAGCCGGCGAACCGTCTATTC	
11[76]	8[90]	GGCACTGATGT	#800000
20[28]	7[28]	GGTATTCTCGGTGCGTGACCCTGATACA	#800000
13[167]	26[167]	CGGCTGCGCGTTCAATAGGACCGTAAAT	#800000
		TGTGACGCTTTCCTCCCTTTTTACGACGGGAATACCGGCACCAGCCC	
7[160]	2[153]	AG	#800000
		CCCACCTTTCACCGAGATCCGTGATTCTCCTGAACGACCAGCTTAGT	
39[97]	27[84]	TCGACGGCA	#800000
		CAAATCCTCACATCGATGATAACGGTCGGCCGAGCGGGTCACTAGG	
21[62]	41[56]	ACT	#800000
		ATTACGGAAACGTTTCGATCAATTCCTCACCTGACCACCGTCATTTCC	
27[55]	15[42]	CGGGAGTT	#800000
15[167]	28[167]	TGTCCGGCGACGATGCAGAGGCCCGGCT	#800000
		TTGTATCTGAACCACACCCAGGTCTGTGCCGTTGTTCTGGGGGAGCA	
23[90]	35[77]	GTTCTGTTTT	#800000
3[34]	6[48]	TTTTTTGGATAGGGTTGAGTGATCGGCACGTTACGCCACCAG	#800000
		CCGTTTTGAGGGGTTCGTCCCGCGGATGGTGGCCCCGCGAAAGGA	
20[133]	4[132]	CAACAGTTGCT	#800000
		CCAGACAGCCGGTAACTTTCGATGACATCCCGGCAGGCTGATTCTG	
43[146]	38[132]	TCATGTCCGC	#800000
18[133]	16[132]	GAATCATAAACAATGGCCCCGTCTTCTT	#800000
		ATACGGCGGCACCATTTCAGCTGTACGGACACGCACATAAAAATGT	
8[98]	16[97]	TATCCAGTTC	#800000

		CAGTCCCGCCTGATGTTAAACGAAAAACAAGCATGGCTCACCGTCG	
27[125]	43[126]	AACCACCACG	#800000
44[161]	42[160]	GCCACAGTTCACCACAAACTCCTGCGGG	#800000
		GTCCTGAGTGCCACCCCGGCACATCGAACAGGTTAACCTGACCAGG	
15[132]	1[133]	GACCGGTGTC	#800000
		CCTTCTGTTACCGGTGGTGCCGCTGTTACCGTGCACCGCCTGTCCT	
36[56]	40[55]	CACGCGTAA	#800000
34[70]	21[70]	CTCCCAGGTGCTGTTCAAGTGTGGAGCCG	#800000
		ATGATTACGCCAGTTCTGCCACGTGTCACATCAGTGGTTCATCATCG	
17[118]	12[118]	GCGCGGGCA	#800000
22[35]	27[35]	GTTTGCAGCTCCTGCCGTGTTGCTGGCT	#800000
29[160]	26[153]	TGCTCCGCCGACACGCCGCCTTCGGCGATTGACAT	#800000
32[35]	41[35]	TATAGGTACATCCCTCTGAAACCGGGCG	#800000
		GTGGTTTTGCCCTTCTGTCTGGTGCGGGCGGCAAAACAGGGAGG	
24[42]	8[55]	GAGGCCGTCG	#800000
		AATTTTCGCTGCCGGCTGACGTTCCCATGTACCGGGTCTCCAGTGGA	
9[111]	1[84]	AGGGAAGAA	#800000
		CCGGCAGAGTTCTTATGCAGCATCAAACCGCCGTCCCACCTCCACGC	
15[125]	31[126]	CGTGGTCTG	#800000
		GATGGCCTTGACGGGCGCTAGAATCCGGCACCTGAGCAGGCAAGC	
3[69]	1[77]	GAAA	#800000
		GGCAACCCATTTTGCGCCCGCAGGCGGTGTACCGGACCTTCATGCC	
16[77]	29[77]	GCGTTATCCA	#800000
41[160]	39[147]	TGCCAGTCGGCATAAGCCCGGCAGCACA	#800000
37[167]	45[154]	CAGACTCGTTTTCAGGTTCTGATGGCGT	#800000
		TCCGCCAACCAGTTCGGCTGACCACGTACGCTGTGCTCGTTCGTTAT	
9[146]	29[140]	GG	#800000
46[42]	47[28]	AGGCCACACGGTGGCTTTTGTTTTATCC	#800000
		ACCGGTTACATCCGGTGGCCAAGGACCAGCGCCATTGCGGCCGTC	
37[139]	19[133]	CGC	#800000
		CACGGAACACATTACGGCAGGCGTCACGGTCATGCACCCAGCAGG	
44[77]	40[76]	ATTTCTTATAA	#800000
		GACGCCAATGCCGGGTGTCTGTACTGTGATGGCACCGTCCCAGAC	
9[62]	29[56]	GTT	#800000
		GCCCGGTGCGACCACAATCCGTACCTGACATCAGGCGGCAATGGTG	
9[69]	22[62]	ATC	#800000
		GTCATCCGGTGATCCAGTCCCGGAATTTTCTGGTCCATAGGTGCAGC	
38[112]	18[118]	AT	#800000
		TCAGGGCATTAAAGAACGTGGCCGGAACCAATCCCCGCCAGACCGA	
3[76]	7[77]	ACCAAAACAG	#800000
		TGACTCCGCCTGTTTTAACGGGCGCGGTTGATCGCTGTCAGCCACCTT	
45[125]	30[118]	GT	#800000

3[132]	10[146]	CACCACATCAGTTGATGCTTCCCAGCTCTTCGCCCCGTGTATTGTCGC	#800000
		CCGGGCTGG	
		ATGGTCATTGCTGCAAAGGGCGAAAAACGTGGCGAGAAATCAGT	
9[97]	3[98]	ATGTCCAGCCA	#800000
		CACGGAGATGGCCACTGGCCTTTACCCAGCTTGATACATGCCGTTTT	
16[112]	2[111]	TCTTCATCG	#800000
7[97]	8[111]	GGCACCGTGCCCCCGGTTAACTTTGATTTTGCGGCTGAGGTT	#800000
		CCTGAGTTTAACTCATCAGGGTCAGCCAGCGCAGAGTTGTCCCACG	
19[97]	22[111]	ACGAACGGAA	#800000
		ACCGGCCATTTTTCCCGCGCAGCGGATTTTTGACCAAGCAACCAAGA	
4[112]	9[126]	CCGCGAAAC	#800000
		GGCGACCGTGCCGTTCCGGAACTGCCCGATAGCTGTCGTCACGCT	
43[90]	39[63]	CCCGGTACAG	#800000
		TGCCGGACCCACCACCCTGTTCCCGACGGAAATCCCGCTGTTACCG	
32[126]	46[125]	GA	#800000
33[104]	44[104]	CAAGCCCGTCATCACTGAAATCCTGCTC	#800000
		GTTCTGCCCTTAAGCACGGCAATACGTGACCGCTGCACACCGATCCA	
25[153]	14[160]	GA	#800000
		GGAAAACTTCGATATGCAGCTGCACGGACGCCGCACTCACCGTGAA	
0[84]	15[98]	GTGTCAAGCT	#800000
16[35]	18[48]	CACGCGCCCTTCGTGCTTCCTTTACCC	#800000
24[35]	33[35]	CTGCGAGAAACGGGATGGCCTGAAGCTG	#800000
10[35]	15[35]	CCCCCGTGTCGCTGCTGGCACCTGGCTT	#800000
32[28]	19[28]	CTGCGGTGCCGAACGTTGTTGGTGGACT	#800000
		TACGGCTCCGCCTGCCAGGTATCGCTGTTCCAGTGTGAGTCCCCGAT	
1[111]	14[125]	CTTTCACCA	#800000
		TCCGTGCCGCCGGAAAGGCTTTAAGCCGCTGCCCCCTCGGCAGTCCG	
23[132]	39[119]	TCAGCCTCGC	#800000
		ACGACCAGTACATGGCGAAGCGTGTGCCTTCAACACGCATCCCACT	
37[48]	21[49]	GTTCTCCGGC	#800000
24[84]	34[104]	CCACCGTTAGGCCCATATCCATGCGGATCCCGCCG	#800000
34[42]	36[41]	GCGTATCTGCCCCCGGCTGGTTCGCCCCG	#800000
26[112]	15[112]	GCAGGGTTTAGAGCGGCAGGATTTTCATC	#800000
		CCATCGTCCGCGTGCCGTGGTGTCTGTGGTCAGAGCGCCATTCTG	
20[98]	28[97]	CGGGTTATCT	#800000
		CGTTAACCCCGCGTATCGTTTCCCGCGTTTGAAGGGGTATCCATCCG	
12[84]	27[98]	GCATAGACT	#800000
		TCTGCGTTTCGGCTCAGCGACCTTCTGAATGCGCTCCGTTTCATCAG	
36[84]	44[97]	GGAGCACTT	#800000
		ATGTCTTTTTCCGTGCAGGCGCATATTTGGTAAAGACCGGCCGCGCC	
24[63]	8[76]	ATGGCGTGA	#800000
26[140]	42[153]	CTTCGGCAACGTACAGCGTTTGCTGAGTGTACGTCCTCCGTGCGCA	#800000

		GAAACAGGCT	
36[77]	46[76]	TCCACGGGTCAGCGACCGAGGTGCTGAGGCAGCAACGCCGTTTAC	#800000
		GGTGTTCACC	
10[77]	22[90]	GTAAATCCCCGTGTACGTGCTGACGTCCACTGCGCCTCCGGCCACTT	#800000
		CCGTACCTT	
9[153]	21[140]	GACAACTAGCCTTCCCACGTATCACCGCTCGAAATCACGAAAATGAT	#800000
		ATGTCTTTC	
3[55]	8[69]	TGAACCAAGTTTGAACAAGAATCTAAACACGATGAAAGTCCGCAC	#800000
20[35]	29[35]	ATCAAATTGC	#800000
		CCCTCAGCAACTGCCTGTATGCGCGGAT	#800000
24[56]	44[62]	AATGGTGCATCCTGAGCTCTGATGGCGGTAGCCGCGCATAGCTACC	#800000
		GAT	
37[76]	18[76]	TCCGGGTCATACTGACCTCAAACGAACGAATACGCGCTGTATATCG	#800000
		GCCACAAAAC	
2[49]	17[56]	CCCCGATTTAGAGCCACTACGCCGAGTGCCCGGCAACAGGCCACC	#800000
27[160]	18[160]	ACA	#800000
		GAAACAAGCAATGCCGTTAATCCGGATC	#800000
18[140]	23[140]	GGTGAACCTACGCGGCCTTCGCTGTCCGTGCCAGTCACCACGACTT	#800000
34[28]	21[28]	GTCACGCTT	#800000
		ACGGGCACGCGGCGACCAAGTGTCTACCA	#800000
20[49]	5[42]	CACCGGCGTATACGCGTACAGTGTCCGTCATCCTAAAGGTACCCC	#800000
22[28]	8[34]	GAA	#800000
		GGTCGATCTGGAATCACCATAACATTTGAGGAATA	#800000
18[56]	23[56]	ACGCTCCATAAATGCTTCGCTTCAGCAGGAGGATTGGCGTGTGACT	#800000
		TCCGACGGAT	
2[12]	1[12]	TTTTCGTAAAGCACTACCACCACACCCGTTTT	#45d511
40[12]	39[12]	TTTTCAGACGCGACCAAGTCAGATCGGCTTTT	#45d511
22[21]	23[8]	CGCAGACCAGTGCCTGTACACCGTTTT	#45d511
34[21]	35[8]	GACCGTAAAGGCATCCATCTTCGTTTT	#45d511
12[12]	11[28]	TTTTTTGGCCCCGGCGACTCTGGTGACGAA	#45d511
0[12]	1[35]	TTTTCCGCGCTTAATGCGCCGCTACAGGGCCACGCTG	#45d511
24[12]	23[28]	TTTTTACCACCGAGGCCAGATATTTCCAC	#45d511
30[8]	33[21]	TTTTTACGCATTACAGGTGAACGCTGTCACATTAACGGCGC	#45d511
6[28]	5[12]	TTCTGGCAAATCCCTTATAAATCAAATTTT	#45d511
34[8]	33[8]	TTTTTAACATTACGCCATCATCTTTT	#45d511
20[8]	19[8]	TTTTTGACACCGGGTCCTTCGTTTTT	#45d511
30[28]	29[12]	TGCGCGGGGGTCAGCATATCCCACAGTTTTT	#45d511
42[28]	41[12]	TCCACGCTCAGGTCTTTAACTTTTGCTTTT	#45d511
22[8]	21[8]	TTTTAAGGTAAAGACACCACCGTTTTT	#45d511
8[8]	7[8]	TTTTGTTTGCTCACGCCTCCAGTTTTT	#45d511
26[12]	25[12]	TTTTACCTGCACGCCGGCAGGTTACCCATTTT	#45d511
14[12]	13[12]	TTTTCACACCACCGAGACGCCACCTGACTTTT	#45d511

4[12]	3[12]	TTTTAGAATAGCCCCGAGGGTCGAGGTGCTTTT	#45d511
16[12]	15[12]	TTTTGAGAAACACGCCAGCATCTGCGCTTTT	#45d511
32[8]	31[8]	TTTTCGGCATCACGTGGTCAGGTTTT	#45d511
18[28]	17[12]	CCCTTACTCTGCCGTGCTGATCTCCTTTTT	#45d511
38[12]	37[12]	TTTTTGAAGTCCACAAGGCATAGTCATCTTTT	#45d511
46[21]	47[8]	CGGTTACGCAGACACCGCACGAATTTT	#45d511
42[8]	45[21]	TTTTCCCTGCTCTCCGGGCACAGCAGCCGTGCGGGACGTCC	#45d511
36[12]	35[28]	TTTTATCGCAGATTTCAATAACAACAGCCA	#45d511
6[8]	9[21]	TTTTATTATCCAGATAGAGAGCCCCGTGTTGTTCTCGGTGT	#45d511
10[8]	9[8]	TTTTGGCCAGTGACCTCTCTCTTTT	#45d511
10[21]	11[8]	CCGGATGACGCCGGGAGCTGTGTTTTT	#45d511
28[12]	27[12]	TTTTACACCAGGCCATTGCTCCGAGTCCTTTT	#45d511
46[8]	45[8]	TTTTAGCTCTTGTCACATTGGTTTTT	#45d511
44[8]	43[8]	TTTTGACTTTCACGCCTGCTGCTTTT	#45d511
18[8]	21[21]	TTTTTTCGCGCGGGGTATGCTCAGGTTATATGTTGCCCGGA	#45d511

Electron Micrographs for pScaf-8064-GHI Brick

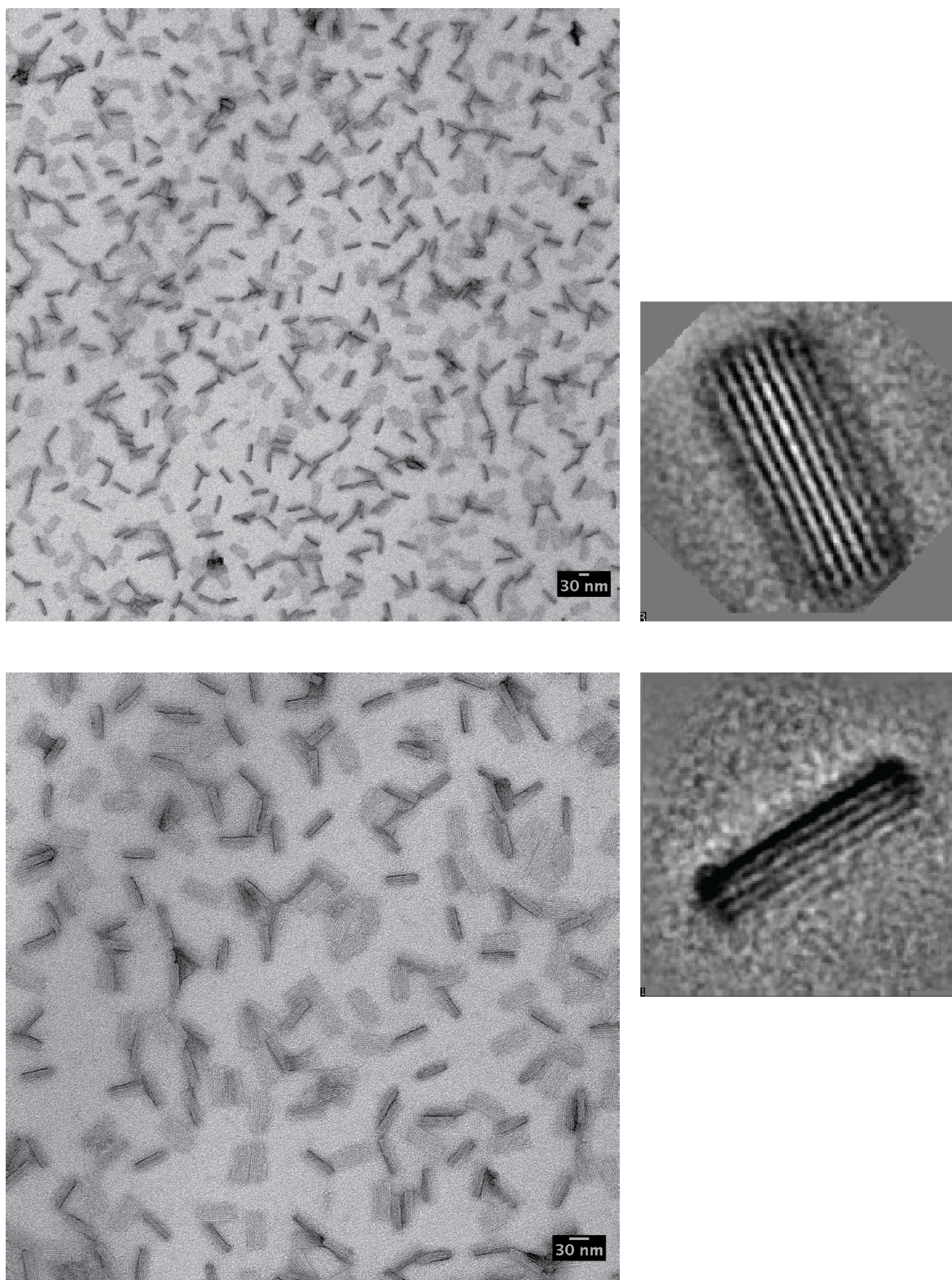


Figure 58: Electron Micrographs and Class Averages for pScaf-8064-GHI Brick

pScaf-10080 Brick

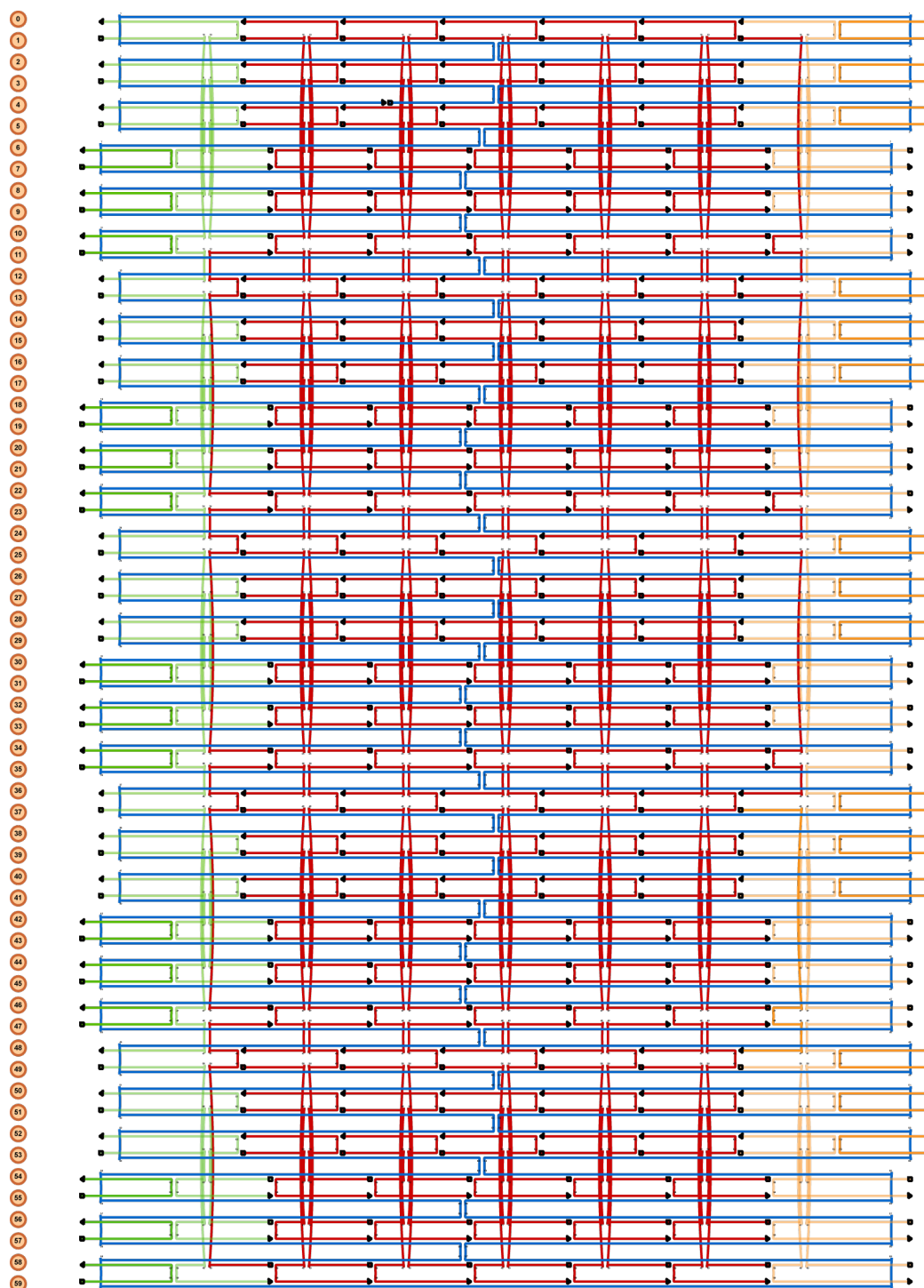


Figure 59: cadnano Design for pScaf-10080 Brick

pScaf-10080-ACKN Scaffold Sequence

AATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGCTATTC
TTTTGATTTATAAGGGATTTTGCCGATTTTCGGGGTACCTTTTTTAGATCTAATGCGG
CATACCTCAGTGGCGTGGAGTGCAGGTATACAGATTAATCCGGCAGCGTCCGTCG
TTGTTGATATTGCTTATGAAGGCTCCGGCAGTGGCGACTGGCGTACTGACGGATTC
ATCGTTGGGGTTCGGTTATAAATTCTGATTAGCCAGGTAACACAGTGTATGACAGC
CCGCCGGAACCGGTGGGCTTTTTTGTGGGGTGAATATGGCAGTAAAGATTTTCAGG
AGTCCTGAAAGACGGCACAGGAAAACCGGTACAGAACTGCACCATTCAGCTGAAA
GCCAGACGTAACAGCACACCGGTGGTGGTGAACACGGTGGGCTCAGAGAATCCG
GATGAAGCCGGGCGTTACAGCATGGATGTGGAGTACGGTCAGTACAGTGTATCC
TGCAGGTTGACGGTTTTCCACCATCGCACGCCGGGACCATCACCGTGTATGAAGA
TTCACAACCGGGGACGCTGAATGATTTTCTCTGTGCCATGACGGAGGATGATGCC
CGGCCGGAGGTGCTGCGTCGTCTTGAAGTATGGTGGAGAGGTGGCGCGTAAC
GCGTCCGTGGTGGCACAGAGTACGGCAGACGCGAAGAAATCAGCCGGCGATGCC
AGTGCATCAGCTGCTCAGGTCGCGGCCCTTGTGACTGATGCAACTGACTCAGCAC
GCGCCGCCAGCACGTCCGCCGGACAGGCTGCATCGTCAGCTCAGGAAGCGTCCT
CCGGCGCAGAAGCGGCATCAGCAAAGGCCACTGAAGCGGAAAAAAGTGCCGCAG
CCGCAGAGTCCTCAAAAAACGCGGCCGCCACCAAGTGCCGGTGCGGCGAAAAACGT
CAGAAACGAATGCTGCAGCGTCACAACAATCAGCCGCCACGTCTGCCTCCACCGC
GGCCACGAAAGCGTCAGAGGCCGCCACTTCAGCACGAGATGCGGTGGCCTCAAA
AGAGGCAGCAAAATCATCAGAAACGAACGCATCATCAAGTGCCGGTCGTGCAGCT
TCCTCGGCAACGGCGGCAGAAAATTCTGCCAGGGCGGCAAAAACGTCCGAGACG
AATGCCAGGTCATCTGAAACAGCAGCGGAACGGAGCGCCTCTGCCGCGGCAGAC
GCAAAAACAGCGGCGGCGGGGAGTGCGTCAACGGCATCCACGAAGGCGACAGAG
GCTGCGGGGAAGTGCGGTATCAGCATCGCAGAGCAAAAGTGCGGCAGAAGCGGCG
GCAATACGTGCAAAAAATTTCGGCAAAACGTGCAGAAGATATAGCTTCAGCTGTGCG
GCTTGAGGATGCGGACACAACGAGAAAGGGGATAGTGACAGCTCAGCAGTGCAAC
CAACAGCACGTCTGAAACGCTTGCTGCAACGCCAAAGGCGGTAAAGGTGGTAATG
GATGAAACGAACAGAAAAGCCCACTGGACAGTCCGGCACTGACCGGAACGCCAAC
AGCACCAACCGCGCTCAGGGGAACAAACAATACCCAGATTGCGAACACCGCTTTT
GTACTGGCCGCGATTGCAGATGTTATCGACGCGTCACCTGACGCACTGAATACGC
TGAATGAACTGGCCGCAGCGCTCGGGAATGATCCAGATTTTGCTACCACCATGAC
TAACGCGCTTGCGGGTAAACAACCGAAGAATGCGACACTGACGGCGCTGGCAGG
GCTTTCCACGGCGAAAAATAAATTACCGTATTTTTCGGGAAATGATGCCGCCAGCC
TGAATGAACTGACTCAGGTTGGCAGGGATATTCTGGCAAAAAATTCCGTTGCAGAT
GTTCTTGAATACCTTGGGGCCGGTGAGAATTCGGCCTTTCCGGCAGGTGCGCCGA
TCCCGTGGCCATCAGATATCGTTCCGTCTGGCTACGTCCTGATGCAGGGGCAGGC
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CGGGTCTTGTTTCGATTAACTGCCGCGGATCCACGCGCCCTGTAGCGGCGCATTA
GCGCGGCGGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCC
TAGCGCCCGCTCCTTTGCTTTCTTCCCTTCTTCTCGCCACGTTGCGCCGGCTTT
CCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACG
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Staple Sequences for pScaf-10080-ACKN Scaffold

Start	End	Sequence	Color
32[21]	35[8]	TGAGCATGGCCCCTTAAATAAGGCTGCCCTGACTGTCTTTT	#4acc2a
48[12]	47[28]	TTTTTTTTAAGTCCTTTTATTAATTCGCAC	#4acc2a
42[28]	41[8]	CTGCTGAGAGCAATTGAGGCAGCGTTTTTT	#4acc2a
16[12]	15[12]	TTTTGCTTTTCGTGGCCCCTTTGCTGATGTTTT	#4acc2a
58[21]	59[8]	ATAAATGAATACACACGCGCTTCTTTT	#4acc2a
34[8]	33[8]	TTTTCGTTTTTCGAGTTGAACCGTTTT	#4acc2a
24[12]	23[28]	TTTTTGACGCGTCGATAACATCTTTCGTTT	#4acc2a
28[12]	27[12]	TTTTGGAACGATATCTCATTTTCCGCAATTTT	#4acc2a
30[28]	29[12]	CAATACACATCAGGACGTAGCCAGACTTTT	#4acc2a
30[8]	31[21]	TTTTCATCCTGTTTCCTGAGAGTGTGCG	#4acc2a
40[8]	39[8]	TTTTGGTGAAGCACGAGCAATGCCCTCGTTTT	#4acc2a
58[8]	57[8]	TTTTCAGCGGAGTAAATGATGGTTTT	#4acc2a
18[8]	21[21]	TTTTGGCATTTCGTCTCGGAGCTGTTGCCGCACTTTTGCA	#4acc2a
54[8]	57[21]	TTTTGTGTAGAAAATTAACGTTTCGCATCCTTTGAACGAAG	#4acc2a
26[12]	25[12]	TTTTAATACGGTAATTCAGTGCGTCAGGTTTT	#4acc2a
44[8]	43[8]	TTTTATTGGTTGCAGCGACAAATTTT	#4acc2a
		TTTTACGGTGATGGTGTGTCCGTTGACTGTGCAATACGCGTATTTATTT	
36[8]	26[34]	TT	#4acc2a
22[21]	23[8]	TGGCGTTCATCCATTACCACCTTTTTT	#4acc2a
52[12]	51[12]	TTTTTTAAAACGATGCTCAAAGGTATAGTTTT	#4acc2a
10[8]	9[8]	TTTTATGGTGGAAGGTGCAGTTTTTT	#4acc2a
38[8]	37[8]	TTTTTAATTAAGTGAACCCGCAGCGTTATTTT	#4acc2a
46[21]	47[8]	GCAACCTTTGATCGAATATTTCTTTTT	#4acc2a
8[8]	7[8]	TTTTCTGTACCGGGAATCCGTCTTTT	#4acc2a
8[21]	11[8]	TTTTCTGCTGAATAACCGTCATGGTCCCGGCGTGCGTTTT	#4acc2a
32[8]	31[8]	TTTTCTCAGACTGACTTAATTCTTTT	#4acc2a
22[8]	21[8]	TTTTAACCGCCTTCGTATTGCCTTTT	#4acc2a
56[8]	55[8]	TTTTGTGATGGTTGAATCGTATTTTT	#4acc2a
20[8]	19[8]	TTTTGCCGCTTCTCAGATGACCTTTT	#4acc2a
6[8]	7[21]	TTTTAGTACGCCAGTCGCCACAACGAT	#4acc2a
50[12]	49[12]	TTTTTAATATCTTTTACATTTACAACCTTTTT	#4acc2a
12[12]	11[28]	TTTTCTGTGCCACCACGGACGCGCACGGTG	#4acc2a
18[28]	17[12]	CGTTTTTTTGAAGTGGCGGCCTCTGACTTTT	#4acc2a
2[12]	1[12]	TTTTCGTAAAGCACTACCACCACACCCGTTTT	#4acc2a
14[12]	13[12]	TTTTCCGCTTCTGCGCGTCTGCCGTACTTTTT	#4acc2a
46[8]	45[8]	TTTTTTAAAAATGCTTAATAGTTTTT	#4acc2a
54[28]	53[12]	AAACCCTCGGGAGTGTCCGGGAATAATTTT	#4acc2a
42[8]	45[21]	TTTTATGAATAAAGAACAATCGCATGGGTAACAAGTTTTCA	#4acc2a

6[28]	5[12]	CTGCCGGAAATCCCTTATAAATCAAATTTT	#4acc2a
4[12]	3[12]	TTTTAGAATAGCCCGAGGGTCGAGGTGCTTTT	#4acc2a
0[12]	1[35]	TTTTCCGCGCTTAATGCGCCGCTACAGGGCCACGCTG	#4acc2a
15[167]	28[167]	ACTCATAAGAGATTTTGGTGAAACCCAT	#800000
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44[77]	56[90]	ATAAGGT	#800000
27[160]	18[160]	GGAGCCAGAATCCCTTTATGCACTTGCA	#800000
		AATGGGTTTCAGGATGCAGGTGTTGTGAATTCGGGACGGTTTTACATTC	
0[126]	15[140]	AAATTAAA	#800000
		CACGAAGGAGTTAGAGAGAACAACCTTGTGCAAGCAGCACCCAGGTTG	
57[146]	42[139]	TC	#800000
2[147]	0[167]	TTTCTTTATAACGCAGTATCTGCATATGATGTCTG	#800000
57[90]	51[70]	CCCATTTTCTGCGCCGCCACAGAGCACAAAAGATT	#800000
56[28]	43[28]	GTGCTACTGAATTAACACAGGGAGCAAG	#800000
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44[63]	58[62]	TTACGAA	#800000
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43[97]	46[111]	GGAGGGCC	#800000
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52[42]	46[55]	ATGTGAT	#800000
		GGGCCAGGGCCAAGGCCCATTTGTTTGAAAGCATCTGTGTTTCCCGACT	
41[139]	36[139]	CCCAGCAG	#800000
23[139]	3[133]	CTCTCAGTCGAGATAAGCTATTTACGTTTTCTTGCTGATATGTAGAGTC	#800000
54[35]	57[42]	AAACAATGAGTTGATTATTCGGTTTTGTTTTCTTC	#800000
57[125]	42[118]	TAAAGCTGAAAATGGCCCAGCAACTCAACCCGTTGCCTCCTCGTGTGTTG	#800000
		CGAATTCTATCCCTAGTGTCGTGGATCATCGCAATCTGGGTATGGCGTT	
28[63]	22[76]	TGCACTA	#800000
44[35]	53[35]	TCCTGCTAGATGAAGTTTAGCTTCTCTG	#800000
15[160]	6[160]	AATTACAGCCAAGTGGGTTTTATCCTCG	#800000
46[42]	48[41]	ACCTTCCACAGTCTAAATCTTCACGGTG	#800000
		TCCCGCATTGCGTCGAAAGGCAGGCTGACCCTTGATTGTCCAGTTTTCG	
20[56]	32[69]	TCGAAAC	#800000
		TATCTGCAAATGCCCTGATGCAGTCAGTCTGATCACTATGCAAAAACAA	
55[132]	58[118]	CAAAGCA	#800000
		CCCAACAGCAAAACATTGGAGCATGAGAAACCCCGATGGATGTATTC	
20[98]	28[97]	AAGAACAG	#800000
58[42]	59[28]	ACCGAGCACTCATGCAATTATTGTGAGC	#800000
		TCATCCAACGATTTGGAAGAACAGAAGAAGCATCTTTATAAGTGTTC	
31[97]	34[111]	ACACAGA	#800000
		ACTGTTCTAAACTTGTGCACCGGCACTCGGGCATGTAAATGCACGAGG	
33[69]	45[56]	GTGTTTTG	#800000
47[111]	37[84]	TGAAGCAATTCTTCAACTCTATCTCTACCTGAATGCCATT	#800000

51[97]	39[84]	GAACGTTATGATTTGTATTGCTGAAATCTCTACGAAATCACCAAAGTCT GTGACAG	#800000
27[125]	43[126]	TGGTGGCTCTCTCTCTGAATCAAATCGACTGACTTAAGAACTTGCACCT AACTTTA	#800000
49[118]	30[118]	TGCACGCTTCCCAATTCCTCAAGAATCTTCTCCACACACTCGAGACTAAC TAAGCT	#800000
38[84]	26[97]	AGATCCCTCATTAACCTCCAGATCTCTGACCATCTGCAACGGATTACCCG CAAGCGA	#800000
41[55]	36[55]	AGGTGTCTCTGGAAATTATTCTAAAACTCTGTTGTTGACGGAATACTG AAAGAAT	#800000
46[28]	33[28]	GAGCCATCGCGTTATTTACAACACCCGC AGCTATTGGTTACACCGCTTTAGCAGGACTGAGGTCTGCAAACCAAGG	#800000
47[118]	28[118]	CCATCGAC CTTCGGTCCGCTCTGCCAAGAAATCTTGACCTTGGCTGATCAACCTGAT	#800000
49[139]	30[139]	AGGATAA CCCCTAACCTTTATTTAAATCCGATGGGAATGCTGGTATTATCTTACTGA	#800000
12[126]	3[140]	ATGAGT CTGTTTTAACAAGCATTAAATGAGCAGTGCAGATAGTGGGTTTAACCTAT	#800000
58[77]	50[76]	GCAAGAT	#800000
50[35]	55[28]	GGATATTATAGTAATGCTGCCCCGATGCTTTTTTGAA GCTATTACGGGGTTGGAGGTCCGTCGTCTTGAAACTGCGGAGCAGTGA	#800000
0[105]	15[119]	TTCTGCCT GCGGTTGGTGCTGTTTGTGTTGGTGGTAGTGGTTGCTGTTCAACAATACC	#800000
23[90]	35[77]	AACTGTA	#800000
25[153]	23[154]	TTTGCCCAAGTCTGAAGGTCTACTTGTC CCCTGGCCCCTGATCATCCAGAATTGTTGCAAAGGAGGACGATATCA	#800000
33[132]	45[119]	TAACCTACA	#800000
8[28]	4[41]	GTGCCGTTTTTTTTGGATAGGGTTGAGTG TTTGTCAAACGCCTCGGCGCAGTTCAGTGAAAGCCGGCCAGTCGGCCA	#800000
29[55]	24[55]	GTACAAAA	#800000
37[160]	32[160]	TCGCTCTTCCAGCATCCAAGACTCGCTG	#800000
17[160]	14[153]	AGGCCATATAACAAGGCGTCATGAGCAATGGCTTG	#800000
7[118]	5[105]	AGGAGAATGTACAACAAAATTATTATCA	#800000
30[35]	33[42]	GCACGACCGCTGGCCCGGATGTTGTCGTGTGTGG	#800000
25[41]	39[35]	TCATTACCTGTGTGCATGAGTATCGTCGCAACTC	#800000
59[111]	49[84]	CTGGAAGGAACCCAGAAGTATTGGTCGTGATGTGATTTCTCT GACTGAGAGGCCAGAGGAGTTAGAGATTGCAAGACCCAGCTCGAGTC	#800000
29[139]	24[139]	AGAGCAGCT GGAAACAAGTGGTCTTCTGACGCTCAGGAAGATCCAAGTGGCCTGGA	#800000
20[140]	32[153]	GTCTGCCA GCCAACC GCCGTAATCCCCAAGTTGACTGAACATATCGTGTAATAAAT	#800000
27[62]	43[63]	AAAAGATT	#800000

35[118]	16[118]	CCAGGAGACAGAGGACTGAGTGAAGTGATGCGCGCATATTGAGATGC	#800000
		TCGCAATGC	
22[63]	38[62]	GCTGAGCCCGTCAAGTCCCGGGCGGTGTTTCCCGACTGTGGAATCCTT	#800000
		AGAACGCG	
12[105]	3[119]	GTCATGAGCGCCGGGGCCTTTAGAAGCCTTCACAGCGTACTCAAACAT	#800000
20[35]	29[35]	CAAAGATT	#800000
		CTGCGATCGCTCCGACGGGATGCCCCTG	#800000
7[62]	21[63]	GTGTTACGTTGTGACCTCTTTGCCGCCGTTGCCGAGCTGTTTGCCTCTG	#800000
		CGCGACA	
20[119]	32[132]	TGCTCTCGATATCTTATAACGCTCCTGGGCTCCAACAGGAGGCGAGTCT	#800000
		CGACCAG	
45[76]	29[63]	GTCTGACGATGTATACGCAGTATAACTGCGATGTGGCCTCGCAGCAAG	#800000
3[41]	17[35]	TTTTGGGT	#800000
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23[55]	3[49]	ACTGTCCCTGTTGGCACTGGCACGATGCACCGTGGGAAATCTTGAACC	#800000
53[160]	50[153]	A	#800000
		GGCGCTTTACCTATCTGAAAGCGATCACCAGGCTA	#800000
10[63]	26[62]	CATCCATAAATCATTAGCGTATCAGTTGCTGATGTTGCACTGCTGAAG	#800000
13[41]	26[41]	CGCCGTC	#800000
		GCTGATTGCGTTTCCGTTTTGCGCCGTG	#800000
10[126]	26[125]	AGTTCGTGACCAGTAACAGGTGATTTACAACATTGGAATGCTTATTTTA	#800000
58[154]	44[160]	TCAACCA	#800000
		CGGCGTGAGAAGACGATCCCCTGGCCTGTCTCTAA	#800000
19[118]	13[105]	TTATGTCGAAAATTAATAATCAGAGGTAAAATTGCGCACTTGAACAGCG	#800000
		ATTCAGGA	
3[97]	7[98]	GAAAGGGCGAAAAACGTGGCGAGAAAAGTGTCAGATTGTGCAAGCG	#800000
		GAGGAGAGCC	
33[153]	45[140]	TCAATAAACATGGTCTGCATTTTCGTCAGAAGATCACTGTACCATGGTGC	#800000
		GCCGCAT	
37[69]	21[70]	ATATAGTAGCACCGATAACAATGCGCATCCTGTAACAAAATCCATTCTT	#800000
		CCTCAAG	
50[140]	53[126]	ATTACTGCCTCGCATGAAGCTATCTCCGCTCTGGTGCTGACACGTGGTG	#800000
		ATATCCG	
17[76]	12[76]	TGATGATTTTGCTGCGCTGCATTGAGGACCGGCGGCTGAGCACAAGAC	#800000
		GACGCAGC	
52[70]	38[69]	CCCGGTGCATACAGTGCTTAATAATTGCATGTATAGATCACCTGTAC	#800000
25[160]	20[160]	GGATGTT	#800000
7[139]	5[126]	TCCCGCGGAACCTCGGTCGCGAAATGCAA	#800000
10[35]	15[35]	ATAAATGTAAACCCCGAGAAATGACTGA	#800000
39[160]	30[160]	AGGATGAACGTCTGCGCTTCTTCCGCT	#800000
21[34]	7[28]	ATGGCCCCGCGATCGCAACATCCAACT	#800000
		CCGAATTTTTTGCTTCAGTGGGCGGTGGCCGACCC	#800000

32[28]	19[28]	GAGCCCCGCGGCATGATGGCCTTCCGCT	#800000
		GAACAAAGTTTACCAAGGACGCATCGATAACTATCCTACAGCATCCTT	
44[147]	58[146]	GCCACCA	#800000
		CCGCGACACGTGCTGAGCCCAACCCCACTCAGGGCATTAAAGAACGTG	
13[76]	5[77]	GCACTGAG	#800000
		AATGACGCACTCCCCCCAAGACGCGACATATCAGGAAGCACAGGTAA	
19[90]	31[77]	TCAAACGA	#800000
4[84]	5[84]	ACTCCAACGTCATCCGGTGTCCGAGTACGGTGGACTCCACGC	#800000
		ACGGCTGCCTTTAAGCGCTGCCTGCCAGCTATATCCCGCACTGCTGCGG	
35[55]	16[55]	GCTGATT	#800000
		ACATATGGAGGCGCTGACAGGTGCGCGTCCTGCAGGCAACAACCAAC	
44[126]	58[125]	ATGTCTGTC	#800000
		CAAAAGAGGAGATCAAGACATGATGCGTCGTTTTCGCCGCCAATTCAA	
16[98]	7[91]	C	#800000
10[154]	2[167]	ATATTCCGTAAAAGACATAAACATTGCT	#800000
		GCCGGGCATGAAGAAGGCGAACACAAACGGACCGCGCATCAGAGGAT	
12[84]	27[98]	TCATGTGGT	#800000
22[28]	9[28]	GCAGCAATCTTCGCCGGAGGAGCTTTCA	#800000
		ACCTGGAGCCTCAACTACCAGCAATATCTGGGCAATCGTCATGGAAGA	
44[98]	52[97]	AACCGGCG	#800000
		TGATATCATCATCATGTCGCTGCCACTCATACATAGGAGGGGAATTCCG	
19[139]	13[126]	ACAGGCC	#800000
		CATGCAGACATCACTACCGTGAACATATAGATGATCACTTCCCACTGGA	
4[112]	9[126]	GTGTAAT	#800000
		GTCTTTCTTGCCATACACCGGCACTGGTCGTGCTGAGTCAAATTCAGGA	
21[97]	8[97]	TTATCGA	#800000
		GGCTATCCAAATGCCAAGGAGTCCCACTATGTGTGGTGGCGCTCGGAG	
41[118]	36[118]	ACATGTTG	#800000
		AATGTTTGAGGGAAAACCACTATTTGGTTGAGTCACCTGCCGTGCCGC	
37[55]	18[55]	GATTTTCT	#800000
		CTATCGACATCATTACGCATCAGAGTCTGCCAGCGTTGACACGCCTCTG	
0[84]	15[98]	AGACATC	#800000
27[41]	41[35]	CAGGCTGCGTGTTACTGGCATGAAGGGCATTCA	#800000
20[70]	5[63]	TCGCCTGCGTTTTGCATTGCGCTGTCAGCCGGATGTATGCCGCATTAG	#800000
		TGTCAGGAAATACATGAAGTACATTATGTCCACAGTATTGTTAATATT	
39[62]	55[63]	AGTAGCA	#800000
		CTAATCAGATGTTGTAGCACCATTTCTATATGCTCAAATCTTCTGACACT	
40[77]	53[77]	GTATTC	#800000
		CCGGATGATGCAAGTATCGTTTTTATTCATGTGAGACGTTGTGGCTAGA	
36[77]	49[77]	TGATTTT	#800000
10[28]	2[41]	AACCTGCCGCGTAAAATCGGAACCCTAA	#800000
37[153]	34[160]	GCCTTCGGGACCAGTTGCCTGTTCGCCGTACGAA	#800000

6[77]	18[90]	TAATCTGTATACCTAGCGGTTCCGGCGGTTTCTGATCGTTTCGCACGAC CGGCACT	#800000
17[55]	12[55]	TGAGGCCACCGCATCGTGGCGCACTTTTTGAGCTGATCGCCGCCACCTC TTCCACC	#800000
35[139]	16[139]	AGGTATGCAGCTCCATCGATCTCCAAGTGCACAGTCAATGAAGCAATA AGACGAGG	#800000
18[35]	21[42]	GCCGCCCTGGCAGAGCAGAGGGCTGATATTCTGCA	#800000
34[42]	45[35]	GCTGGTTTACCGGACAATGAGGTGAACCTGTTTTCTGGTAAACAAATAT GTCCACTGATGGCCTTGACGGGCGCTAGTCGAACAAGACCCGTTAGTA	#800000
4[63]	1[77]	AAGCGAAA AGGAGTGAGAGATGAGTTATTGAATCAAACCTTGCACGACTGGGGCGT	#800000
23[118]	3[112]	T	#800000
36[84]	50[104]	CTGGTGCGCCTGGCGTGGTGGACCTACTCCGACCTACCAAATTCATCTC AGGCGTATGTTACGGCTGACGAATACCTGAGCAAAGCATTGCACGAAA	#800000
19[97]	22[111]	AGAACACA	#800000
37[167]	50[167]	TTGAAGAGGGAGTTTTCTCTCAGAAATC	#800000
51[167]	54[160]	TTTACTGTTACTGAATGAGCAGAGTCACTTCAAAG	#800000
4[126]	1[126]	GAAGGTGTGAGCAAGGGTTGAAATTTTCG AGTTTGGAACAAGAATCTAAACAACGACGGACGCTTAACACTCATATT	#800000
4[49]	9[63]	CCCGTGTT CCAACGCTGTGTAGTGCTTTACATAGGATGAATATTATCTGTGACGTTT	#800000
52[63]	46[76]	TTTGCAT	#800000
6[91]	10[111]	GCAAACGATACTTGCCCTCTCTGGAATGAACATCAGATGTACACGTCCA CGGTTGTATTTTTTTGTTTTCGTCCCAGATCATACTGACTCCCATCACTA	#800000
26[77]	39[77]	GCCAAC CCTCACACATTCTTAGCCAGATGCCTGTGCCAGAATCACCGGCGCCGCC	#800000
37[76]	18[76]	GGAAGCT	#800000
51[104]	55[91]	GGCGCCGCCATTACGCTGTTAATCATT	#800000
37[139]	28[139]	AGACTCTTGGCTAAAGGTTCTGAGCGCGGTTGCGCTCGCCTG GAAGGTCCTGCAAGGACACCTCCGAAGTGCTTGGCGATTGATGCCTGA	#800000
38[140]	54[153]	TTGCAAAA	#800000
22[154]	9[154]	CAACCCTACAGGCGATCGCTAAAGCCGC	#800000
54[91]	58[111]	TGTTCACTGCCGGGCGCTGGTGTCTATGGCTACAAAATGCTCTTGACAT	#800000
41[160]	38[153]	ATGTTACCACCAGGTCCAGGATCTTGCAGCTTGT GGCGCAGGGAGATGGCAAGGCCAAACCACCATGTTGGCTCAGGAAAT	#800000
8[140]	20[153]	GACTTGATA GTCTGTTAATTTTGTGTTTGCAGTTGCCCATATCGATGGGCAAATCCAT	#800000
56[63]	49[49]	ATAAGAT ACCCTCTACGCATAAACAAGGTTATTTCTTGCAATTCCTGGCTGCCTGTT	#800000
27[146]	43[147]	TGACAG	#800000
8[154]	4[167]	ATGCAGATATAAATATATAGAGTCGGCA	#800000
42[35]	45[42]	TGATCCCTCCGTGGAACCGCTGGCATTCTTTATGT	#800000

57[62]	42[55]	GCTGCATCGACAGTTTTGAACAATGCTCCGTATTGATATTTTATCTGAT	#800000
		AAACTCCTGTTCTGAGTAAACTTTTTTTCATGGTGAATTTACCCTGACG	
50[56]	53[42]	GGGAAC	#800000
		ATTTCTGGAAGTCCATGCGCCACCTATGTGGGATTCAGGCAAGTTCG	
38[119]	54[132]	AGCATAAG	#800000
51[160]	42[160]	ATTACTCACTTCTCGCTCTGGCTTGGAG	#800000
		CGCGATTGGCGAACC AAAATACCGCCTCTATTTACCCTCAGAGAGAG	
55[153]	58[139]	GGCGTGAA	#800000
49[41]	57[28]	TCTTGAGTAATAAATGCCCAATTCCAGA	#800000
		AGGAATCTCCGCCCGGCTGTATCAAGGAATGCGAGTTACCCTTTCAA	
32[98]	40[97]	CTATTTGA	#800000
		GCCTACCCACGCTATCCTGGGCTGCCTGGAAGTCCATCTTTCTTCGACT	
52[126]	46[139]	CTCTTTG	#800000
35[111]	25[84]	AACGAATGGGATACCTTCAACGCAGGATTTGCGTTAGTCATG	#800000
		CTCCGCAAACCAAGCGACAAATCATCGTCTGCCTTATGGTCATCGACC	
50[119]	53[105]	GTTGCAG	#800000
		CTGGCTAATATCAAAAAGGTACCCCGAATTGTTCTCACCCAAGGGAG	
7[55]	1[42]	CTAGCGGT	#800000
5[160]	3[147]	AAATCCCCATTCTGGGTATAGGAGTATC	#800000
23[111]	13[84]	GACCAAGTCTGCCGGCCTGGCGAGATCCACGTTGCATCAGTC	#800000
11[55]	8[62]	CCCCGGTCTGACCGGCAAGTGCCCCGATTTAGAGCCACTACGTTACTGC	#800000
25[167]	38[167]	CTTGCAATACATCTTGACCTCGATCCTC	#800000
3[167]	16[167]	AGGAGTTCGTAACCCTACGGCTAATCAG	#800000
1[167]	14[167]	CGGTTTTACCTCCGAACACCCGAAAAA	#800000
		ACCGCTTGAGCTGGCGCTGCACCTGAGTGATCTGGTTTCCTCCTGCAGC	
21[153]	33[140]	TATGTAC	#800000
44[28]	31[28]	AGTGCGGAATTACTTAATAATACTGTGG	#800000
		TTTATGTAGATGAACAATGTGAATATTACATAACAGTTTTATATAGCAC	
4[133]	9[147]	CTGATGG	#800000
10[42]	12[41]	CACTGTATGTGAATCTTCATATTACGCG	#800000
		CGCCCGGCTTCAGGTCCTCCGTCATGGCACCTCCGACAAGGGTCCCTT	
10[77]	22[90]	TCTCGTC	#800000
58[28]	45[28]	CCTAAAGAATTTAATGTTCATTAGGAAT	#800000
52[105]	48[118]	CTTCTTTATCAACAAGGTGATTCATCACCGTGTTCAATCAGC	#800000
		GCGTGGATCCGCGGCAGTTAAGGCGCTGTACTCCACACCACCAGCCTG	
0[42]	15[56]	TCTCTGCG	#800000
		GTTCCGGATTCTCTGGCGGGGCGCCCGTGGATGCCGTAACTTTGT	
9[90]	21[77]	GTCCGCAT	#800000
11[111]	1[84]	TGAAGAGGATCAGAAATGGCAAACGCACGTGGAAGGGAAGAA	#800000
4[105]	1[105]	TTGTCTCATCAATGTGATGACCGATGCC	#800000
39[167]	52[167]	GTCAGCTGAAAGATCTTTCAGTCCGAAA	#800000
29[160]	26[153]	ATTTACAACCACCGCACTAGGTTCTGTTGCCCTG	#800000

11[76]	8[90]	ACAGAGAGCTGTAAGGAGCGGGGAAAGCCGGCGAACCGTCTAAAAA AAGCCACAG	#800000
21[132]	33[119]	ATCAAGGCAGGTCGAAAGACATGTCTGGCTGCACCACAGGCTATTCAA TCGCCTTT	#800000
22[42]	24[41]	AGACGTGAGTGGGCTTTTCTGGCAATCG	#800000
49[167]	56[160]	AGATTCCGATAATGTGCTGCGCTCGATGGCGGCAA TATGTCAGGTGCGACCCAGGGCCAGCGCAGTAGCGATTAATGCCGGAT	#800000
54[77]	53[63]	TAACATG	#800000
5[41]	9[42]	ATCGGCAAGCCTTCATAAGCAATCAGAAGACTCCTTGCTGTT CTACGACGAGTCACTCAATGGGCGCAGCTATCAAGCAGCAGAGTAGAT	#800000
8[119]	20[132]	GGGAATAT	#800000
13[153]	11[154]	AATCGCAACAATGGATATTACGAATCAG CCCTCACCATCCAAATAAGATCCACGCTCAGGCTGGGCTGAACTGAGA	#800000
29[118]	24[118]	AGATTCT CATCGGATTGATGATGGAGGGAAAGTGGCCTGGTGATCCGTAGGGTT	#800000
50[49]	30[55]	TC	#800000
21[167]	31[161]	TCTACTCCCGTCCGATGGCGTAGTCTGCTCTCAGG TTCCCTGAGAGAAGTTTGACGACTCACTCTGAGAAAGGGCACAACAG	#800000
24[84]	39[98]	CCAATCTC TAGTAAATATAGCGTCATTGAAGCGCATCGTCGGTGCGTGATGATGTT	#800000
48[84]	56[97]	GGATAACG ATGCAGGCGGTAAAGGTTGACATAAGAAATCACTGTACTGAGTCATCC	#800000
10[147]	26[146]	GTCATCAC	#800000
49[153]	46[160]	GATAAGCCGTGAGCCTGCCACGGAGGCCGTGCAAT	#800000
51[187]	52[187]	TTTTGTAGGCGGACGCTGTGGTTTT	#d79035
15[187]	16[187]	TTTTGAATCAAGCCTGAGCGATTTTT	#d79035
31[183]	32[183]	TTTTACATTGGCTACTTTCAAAGATGTATTTT	#d79035
35[183]	36[167]	TTTTCAAGGCCGACCAGCTGATTCATGGA	#d79035
13[187]	14[187]	TTTTTAAAGAGTTCCAGCAGAGTTTT	#d79035
21[183]	22[183]	TTTTACCATAATTGCAAGGGCTCAGTTGTTTT	#d79035
29[167]	30[183]	GAGGTTACGAAGAATCTCTGCTGGAGTTTT	#d79035
55[183]	56[183]	TTTTAGAGATTGATGTCATGCAGATGCGTTTT	#d79035
43[183]	44[183]	TTTTAACCCAAAGACAAACACTCACCGCTTTT	#d79035
53[167]	54[183]	GGAGCGTAAGCGGGCGGAACGGTCAGTTTT	#d79035
17[167]	18[183]	TAGCTGAATGGAAGTGGCTGTTGTTATTTT	#d79035
37[187]	38[187]	TTTTCTGGGCTGTAGGTGCTCATTTT	#d79035
23[183]	24[167]	TTTTGCCGACGCTTTATCACCTGCCCAGC	#d79035
40[161]	42[183]	CCTCCACAGGAGAAAGGAACTGAACACCTTGCTTTT	#d79035
53[187]	50[174]	TTTTCATTGCAGCAGATTAAAGTCAGGAGAGCTAATGGTTA	#d79035
49[187]	50[187]	TTTTAGGGCTGTCTGACGTCATTTTT	#d79035
39[187]	40[187]	TTTTGGCCAGATACAGGAGCTCTTTT	#d79035

59[183]	59[154]	TTTTGACTGGCAGACACCGCTGAACGGGAT	#d79035
1[174]	0[187]	GTGAGATACGCTGGCATTTCGCATTTTT	#d79035
27[187]	28[187]	TTTTATCATCCAGACCAGACGCTTTT	#d79035
25[187]	26[187]	TTTTAGAACGCACAAGACCTTGTTTT	#d79035
45[183]	46[183]	TTTTGCACATGCTCTGTGTCCATGGTCTTTTT	#d79035
47[183]	48[167]	TTTTGCTGGAGAACGCCATCTACAAAGCTG	#d79035
1[187]	2[187]	TTTTCAAAGGAGATTAGCTGATTTT	#d79035
5[167]	6[183]	TGAAGACCACTCGCGGGGATTTATTTTTTT	#d79035
57[183]	58[183]	TTTTTCAGCGTGATGTCAGCCTCCCCCTTTT	#d79035
37[174]	36[187]	GGCAACCTCGATCAAGAGAGATCTTTT	#d79035
49[174]	48[187]	ATTGAGACAGCATTCTGGATTACTTTT	#d79035
11[183]	22[167]	TTTTAAGAGAGTATCTGTTCCACTGCTACAGGCAGAGCCCCAAAG	#d79035
5[187]	2[174]	TTTTAGGTTTATAAGTCTAATACAAATCATTGCAGATACCG	#d79035
41[187]	38[174]	TTTTGCCAACGCCGAAAGATGGAACCTCGATTGCAGTCGTGC	#d79035
26[168]	24[187]	CTTCGGCTGGTTATGATGACCTCTCAATCGTTTT	#d79035
13[174]	12[187]	TGGATTAGGGACCCAAGGACGGGTTTT	#d79035
29[187]	27[168]	TTTTTGCCAGCATCGTCACATCATTCCGAGATGG	#d79035
9[183]	10[183]	TTTTGAAGAACTGAAAGGTTAAGGATGGTTTT	#d79035
33[183]	34[183]	TTTTCTTCATTATCTCGCCGCTGCCTGTTTTT	#d79035
19[183]	20[183]	TTTTCCGGGCAACGAGCTTGCGGAGAATTTT	#d79035
3[187]	4[187]	TTTTAACGACATAATTCCATGATTTT	#d79035
7[183]	8[183]	TTTTTATCTGAACTCGAATATTCGAATTTTTT	#d79035
17[187]	14[174]	TTTTGCATTCCGAGAGGCAAATCAACAAGAAGACATCCTGG	#d79035

Electron Micrographs for pScaf-10080-ACKN Brick

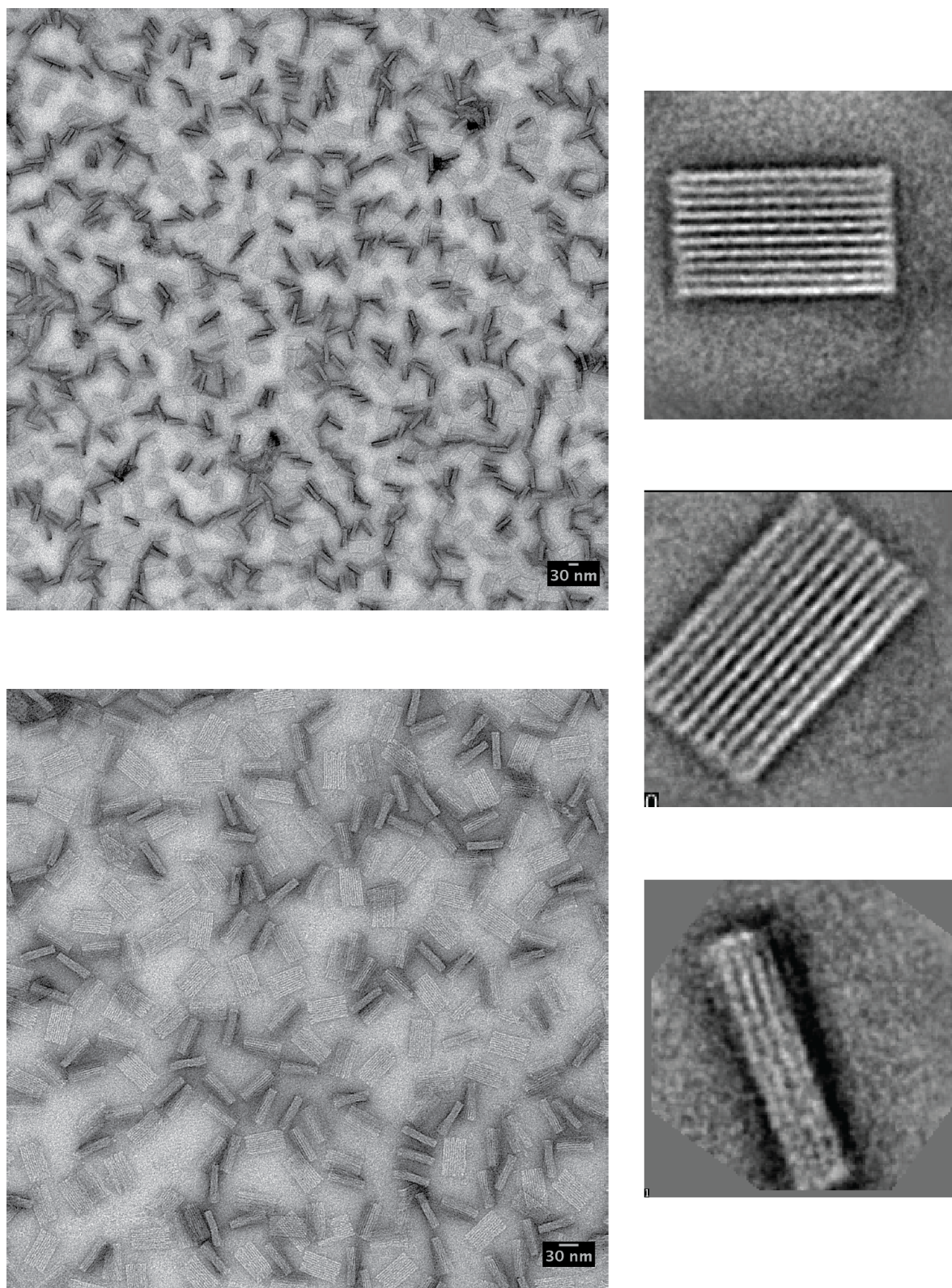


Figure 60: Electron Micrographs and Class Averages for pScaf-10080-ACKN Brick

VIII. Protocols

Instruments / Equipment

- Innova incubator shaker
- Eppendorf microcentrifuge
- Typhoon laser gel scanner
- Ultrospec 10 cell density meters
- Safe Imager™ 2.0 Blue Light Transilluminator

Tools

- Rainin LTS Pipette
- Pipette aid (Drummond)

Glassware & Consumables (manufacturer & part number)

- Pipette tips (Rainin, RT-L10S, RT-L200S, RT-L1000S)
- Petri dishes (BD Falcon, 351029)
- Culture tubes (Spectrum Chemical, 8597)
- Ultra-yield shake flasks (Thompson Instrument Company, 931141)
- Microcentrifuge tubes (Axygen, MCT-150-C)
- Centrifuge bottles, 500-mL (Nalgene, 3141-0500)
- Oak Ridge centrifuge tubes, 50-mL (3139-0050)
- Centrifuge tubes, 15-mL (Corning, 430791)
- Centrifuge tubes, 50-mL (Corning, 430829)
- Nalgene large polypropylene sterilizing pan (Thermo Scientific 69000020)
- Microtube pestles (USA Scientific 1415-5390)
- Microtube cutter (Sigma-Aldrich Z217085)
- Freeze 'N Squeeze DNA gel extraction spin column (Bio-Rad 7326165)
- Acrodisc® Ion Chromatography Syringe Filters (VWR 28144-030)
- Formvar/Carbon-Coated Grids (Structure Probe, 3440C-MB)
- Dumont N4 Tweezer (Fisher Scientific 5024125)
- Dumont N4 Anti-Capillary Tweezer (Fisher Scientific 5024210)

Materials

E.coli Strains

- K91 (Yale Ecoli Stock Center)
- XL1-Blue MRF' Kan (Agilent Genomics)

DNA vectors

- M13cp

- pScaf

Chemicals

- Yeast extract powder (Sigma-Aldrich)
- Tryptone powder (Sigma-Aldrich)
- Sodium chloride (Sigma-Aldrich)
- Sodium hydroxide (Sigma-Aldrich)
- PEG8000 (Fisher Scientific)
- Sodium phosphate monobasic (Fisher Scientific)
- Potassium phosphate dibasic (Fisher Scientific)
- Ethanol (VWR)
- Tris base (Fisher Scientific)
- EDTA (Thermo Fisher Scientific)
- Chloramphenicol (Fisher Scientific)
- Carbenicillin (Teknova)
- Kanamycin (VWR)
- Magnesium chloride (Fisher Scientific)
- Glucose (Sigma-Aldrich)
- Sodium dodecyl sulfate, 20% solution (Sigma-Aldrich)
- Potassium acetate (Sigma-Aldrich)
- Glacial acetic acid (Fisher Scientific)
- Tris-EDTA (TE) Buffer, pH 8.0 (Thermo Fisher Scientific)
- DMSO (Thermo Fisher Scientific)
- SOC medium (Teknova)
- LB Lennox agar (VWR)
- Antifoam 204 (Sigma-Aldrich)
- Agarose (VWR)
- SYBR Safe DNA gel stain (Thermo Fisher Scientific)
- 10x CutSmart Buffer (NEB)
- BamHI-HF (NEB)
- KpnI-HF (NEB)
- Calf intestinal phosphatase (NEB)
- 6x Purple Gel Loading Dye (NEB)

Methods

Preparation of Buffers and Stocks

2xYT with carbenicillin

- Get 1 clean, empty 1-L glass bottle
- Pour 8g tryptone into bottle
- Pour 5g yeast extract into bottle
- Pour 1.25g sodium chloride into bottle
- Pour 500-mL deionized water into bottle

- Place cap on bottle and swirl to mix
- Affix autoclave tape to cap
- Loosen cap and autoclave bottle on liquid cycle for 45 minutes
- After autoclaving is complete, carefully remove bottle and place on counter to cool
- Once the media has reached room temperature, add 500 uL of 100 mg/mL carbenicillin
- Store media at 4C

Phosphate buffer

- Get 1 clean, empty 600-mL beaker
- Place 1 magnetic stir bar in beaker
- Pour approximately 400 mL deionized water into beaker
- Place beaker on stir plate and turn on stirring
- Pour 35g potassium phosphate dibasic into beaker and wait for it to dissolve
- Pour 15g sodium phosphate monobasic into beaker and wait for it to dissolve
- While the solution continues to stir, use a pH probe to measure the pH of the solution
- Using a dropper, slowly add 5N sodium hydroxide until pH reaches 7.0
- Pour water into beaker until volume reaches 500 mL
- Get 1 clean, empty 1-L glass bottle
- Transfer solution from beaker to the empty glass bottle
- Place cap loosely on bottle, affix autoclave tape to cap, and autoclave bottle on liquid cycle for 45 minutes
- After autoclaving is complete, carefully remove bottle and place on counter to cool
- Store buffer at room temperature

Lysis buffer (0.2M NaOH, 1%SDS)

- Get 1 clean, empty 250-mL bottle
- Pour 5 mL of 20% SDS into bottle
- Pour 8 mL 5N sodium hydroxide into bottle
- Pour 187 mL deionized water into bottle
- Place cap on bottle and swirl to mix
- Store buffer at room temperature

Neutralization buffer (3M KOAc, pH 5.5)

- Get 1 clean, empty 600-mL beaker
- Place 1 magnetic stir bar in beaker
- Pour approximately 250 mL deionized water into beaker
- Place beaker on stir plate and turn on stirring
- Pour 147g potassium acetate into beaker and wait for it to dissolve
- While the solution continues to stir, use a pH probe to measure the pH of the solution
- Using a dropper, slowly add glacial acetic acid until pH reaches 5.5
- Pour water into beaker until volume reaches 500 mL
- Get 1 clean, empty 1-L glass bottle
- Transfer solution from beaker to the empty glass bottle

TSS Buffer

- Get 1 new 50-mL centrifuge tube
- Pour 5g PEG-8000 into tube
- Pour 1.5 mL of 1M magnesium chloride into tube
- Pour 2xYT media into tube up to 50 mL
- Confirm that pH of buffer is 6.5; if not, adjust
- Get 1 new 50-mL centrifuge tube, 1 0.22 μ M syringe filter, and 1 50-mL syringe
- Remove plunger from syringe, screw on filter, and place over new tube
- Pour solution into top of syringe
- Apply plunger to force solution through syringe filter and into new tube
- Pour 2.5 mL DMSO into new tube with solution (?)
- Store buffer at 4°C

LB Agar Plates

- Get 1 clean, empty 500-mL bottle
- Pour 16 g LB Lennox agar powder into bottle
- Pour 484 mL deionized water into bottle
- Screw cap onto bottle
- Place cap on bottle and swirl to mix
- Affix autoclave tape to cap
- Loosen cap, affix autoclave tape to cap, and autoclave bottle on liquid cycle for 45 minutes
- After autoclaving is complete, carefully remove bottle and place on counter to cool
- Once the bottle is cool-to-the-touch, add the desired antibiotics (if any) at the following volumes and concentrations
 - Kanamycin: 500 μ L at 50 mg/mL
 - Carbenicillin: 500 μ L at 100 mg/mL
 - Chloramphenicol: 500 μ L at 34 mg/mL
- Place cap on bottle and swirl to mix
- Get 1 sleeve of 20 sterile Petri dishes and lay them out on a bench top
- For each plate, briefly lift the lid, pour approximately 25 mL of solution into the plate, and replace the lid
- Allow the plates to sit for 5 minutes until they have solidified
- Flip the plates over and label each with the names of the appropriate antibiotics
- Return the plates to the original sleeve, and seal with tape
- Store plates at 4°C

50x TAE Buffer

- Get 1 1-L bottle
- Place stir bar in bottle
- Pour approximately 600 mL deionized water into bottle
- Begin stirring bottle on stir plate
- Add 242 g Tris base to bottle

- Add 100 mL 0.5 M EDTA to bottle
- Add 57.1 mL glacial acetic acid to bottle
- Add deionized water to bottle until total volume reaches 1 L
- Remove bottle from stir plate and cap
- 50x buffer may be stored at room temperature
- 1x TAE buffer may be prepared by diluting 10 mL of 50x TAE buffer in 490 mL of deionized water

5x TBE

- Get 1 1-L bottle
- Place stir bar in bottle
- Pour approximately 600 mL deionized water into bottle
- Begin stirring bottle on stir plate
- Add 54 g Tris base to bottle
- Add 27.5 g boric acid to bottle
- Add 20 mL 0.5 M EDTA to bottle
- Add deionized water to bottle until total volume reaches 1 L
- Remove bottle from stir plate and cap
- 5x buffer may be stored at room temperature
- 0.5x TBE buffer may be prepared by diluting 50 mL of 5x TBE buffer in 450 mL of deionized water

5x TBE + 110 mM Magnesium Chloride Buffer

- Get 1 1-L bottle
- Place stir bar in bottle
- Pour approximately 600 mL deionized water into bottle
- Begin stirring bottle on stir plate
- Add 54 g Tris base to bottle
- Add 27.5 g boric acid to bottle
- Add 22.36 g magnesium sulfate
- Add 20 mL 0.5 M EDTA to bottle
- Add deionized water to bottle until total volume reaches 1 L
- Remove bottle from stir plate and cap
- 5x buffer may be stored at room temperature
- 0.5x TBE + 11 mM MgCl_2 buffer may be prepared by diluting 50 mL of 5x TBE + 110 mM MgCl_2 buffer in 450 mL of deionized water

10x Folding Buffer

- Get 1 1.5-mL microcentrifuge tube
- Add 930 μL water to tube
- Add 50 μL 1M Tris to tube
- Add 20 μL 0.5M EDTA to tube
- Vortex tube

PEG Precipitation Buffer

- Get 1 15-mL centrifuge tube
- Weigh 1.5 g PEG-8000
- Add PEG-8000 to tube
- Add 100 μ L 1 M Tris
- Add 200 μ L 1 M $MgCl_2$
- Add 1 mL 5 M NaCl
- Add water up to 10 mL
- Vortex until PEG is dispersed in solution
- Incubate at 42°C for 5 minutes to dissolve PEG
- Vortex again to mix fully

1x Folding Buffer with Magnesium

- Get 1 1.5-mL microcentrifuge tube
- Add 100 μ L 10x folding buffer
- Add 13 μ L 1 M $MgCl_2$
- Add 887 μ L water
- Vortex to mix

Preparation of Chemically Competent Cells

- Get 1 16-mL culture tube
- Pour 5 mL 2xYT into tube
- Inoculate tube with a single colony from a culture plate
- Grow culture for 18 hours in incubator shaker (37C, 225 rpm)
- Get 1 sterile 500-mL flask
- Pour 60-mL 2xYT into flask
- Inoculate flask with 600 μ L of overnight culture
- Grow flask in incubator shaker (37C, 225 rpm)
- Monitor growth by measuring OD 600 every 30 minutes
- Once OD 0.5 has been reached, halt growth
- Get 2 new 50-mL centrifuge tubes
- Pour culture from flask into tubes, splitting evenly between them
- Get 1 bucket filled with ice
- Chill tubes on ice for 10 minutes
- Centrifuge tubes at 3000 rpm for 10 minutes at 4C
- During centrifugation, prepare 50 microcentrifuge tubes and number them 1-50
- Place numbered tubes in ice bucket
- After centrifugation, discard supernatant from tubes, retaining cell pellet at bottom
- Resuspend each pellet in 2.5 mL of chilled TSS buffer and place tubes in ice bucket
- Transfer 100 μ L of resuspended cell solution into each of the numbered tubes
- Prepare a shallow tray with a bath of dry ice and 70% ethanol
- Place each numbered tube briefly in dry ice bath to freeze contents, then immediately transfer into storage box and place in -80C freezer

Introduction of Plasmid into E.coli by Chemical Transformation

- Get desired number of tubes of competent cells from freezer, such that there is enough total volume for 50 μ L per transformation
- Thaw competent cell tubes on ice
- Get plasmid solutions and chill approximately 10 ng in a 1.5-mL microcentrifuge tube on ice
- Add 50 μ L thawed competent cells to each chilled tube
- Chill the resulting solution on ice for 30 minutes
- Meanwhile, transfer a bottle of SOC medium to a 37°C incubator
- Heat shock the solution by incubating it in a 42°C water bath for 45 seconds
- Incubate the solution on ice for 2 minutes
- Add 250 μ L of pre-warmed SOC medium to each tube
- Position each tube inside of a 15-mL centrifuge tube, and place in 37°C incubator shaker (250 rpm) for 60 minutes
- Meanwhile, get n LB agar plates with the appropriate antibiotic, where n is the number of transformations; Place agar plates in 37°C incubator
- Once incubation is complete, remove the tubes and plates from their respective incubators
- Pour the entirety of each solution onto a separate plate
- Get 4 sterile glass beads and add onto each plate
- Shake the plates back and forth on a flat surface to spread the solution across the plate
- Transfer used beads to a 70% ethanol solution
- Incubate plates overnight in a 37°C incubator
- Once colonies have grown, remove plate from incubator, wrap in Parafilm, and store at 4°C

1% Agarose (TAE) gel for plasmid and PCR product

- Get 1 clean, empty beaker
- Add 1.2 g agarose to beaker
- Add 120 mL 1x TAE buffer to beaker
- Add 35 mL water to beaker
- Heat beaker in microwave for 2.5 minutes
- Using thick gloves, carefully remove beaker and swirl to mix
- Return beaker to microwave and heat for 1.5 minutes
- Using thick gloves, carefully remove beaker and swirl to mix
- Get 1 bucket filled with ice and water
- Place beaker in water bath and swirl for 120 seconds until it is cool to the touch
- Add 8 μ L 10,000x SYBR Safe DNA gel stain to beaker and swirl to mix
- Get 1 gel box and fit gel tray snugly into box
- Pour contents of beaker evenly into gel tray
- Use butane torch to eliminate bubbles from gel solution
- Insert the thin-well side of a 20-tooth comb into the gel
- Allow it to sit at room temperature for 30 minutes until solid
- Carefully remove comb from gel, pulling directly upward

- Remove gel tray from box, rotate 90 degrees and place back into gel box
- Pour 570 mL 1x TAE buffer over gel and into gel box
- Load ladder and samples into gel
- Apply 110 V potential across gel for 45 minutes
- Visualize gel using Typhoon gel scanner with SYBR Safe method at 450 PMT voltage

2% Agarose (TBE) gel for ssDNA and origami product

- Get 1 clean, empty beaker
- Add 1.2 g agarose to beaker
- Add 120 mL 0.5x TBE buffer to beaker
- Add 35 mL water to beaker
- Heat beaker in microwave for 2.5 minutes
- Using thick gloves, carefully remove beaker and swirl to mix
- Return beaker to microwave and heat for 1.5 minutes
- Using thick gloves, carefully remove beaker and swirl to mix
- Get 1 bucket filled with ice and water
- Place beaker in water bath and swirl for 120 seconds until it is cool to the touch
- Add 1 mL 1.3 M magnesium chloride to beaker and swirl to mix
- Add 8 μ L 10,000x SYBR Safe DNA gel stain to beaker and swirl to mix
- Get 1 gel box and fit gel tray snugly into box
- Pour contents of beaker evenly into gel tray
- Use butane torch to eliminate bubbles from gel solution
- Insert the thin-well side of a 20-tooth comb into the gel
- Allow it to sit at room temperature for 30 minutes until solid
- Carefully remove comb from gel, pulling directly upward
- Remove gel tray from box, rotate 90 degrees and place back into gel box
- Pour 570 mL 0.5x TBE + 11 mM $MgCl_2$ buffer over gel and into gel box
- Place gel box large sterilizing pan
- Put ice in pan until entire gel box is surrounded
- Pour water over ice until water level reaches the height of the gel
- Load ladder and samples into gel
- Apply 70 V potential across gel for 3 hours
- Visualize gel using Typhoon gel scanner with SYBR Safe method at 450 PMT voltage

Phagemid growth with Helper Plasmid

- Get 1 16-mL culture tube
- Pour 5 mL 2xYT into tube
- Add the following antibiotics into the tube:
 - 5 μ L 100 mg/mL carbenicillin into tube
 - 5 μ L 50 mg/mL kanamycin
 - 5 μ L 34 mg/mL chloramphenicol
- Inoculate tube with a single colony of phagemid-containing X11-Blue.M13cp from a culture plate
- Grow culture for 18 hours in incubator shaker (30°C, 225 rpm)
- Get 1 500-mL ultra-yield shake flask

- Add the following ingredients into the shake flask
 - 100 mL 2xYT medium
 - 10 mL phosphate buffer
 - 1 mL 50% glucose
 - 500 μ L 1M magnesium chloride
 - 115 μ L 100 mg/mL carbenicillin
 - 115 μ L 50 mg/mL kanamycin
 - 115 μ L 34 mg/mL chloramphenicol
 - 5 mL of tube culture
- Grow the flask for 24 hours in a incubator shaker (30°C, 225 rpm)

Phagemid growth with Helper Phage

- Get 1 16-mL culture tube
- Pour 5 mL 2xYT into tube
- Add 5 μ L 100 mg/mL carbenicillin into tube
- Inoculate tube with a single colony of phagemid-containing K91.A from a culture plate
- Grow culture for 18 hours in incubator shaker (37C, 225 rpm)
- Get 1 500-mL ultra-yield shake flask
- Add the following ingredients into the shake flask
 - 100 mL 2xYT medium
 - 10 mL phosphate buffer
 - 1 mL 50% glucose
 - 500 μ L 1M magnesium chloride
 - 115 μ L 100 mg/mL carbenicillin
 - 2 mL of tube culture
 - 5 μ L VCSM13 helper phage at a concentration of 2×10^{12} phage/mL
- Grow the flask for 24 hours in incubator shaker (37C, 225 rpm)

Phagemid ssDNA recovery

Cell removal

- Grow flask culture using one of the above phagemid growth protocols
- Once growth is complete, remove flask from incubator shaker
- Remove 1 mL of culture and dilute 20-fold in 2xYT; measure OD₆₀₀ using cell density meter
- Prepare a 1:5 dilution of antifoam 204 in water
- Add 5 drops of dilute antifoam to flask
- Swirl flask until foam layer disappears
- Get 1 clean, empty 500-mL centrifuge bottle
- Transfer contents of flask into bottle
- Incubate bottle on ice for 30 minutes
- Centrifuge bottle at 7000g for 15 minutes at 4°C to pellet cells
- Get 1 clean, empty 500-mL centrifuge bottle
- Transfer the phage-containing supernatant into the new bottle

Phage recovery

- Pour 4 g PEG-8000 and 3 g sodium chloride into bottle
- Shake bottle until PEG-8000 and sodium chloride are dissolved
- Incubate bottle on ice for 30 minutes
- Centrifuge bottle at 9000g for 15 minutes at 4°C to precipitate phage
- Decant supernatant into waste beaker, retaining precipitated phage in bottle
- Rest bottle upside down on paper towels for 2 minutes to remove additional liquid
- Resuspend the phage in 6 mL TE buffer
- Centrifuge bottle at 15000g for 15 minutes at 4°C to pellet residual cells
- Get 1 new 15-mL centrifuge tube
- Transfer supernatant (concentrated phage solution) from bottle into tube

Phage lysis

- Get 1 clean, empty 50-mL Oak Ridge centrifuge tube
- Transfer 3 mL of concentrated phage solution to ultracentrifuge tube
- Add 6 mL lysis buffer into tube
- Vortex gently to mix
- Add 4.5 mL neutralization buffer into tube
- Vortex gently to mix
- Incubate tube on ice for 15 minutes
- Centrifuge tube at 15000g for 15 minutes at 4°C to pellet viral protein
- Get 1 clean, empty 50-mL Oak Ridge centrifuge tube
- Decant DNA-containing supernatant into new tube
- Add 27 mL pure ethanol to tube
- Invert tube 5 times to mix
- Incubate the tube at -20 °C for 12-24 hours to precipitate DNA

DNA recovery

- Centrifuge tube at 16000g for 15 minutes at 4 °C to pellet DNA
- Decant supernatant into waste beaker, leaving DNA pellet at the bottom of tube
- Add 9 mL ice-cold 70% ethanol
- Centrifuge tube at 16000g for 5 minutes at 4 °C
- Decant supernatant into waste beaker
- Rest tube upside down on paper towels for 2 minutes to remove additional liquid
- Dry DNA pellet by blowing air into tube
- Resuspend DNA in 1 mL TE

Phagemid vector construction

Computer-aided design (PCR amplification of pre-existing sequence)

- Open .dna file for plasmid containing sequence to be inserted in SnapGene
- Design forward PCR primer at start of desired insert sequence
- Modify forward PCR primer to include "TTTTT" spacer followed by KpnI and BglII restriction sites at 3' end

- Design reverse PCR primer at end of insert sequence
- Modify reverse PCR primer to include "TTTTT" spacer followed by BamHI restriction site at the 3' end
- Use SnapGene to perform simulated PCR
- Confirm that expected product is produced and save resulting file
- Open pScaf.dna file in SnapGene
- Use SnapGene to simulate one-fragment restriction cloning of PCR product into pScaf vector at BamHI and KpnI sites
- Confirm that insert is successfully placed between initiator and terminator blocks in vector

Computer-aided design (de novo synthesis of sequence)

- In SnapGene, create a file containing the desired insert sequence
- Place a KpnI restriction site at the 3' end of the sequence and a BamHI restriction site at the 5' end of the sequence. Ensure that these sites are not present elsewhere in the sequence
- Using a gene synthesis company, order the sequence, either as a linear fragment or cloned into a standard cloning plasmid (such as pUC19) at KpnI and BamHI sites
- In SnapGene, open the .dna for either the linear fragment or sequence in the cloning plasmid
- In SnapGene, open pScaf.dna file
- Use SnapGene to simulate one-fragment restriction cloning of the synthesized sequence into pScaf vector at BamHI and KpnI sites
- Confirm that insert is successfully placed between initiator and terminator blocks in vector

Phagemid vector cloning

PCR-amplification of insert (if necessary)

- From an oligonucleotide vendor, order forward and reverse primers designed in SnapGene
- Resuspend primers to 100 μ M in TE buffer
- Dilute 10 μ L of primer stock in 90 μ L water to obtain 10 μ M working stock
- Prepare a 50 μ L PCR based on the polymerase manufacturer's protocol; for example prepare a reaction with the following components for Phusion polymerase from NEB:
 - 10 μ L 5x Phusion HF or GC Buffer
 - 1 μ L 10 mM dNTPs
 - 2.5 μ L 10 μ M forward primer
 - 2.5 μ L 10 μ M reverse primer
 - 1 μ L 1pg/ μ L-10ng/ μ L template DNA
 - 28 μ L water
 - 5 μ L 10x dilution of Phusion DNA polymerase (added last to reaction and dilution performed immediately prior to addition)
- Pipet up and down to mix reaction
- Transfer to PCR machine and begin thermocycling using conditions recommended by

polymerase manufacturer

- Evaluate PCR product by running on 1% agarose (TAE) gel alongside ladder
- If PCR is successful and displays a single correctly sized product, purify using a PCR purification kit (see manufacturer's instructions for protocol); else if the correctly sized product is displayed alongside other products, purify using a gel purification kit (see manufacturer's instructions for protocol); else if no correctly sized product is displayed, troubleshoot per polymerase manufacturer's suggestions

Restriction digest of insert and pScaf vector

- Get 1 100 ng/μL stock solution of pScaf vector and 1 100 ng/μL stock solution of PCR-amplified or synthesized insert
- Get 2 200-μL PCR tube; label 1 "vector" and the other "insert"
- Add 10 μL of 100 ng/μL vector to "vector" tube and 10 μL of 100 ng/μL insert to the "insert" tube
- Add 2 μL 10x CutSmart Buffer to each tube
- Add 6 μL nuclease-free water to each tube
- Add 1 μL BamHI-HF to each tube
- Add 1 μL KpnI-HF to each tube
- Pipet up and down in each tube to mix solutions
- Incubate tubes at 37°C for 1 hour
- Remove "insert" tube and purify contents using a PCR purification kit (see manufacturer's instructions for protocol)
- Add 1 μL calf intestinal phosphatase to "vector" tube
- Incubate "vector" tube at 37°C for 30 minutes
- Remove "vector" tube and purify contents using a gel purification kit (see manufacturer's instructions for protocol)

Ligation of insert into vector

- Get 1 purified solution of vector and 1 purified solution of insert
- Measure concentration of purified products using a micro-volume spectrophotometer (e.g. Nanovue Plus)
- Calculate volume needed for 50 ng of vector
- Calculate insert mass needed for 3:1 insert:vector molar ratio, where required mass insert (ng) = desired insert:vector molar ratio x mass of vector (ng) x ratio of insert to vector lengths
- Calculate insert volume needed for calculated insert mass
- Get 1 1.5-mL microcentrifuge tube
- Add calculated volumes of vector and insert stock to tube
- Add 2 μL 10x T4 DNA ligase buffer to tube
- Add nuclease-free water up to 19 μL
- Add 1 μL T4 DNA ligase
- Mix reaction by pipetting up and down
- Incubate tube at room temperature for 10 minutes
- Heat inactivate by incubating at 65°C for 10 minutes
- Chill on ice for 2 minutes

- Transform 5 μL of reaction into 50 μL Mach1 competent cells and plate on carbenicillin agar (see "Introduction of Plasmid into E.coli by Chemical Transformation")

Analysis of ligation products

- Get 6 16-mL culture tubes
- Add 4 mL 2xYT-carbenicillin to each tube
- Using a sterile pipet tip pick 1 colony from the transformation plate and release it into the media in the tube; repeat this with 5 additional colonies in the other 5 tubes
- Grow the culture in an incubator shake at 37°C and 225 rpm for 18 hours
- Recover and purify the plasmid from each tube using a commercial plasmid miniprep kit (see manufacturer's instructions for protocol)
- Digest a small amount of each purified plasmid by combining 2 μL plasmid with 1 μL 10x CutSmart buffer, 6 μL nuclease-free water, and 1 μL BamHI-HF in a 200- μL PCR tube; incubate the PCR tube at 37°C for 1 hour
- Remove the PCR tubes and add 2 μL 6x purple gel loading dye to each tube
- Load the samples into a 1% agarose (TAE) gel containing SYBR Safe alongside a 1 kb ladder
- Run the gel at 110V for 35 minutes
- Scan the gel to determine the location of the bands
- Successful products (running at a size consistent with the expected product) may be transformed into K91.A or XL1-Blue.M13cp for phage production; If products are unsuccessful, additional minipreps may be performed to identify a correct clone, or cloning may be repeated from an early step

Preparation of phagemid-producing cells

- Get 2 1.5-mL tubes for each successful phagemid construct
- Transform each successful phagemid construct into K91.A (plate on carbenicillin) and XL1-Blue.M13cp (plate on Kanamycin/Carbenicillin/Chloramphenicol) using the established transformation protocol (see "Introduction of Plasmid into E.coli by Chemical Transformation")
- K91.A transformants may be grown with helper phage to produce custom phage, while XL1-Blue.M13cp constructs may be grown alone to produce custom phage (see "Phagemid growth with helper phage" and "Phagemid growth with helper plasmid")

DNA origami preparation

- Get 1 100 nM stock of desired scaffold, 1 500 nM stock of desired staple pool, 1 10x stock of folding buffer, and 1 130 mM stock of MgCl_2
- Get PCR tubes or PCR strip tubes (1 tube per reaction)
- For a 50- μL reaction, mix 5 μL scaffold, 10 μL staples, 5 μL folding buffer, 5 μL MgCl_2 , and 25 μL water (Volumes can be scaled for different reaction volumes) in a PCR tube.
- Pipette up and down to mix
- Centrifuge briefly to ensure all liquid is at the bottom of the tube

- Place tube(s) in PCR block and select 60-38_18hour ramp (65°C for 15 minutes, then cooling down from 60°C to 38°C at 1°C per 50 minutes, then hold at 4°C)

DNA origami purification

Gel purification

- Prepare a 2% agarose (TBE) gel, as outlined above, loading wells with DNA origami samples mixed with gel loading buffer
- Run and scan gel, as outlined above
- Prepare microcentrifuge tubes, label with sample name (1 per sample)
- Remove blue-light transilluminator lid
- Place saran wrap over blue-light transilluminator surface
- Place gel on saran wrap
- Place lid back on blue-light transilluminator
- Turn off room light
- Wear blue-light transilluminator viewing glasses
- Turn on blue-light transilluminator light
- Get 1 razor blade
- Cut out one band at a time and place in appropriate tube
- Turn of blue-light transilluminator
- Discard gel and saran wrap
- Wipe blue-light transilluminator surface dry
- Get disposable pestles (1 per sample)
- Muddle gel in tube using pestle until it is liquefied
- Get Freeze 'N Squeeze columns (1 per sample)
- Get microtube cutter
- Cut each microtube above liquefied gel and place open-side down in Freeze 'N Squeeze column
- Spin columns at 16000g for 30 seconds
- Discard column insert

PEG purification

- Prepare microcentrifuge tubes (1 per sample)
- Add 50 µL assembly product to appropriate tube
- Add 50 µL PEG precipitation buffer
- Pipette up and down to mix
- Place tubes in microcentrifuge and spin at 16000g for 25 minutes at 20°C, making note of tube orientation
- Remove tubes from centrifuge
- Carefully remove supernatant with a pipette
- Resuspend pellet (even if it is invisible) with 50 µL 1x folding buffer with magnesium

DNA origami imaging

1.5% Uranyl Formate stain preparation

- Get 1 5-mL beaker
- Add 2.5 mL water to beaker
- Microwave beaker for 1 minute
- Get another 5-mL beaker
- Weigh 30 mg uranyl formate in beaker
- Add stir bar to uranyl formate beaker
- Pour hot water from first beaker into uranyl formate beaker
- Place beaker on stir plate and cover
- Stir until uranyl formate is completely dissolved (approximately 5 minutes)
- Prepare 1 2-mL microcentrifuge tube and 2 1.5-mL microcentrifuge tubes
- Prepare 2 2-mL syringes and 2 syringe filters
- Using 1 syringe, pass the uranyl formate solution through 1 filter into the 2-mL tube
- Transfer 1 mL of filtered uranyl formate solution into a 1.5-mL tube
- Add 5 μ L 5 M NaOH to the transferred uranyl formate solution
- Vortex immediately and vigorously
- Using the second syringe, pass the NaOH-added uranyl formate solution through the second filter into the second 1.5-mL tube
- Store the twice-filtered solution covered in a drawer

Grid preparation

- Prepare a glass slide wrapped with parafilm
- Get carbon-coated 400-mesh formvar grids (1 for each sample) and transfer carbon-side-up onto slide using a Dumont N4 tweezer
- Glow-discharge grids
- Pick glow-discharged grids up with Dumont N4 anti-capillary tweezers and rest tweezers on a pipette tip box lid such that carbon-coated side of grids is facing up
- Prepare a piece of parafilm with 2 15- μ L droplets of 1x folding buffer with magnesium and 2 15- μ L droplets of freshly made 1.5% uranyl formate solution
- Apply 5 μ L of 25 ng/ μ L purified origami solution to carbon side of grid
- Allow 1.5 minutes for origami to deposit on grid
- Blot grid dry with Whatman paper
- Touch carbon side of grid to the first 1x folding buffer droplet
- Blot grid dry with Whatman paper
- Touch carbon side of grid to the second 1x folding buffer droplet
- Blot grid dry with Whatman paper
- Touch carbon side of grid to the first uranyl formate droplet
- Blot grid dry with Whatman paper
- Hold carbon side of grid to the second uranyl formate droplet for 45 seconds
- Blot grid dry with Whatman paper
- Allow grid to air dry for 10 minutes prior to imaging

Imaging

- Load grids into FEI TECNAI T12 transmission electron microscope
- Use imaging software to capture images at 26000x and 56000x magnification

Class Averaging of Origami Particles

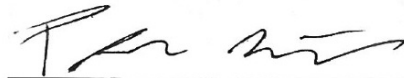
- Install EMAN2 software
- Import raw 56000x magnification micrographs into EMAN2
- Use EMAN2 particle-picker tool to manually box origami particles on micrograph
- Use EMAN2 class-averaging tool to generate reference-free class averages of the particle

Publishing Agreement

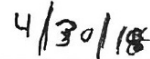
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