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VIEWS & NEWS

The Technologist's Dilemma

AU1 ▶ Benjamin J. Lesch[1,2] and M. Kyle Cromer[1–3,*] ◀ AU2

An exciting new approach for genome editing, dubbed PASTE, offers a means to site-specifically integrate large custom DNA sequences into the genome.

The field of genome editing is rapidly evolving with new tools seemingly developed every month. It is great to see creative solutions that expand the collective synthetic biology toolbox. However, with each new technology, we are faced with the same technologist's dilemma: Will the particular advantages of a new technology outweigh the potentially significant time and effort it may take to add it to the toolbox, multiplied by the distinct possibility that we simply won't be able to get the technology to work?

Early adopters may be able to snatch up the most obvious low-hanging fruit, but they may also spend an inordinate amount of time working out the kinks. With most new tools, researchers tend to opt for a wait-and-see approach, but every so often there is a new technology that makes us seriously re-evaluate. That appears to be the case regarding PASTE (*P*rogrammable *A*ddition via *S*ite-specific *T*argeting *E*lements), a new genome editing platform recently described in *Nature Biotechnology* by Abudayyeh, Gootenberg, and colleagues, which makes it possible to introduce enormous DNA sequences into specific locations in the genome.[1]

In its native form, the most basic function of the CRISPR-Cas9 enzyme is to cleave specific DNA sequences. This technology instantly transformed basic and translational biology by allowing scientists to perform genome-wide knockout screens and to cleave and correct pathogenic mutations. Owing to its efficiency and modularity—where a user-defined guide RNA sequence can be directed to extremely specific regions of the genome—the original Cas9 enzymes are now serving as the backbone on top of which the next generation of genome editing tools is being developed.

For instance, base editors[2] and prime editors[3] as well as CRISPR epigenetic activators and inhibitors[4] have been created by tethering additional enzymes to Cas9 to direct them to specific sites in the genome. Each platform has particular strengths and weaknesses in specific use cases (Table 1). However, a conspicuous issue with all of them is the inability to introduce sequences larger than a few kilobases.

To address this previously unmet need, Yarnall et al have described a new technology termed PASTE.[1] This platform combines the site specificity of CRISPR with the ability of natural transposable elements called integrases to insert large segments of DNA into the genome (Fig. 1). ◀ F1

In their native form, integrases are able to integrate cargo containing a specific DNA sequence (referred to as an *attP* attachment site) into regions of the genome containing bona fide or pseudo target sites (called an *attB* target site). The MIT group evaluated the efficiency of tethering various integrases to a prime editor backbone (which already comprises a Cas9 nickase tethered to a reverse transcriptase). This allows the prime editor to introduce an *attB* target site at custom locations throughout the genome and for the tethered integrase to drag-and-drop custom DNA templates containing *attP* attachment sites into these specific loci. ◀ T1

Table 1. Summary of strengths and weaknesses of genome modifying platforms

Platform	*Site specific*	*Gene KO*	*Gene correction*	*Gene addition*	*Permanent changes*	*Scarless*	*Involve DSBs*	*Active in nondividing cells*	*Insertion size limit*
Viral-mediated integration	No	No	No	Yes	Yes	No	No	Yes	10 kb for lentivirus
CRISPR nucleases	Yes	Yes	No	No	Yes	No	Yes	Yes	N/A
Base editors	Yes	Yes	Yes	No	Yes	Yes	No	Yes	~1–5 bp
Prime editors	Yes	Yes	Yes	No	Yes	Yes	No	Yes	<50 bp
CRISPRa	Yes	No	No	No	No	Yes	No	Yes	N/A
CRISPRi	Yes	No	No	No	No	Yes	No	Yes	N/A
Nucleases+repair template	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	4.5 kb for AAV
PASTE	Yes	Yes	Yes	Yes	Yes	No	No	Yes	>35 kb

AAV, adeno-associated virus; CRISPRa, CRISPR activator; CRISPRi, CRISPR inhibitor; DSB, double-strand break; KO, knockout; PASTE, *P*rogrammable *A*ddition via *S*ite-specific *T*argeting *E*lements.

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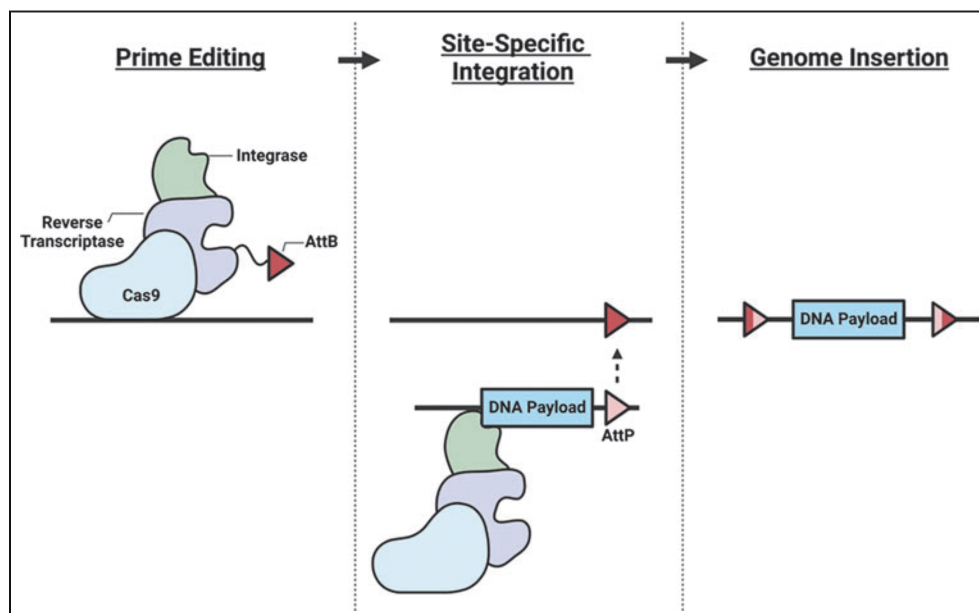

FIG. 1. How PASTE is used to insert large sequences into the genome.
The PASTE enzyme—comprising a Cas9 nickase, reverse transcriptase, and integrase—first introduces an *attB* target site into a specific location in the genome through the prime editing mechanism. The tethered integrase then drags the DNA payload containing an *attP* attachment site to the intended locus and inserts the payload into the prime-introduced *attB* site. PASTE, *P*rogrammable *A*ddition via *S*ite-specific *T*argeting *E*lements. (Credit: Benjamin J. Lesch; created by BioRender)

After several design–build–test cycles, Yarnall et al developed a system that could be delivered as a single transfection (containing the PASTE enzyme, Cas9 guide RNAs, and DNA integration template) and achieve integrations of up to 36kb at integration frequencies of 10–20%. Not only does this technology set a new high-water mark for the size limit of site-specific integration into the genome, but also it is able to introduce these templates in the absence of DNA double-strand breaks. This, therefore, avoids many of the significant safety risks, such as large-scale genomic deletions and rearrangements, that have been reported with traditional CRISPR-Cas nucleases.[5,6]

Furthermore, without relying on homology-directed repair, which may only integrate repair templates in cells that are actively cycling, PASTE has the potential to achieve higher integration frequencies in clinically relevant postmitotic cells such as neurons and myocytes as well as largely quiescent stem cell populations.

Although the development of PASTE certainly expands the genome editing toolbox and has distinct advantages over previous generations of CRISPR-based editors, there are limited use cases in which we currently need to integrate sequences larger than a few kilobases (cystic fibrosis and muscular dystrophy being the most obvious examples).[7,8] Furthermore, compared with the more established genome editing systems that have already successfully corrected disease in clinical trials, there remain questions about the ability of PASTE to edit clinically relevant stem cell populations without causing considerable toxicity and loss of stemness.

And for a system with as many moving parts as PASTE—a Cas9 nickase, reverse transcriptase, Bxb1 integrase, multiple guide RNAs, and DNA repair template—troubleshooting might be almost impossible without expert help. With nascent technologies, there are always a limited number of experts in the field and few resources are available online to provide assistance (e.g., there is currently only some code on GitHub to design PASTE-compatible guide RNAs).

Despite these concerns, PASTE addresses many of the current bottlenecks for site-specific integration in both dividing and nondividing cells without the risks associated with DNA double-strand breaks. There will likely be a growing number of use cases for this technology as genome engineers begin multiplexing editing events and introducing increasingly large pieces of DNA code into the genome.

In the future, it seems likely that either *this* version of PASTE, the *next* version of PASTE, or something similar but fundamentally different from PASTE will be widely adopted by genome editors. In terms of this specific dilemma—whether to become an early adopter of PASTE version 1—we plan to wait and see if the technology matures and disseminates. This should serve as a reasonable proxy for its modularity and ease of use.

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