UNIVERSITY OF CALIFORNIA SAN DIEGO

In search of memory: can eDNA be used in storage sediment samples from Borikén?

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by

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The Thesis of Javier Jomar García-Colón is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

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ABSTRACT OF THE THESIS

In search of memory: can eDNA be used in storage sediment samples from Borikén?

by

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Master of Arts in Anthropology

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The application of environmental DNA (eDNA) methods in tropical environments has been limited due to assumptions of poor preservation of genomic material given drastic climate variations (high temperature, soil acidity, high precipitation). However, improvements in technologies and methods (Orlando et al 2021; Shapiro et al 2019; Rizzi et al 2012; Mumy et al 2004; Miller et al 1999) have made it possible to extract and sequence highly degraded DNA (aDNA) from some tropical environments, drastically changing our previous understanding of DNA preservation capacities. Addressing the need to reclaim material from archaeological

collections, I visited the Paleogenomics lab at the University of California Santa Cruz (UCSC) and performed DNA extraction experiments on sediment samples from three archaeological sites in Borikén (Puerto Rico) - Tierras Nuevas, Cueva María de la Cruz, and Puerto del Rey - in search of the presence of aDNA, and the levels of degradation they present. The ability to extract genomic material from tropical sediments, could provide high value proxy data to inquiry past climate, migration patterns, biomonitoring, and landscape formation processes, in addition to providing mitigation strategies to deal with the ongoing curation crisis exacerbated by the colonial system.

Keywords: molecular archaeology, tropical climate, eDNA, curation crisis

Introduction

In a verse from her song, “Contra todo", Grammy award winning singer and composer Ilé Cabra, states - “Yo soy terreno invadido, naturaleza robada” (“I am invaded soil, stolen land”). This verse aptly captures the nature of the relationship that we Boricuas (people of Borikén/Puerto Rico) have with the island; a connection that reinforces our identity without a distinction from the land. We are de aquí, de e’jta tierra (from this land); a land akin to an SD card, a preserver of memory that can be made accessible with environmental DNA technologies.

The application of environmental DNA (eDNA) methods in tropical environments has been limited due to assumptions of poor preservation of genomic material given to drastic climate variations (high temperature, soil acidity, high precipitation) and reinforced by Western/Colonial mindsets. Laboratory work has drastically changed since the development of Next Generation Sequencing (NGS) technologies and now highly degraded DNA (aDNA) is extractable and sequenceable for research (Orlando et al 2021,Taberlet et al 2018). The application of these technologies to archaeological context materials has the potential to access data that was not previously available (Hassan et al 2022; Jia et al 2022; Jansson et al 2019; Eisenmann et al 2018; Han et al 2017).

Not unsurprisingly, the struggles of the ongoing curation crisis of archaeological deposits around the world, are exacerbated for geographies marked by colonization. The application of eDNA technologies could mitigate this crisis by offering less invasive ways to extract data for

research. The application of the technologies to existing collections also poses a mitigation strategy for the crisis (Schiappacasse 2019; Rizzi et al 2012).

For this project, the sediment samples were previously collected for geoarchaeological analysis during archaeological excavations from 2009-2019. This is important to consider for we expect to have a high level of contamination and degradation of DNA. This research has a primary question, *can we extract eDNA from archived sediment samples?*As a way to have a basic understanding of how DNA behaves under these conditions and further develop protocols for possible application of these techniques on tropical environments. These techniques could be of use for biomonitoring, environmental reconstructions, and archaeological research (Pascher et al 2022; Mohammed et al 2022; Orr et al 2021; Wibowo et al 2021; Zhang et al 2021, 2020; Nägele et al 2020; Suleymanov et al 2020; Sun et al 2020; Siles et al 2018; Warinner et al 2017; Watzinger et al 2015; Nerlich et al 2009).

What is DNA?

Deoxyribonucleic Acid (DNA) is a biopolymer composed of nucleotides. Each nucleotide is composed of a sugar (deoxyribose), a phosphate and a nitrogenous base: these could be adenine(A), guanine (G), cytosine (C), or thymine (T). In living cells, DNA exists as double-stranded, where two complementary DNA polymers bond through hydrogen bonds between A and T and between G and C respectively (Figure 1; Campana, et. al, 2013). Codons (groups of three nucleotides) sequence the instructions for protein assembly (coding DNA), these regions are called genes. In eukaryotic organisms the majority of DNA (>99%) is located in the nucleus as a mixture of proteins and DNA called chromosomes (Figure 2). Eukaryotic organisms

also possess mitochondria (chemical energy generators) in which a short circular strand of DNA exists (mtDNA). This means there can be thousands of copies of the mitochondrial genome per eukaryotic cell (Matisoo-Smith, E. 2008).

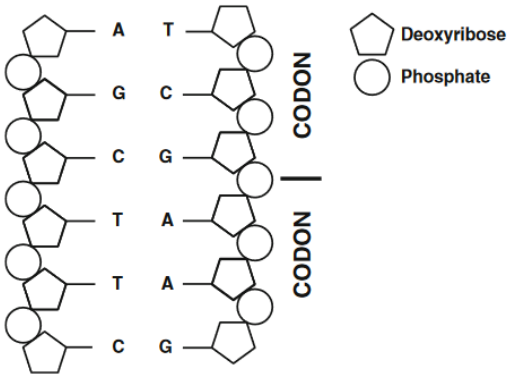


Figure 1. Some groups of codons (genes) can sequence the instructions for protein assembly; also known as coding DNA. (Campana, et. al, 2013).

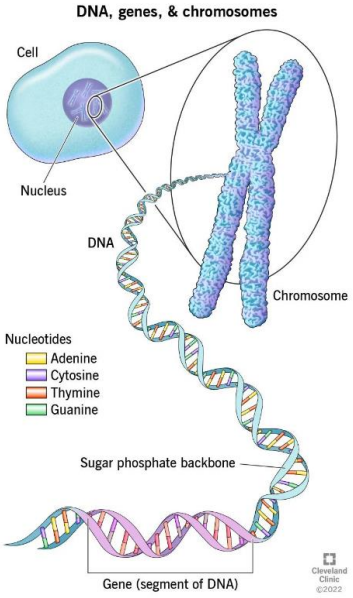


Figure 2. Gene is composed of DNA, groups of genes and proteins form chromosomes. In living eukaryotic organisms each cell nucleus contains two copies of each autosome (non sex chromosomes). The number of autosomes varies by species. In addition, cell nuclei may contain a pair of sex chromosomes depending on the species.

Where can you find environmental DNA? How can it be used?

All organisms leave traces of DNA in their surrounding environments through the shedding of skin cells and hair or by the discharge of feces and urine.The complex mixture of DNA from different organisms in nature is called environmental DNA (eDNA). It can be extracted from environmental samples (e.i. soil, sediments, water, coprolites,air, etc.), and it can be used to obtain taxonomic and/or functional information of the organisms and biomonitoring (Taberlet et al 2018). These traces of DNA can be found in the environment as intracellular (originating from living cells or multicellular organisms) or extracellular (results from cell death and subsequent destruction of cell structures) DNA.

Extracellular DNA is susceptible to degradation and the rate in which this happens is dependent on the environment, the soil pH, the temperature, the soil microbiome, the sediment mineral composition (Taberlet et al 2018), and it is best stable (preserved) in cold, dry environments with slightly basic soil and high salt concentrations (Campana, et.al. 2013). The degraded fragments of DNA or genomic material obtained from anything other than fresh tissue is called ancient DNA (aDNA) (Zhang et al 2021; Matisoo-Smith 2008).

Since the awareness of the possibility to apply DNA analysis to archaeological materials during the 1980s, the first report of an extraction protocol for eDNA from sediments (Ogram et.al 1987) and the development of metagenomics in the late 1990s and early 2000s (Giovannoni et al. 1990; Handelsman et al. 1998; Willerslev et al. 2003; Shendure & Ji 2008), insights on population migration, location, timing and processes of domestication, environmental and landscape formation and change processes, biodiversity and biomonitoring, and other research

areas have become more accessible. The identification of species or taxa present on environmental samples (eDNA or aDNA, it depends on the level of degradation; see Figure 3) can be achieved mainly by two approaches, both based on PCR (polymerase chain reaction). Quantitative PCR to determine the presence or absence of a single species (1) and metabarcoding to identify the many taxa available on the environmental sample (2), which is usually approached through shotgun sequencing - the random sequencing (reading or amplification) of DNA fragments - generally using NGS (next generation sequencing), a technologie that pushed forward the discipline (Pierre Taberlet, Aurélie Bonin, Lucie Zinger, Eric Coissac. 2018; Nicolas Arning and Daniel Wilson. 2020., Arriola, L. A., Cooper, A., & Weyrich, L. S. 2020., Ávila-Arcos, M. C., de la Fuente Castro, C., Nieves-Colón, M. A., & Raghavan, M. 2022).

Thousands of years old DNA has been successfully extracted from permafrost (permanently frozen) sediments (Slon et al 2017, Pedersen et al 2016; Willerslev et al 2014; Meyer et al 2014; Carrigg et al 2007; Hebert et al 2003). Likewise, traces of aDNA have also been found in non-frozen sediments and soils, even in the absence of macrofossils (Nieves-Colón et al 2022; Pérez et al 2022; Capo et al 2022; Crump 2021; Arning et al 2020; Arriola et al 2020; Berkelmann et al 2020; Domain et al 2020; Nieves-Colón et al 2019, 2018; Foley et al 2011; Haile, et.al, 2007). Based on laboratory and field experiments, acidic and warm conditions are known to promote DNA hydrolysis (the rupture of chemical bonds due to water molecules) and are non-conductive to long-term DNA preservation (Dommain, et.al. 2018). This has limited the application of these techniques to tropical regions of the planet, although these areas hold the greatest biodiversity on Earth containing about three quarters of all species. These technologies have the potential to open a window into present and past composition of ecosystems, climate,

etc (Ávila-Arcos et al 2022; Liu et al 2022; Lipson, et.al, 2021; Blong et at 2021; Li et al 2021; Li et al 2020; Khomutova et al 2019; Lacerda-Júnior et al 2019; Benn-Torres 2019, 2018; Dommain, et.al, 2018; Bohmann et al 2014;Boessenkool et al 2013; Rivera-Collazo, 2015; Haile, et.al, 2007).

In 2021 the *sedaDNA Scientific Society,* established as a collaborative effort to promote best practices and increase collaborations between research groups, formed the *African sedaDNA Working Group*. They shared a paper published in 2018 where they address the challenges of applying sedimentary aDNA research methods in an unaltered tropical forest swamp in Uganda (Dommain, et.al. 2018). Their conclusions indicated that (1)metagenomic sedimentary DNA can provide valuable insights into past tropical biodiversity, but that further development of genomic databases are necessary to provide robust, detailed community reconstruction, (2) the actual taxonomic composition and resolution of DNA recovery would likely change due to little sequencing of tropical species genomes, and (3) that until taxonomically representative databases are generated and further DNA taphonomic studies are completed, sedaDNA cannot be fully utilized for biodiversity studies in the tropics (Dommain et al., 2018).

Other studies (Bremond et al., 2017; Gomez Cabrera et al., 2019; Mergeay et al., 2007; Epp et al., 2010, 2011; Stoof-Leichsenring et al., 2012; Boessenkool et al., 2014; Bremond et al., 2017; Ávila-Arcos et al., 2022; Berkelmann et al., 2020; Borry et al., 2020; Bravo-Lopez et al., 2020;Gutiérrez-García et al., 2014; Kehlmaier et al., 2017; Lewis et al., 2012; Nieves-Colón et al., 2019; Haile 2011; Hagan et al 2019) have shown that DNA can also persist in tropical lacustrine and marine sediments under high temperatures for hundreds to thousands of years. Yet

a better understanding of the processes of transport, deposition, preservation and degradation of genetic material is needed, hence DNA preservation is dependent on the different environmental conditions (Dommain et al., 2018).

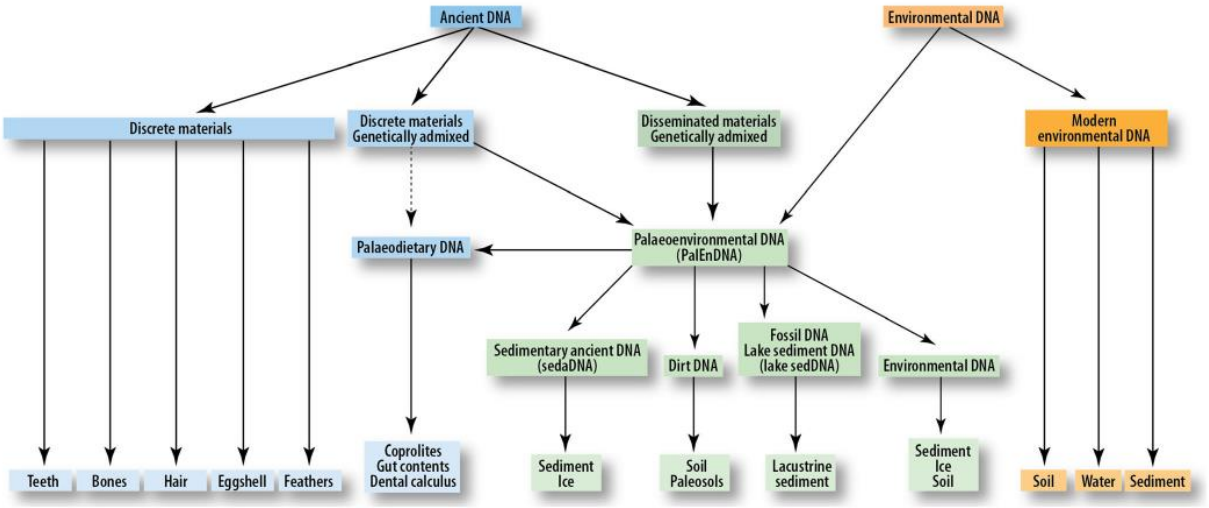


Figure 3: Ancient and Environmental DNA, where to find them (Rawlence, et al., 2014).

Following this train of thought, the gap of information regarding the degradation and preservation processes of environmental DNA in tropical settings is still not well understood. But it does not mean that it is impossible for DNA to be preserved in these settings. It is dependable on the context of those sediments (caves, lakes, etc) and the sampling methods, hence the contamination of the samples with modern DNA is possible if the correct protocols are not followed (Capo et al 2022; Crump et al 2021; Epp et al 2019; Eisenmann et al 2018; Straube et al 2013; Dabney et al 2013; Bollongino et al 2008; Bürgmann et al 2001).

Molecular Biology, Sediments, and Caribbean Archaeology

The integration of molecular biology techniques for DNA extraction of environmental samples in archaeological contexts can contribute to reconstruction of past environments and the ability to track environmental change through time, population origins and dispersals, domestication and other anthropogenic processes (Matisoo-Smith, 2008). For archaeological research, the application of these technologies can challenge master narratives established during the 19th and early 20th centuries (Martin-Laurent et al 2001; Miller et al 1999;**)**; and although the identity of social groups cannot be reflected or demonstrated by genetic diversity, the reconstruction of the environment and the ecosystems surrounding these communities can better promote a more regional research focus of human decision making processes (Hassan et al 2022; Vernot et al 2021; Cajete 2020; Domain et al 2020; Sun et al 2020; Wiscovitch-Russo et al 2020; Epp et al 2019; Hagan et al 2019; Brather 2016;Gansauge et al 2013, 2020; Smith 2011; Bürgmann et al 2001).

In the Caribbean, just like in sub-Saharan Africa and other tropical regions, little genomic investigations of the ancient environment, biodiversity, and peoples have been produced. Our stories, disrupted by past and modern demographic transformations, colonialism, imperialism, enslavement, and socio-political reorganization could benefit from the advent of genome-wide DNA technologies that hold the promise for a deeper holistic understanding of our environment and how humans interacted and reacted on these scenarios (Lipson et al 2022). As a dataset, eDNA - and aDNA - can be used as proxies in combination with radiocarbon dates, ethnobotanical remains, zooarchaeology and other datasets in order to address deep-time anthropogenic dynamics and long-term environmental change (Rivera-Collazo 2015; Rivera-

Collazo et al 2018; Eaton et al 2021; Dussex et al 2021; Eisenmann et al 2018; Gougoulias et al 2014; Griffin et al 2019; Grund et al 2014; Gutiérrez-García et al 2014; Jia et al 2022; Stilling et al 2014; Smith 2011).

The Anthropocene, suggested by Rivera-Collazo (2015) to be used interchangeably as a synonym of the Holocene, refers to the period after 10ka were human impacts transformed the environments and created landscapes useful for them. The landscapes we appreciate today (in modernity) are not the same as past individuals experienced since humans continue to transform their surroundings. In the Caribbean, the impacts of humans and environmental changes are considered significant only after the European colonization ca. 1490. But these changes and environmental information accumulates on the landscape as palimpsests of data, and the application of molecular biology could provide insights to information otherwise invisible in the archaeological record (Massilani et al 2021; Dussex et al 2021; Fernandes et al 2020; García del Amo et al 2020; Zhang et al 2020; Sun et al 2020; Ficetola et al 2019; Rivera-Collazo et al 2018; Siles et al 2018; Rivera-Collazo 2015).

Technology developments on eDNA extraction provide the opportunity to address those palimpsests of information on tropical environments. Giving us another dataset useful for past environmental reconstructions and so, better understand human decision-making processes. The ability to extract genomic material from sediments (sedaDNA) pushes the boundaries of what we know about the modification of island landscapes by providing us with a resource that is less destructive to the environmental and archaeological setting. Similar to the metaphor of tearing pages of a book that can never be recovered to address the practice of archaeological digging, I make a comparison of the sediments (the earth beneath our feet, *ej'ta tierra)* with a memory

card. This comparison comes from the capacity of the soils to preserve memory through remnants of eDNA (Foucher et al 2020) and ecofacts (Li et al 2021; Dussex et al 2021; Demko et al 2021; Eaton et al 2021; Escalera-Reyes 2020; Fernandes et al 2020; Foucher et al 2020; Frindte et al 2020; Lyons 2020; Griffin et al 2019; Davidson et al 2018; Dutta et al 2016; Deng et al 2014; Demkina et al 2008).

Thus, to access that memory stick we must increase our comprehension of eDNA behavior in tropical settings. Including the way it behaves in different scenarios, environments, and collection methods. Hence eDNA can be unstable and the samples can be easily contaminated, the sampling, extraction and analyzing methods should be specific for the settings and contexts under research. Because of this, this project functions as an initial experiment to start understanding eDNA behavior and start building methods to better integrate these techniques.

The Curation Crisis

The curation crisis, that is, too much stuff with too little research, analysis, and public interpretation (Allen and Ford 2019) is a complex issue that affects many countries, particularly those with a colonial history. In December 2005 a nonprofit organization from Washington D.C., (Heritage Preservation) made the first comprehensive survey of U.S. archaeological collections held in the public trust and found that roughly 20% of them need better care and that more than 40% of bulk cataloged collections have an unknown status, meaning that they had not recently been inspected by archaeological staff (Bayawa 2007). Insufficient space, inadequate funding management, and a growing collection due to Federal and State laws for archaeological

surveys before construction work has unbalanced the conservation system for more than 30 years, risking the artifacts research and educational value (Childs 2022; Allen et al, 2019; Bawaya 2007). Some scholars suggest the reclamation of these ‘old’ collections to mitigate the effects of the crisis (Childs 2022; Schiappacasse 2019; Williams et al 2019; Allen and Ford 2019; Benden et al 2019; Bremong et al 2017; Bawaya 2007)

In the Caribbean, colonialism and imperialism have worsened the archaeological curation crisis. During the eighteenth and nineteenth centuries, the art of collecting was intertwined with antiquarianism and some of those collections eventually found their way to museums all over the world (Schiappacasse 2019). The discarding of material that is considered to hold little research value (e.i. soil samples) has become another threat to these ‘orphan’ collections - the term orphan or orphaned connote a lack of intellectual guidance, no longer accessible or actively contributing to archaeological research (Williams et al 2019). Now, the discipline of archaeology has many tools, like environmental DNA, to ask new questions of existing archaeological collections and new methodological and theoretical approaches that can be applied in order to reclaim these valuable data from these collections (Schiappacasse 2019; Allen and Ford 2019; Bremong et al 2017; Bawaya 2007 Camp 2003;).

Case Study

I will use my training on the application of eDNA technologies on sediment samples from the archaeological collection at the Human Ecology Lab at the University of California in

San Diego (UCSD) as my case study. With this, I intend to answer an important question: *are there sequenceable fragments of DNA on these sediment samples?* As mentioned earlier, the improvement of methods for the extraction of highly degraded DNA (Kapp et al 2021; Gansauge et al 2020; Dabney et al 2019; Epp et al 2019; Hagan et al 2019; Gansauge et al 2013; Haile et al 2011; Bollongino et al 2008; Bürgmann et al 2001;) and better insights on the preservation of DNA in tropical climate environments (Dommain et al 2020; Ficetola et al 2019; Bremond et al 2017; Gutiérrez-García et al 2014; Boessenkool et al 2013; Stoof-Leichsenring et al 2012; Epp et al 2011; Epp et al 2010; Mergeay et al 2007) provide a strong framework for the application of these techniques in tropical contexts.

If the null hypothesis is confirmed, where no DNA is successfully extracted from the sediment samples, I would first suggest the experiments be repeated to address possible errors on the different extraction steps. There should also be experimentation between different extraction kits and genetic library protocols to know which combination has the best results. In the case where the alternative hypothesis is confirmed and genomic material (DNA) is successfully extracted from the samples, I suggest addressing the sediment samples on archaeological collections to test for DNA before discarding them.

The datasets

The sediment samples used (n=27) belong to three archaeological sites on the island of Borikén (Puerto Rico): Tierras Nuevas (TN; n=14), Cueva María de la Cruz (CMLC; n=11), and La Gallera (LG; n=2). The sites, respectively located in the municipalities of Manatí, Loíza, and

the boundaries of Ceiba and Fajardo (Figure4&5), are all located in coastal tropical environments (Rivera-Collazo et al 2019; Oliver et al 2012a, 2012b; Muñoz-Guevara 2020). The samples were collected for geoarchaeological analysis; hence we are expecting a high level of human contamination due to inadequate sampling methodology.

Tierras Nuevas is a site located on the eastern side of the Grande Manatí river mouth on an aeolianite terrace on the north of the island. The site is considered the only surviving coastal archaeological site with ball courts in Puerto Rico given the impact of agriculture over most of the other reported sites (Rivera-Collazo et al 2019-report in progress). Approximately 70 km from this site we find Cueva María de la Cruz, a cave located near the Grande de Loíza river around 1500m inland from the coast. The last site, La Gallera, is located at the east of the island, in the vicinity of the Damajagua river near the Puerto del Rey marine (Muñoz-Guevara, L. V. 2020).

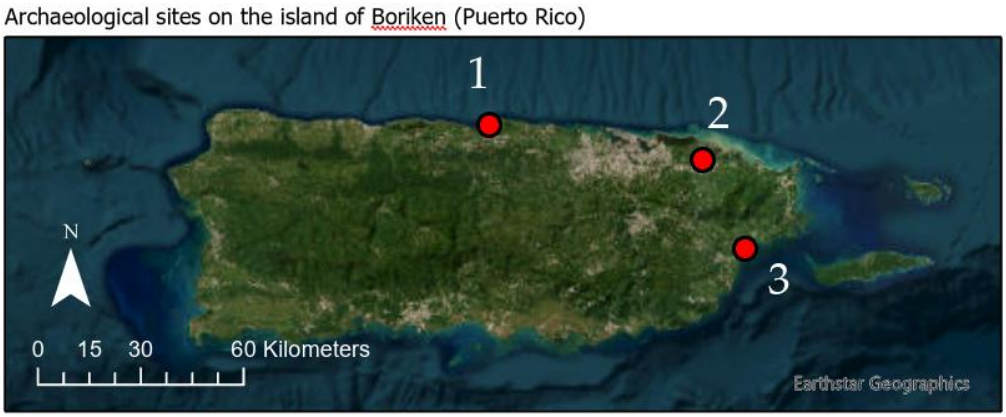


Figure 4: The distribution of the archaeological sites on the island. 1. Tierras Nuevas, Manatí. 2. Cueva María de la Cruz, Loiza. 3. La Gallera, Ceiba.

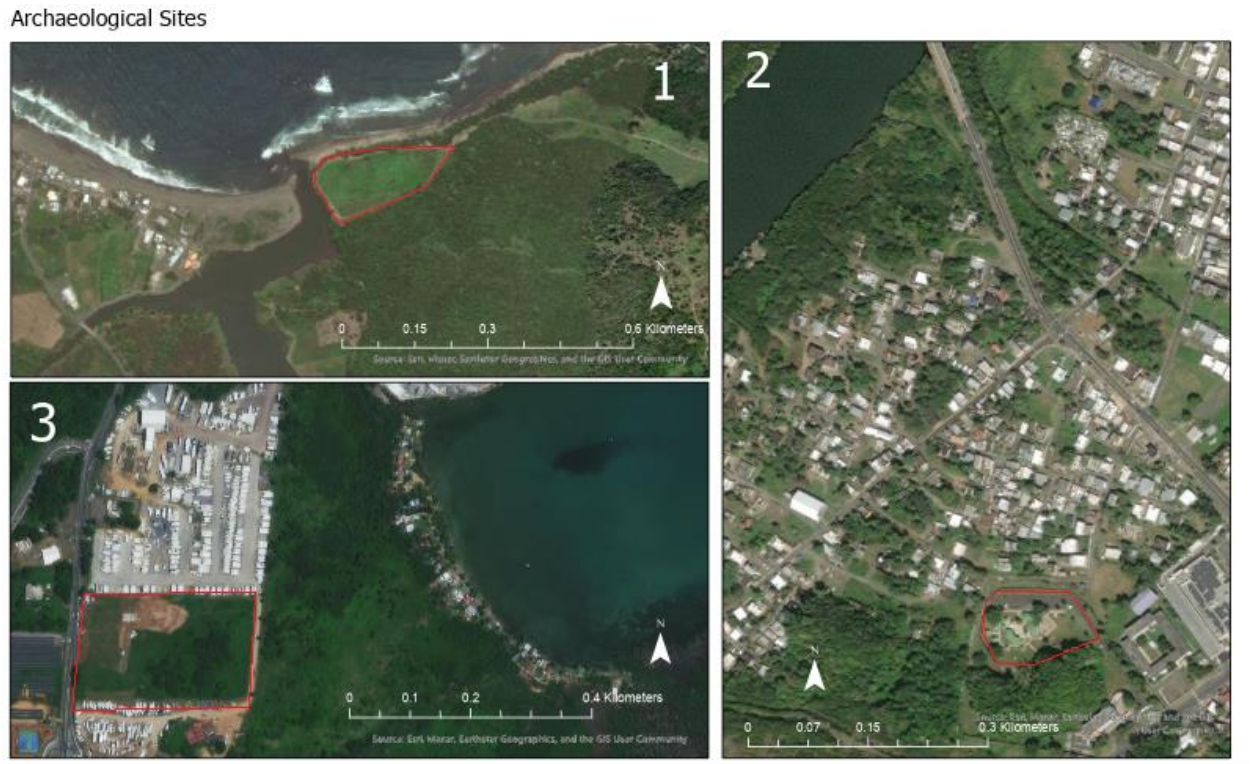


Figure 5: The archaeological sites. 1. Tierras Nuevas, Manatí. 2. Cueva María de la Cruz, Loíza. 3. La Gallera, Ceiba.

Methods

All sediment samples were stored at the Human Ecology Lab facilities at UCSD. Under the supervision of Dr. Isabel Rivera-Collazo, the sediments resided in the lab's fridge at 6ºC. Approximately 3 g were collected for each sample, and they were transported to UC Santa Cruz's (UCSC) Paleogenomics Lab, where Dr. Rachel Meyer mentored me on the techniques and methods used in environmental DNA and guided me through the entire process.

For the first step, DNA extraction, approximately 50 mg of each sediment sample were mixed with a lysis buffer in a 2 ml screw cap tube to tear the cell apart and free the genomic material. This happens overnight while incubating at 37 ºC. Next day, the Rohland protocol was followed to extract and separate the genomic material from the sediment. Quantification using

the QuietFlex Fluorometer and Agilents 5200 Fragment Analyzer (See fragment Analyzer data reports) were performed and DNA was successfully extracted from the sediments. The DNeasy PowerSoil Pro Kit from Qiagen was selected in order to increase the efficiency in the isolation of DNA. After this, single-stranded DNA library preparation was realized following the Spotlight protocol (Kapp et al 2021).

Kapp's Spotlight protocol was designed thinking of applying it on the extraction of very degraded DNA from rootless hairs on forensic settings. Considering that the sediment samples were taken for geoarchaeological analysis - not for genomic analysis -, spent some time in the HELab's fridge storage and they come from a tropical climate context, among other issues, we expect that the DNA present is highly degraded and to present a high level of modern DNA contamination (See Table 1). Quantitative PCR, to know the amount of DNA present in the sample, and Indexing PCR proceeded in order to attach an adapter design to interact with a specific sequencing platform, finalizing the genomic library preparation process for 16 samples having them ready for sequencing. Quantification using the QuietFlex Fluorometer and Agilents 5200 Fragment Analyzer was performed again (See Table 2).

Back at UCSD, I received guidance from my mentor Dr. Kelly Fox and Dr. Elsa Molina - director of the Next Generation Sequencing Core at the Salk Institute - in order to better address the data collected during the experiment. In collaboration with personnel from Agilent, we have identified the presence of highly degraded DNA on some of the samples, yet it is still impossible to say if they come from ancient or degraded modern DNA. To fully know this, sequencing must be performed. Sequencing using Illumina's NextSeq was recommended because it is cost-

effective and it has the option to do 2x100bp reads - which means each library is sequenced 2 times, from 5' to 3' and reverse. This would give us a basic overview of the genomic material present in each library.

The sequencing raw data was shared through SFTP server, from where it was downloaded and uploaded into mBRAVE (https://mbrave.net/), a multiplex barcode research and visualization environment (Ratnasingham 2019). This platform is a cloud based data storage and analytics platform with standardized pipelines and a sophisticated web interface for transforming raw high-throughput sequencing (HTS) data into biological insights by integrating analytical methods and links to the BOLD system (Barcode of Life Data-Bold Systems v4) for reference datasets (Ratnasingham 2019).

Table 1: Sample description and values for Qubit and Fragment Analysis after extraction (green) and after library preparation (yellow). Only 16 of the initial 27 samples were selected for sequencing because as can be seen from the Qubit data, after library preparation some samples did not present enough DNA. Samples 18 and 27 are the same samples, yet present different values. This could suggest possible contamination during the laboratory experimentation. In addition, you can see sample 30 (control) which indicates Qubit value after library preparation, which also suggests contamination of the samples.

Sample	Archaeological Site	Description	Qubit (ng/uL) after extraction	Fragment Analyzer *Peaks*	Fragment Analyzer (ng/uL)	Qubit (ng/uL) after Library Preparation	Fragment Analyzer *Peaks*	Fragment Analyzer (ng/uL)
S1	La Gallera	45cmbd	5.2	56	2.1568	41.8	203	45.0733
S2	La Gallera	4cmbd	2.36	51	0.906	5.04	192	89.0562
S6	TNAP Bloque 1	Strata B 23-30cmbd	3.33	57	1.4423	8.92	200	8.6786
S7	TNAP Bloque 1	Strata C 30-35cmbd	4.46	55	1.3614	<0.05	-	-
S8	TNAP Bloque 1	Strata D1	2.16	51	0.5948	<0.05	-	-
S9	TNAP Bloque 1	Strata D2	2.97	52	0.9102	4.02	194	1.5619

S10	TNAP Bloque 1	Strata D3	2.22	51	0.6063	4.89	192	276.8994
S11	TNAP Bloque 1	Strata D4	2.81	54	0.8126	<0.05	-	-
S12	TNAP Bloque 1	Strata E	1.77	51	0.3594	<0.05	-	-
S13	TNAP Bloque 1	Strata F	0.844	449	0.2761	<0.05	-	-
S14	TNAP Bloque 1	Strata G	0.636	50	0.2135	<0.05	-	-
S3	TNAP Bloque 2	Level 4	1.01	51	0.2225	36.1	207	39.9557
S4	TNAP Bloque 2	Level 5	1.23	52	0.4465	39.7	205	37.3056
S5	TNAP Bloque 2	Level 6	0.714	52	0.512	16.1	203	7.1917
S27	TNAP Bloque 2	Level 1	6.79	67	32.4908	2	221	0.737
S28	TNAP Bloque 2	Level 3	2.06	51	0.5408	2.43	221	0.5493
S29	TNAP Bloque 2	Level 2	4.14	64	2.2538	5.51	222	2.7361
S15	CMDLC	level 6 unscreened soil	3.05	436	0.4048	0.577	error	-
S16	CMDLC	Strata A	3.05	70	1.4599	28.8	210	37.2652
S17	CMDLC	Strata B	2.55	61	1.1381	<0.05	-	-
S18	CMDLC	Strata C	0.999	55	0.5779	<0.05	-	-
S19	CMDLC	Strata C arriba	1.64	56	0.6581	<0.05	-	-
S20	CMDLC	Strata C abajo	0.809	58	0.3271	<0.05	-	-
S21	CMDLC	Strata D	0.24	50	0.0689	<0.05	-	-
S22	CMDLC	Strata E; associated to manatee bone	0.929	55	0.6006	<0.05	-	-
S23	CMDLC	Strata E	0.936	56	0.3672	<0.05	-	-
S24	CMDLC	Strata E; fondo del elemento	1.36	-	-	<0.05	-	-
S25	CMDLC	Strata C	1.08	56	0.5308	5.56	221	2.4846
S26	CMDLC	Superior Strata 9	11.6	28	0.2515	0.849	error	-
S30	BLANK	Control	<0.05	-	-	0.586	142	0.3027

Table 2: Library samples description, Qubit concentration, Fragment Analyzer peaks and concentration recorded. Sample 15 and 26 did not seem to have sufficient DNA for the Fragment Analyzer to record. This could suggest extreme levels of DNA degradation for these samples. Yet, sample 15 was validated by the mBRAVE platform while sample 26 was not. Similarly, for sample 30 (control), only the first read (R1) was accepted by the mBRAVE platform. The reasons for the reasoning of the platform to accept or reject sample data is still unclear.

Sample #	Archaeological Site	Description	Depth (cmbd)	Qubit (ng/uL)	Fragment Analyzer *Peaks*	Fragment Analyzer (ng/uL)
1	La Gallera	Soil in related to human burial	45	41.8	203	45.0733
2	La Gallera	Sample from inside ceramic	4	5.04	192	89.0562
6	TNAP Bloque 1	Strata B	20-25	8.92	200	8.6786
9	TNAP Bloque 1	Strata D2	60-65	4.02	194	1.5619
10	TNAP Bloque 1	Strata D3	65-70	4.89	192	276.8994
3	TNAP Bloque 2	Level 4	26-34	36.1	207	39.9557
4	TNAP Bloque 2	Level 5	40-48	39.7	205	37.3056
5	TNAP Bloque 2	Level 6	54-59	16.1	203	7.1917
27	TNAP Bloque 2	Level 1	0-4	2	221	0.737
28	TNAP Bloque 2	Level 3	18-23	2.43	221	0.5493
29	TNAP Bloque 2	Level 2	8-16	5.51	222	2.7361
15	CMDLC	Level 6 - unscreened bulk soil	60	0.577	error	-
16	CMDLC	Strata A	0-20	28.8	210	37.2652
25	CMDLC	Strata C	60-90	5.56	221	2.4846
26	CMDLC	Strata C9	0-13	0.849	error	-
30	BLANK	BLANK	BLANK	0.586	142	0.3027

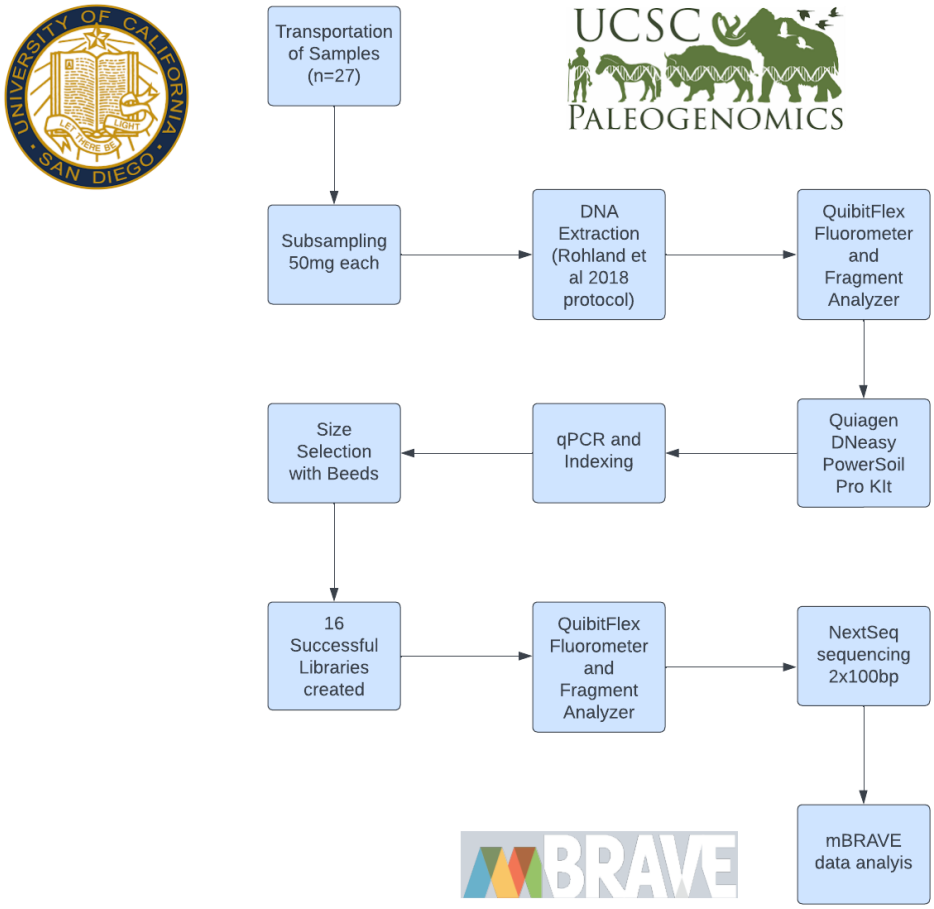


Figure 6: **Workflow chart:** In order to experiment on DNA extraction from sediments, the samples had to be transported from UCSD to UCSC to use the Paleogenomics Lab facilities. Extraction (Rohland et al 2018) and genomic library preparation protocols (Kapp et al 2021) were consulted with Dr. Rachel Meyer (UCSC) to increase the chances of extracting genomic material. The Spotlight protocol was designed to extract highly degraded DNA from hair in forensic settings. Hence the genomic material found on the sediment samples were very degraded, this library preparation protocol seemed like the best technique to apply. Sequencing was performed using Ilumina's NextSeq sequencer and 2x100bp reads were performed. Finally, the data analysis was conducted using the mBRAVE metagenomic platform.

Results

The extraction of genomic material from the genomic libraries from 15 of the 16 libraries was successful. As expected, the sequences present high levels of degradation with 202.357 as an average pick size recorded by the fragment analyzer. For the bioinformatics data analysis portion of research, I used the mBRAVE platform which filtered and processes the data automatically once all the sequence data was uploaded to the system. Sample 26 was unable to upload into mBRAVE because of an *unable to validate the sample* error, while for sample 30 only the first read (R1) was uploaded successfully. Sample 26 had no fragment analyzer peaks recorded and a 0.849 Qubit concentration value, indicating that the very small amount of DNA presented high levels of degradation.

The rest of the data set was successfully uploaded to the software; and although they present a high number of reads - which reflects the raw sequence data obtained from each metagenomic sample - have not yet confirmed the presence of any Operational Taxonomic Unit (OTU). In other words, it was unable to find clusters of sequence similarities that could represent a taxonomic unit of a species or genus (Figure 7**)**.

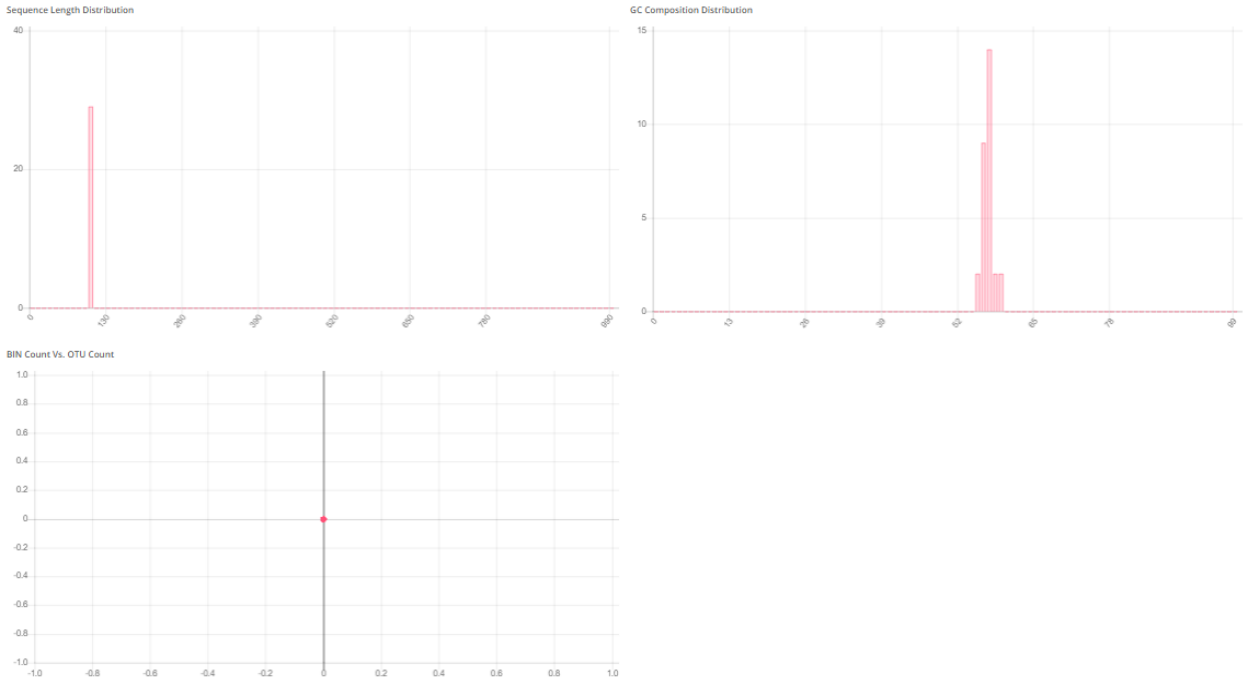


Figure 7- For the overall samples, the mBRAVE platform was unable to identify any OTU. Indicating that although there was genomic material sequenced, they are far too degraded for them to be useful for taxonomic matching.

Conclusion

As a recap, the application of environmental DNA is in constant improvement. Since the development of PCR and NGS technologies the amount of research projects engaging with these methods have increased exponentially (Taberlet et al 2018;**)** although focusing on temperate and polar regions of the globe. However, the application of these methods on tropical environments demands more attention in order to close the knowledge gap exacerbated by global north biases and colonia/imperial legacies. Therefore, focused regional research is needed to better

understand the taphonomy of sedimentary DNA considering diverse sampling regions in tropical environments (Gagelidze et al 2018).

The use of the Spotlight genomic library preparation protocol (Kapp et al 2021) seems to be a useful tool when working with highly degraded fragments of DNA. The continued development of methods and protocols like this must continue in order to improve biomolecular analysis. The bioinformatic analysis of the sequences determined reads for the genomic material, although it was unable to match OTU's. I understand this in two ways: first, it could be that hence the levels of degradation of the samples were drastic, the platform simply cannot find taxonomic matches for us to identify. Yet, considering that research biases have excluded the tropical regions of the planet, there could be a limited database collection for the platform to make those taxonomic connections.

More research must be done in order to improve the analysis of environmental DNA in tropical regions. We can say that a portion of the hypothesis was somehow confirmed, we were able to extract genomic material from the archived sediment samples. Improvements in protocols of collection and sampling must be considered to minimize the possibilities of contamination with the use of gloves and disinfected equipment. Storage of the samples in an ice cooler or cold environment after collection must be considered unless DNA extraction is conducted at the time of sample collection.

A lot is yet unknown, and although the discipline has improved exponentially, there is still space for improvement. The biases regarding the possible preservation of DNA in tropical

contexts ignore micro-climate regions that could have better possibilities of eDNA preservation. But unless we do the experiments, we will simply continue to replicate over simplistic generalizations that marginalize these regions once again. The soil preserves our memory, we just need to find the correct way to access it.

Future Considerations

Due to limitations during this MA research project, there are analyses yet to be applied to these samples - like degradation pattern analysis, which could give us better insight on the quality of the DNA samples and could help us better determine if the genomic material could be considered ancient or just highly degraded/contamination. In addition, I would like to continue my academic development in environmental DNA analysis considering different sampling methods in different tropical contexts like cave, mangrove, and lacustrine sediments, in order to compare and have a better understanding of the taphonomy behavior of DNA on tropical environments.

Appendix

1. Fragment Analyzer data for the genomic libraries (August 25, 2022)

Fragment Analyzer **Run Summary:**

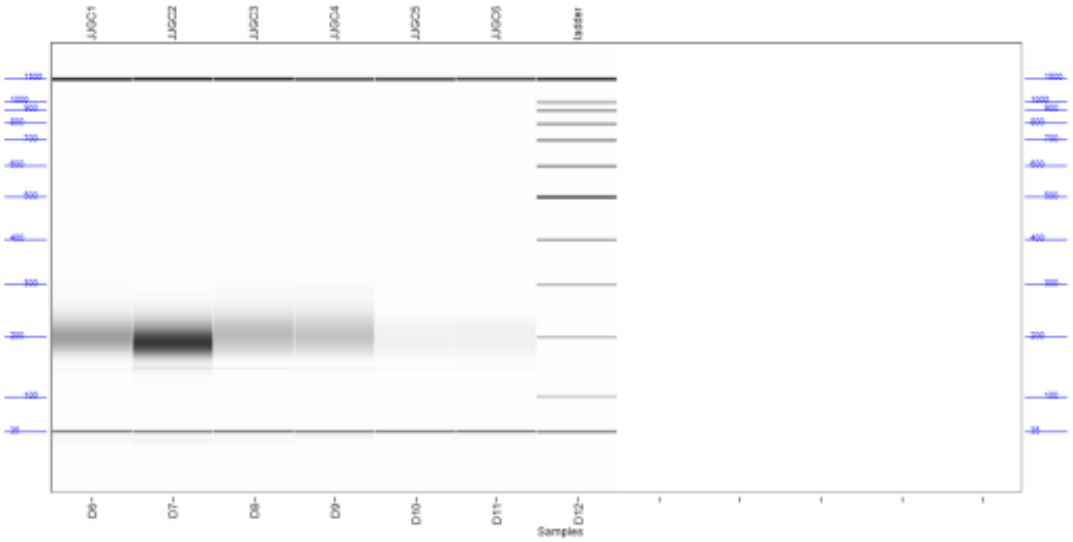
Filename and Data Path: C:\AATI\Data\2022 08 25\16-41-50\2022 08 25 16H 41M.raw
Created: Thursday, August 25, 2022 4:57:54 PM
# of Capillaries: 7
Array Serial #: 030521-49SFS
Effect Length: 33 cm
Array Usage Count: 552
FA Version #: 1.2.0.11
Device Serial #: 4088

METHOD INFORMATION
Method Name: DNF-910-33 - DNA 35-1500bp.mthds
Gel Prime: No
Full Conditioning: Yes
Gel Prime to Buffer: No
Gel Selection: Gel 1
Perform Prerun: 6.0 kV, 30 sec.
Rinse: No
Marker 1: Row: A, 5.0 kV, 10 sec.
Rinse: No
Sample Injection: 5.0 kV, 10 sec.
Separation: 6.0 kV, 45.0 min.
Tray Name: Tray-1

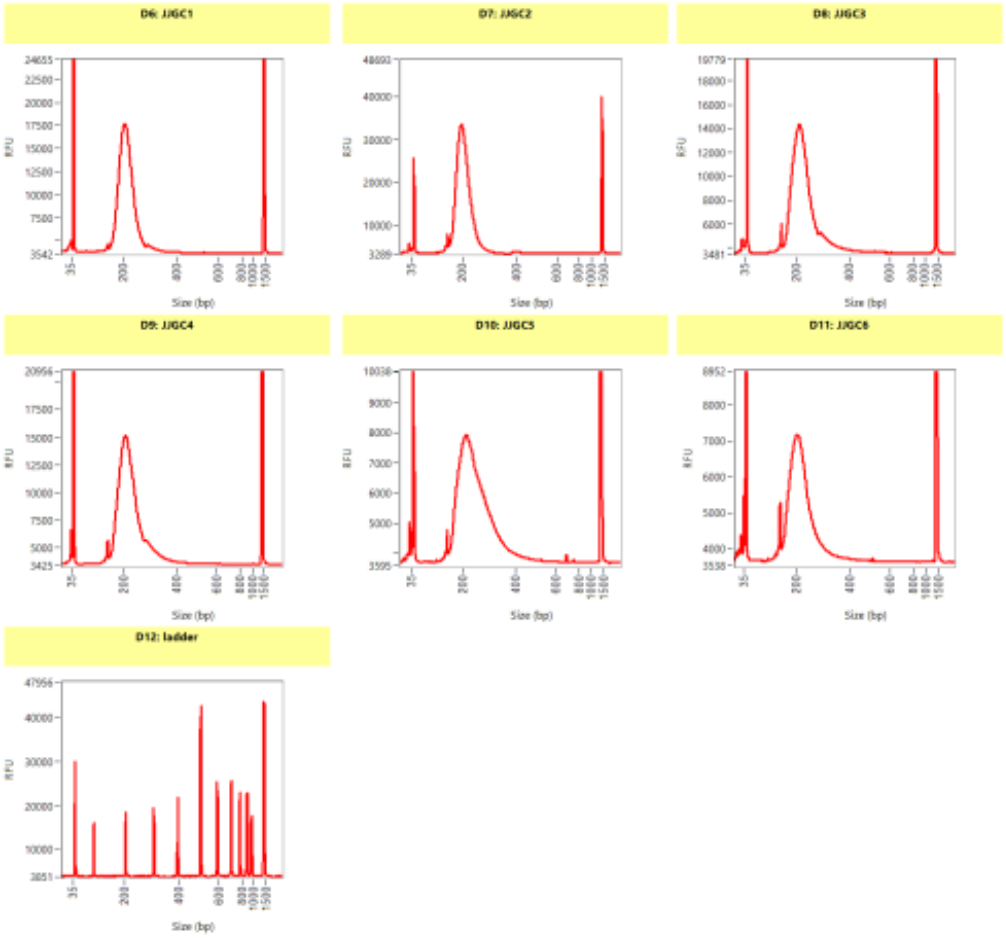
Analysis Mode: DNA

NOTES

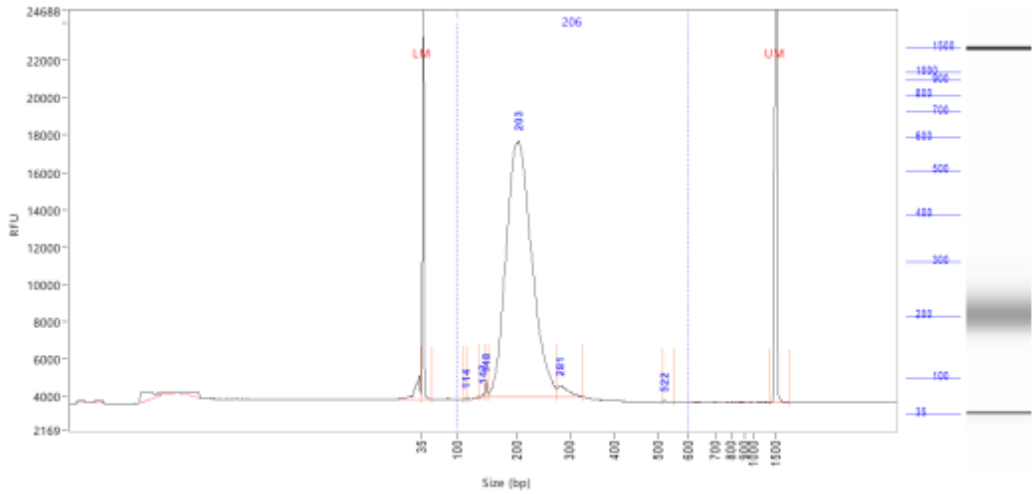
Gel Image



Filename and Data Path: C:\AATI\Data\2022 08 25\16-41-50\2022 08 25 16H 41M.raw



Sample: JJGC1
Well Location: D6
Created: Thursday, August 25, 2022 4:57:54 PM



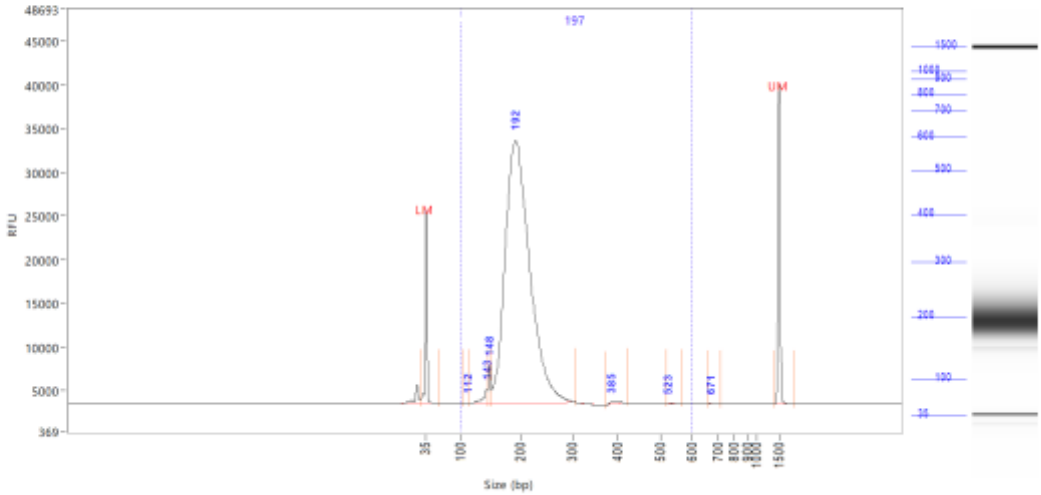
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4144	31	53	35		21617	100.583
2	114	0.0108	110	116	114	0.0	77	0.218
3	142	0.0728	136	145	142	0.2	225	1.473
4	148	0.1992	145	151	148	0.4	820	4.030
5	203	45.0733	151	274	204	97.7	13735	911.641
6	281	0.7863	274	328	290	1.7	635	15.904
7	522	0.0099	518	554	527	0.0	74	0.199
8	1500 (UM)	0.5000	1362	1800	1494		35208	121.354

TIC:	46.1524	ng/uL
TIM:	370.9982	nmole/L
Total Conc.:	46.2395	ng/uL

Smear Analysis 100 bp to 600 bp 46.1724 ng/ul 99.9 %Total 369.0003 nmole/L 206 Avg. Size (bp) 12.69 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V?: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V?: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC2
Well Location: D7
Created: Thursday, August 25, 2022 4:57:54 PM



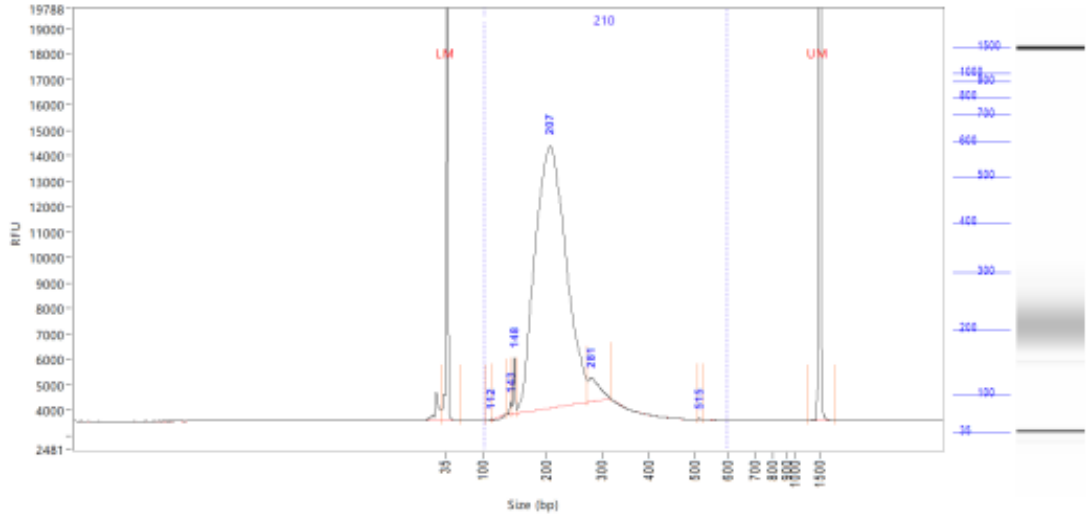
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4419	24	59	34		22192	113.029
2	112	0.0142	104	114	112	0.0	80	0.304
3	143	0.9091	114	145	138	1.0	1617	19.379
4	148	1.1290	145	151	148	1.2	4323	24.066
5	192	89.0562	151	306	198	97.5	29962	1898.340
6	385	0.2372	371	422	393	0.3	272	5.056
7	523	0.0109	518	571	535	0.0	62	0.232
8	671	0.0111	663	720	677	0.0	53	0.237
9	1500 (UM)	0.5000	1375	1832	1498		36500	127.897

TIC:	91.3678	ng/uL
TIM:	764.1528	nmole/L
Total Conc.:	91.3724	ng/uL

Smear Analysis	100 bp to 600 bp	91.2710 ng/ul	99.9 %Total	761.0812 nmole/L	197 Avg. Size (bp)	13.21 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V?: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V?: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC3
Well Location: D8
Created: Thursday, August 25, 2022 4:57:54 PM

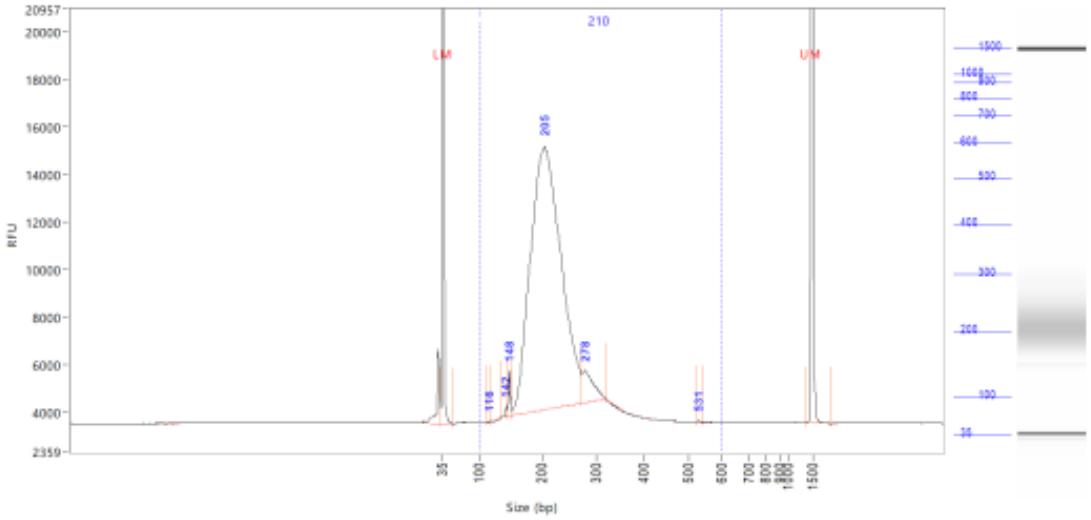


Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4557	27	59	35		22717	115.094
2	112	0.0083	104	114	111	0.0	64	0.174
3	143	0.1251	138	146	143	0.3	475	2.633
4	148	0.4656	146	152	148	1.2	2222	9.800
5	207	36.9557	152	274	209	95.6	10293	777.834
6	281	1.0969	274	318	288	2.8	952	23.088
7	515	0.0058	510	523	514	0.0	68	0.122
8	1500 (UM)	0.5000	1264	1845	1496		36494	126.286
	TIC:	38.6574	ng/uL					
	TIM:	303.7645	nmole/L					
	Total Conc.:	38.6989	ng/uL					

Smear Analysis 100 bp to 600 bp 38.6377 ng/ul 99.8 %Total 302.6799 nmole/L 210 Avg. Size (bp) 13.79 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V?: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V?: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC4
Well Location: D9
Created: Thursday, August 25, 2022 4:57:54 PM

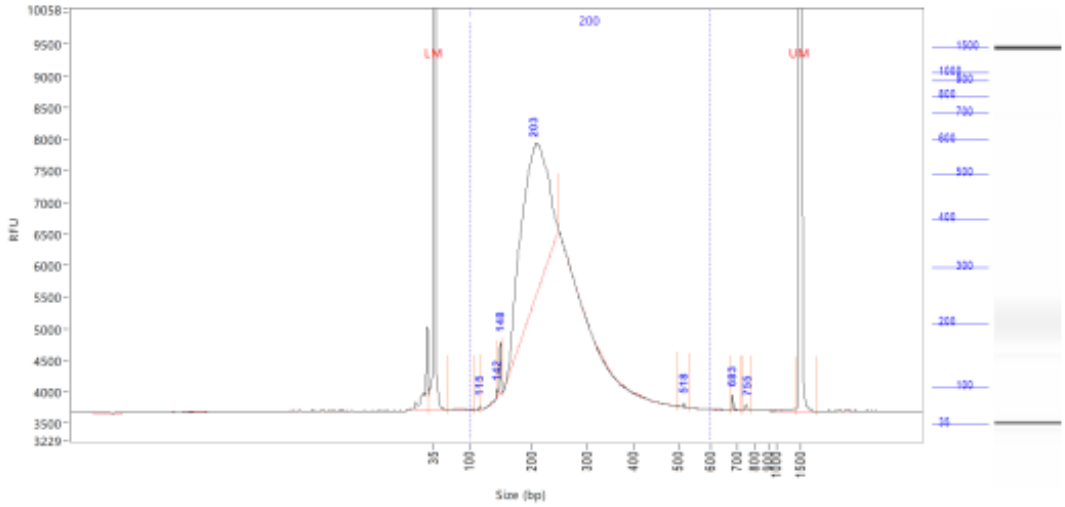


Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4970	31	53	35		27407	132.393
2	116	0.0074	112	118	116	0.0	64	0.164
3	142	0.1060	134	144	142	0.3	421	2.354
4	148	0.3899	144	152	147	1.0	1894	8.654
5	205	37.3056	152	271	208	94.7	11032	828.123
6	278	1.5664	271	319	285	4.0	1343	34.772
7	531	0.0065	523	540	531	0.0	70	0.145
8	1500 (UM)	0.5000	1362	1878	1492		38051	133.190
	TIC:	39.3819	ng/uL					
	TIM:	309.6639	nmole/L					
	Total Conc.:	39.4057	ng/uL					

Smear Analysis 100 bp to 600 bp 39.3500 ng/ul 99.9 %Total 307.4896 nmole/L 210 Avg. Size (bp) 14.20 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V?: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V?: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC5
Well Location: D10
Created: Thursday, August 25, 2022 4:57:54 PM

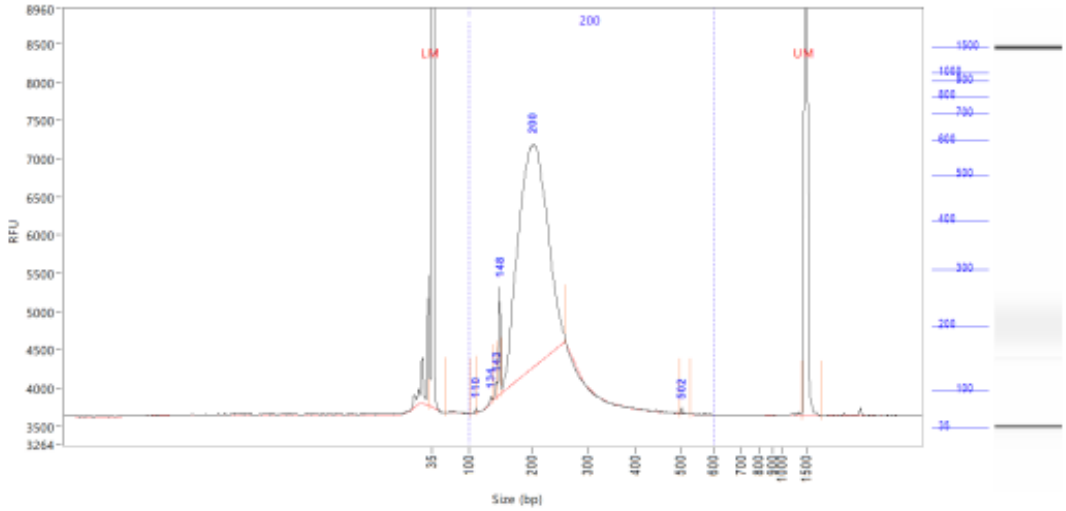


Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4990	26	60	35		27912	135.563
2	115	0.0091	107	117	114	0.1	70	0.205
3	142	0.0311	117	144	139	0.4	125	0.705
4	148	0.1448	144	152	148	1.9	827	3.277
5	203	7.1917	152	248	200	96.8	2424	162.799
6	518	0.0081	498	540	517	0.1	71	0.184
7	683	0.0341	676	725	685	0.5	237	0.771
8	755	0.0129	738	789	755	0.2	86	0.293
9	1500 (UM)	0.5000	1408	1865	1493		38857	135.823
	TIC:	7.4318	ng/uL					
	TIM:	61.3658	nmole/L					
	Total Conc.:	7.5184	ng/uL					

Smear Analysis 100 bp to 600 bp 7.4050 ng/ul 98.5 %Total 60.9196 nmole/L 200 Avg. Size (bp) 12.99 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V?: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V?: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC6
Well Location: D11
Created: Thursday, August 25, 2022 4:57:54 PM

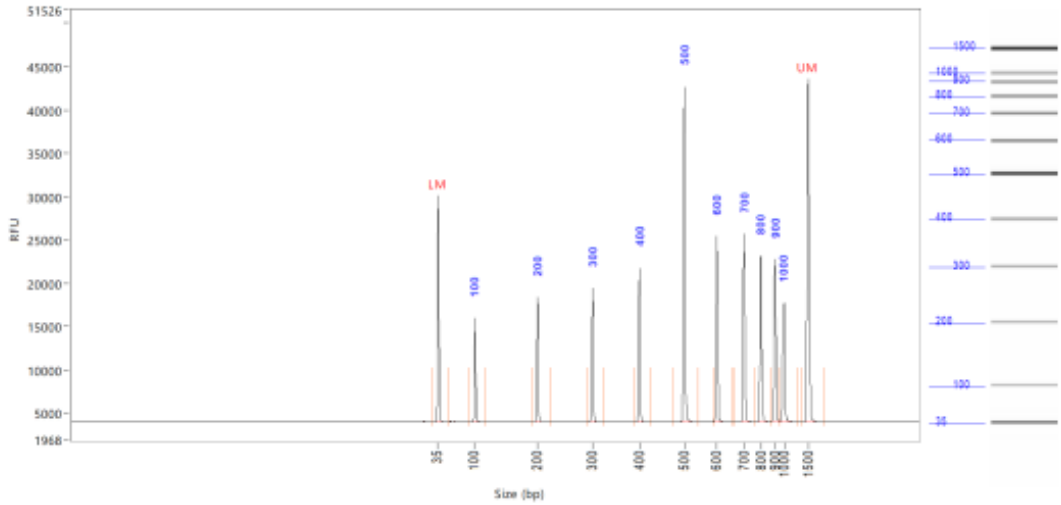


Peak	Size (bp)	Conc. (ng\uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng\uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4900	31	58	35		30691	143.701
2	110	0.0068	103	112	110	0.1	65	0.166
3	134	0.0157	112	137	132	0.2	67	0.385
4	143	0.0445	137	144	142	0.5	206	1.088
5	148	0.2504	144	152	148	2.8	1402	6.120
6	200	8.6786	152	260	201	96.4	2919	212.086
7	502	0.0082	499	531	507	0.1	69	0.201
8	1500 (UM)	0.5000	1415	1819	1498		41015	146.628
	TIC:	9.0043	ng\uL					
	TIM:	74.6131	nmole/L					
	Total Conc.:	9.0674	ng\uL					

Smear Analysis 100 bp to 600 bp 9.0225 ng\ul 99.5 %Total 74.2223 nmole/L 200 Avg. Size (bp) 14.54 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V?: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V?: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: ladder
Well Location: D12
Created: Thursday, August 25, 2022 4:57:54 PM

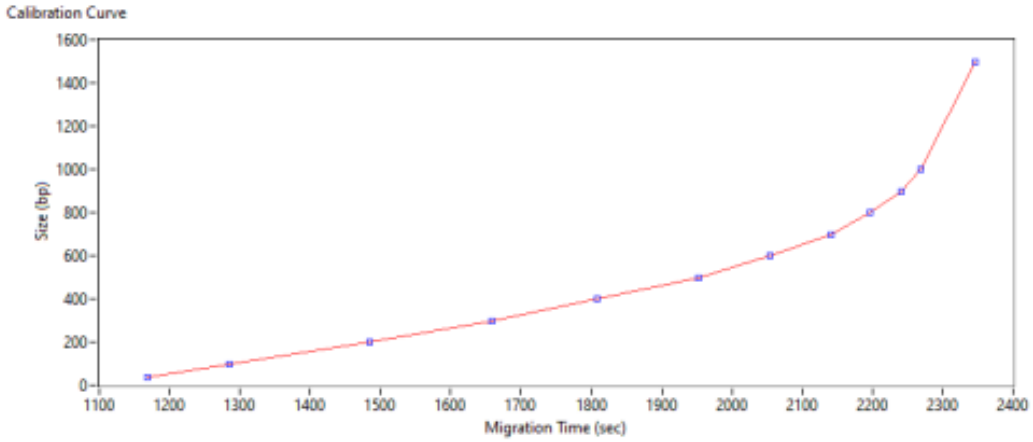


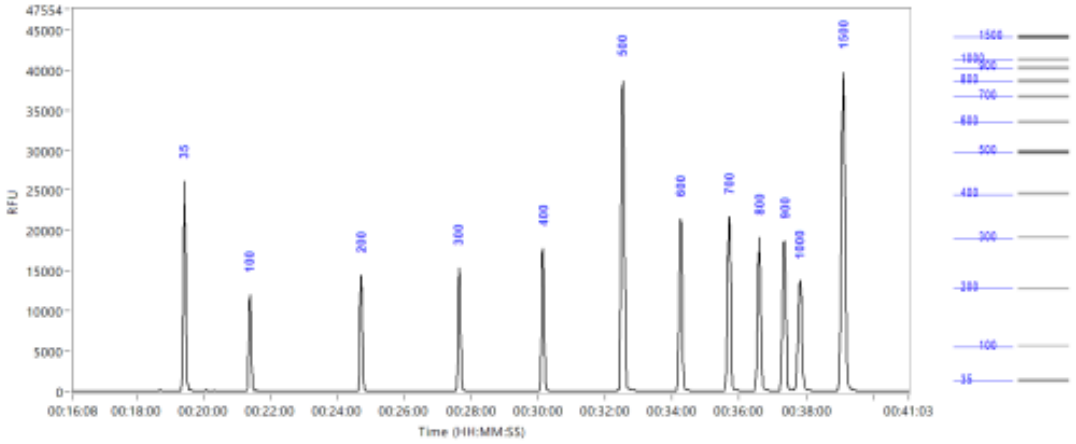
Peak	Size (bp)	Conc. (ng\uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng\uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4423	25	53	34		26087	121.170
2	100	2.0637	89	118	99	7.4	12057	47.109
3	200	2.2038	192	225	199	7.9	14443	50.306
4	300	2.2337	291	326	300	8.0	15421	50.990
5	400	2.4531	388	427	399	8.8	17765	55.997
6	500	5.6269	475	542	499	20.2	38615	128.446
7	600	2.9271	590	660	600	10.5	21416	66.817
8	700	2.9135	664	761	699	10.5	21700	66.507
9	800	2.6315	761	871	798	9.4	19136	60.070
10	900	2.5759	871	956	897	9.2	18709	58.801
11	1000	2.2193	956	1270	998	8.0	13762	50.660
12	1500 (UM)	0.5000	1362	1819	1496		39629	136.963

TBC:	27.8485	ng\uL
TIM:	122.0585	nmole/L
Total Conc.:	28.0015	ng\uL

```
Sample Peak Width (sec): 5     Sample Min Peak Height: 500    Sample Baseline V to V?: Y     Sample Baseline V to V pts: 3
Sample Filter: Binomial        # of Pts for Filter: 3         Sample Start Region (min): 0   Sample End Region (min): 45
Marker Peak Width (sec): 5     Marker Min Peak Height: 500    Marker Baseline V to V?: Y     Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                  Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```

Sample: ladder
Well Location: D12
Created: Thursday, August 25, 2022 4:57:54 PM
Fit Type: Point to Point





Fragment Analyzer Run Summary:

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Created: Thursday, August 25, 2022 5:59:07 PM
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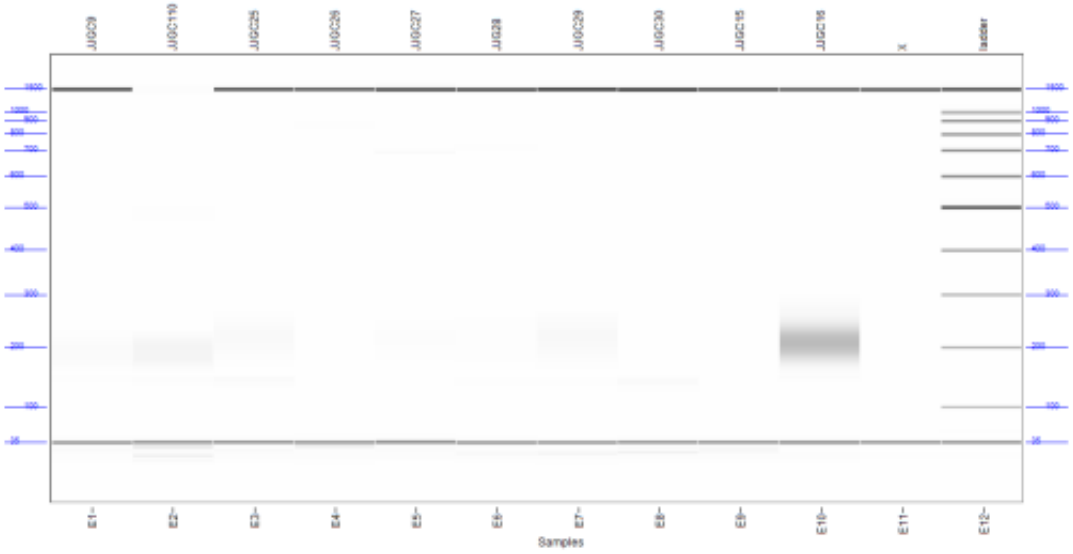
Array Serial #: 030521-49SFS
Effect Length: 33 cm
Array Usage Count: 553
FA Version #: 1.2.0.11
Device Serial #: 4088

METHOD INFORMATION
Method Name: DNF-910-33 - DNA 35-1500bp.mthds
Gel Prime: No
Full Conditioning: Yes
Gel Prime to Buffer: No
Gel Selection: Gel 1
Perform Prerun: 6.0 kV, 30 sec.
Rinse: No
Marker 1: Row: A, 5.0 kV, 10 sec.
Rinse: No
Sample Injection: 5.0 kV, 10 sec.
Separation: 6.0 kV, 45.0 min.
Tray Name: Tray-1

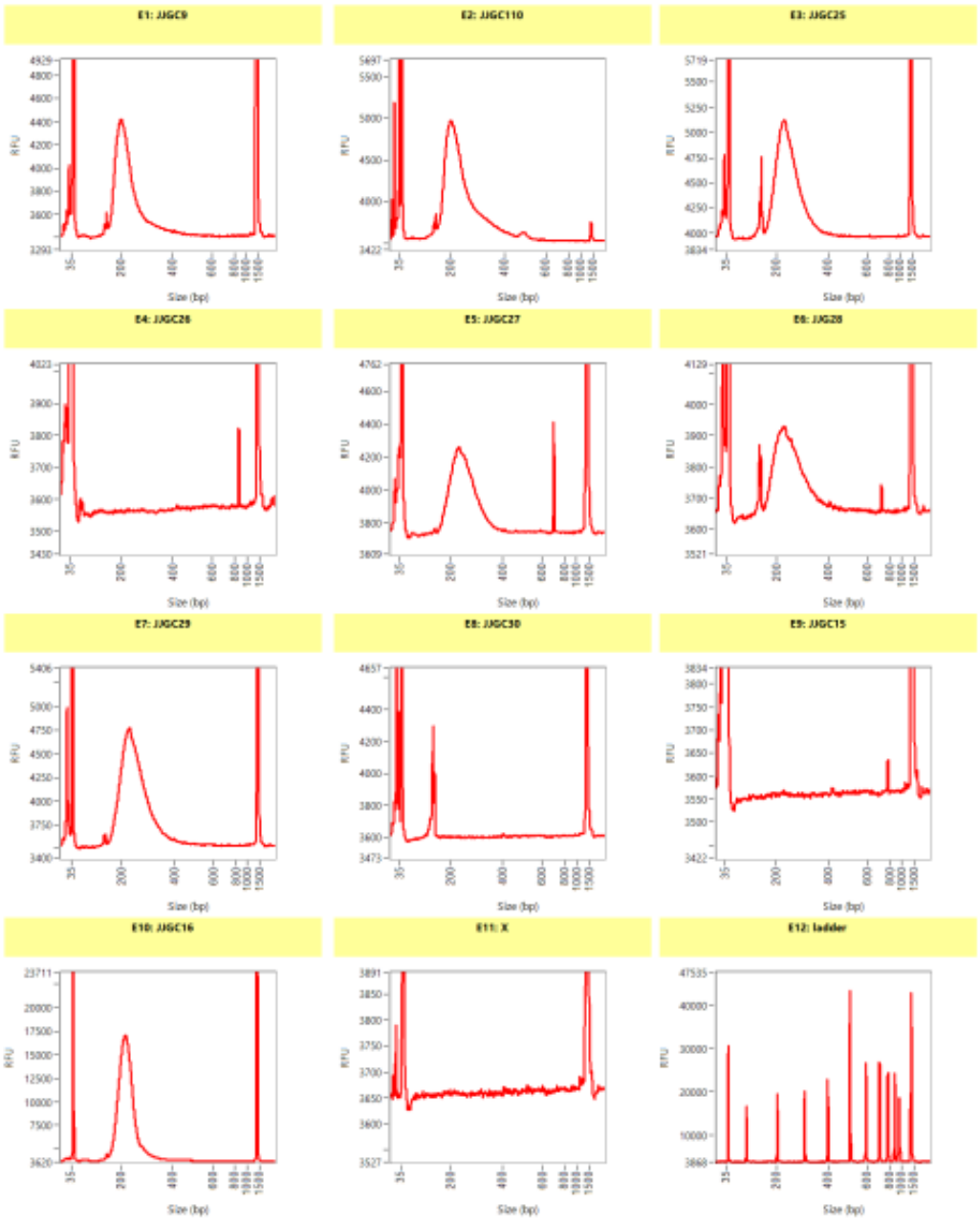
Analysis Mode: DNA

NOTES

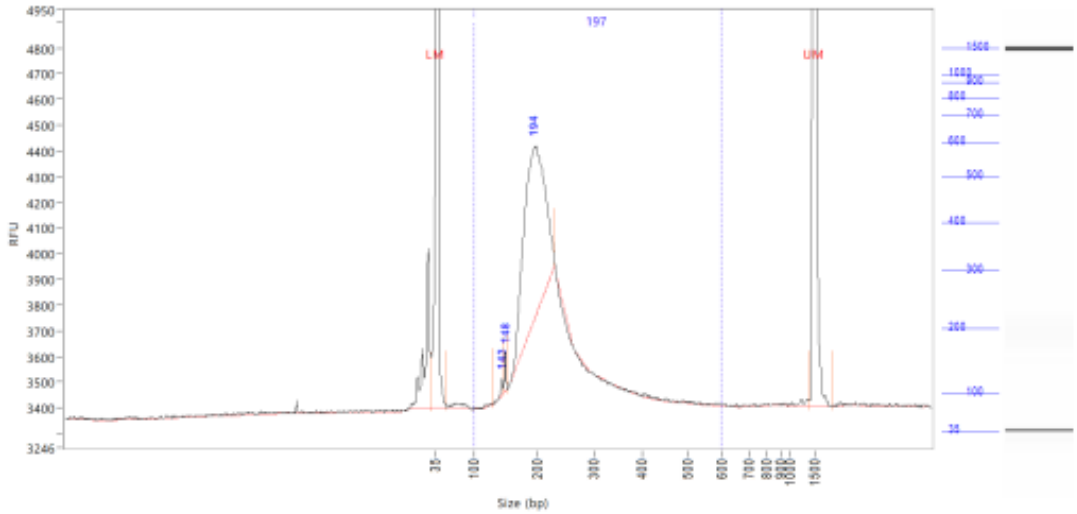
Gel Image



Filename and Data Path: C:\AATI\Data\2022 08 25\17-43-07\2022 08 25 17H 43M.raw



Sample: JJGC9
Well Location: E1
Created: Thursday, August 25, 2022 5:59:07 PM



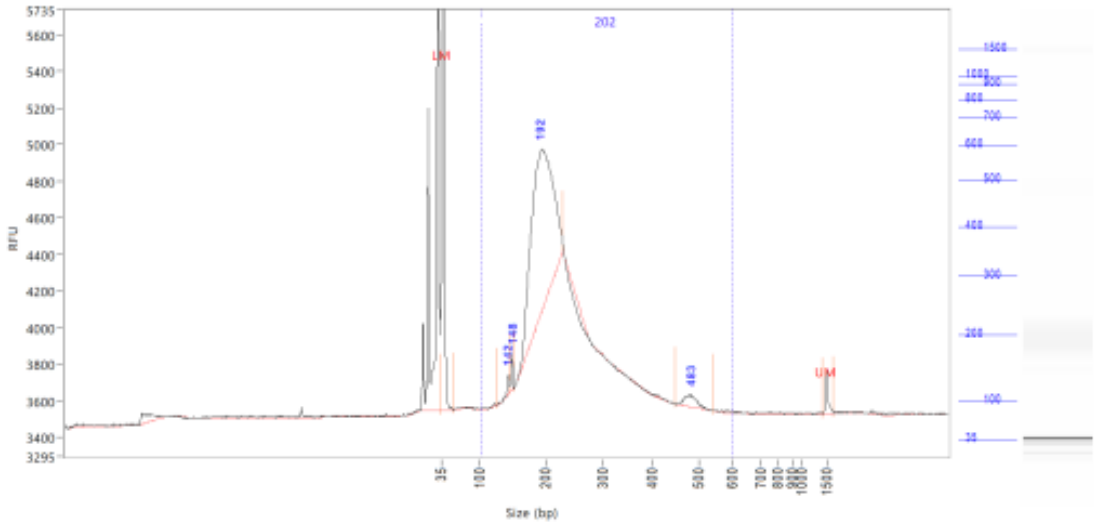
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4699	25	52	34		26066	128.460
2	143	0.0153	130	145	142	1.0	63	0.349
3	148	0.0278	145	152	148	1.7	151	0.634
4	194	1.5619	152	230	193	97.3	674	35.585
5	1500 (UM)	0.5000	1396	1854	1496		38951	136.697

TIC:	1.6051	ng/uL
TIM:	13.7920	nmole/L
Total Conc.:	1.7103	ng/uL

Smear Analysis	100 bp to 600 bp	1.6446 ng/ul	96.2 %Total	13.7392 nmole/L	197 Avg. Size (bp)	22.34 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V7: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V7: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC110
Well Location: E2
Created: Thursday, August 25, 2022 5:59:07 PM



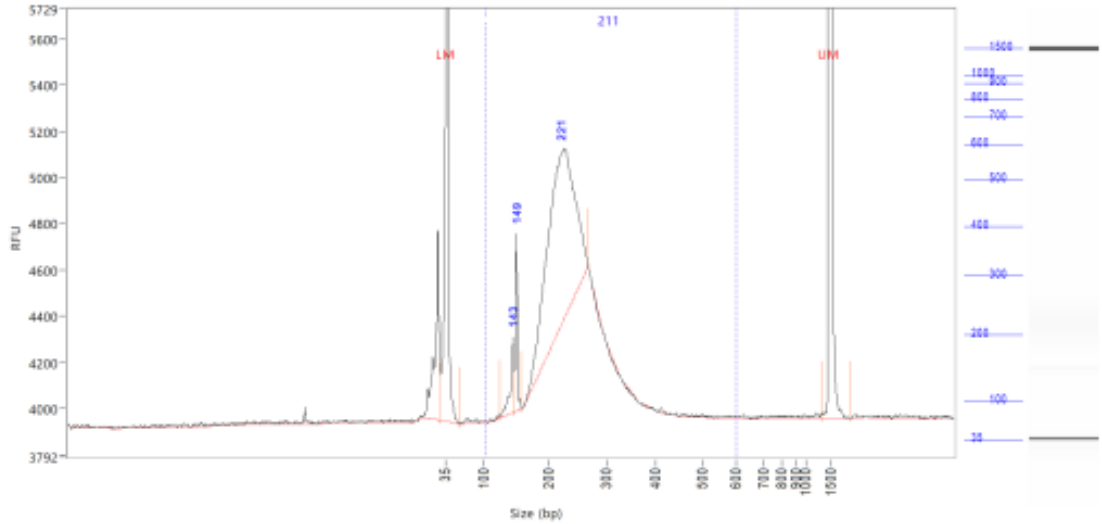
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	29.8179	31	54	35		12744	61.386
2	142	3.5542	124	145	141	1.2	104	0.610
3	148	4.6035	145	151	148	1.6	196	0.790
4	192	276.8994	151	231	193	94.2	890	47.505
5	483	8.9485	451	542	485	3.0	65	1.535
6	1500 (UM)	0.5000	1422	1635	1505		234	1.029

TIC:	294.0056	ng/uL
TIM:	2481.7810	nmole/L
Total Conc.:	302.4505	ng/uL

Smear Analysis: 100 bp to 600 bp | 296.9659 ng/ul | 98.2 %Total | 2413.0491 nmole/L | 202 Avg. Size (bp) | 28.86 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V7: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V7: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC25
Well Location: E3
Created: Thursday, August 25, 2022 5:59:07 PM

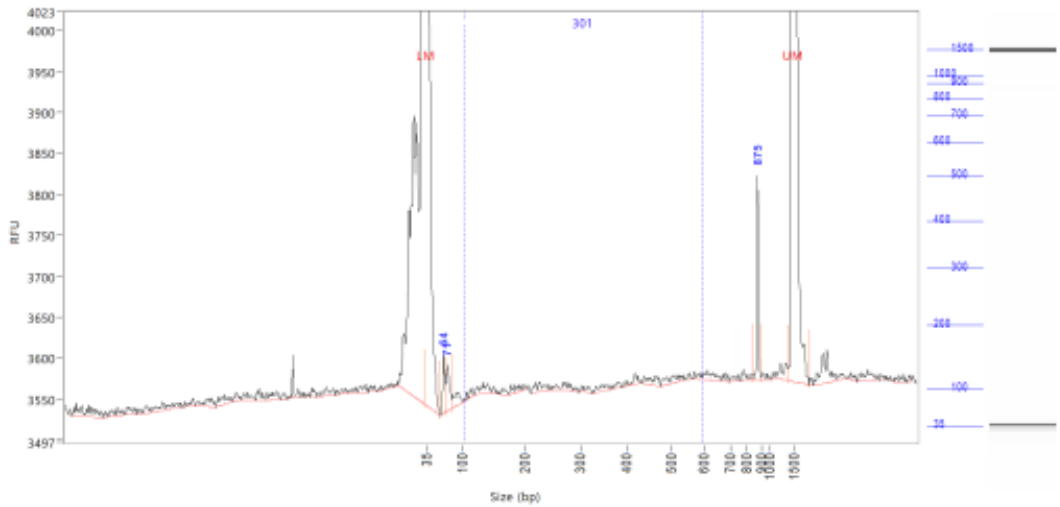


Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4988	25	56	35		23786	118.677
2	143	0.1490	124	146	140	5.3	324	2.953
3	149	0.1839	146	158	148	6.5	766	3.647
4	221	2.4846	158	267	217	88.2	744	49.264
5	1500 (UM)	0.5000	1357	1892	1496		35017	118.968
	TIC:	2.8174	ng/uL					
	TIM:	22.6183	nmole/L					
	Total Conc.:	2.9216	ng/uL					

Smear Analysis 100 bp to 600 bp 2.8548 ng/ul 97.7 %Total 22.2080 nmole/L 211 Avg. Size (bp) 20.53 %CV

```
Sample Peak Width (sec): 5     Sample Min Peak Height: 50     Sample Baseline V to V7: Y     Sample Baseline V to V pts: 3
Sample Filter: Binomial        # of Pts for Filter: 3         Sample Start Region (min): 0   Sample End Region (min): 45
Marker Peak Width (sec): 5     Marker Min Peak Height: 500    Marker Baseline V to V7: Y     Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                  Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC26
Well Location: E4
Created: Thursday, August 25, 2022 5:59:07 PM



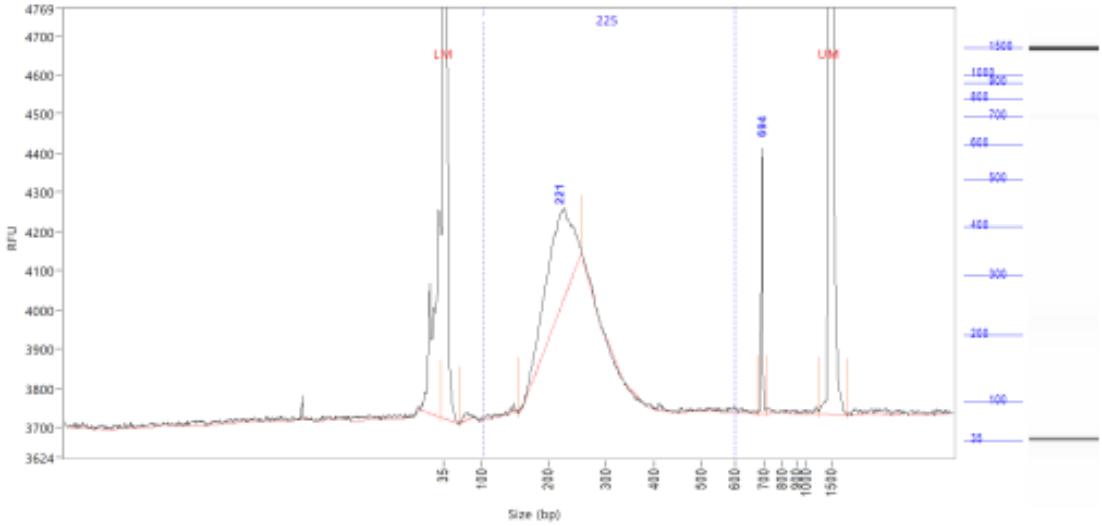
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.5041	31	58	35		26281	127.338
2	64	0.0242	58	68	64	28.2	71	0.510
3	71	0.0269	68	79	72	31.3	56	0.565
4	875	0.0348	844	905	874	40.5	249	0.733
5	1500 (UM)	0.5000	1377	1815	1497		36553	126.291

TIC:	0.0859	ng/uL
TIM:	1.2979	nmole/L
Total Conc.:	0.2443	ng/uL

Smear Analysis	100 bp to 600 bp	0.1124 ng/ul	46.0 %Total	0.6150 nmole/L	301 Avg. Size (bp)	47.39 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V7: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V7: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```

Sample: JJGC27
Well Location: E5
Created: Thursday, August 25, 2022 5:59:07 PM

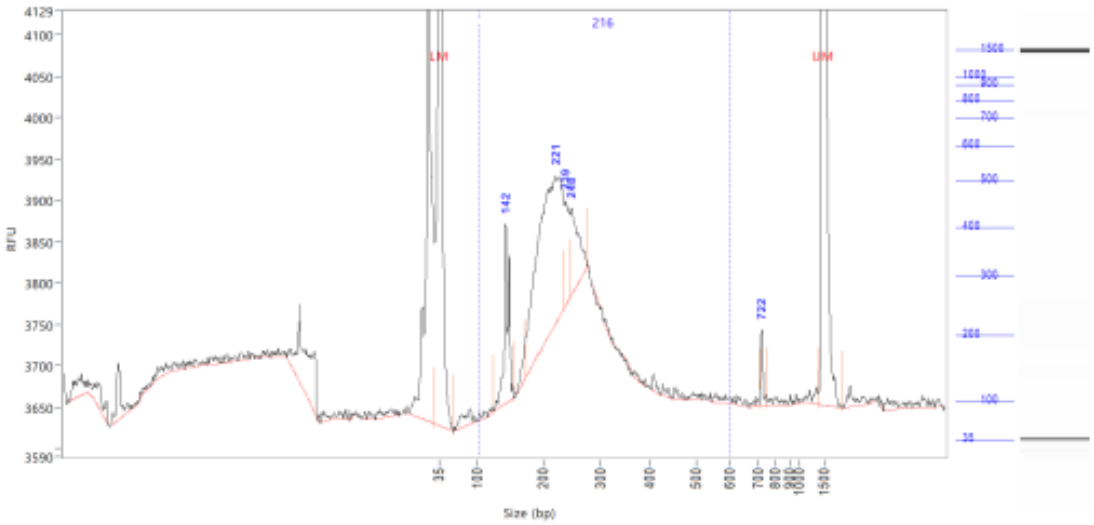


Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.5024	30	60	35		22326	124.769
2	221	0.7370	155	258	214	88.9	241	15.253
3	694	0.0920	680	714	693	11.1	675	1.905
4	1500 (UM)	0.5000	1273	1828	1494		34808	124.184
	TIC:	0.8290	ng/uL					
	TIM:	5.8813	nmole/L					
	Total Conc.:	0.9508	ng/uL					

Smear Analysis 100 bp to 600 bp 0.8120 ng/ul 85.4 %Total 5.9260 nmole/L 225 Avg. Size (bp) 29.03 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V7: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V7: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJG28
Well Location: E6
Created: Thursday, August 25, 2022 5:59:07 PM

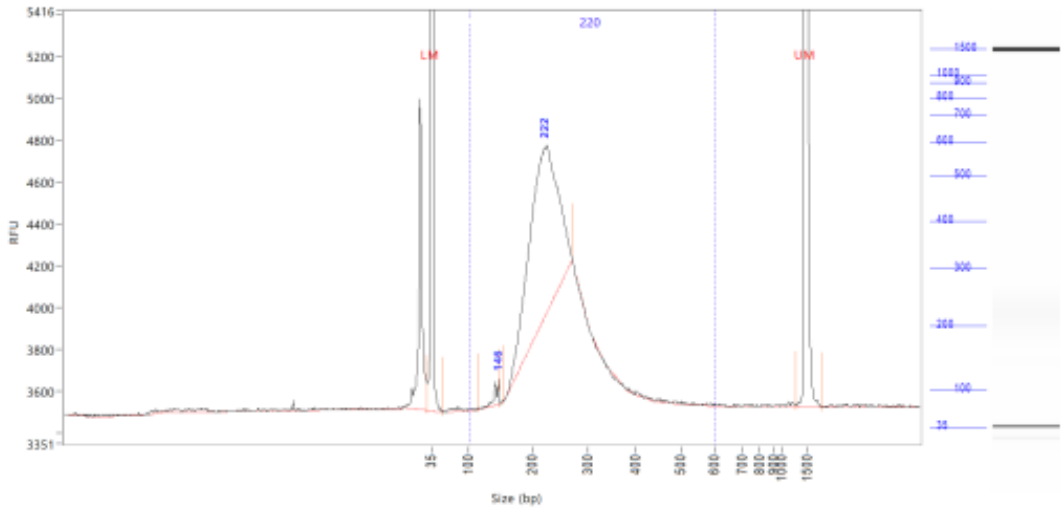


Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4601	26	57	34		22932	114.184
2	142	0.1184	123	154	142	14.1	217	2.448
3	221	0.5493	172	237	207	65.6	179	11.358
4	239	0.0641	237	247	242	7.7	126	1.326
5	248	0.0901	247	278	256	10.8	105	1.862
6	722	0.0150	710	755	724	1.8	91	0.309
7	1500 (UM)	0.5000	1383	1873	1492		35349	124.078
	TIC:	0.8368	ng/ul.					
	TIM:	6.7812	nmole/L					
	Total Conc.:	0.9550	ng/ul.					

Smear Analysis 100 bp to 600 bp 0.8836 ng/ul 92.5 %Total 6.7300 nmole/L 216 Avg. Size (bp) 30.77 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V7: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V7: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC29
Well Location: E7
Created: Thursday, August 25, 2022 5:59:07 PM

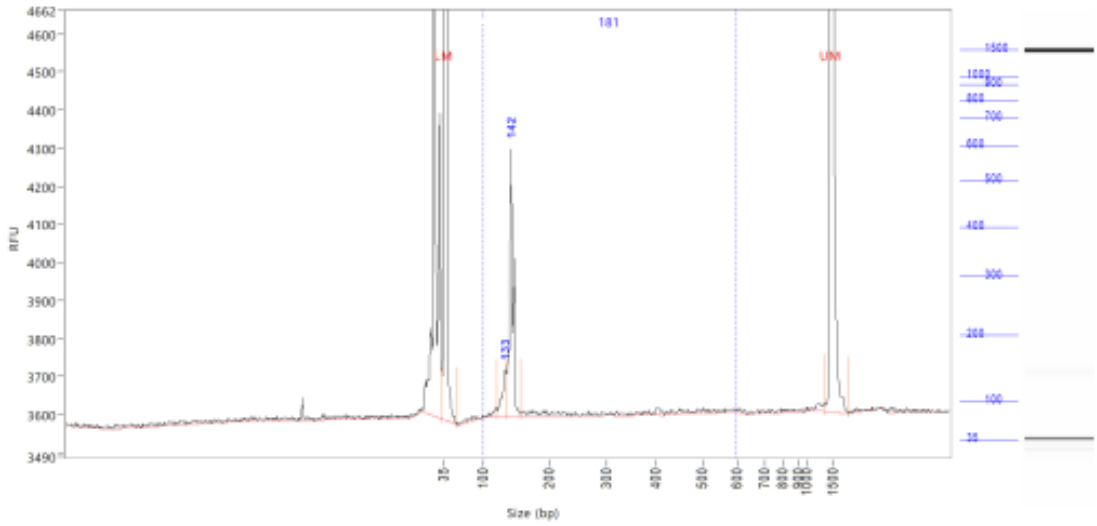


Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4048	27	53	34		21400	101.185
2	146	0.0658	115	150	140	2.3	117	1.371
3	222	2.7361	154	271	217	97.7	803	56.999
4	1500 (UM)	0.5000	1293	1802	1495		36039	124.993
	TIC:	2.8019	ng/uL					
	TIM:	21.5062	nmole/L					
	Total Conc.:	2.9135	ng/uL					

Smear Analysis	100 bp to 600 bp	2.8577 ng/ul	98.1 %Total	21.3936 nmole/L	220 Avg. Size (bp)	19.37 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V7: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V7: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC30
Well Location: E8
Created: Thursday, August 25, 2022 5:59:07 PM

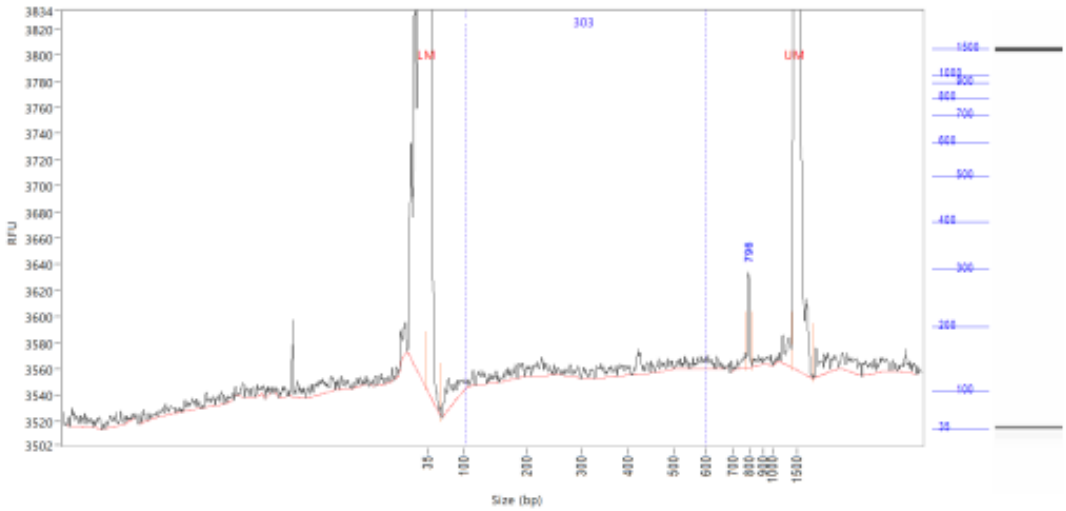


Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.3891	31	57	35		21613	100.424
2	133	0.0657	121	135	130	17.8	120	1.413
3	142	0.3027	135	156	143	82.2	699	6.512
4	1500 (UM)	0.5000	1370	1828	1492		36688	129.060
	TIC:	0.3684	ng/uL					
	TIM:	4.3094	nmole/L					
	Total Conc.:	0.5248	ng/uL					

Smear Analysis 100 bp to 600 bp 0.4769 ng/ul 90.9 %Total 4.3429 nmole/L 181 Avg. Size (bp) 54.72 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V7: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V7: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC15
Well Location: E9
Created: Thursday, August 25, 2022 5:59:07 PM

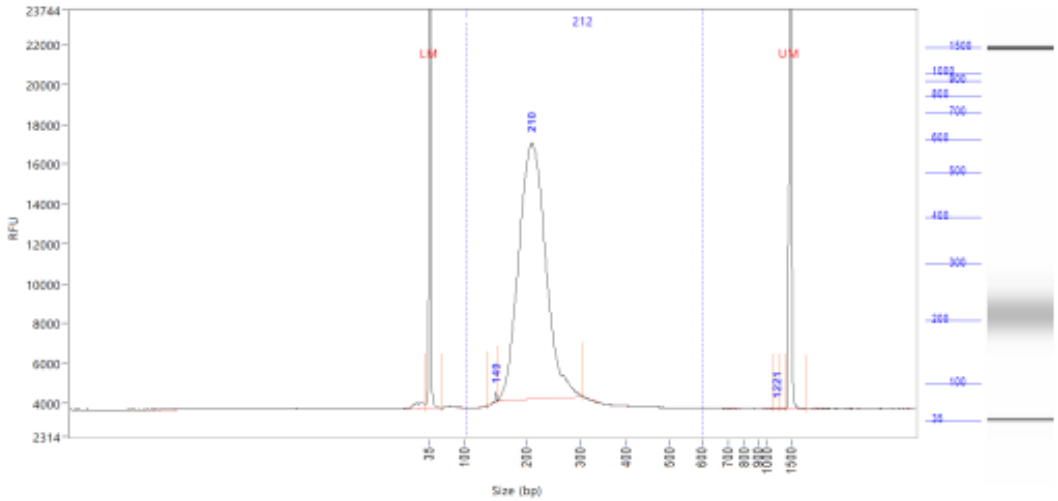


Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4425	31	59	35		24867	116.844
2	796	0.0109	781	819	795	100.0	73	0.241
3	1500 (UM)	0.5000	1403	1841	1494		37564	132.031
	TIC:	0.0109	ng/uL					
	TIM:	0.0226	nmole/L					
	Total Conc.:	0.1846	ng/uL					

Smear Analysis	100 bp to 600 bp	0.1138 ng/ul	61.6 %Total	0.6169 nmole/L	303 Avg. Size (bp)	45.56 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V7: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V7: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: JJGC16
Well Location: E10
Created: Thursday, August 25, 2022 5:59:07 PM



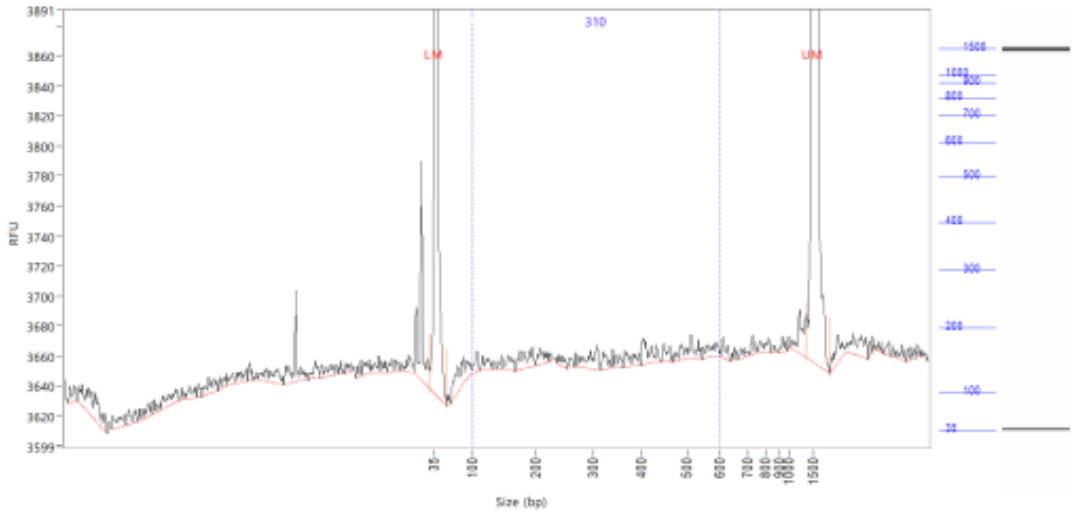
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.5487	26	57	35		28707	153.541
2	149	0.0938	137	152	148	0.3	486	2.187
3	210	37.2652	152	306	211	99.7	12849	869.043
4	1221	0.0098	1144	1293	1215	0.0	57	0.228
5	1500 (UM)	0.5000	1403	1828	1494		38978	139.923

TIC:	37.3688	ng/uL
TIM:	291.4649	nmole/L
Total Conc.:	37.4523	ng/uL

Smear Analysis 100 bp to 600 bp 37.3883 ng/ul 99.8 %Total 290.6630 nmole/L 212 Avg. Size (bp) 11.50 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V7: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V7: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: X
Well Location: E11
Created: Thursday, August 25, 2022 5:59:07 PM



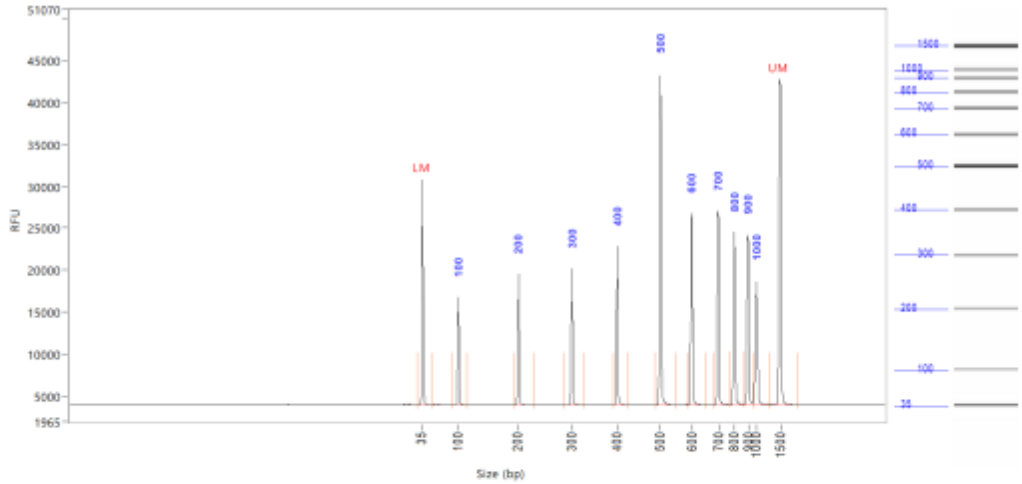
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4631	26	54	35		29574	135.764
2	1500 (UM)	0.5000	1377	1834	1492		40439	146.567

TIC:	0.0000	ng/uL
TIM:	0.0000	nmole/L
Total Conc.:	0.1719	ng/uL

Smear Analysis	100 bp to 600 bp	0.1142 ng/ul	66.4 %Total	0.6064 nmole/L	310 Avg. Size (bp)	44.84 %CV

```
Sample Peak Width (sec): 5    Sample Min Peak Height: 50    Sample Baseline V to V7: Y    Sample Baseline V to V pts: 3
Sample Filter: Binomial       # of Pts for Filter: 3        Sample Start Region (min): 0  Sample End Region (min): 45
Marker Peak Width (sec): 5    Marker Min Peak Height: 500   Marker Baseline V to V7: Y    Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```


Sample: ladder
Well Location: E12
Created: Thursday, August 25, 2022 5:59:07 PM

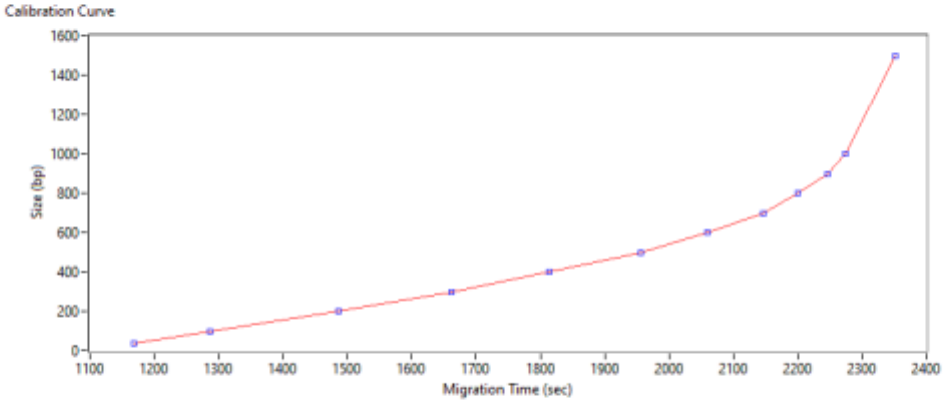


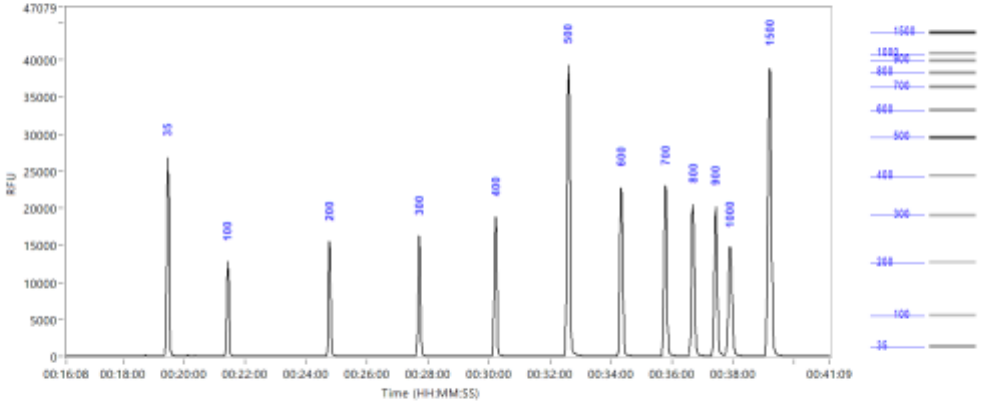
Peak	Size (bp)	Conc. (ng/uL)	From (bp)	To (bp)	Avg. Size (bp)	Rel. Conc. % (ng/uL)	RFU	Corr. Peak Area
1	35 (LM)	0.4453	26	52	35		26787	121.542
2	100	2.1891	91	116	100	7.3	12750	49.791
3	200	2.3669	192	229	200	7.8	15489	53.834
4	300	2.4300	286	328	299	8.0	16211	55.270
5	400	2.6704	391	426	399	8.8	18816	60.739
6	500	5.9154	487	549	500	19.6	39233	134.545
7	600	3.2077	589	654	600	10.6	22742	72.958
8	700	3.1946	684	772	700	10.6	23005	72.661
9	800	2.9049	772	871	799	9.6	20511	66.070
10	900	2.8574	871	960	899	9.5	20204	64.992
11	1000	2.4501	960	1280	1001	8.1	14669	55.727
12	1500 (UM)	0.5000	1280	1879	1496		38827	136.469

TIC:	30.1866	ng\uL
TIM:	130.8060	nmole/L
Total Conc.:	30.3444	ng\uL

```
Sample Peak Width (sec): 5     Sample Min Peak Height: 500    Sample Baseline V to V7: Y     Sample Baseline V to V pts: 3
Sample Filter: Binomial        # of Pts for Filter: 3         Sample Start Region (min): 0   Sample End Region (min): 45
Marker Peak Width (sec): 5     Marker Min Peak Height: 500    Marker Baseline V to V7: Y     Marker Baseline V to V pts: 3
Lower Marker Selection: First Peak > 500 RFU                  Upper Marker Selection: Last Peak > 500 RFU
Ladder Size (bp)35, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500
Quantification Using: Upper Marker          Final Concentration (ng/uL): 0.5000          Dilution Factor: 12.0
```

Sample: ladder
Well Location: E12
Created: Thursday, August 25, 2022 5:59:07 PM
Fit Type: Point to Point





BIBLIOGRAPHY

Allahr, A. (2005). Identity and Erasure: Finding the Elusive Caribbean. *European Review of Latin American and Caribbean Studies | Revista Europea De Estudios Latinoamericanos Y Del Caribe*, *0*(79), 125. https://doi.org/10.18352/erlacs.9668

Allen, R; Ford, B; Kennedy, J.R. (2019). Reclaiming the research potential of archaeological collections. In *New life for archaeological collections*, edited by Rebecca Allen and Ford. lincoln, NE: University of Nevraska Press.

Arning, N; Wilson, D. J. (2020). The past, present, and future of ancient bacterial DNA. *Microbial Genomics*, *6*(7). https://doi.org/10.1099/mgen.0.000384

Arriola, L. A; Cooper, A; Weyrich, L. S. (2020). Palaeomicrobiology: Application of Ancient DNA Sequencing to Better Understand Bacterial Genome Evolution and Adaptation. *Frontiers in Ecology and Evolution*, *8*. https://doi.org/10.3389/fevo.2020.00040

Ávila-Arcos, M. C; De la Fuente-Castro, C; Nieves-Colón, M. A; Raghavan, M. (2022). Recommendations for Sustainable Ancient DNA Research in the Global South: Voices From a New Generation of Paleogenomicists. *Frontiers in Genetics*, *13*. https://doi.org/10.3389/fgene.2022.880170

Bach, E. M; Williams, R. J; Hargreaves, S. K; Yang, F; Hofmockel, K. S. (2018). Greatest soil microbial diversity found in micro-habitats. *Soil Biology and Biochemistry*, *118*, 217–226. https://doi.org/10.1016/j.soilbio.2017.12.018

Bailey, V. L; Bond-Lamberty, B; DeAngelis, K; Grandy, A. S; Hawkes, C. V; Heckman, K., Lajtha, K; Phillips, R. P; Sulman, B. N; Todd-Brown, K. E. O; Wallenstein, M. D. (2017). Soil carbon cycling proxies: Understanding their critical role in predicting climate change feedbacks. *Global Change Biology*, *24*(3), 895–905. https://doi.org/10.1111/gcb.13926

Bardgett, R. D; Freeman, C; Ostle, N. J. (2008). Microbial contributions to climate change through carbon cycle feedbacks. *The ISME Journal*, *2*(8), 805–814. https://doi.org/10.1038/ismej.2008.58

Barnes, M.A; Turner, C.R. (2016). The Ecology of Environmental DNA and implications for Conservation genetics. *Conservation Genetics, 17,*1-17. https://doi.org/10.1007/s10592-015-0775-4

Bastida, F; Eldridge, D. J; García, C; Kenny Png, G; Bardgett, R. D; Delgado-Baquerizo, M. (2021). Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. *The ISME Journal*, *15*(7), 2081–2091. https://doi.org/10.1038/s41396-021-00906-0

Bawaya, M. 2007. Curation in Crisis. *American Archaeology*, 2007.

Bell, M. A; Blais, J. M. (2020). Paleolimnology in support of archeology: a review of past investigations and a proposed framework for future study design. *Journal of Paleolimnology*, *65*(1), 1–32. https://doi.org/10.1007/s10933-020-00156-8

Benden, D.M; Taft, M.C. (2019). A long view of archaeological collections care, preservation, and management. *Advances in archaeological practice, 7(3),*217-223. https://doi.org/10.1017/aap.2019.22

Beng, K.C; Corlett, R.T. (2020) Application of Environmental DNA (eDNA) in Ecology and Conservation: opportunities, challenges and prospects. *Biodiversity and conservation, 29,*2089-2121. https://doi.org/10.1007/s10531-020-01980-0

Benn Torres, J. (2019). Anthropological perspectives on genomic data, genetic ancestry, and race. *American Journal of Physical Anthropology*, *171*(S70), 74–86. https://doi.org/10.1002/ajpa.23979

Benn Torres, J. (2018). 'Reparational' Genetics: Genomic Data and the Case for Reparations in the Caribbean. *Genealogy*, *2*(1), 7. https://doi.org/10.3390/genealogy2010007

Benn Torres, J. (2014). Prospecting the past: genetic perspectives on the extinction and survival of indigenous peoples of the Caribbean. *New Genetics and Society*, *33*(1), 21–41. https://doi.org/10.1080/14636778.2013.873245

Benn Torres, J. (2016). Genetic Anthropology and Archaeology: Interdisciplinary Approaches to Human History in the Caribbean. *PaleoAmerica*, *2*(1), 1–5. https://doi.org/10.1080/20555563.2016.1139859
Berkelmann, D; Schneider, D; Meryandini, A; Daniel, R. (2020). Unraveling the effects of tropical land use conversion on the soil microbiome. *Environmental Microbiome*, *15*(1). https://doi.org/10.1186/s40793-020-0353-3

Blong, J. C; Shillito, L. M. (2021). Coprolite research: archaeological and paleoenvironmental potentials. *Archaeological and Anthropological Sciences*, *13*(1). https://doi.org/10.1007/s12520-020-01242-8

Boessenkool, S; McGlynn, G; Epp, L. S; Taylor, D; Pimentel, M; Gizaw, A; Nemomissa, S; Brochmann, C; Popp, M. (2013). Use of Ancient Sedimentary DNA as a Novel Conservation Tool for High-Altitude Tropical Biodiversity. *Conservation Biology*, *28*(2), 446–455. https://doi.org/10.1111/cobi.12195

Bohmann, K; Evans, A; Gilbert, M. T. P; Carvalho, G. R; Creer, S; Knapp, M; Yu, D. W; De Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. Trends in Ecology &Amp; Evolution, 29(6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003

Bollongino, R; Tresset, A; Vigne, J. D. (2008). Environment and excavation: Pre-lab impacts on ancient DNA analyses. *Comptes Rendus Palevol*, *7*(2–3), 91–98. https://doi.org/10.1016/j.crpv.2008.02.002

Borry, M; Cordova, B; Perri, A; Wibowo, M; Prasad Honap, T; Ko, J; Yu, J; Britton, K; Girdland-Flink, L; Power, R. C; Stuijts, I; Salazar-García, D. C; Hofman, C; Hagan, R; Samdapawindé Kagoné, T; Meda, N; Carabin, H; Jacobson, D; Reinhard, K; Warinner, C. (2020). CoproID predicts the source of coprolites and paleofeces using microbiome composition and host DNA content. *PeerJ*, *8*, e9001. https://doi.org/10.7717/peerj.9001

Boschker, H; Middelburg, J. (2002). Stable isotopes and biomarkers in microbial ecology. *FEMS Microbiology Ecology*, *40*(2), 85–95. https://doi.org/10.1111/j.1574-6941.2002.tb00940.x

Bravo-Lopez, M; Villa-Islas, V; Rocha Arriaga, C; Villaseñor-Altamirano, A. B; Guzmán-Solís, A; Sandoval-Velasco, M; Wesp, J. K; Alcantara, K; López-Corral, A; Gómez-Valdés, J; Mejía, E; Herrera, A; Meraz-Moreno, A; Moreno-Cabrera, M. D. L. L; Moreno-Estrada, A; Nieves-Colón, M. A; Olvera, J; Pérez-Pérez, J; Iversen, K. H; Ávila-Arcos, M. C. (2020). Paleogenomic insights into

the red complex bacteria *Tannerella forsythia* in Pre-Hispanic and Colonial individuals from Mexico. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1812), 20190580. https://doi.org/10.1098/rstb.2019.0580

Bremong, L; Favier, C; Ficetola, G.F; Tossou, M.G; Akouégninou, A; Guelly, L; Ciguet-Covex, C; Oslisly, R; Salzmann, U. (2017). Five thousand years of tropical lake sediment DNA records from Benin. *Quaternary Science Reviews, 170,* 203-211. https://doi.org/10.1016/j.quascirev.2017.06.025

Bürgmann, H; Pesaro, M; Widmer, F; Zeyer, J. (2001). A strategy for optimizing quality and quantity of DNA extracted from soil. *Journal of Microbiological Methods*, *45*(1), 7–20. https://doi.org/10.1016/s0167-7012(01)00213-5

Butzer, K. W. (1982). *Archaeology as Human Ecology: Method and Theory for a Contextual Approach* (paper edition). Cambridge University Press.

Cajete, G. A. (2020). Indigenous Science, Climate Change, and Indigenous Community Building: A Framework of Foundational Perspectives for Indigenous Community Resilience and Revitalization. *Sustainability*, *12*(22), 9569. https://doi.org/10.3390/su12229569

Cano, R. J; Rivera-Perez, J; Toranzos, G. A., Santiago-Rodriguez, T. M; Narganes-Storde, Y. M; Chanlatte-Baik, L; García-Roldán, E; Bunkley-Williams, L; Massey, S. E. (2014). Paleomicrobiology: Revealing Fecal Microbiomes of Ancient Indigenous Cultures. *PLoS ONE*, *9*(9), e106833. https://doi.org/10.1371/journal.pone.0106833

Capo, E; Monchamp, M; Coolen, M. J. L; Domaizon, I; Armbrecht, L; Bertilsson, S. (2022). Environmental paleomicrobiology: using DNA preserved in aquatic sediments to its full potential. *Environmental Microbiology*, *24*(5), 2201–2209. https://doi.org/10.1111/1462-2920.15913

Childs, S. T. 2022. Finding space to store archaeological collections: Challenges and progress in the United States. In *The Oxford handbook of Museum Archaeology*, edited by A. Stevenson, pp. 221–247. Oxford University Press, Oxford, England.

Chowdhury, T. R; Dick, R. P. (2012). Standardizing methylation method during phospholipid fatty acid analysis to profile soil microbial communities. *Journal of Microbiological Methods*, *88*(2), 285–291. https://doi.org/10.1016/j.mimet.2011.12.008

Coolen, M. (2004). Combined DNA and lipid analyses of sediments reveal changes in Holocene haptophyte and diatom populations in an Antarctic lake. *Earth and Planetary Science Letters*, *223*(1–2), 225–239. https://doi.org/10.1016/j.epsl.2004.04.014

Coolen, M. J. L.(Overmann, J. (2007). 217 000-year-old DNA sequences of green sulfur bacteria in Mediterranean sapropels and their implications for the reconstruction of the paleoenvironment. *Environmental Microbiology*, *9*(1), 238–249. https://doi.org/10.1111/j.1462-2920.2006.01134.x

Crump, S. E. (2021). Sedimentary ancient DNA as a tool in paleoecology. *Nature Reviews Earth &Amp; Environment*, *2*(4), 229–229. https://doi.org/10.1038/s43017-021-00158-8

Dabney, J; Meyer, M; Paabo, S. (2013). Ancient DNA Damage. *Cold Spring Harbor Perspectives in Biology*, *5*(7), a012567–a012567. https://doi.org/10.1101/cshperspect.a012567

Dabney, J; Meyer, M. (2019). Extraction of Highly Degraded DNA from Ancient Bones and Teeth. *Methods in Molecular Biology*, 25–29. https://doi.org/10.1007/978-1-4939-9176-1_4

Darling, M. I; Donoghue, H. D. (2014). Insights from paleomicrobiology into the indigenous peoples of pre-colonial America - A Review. *Memórias Do Instituto Oswaldo Cruz*, *109*(2), 131–139. https://doi.org/10.1590/0074-0276140589

Davison, J; Moora, M; Öpik, M; Ainsaar, L; Ducousso, M; Hiiesalu, I; Jairus, T; Johnson, N; Jourand, P; Kalamees, R; Koorem, K; Meyer, J. Y; Püssa, K; Reier, L; Pärtel, M; Semchenko, M; Traveset, A; Vasar, M; Zobel, M. (2018). Microbial island biogeography: isolation shapes the life history characteristics but not diversity of root-symbiotic fungal communities. *The ISME Journal*, *12*(9), 2211–2224. https://doi.org/10.1038/s41396-018-0196-8

De Schepper, S; Ray, J. L; Skaar, K. S; Sadatzki, H; Ijaz, U. Z; Stein, R; Larsen, A. (2019). The potential of sedimentary ancient DNA for reconstructing past sea ice evolution. *The ISME Journal*, *13*(10), 2566–2577. https://doi.org/10.1038/s41396-019-0457-1

Demkina, T. S; Khomutova, T. E; Kashirskaya, N. N; Demkina, E. V; Stretovich, I. V; El-Registan, G. I; Demkin, V. A. (2008). Age and activation of microbial communities in soils under burial mounds and in recent surface soils of steppe zone. Eurasian Soil Science, 41(13), 1439–1447. https://doi.org/10.1134/s1064229308130139

Demko, A. M; Patin, N. V; Jensen, P. R. (2021). Microbial diversity in tropical marine sediments assessed using culture-dependent and culture-independent techniques. *Environmental Microbiology*, *23*(11), 6859–6875. https://doi.org/10.1111/1462-2920.15798

Deng, Y; Cui, X; Hernández, M; Dumont, M. G. (2014). Microbial Diversity in Hummock and Hollow Soils of Three Wetlands on the Qinghai-Tibetan Plateau Revealed by 16S rRNA Pyrosequencing. *PLoS ONE*, *9*(7), e103115. https://doi.org/10.1371/journal.pone.0103115

Díaz-Zabala, H. J; Nieves-Colón, M. A; Martínez-Cruzado, J. C. (2017). A Mainly Circum-Mediterranean Origin for West Eurasian and North African mtDNAs in Puerto Rico with Strong Contributions from the Canary Islands and West Africa. *Human Biology*, *89*(2), 125. https://doi.org/10.13110/humanbiology.89.2.04

Di Leo, P; Bavusi, M; Corrado, G; Danese, M; Giammatteo, T; Gioia, D; Schiattarella, M. (2017). Ancient settlement dynamics and predictive archaeological models for the Metapontum coastal area in Basilicata, southern Italy: from geomorphological survey to spatial analysis. *Journal of Coastal Conservation*, *22*(5), 865–877. https://doi.org/10.1007/s11852-017-0548-y

Dommain, R; Andama, M; McDonough, M. M; Prado, N. A; Goldhammer, T; Potts, R; Maldonado, J. E; Nkurunungi, J. B; Campana, M. G. (2020). The Challenges of Reconstructing Tropical Biodiversity With Sedimentary Ancient DNA: A 2200-Year-Long Metagenomic Record From Bwindi Impenetrable Forest, Uganda. *Frontiers in Ecology and Evolution*, *8*. https://doi.org/10.3389/fevo.2020.00218

Donatuto, J; Grossman, E. E; Konovsky, J; Grossman, S; Campbell, L. W. (2014). Indigenous Community Health and Climate Change: Integrating Biophysical and Social Science Indicators. *Coastal Management*, *42*(4), 355–373. https://doi.org/10.1080/08920753.2014.923140

Dutta, H; Dutta, A. (2016). The microbial aspect of climate change. *Energy, Ecology and Environment*, *1*(4), 209–232. https://doi.org/10.1007/s40974-016-0034-7

Dussex, N; Bergfeldt, N; De Anca Prado, V; Dehasque, M; Díez-del-Molino, D; Ersmark, E; Kanellidou, F; Larsson, P; Lemež, P; Lord, E; Mármol-Sánchez, E; Meleg, I. N; Måsviken, J; Naidoo, T; Studerus, J; Vicente, M; Von Seth, J; Götherström, A; Dalén, L; Heintzman, P. D. (2021). Integrating multi-taxon palaeogenomes and sedimentary ancient DNA to study past ecosystem dynamics. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1957), 20211252. https://doi.org/10.1098/rspb.2021.1252

Eaton, W. D; McGee, K. M; Larimer, M; Hoke, E; Karas, O; Hernandez, B; Wayland, N. A. (2021). Changes in soil bacterial communities, and carbon and nitrogen metrics as potential indicators of land use effects in a humid tropical forest. *Pedobiologia*, *85–86*, 150730. https://doi.org/10.1016/j.pedobi.2021.150730

Eisenmann, S; Bánffy, E; Van Dommelen, P; Hofmann, K. P; Maran, J; Lazaridis, I; Mittnik, A; McCormick, M; Krause, J; Reich, D; Stockhammer, P. W. (2018). Reconciling material cultures in archaeology with genetic data: The nomenclature of clusters emerging from archaeogenomic analysis. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-31123-z

Epp, L. S; Zimmermann, H. H; Stoof-Leichsenring, K. R. (2019). Sampling and Extraction of Ancient DNA from Sediments. *Methods in Molecular Biology*, 31–44. https://doi.org/10.1007/978-1-4939-9176-1_5

Epp, L. S; Stoof, K. R; Trauth, M. H; Tiedemann, R. (2010) Historical genetics on a sediment core from a Kenyan lake: intraspecific genotype turnover in a tropical rotifer is related to past environmental changes. *Paleolimnology*, *43*, 939-954. http://doi.org/10.007/s10933-009-9379-7

Epp, L. S; Stoof-Leuchsenring, K. R; Trauth, M. H; Tiedemann, R. (2011). Molecular profiling of diatom assemblages in tropical lake sediments using taxon-specific PCR and denaturing high-performance liquid chromatography (PCR-DHPCL). *Molecular Ecology Resources*, *11*, 842-853. https://doi.org/10.1111/j.1755-0998.2011.03022.x

Escalera-Reyes, J. (2020). Place Attachment, Feeling of Belonging and Collective Identity in Socio-Ecological Systems: Study Case of Pegalajar (Andalusia-Spain). *Sustainability*, *12*(8), 3388. https://doi.org/10.3390/su12083388

Fernandes, D. M; Sirak, K. A; Ringbauer, H; Sedig, J; Rohland, N; Cheronet, O; Mah, M; Mallick, S; Olalde, I; Culleton, B. J; Adamski, N; Bernardos, R; Bravo, G; Broomandkhoshbacht, N; Callan, K; Candilio, F; Demetz, L; Carlson, K. S. D; Eccles, L; Reich, D. (2020). A genetic history of the pre-contact Caribbean. *Nature*, *590*(7844), 103–110. https://doi.org/10.1038/s41586-020-03053-2

Ficetola, G. F; Manenti, R; Taberlet, P. (2019). Environmental DNA and metabarcoding for the study of amphibians and reptiles: species distribution, the microbiome, and much more. *Amphibia-Reptilia*, *40*(2), 129–148. https://doi.org/10.1163/15685381-20191194

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, *15*(10), 579–590. https://doi.org/10.1038/nrmicro.2017.87

Fleitmann, D; Burns, S. J; Matter, A; Cheng, H; Affolter, S. (2022). Moisture and seasonality shifts recorded in Holocene and Pleistocene speleothems from southeastern Arabia. Geophysical Research Letters, 49, e2021GL097255. https://doi.org/10.1029/2021GL097255

Foucher, A; Evrard, O; Ficetola, G. F; Gielly, L; Poulain, J; Giguet-Covex, C; Laceby, J. P; Salvador-Blanes, S; Cerdan, O; Poulenard, J. (2020). Persistence of environmental DNA in cultivated soils: implication of this memory effect for reconstructing the dynamics of land use and cover changes. *Nature* 10(10502). https://doi.org/10.1038/s41598-020-67452-1

Frindte, K; Lehndorff, E; Vlaminck, S; Werner, K; Kehl, M; Khormali, F; Knief, C. (2020). Evidence for signatures of ancient microbial life in paleosols. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-73938-9

Gagelidze, N. A; Amiranashvili, L. L; Sadunishvili, T. A; Kvesitadze, G. I; Urushadze, T. F; Kvrivishvili, T. O. (2018). Bacterial composition of different types of soils of Georgia. *Annals of Agrarian Science*, *16*(1), 17–21. https://doi.org/10.1016/j.aasci.2017.08.006

Gansauge, M. T; Meyer, M. (2013). Single-stranded DNA library preparation for the sequencing of ancient or damaged DNA. *Nature Protocols*, *8*(4), 737–748. https://doi.org/10.1038/nprot.2013.038

Gansauge, M. T; Aximu-Petri, A; Nagel, S; Meyer, M. (2020). Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. *Nature Protocols*, *15*(8), 2279–2300. https://doi.org/10.1038/s41596-020-0338-0

García-del-Amo, D; Mortyn, P. G; Reyes-García, V. (2020). Including indigenous and local knowledge in climate research: an assessment of the opinion of Spanish climate change researchers. *Climatic Change*, *160*(1), 67–88. https://doi.org/10.1007/s10584-019-02628-x

Glaser, B; Turrión, M. B; Alef, K. (2004). Amino sugars and muramic acid—biomarkers for soil microbial community structure analysis. *Soil Biology and Biochemistry*, *36*(3), 399–407. https://doi.org/10.1016/j.soilbio.2003.10.013

Gougoulias, C; Clark, J. M; Shaw, L. J. (2014). The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food and Agriculture*, *94*(12), 2362–2371. https://doi.org/10.1002/jsfa.6577

Griffin, J. S; Haug, L. A; Rivera, V. A; Gonzalez, L. M. H; Kelly, J. J; Miller, W. M; Wells, G. F; Packman, A. I. (2019). Soil hydrology drives ecological niche differentiation in a native prairie microbiome. *FEMS Microbiology Ecology*, *96*(1). https://doi.org/10.1093/femsec/fiz163

Grund, B. S; Williams, S. E; Surovell, T. A. (2014). Viable paleosol microorganisms, paleoclimatic reconstruction, and relative dating in archaeology: a test case from Hell Gap, Wyoming, USA. *Journal of Archaeological Science*, *46*, 217–228. https://doi.org/10.1016/j.jas.2014.02.010

Gutiérrez-García, T. A; Vázquez-Domínguez, E; Arroyo-Cabrales, J; Kuch, M; Enk, J; King, C; Poinar, H. N. (2014). Ancient DNA and the tropics: a rodent's tale. *Biology Letters*, *10*(6), 20140224. https://doi.org/10.1098/rsbl.2014.0224

Hagan, R. W; Hofman, C. A; Hübner, A; Reinhard, K; Schnorr, S; Lewis, C. M; Sankaranarayanan, K; Warinner, C. G. (2019). Comparison of extraction methods for recovering ancient microbial DNA from paleofeces. *American Journal of Physical Anthropology*, *171*(2), 275–284. https://doi.org/10.1002/ajpa.23978

Haile, J. (2011). Ancient DNA Extraction from Soils and Sediments. *Methods in Molecular Biology*, 57–63. https://doi.org/10.1007/978-1-61779-516-9_8

Haldeman, D. L; Amy, P. S; Ringelberg, D; White, D. C; Garen, R. E; Ghiorse, W. C. (1995). Microbial growth and resuscitation alter community structure after perturbation. *FEMS Microbiology Ecology*, *17*(1), 27–38. https://doi.org/10.1111/j.1574-6941.1995.tb00124.x

Han, D; Nam, S. I; Kim, J. H; Stein, R; Niessen, F; Joe, Y. J; Park, Y. H; Hur, H. G. (2017). Inference on Paleoclimate Change Using Microbial Habitat Preference in Arctic Holocene Sediments. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-08757-6

Hassan, S; Khurshid, Z; Sabreena, Bali, B. S; Ganai, B. A; Sayyed, R. Z; Poczai, P; Zaman, M. (2022). A Critical Assessment of the Congruency between Environmental DNA and Palaeoecology for the Biodiversity Monitoring and Palaeoenvironmental Reconstruction. *International Journal of Environmental Research and Public Health*, *19*(15), 9445. https://doi.org/10.3390/ijerph19159445

Jansson, J. K; Hofmockel, K. S. (2019). Soil microbiomes and climate change. *Nature Reviews Microbiology*, *18*(1), 35–46. https://doi.org/10.1038/s41579-019-0265-7

Jia, W; Anslan, S; Chen, F; Cao, X; Dong, H; Dulias, K; Gu, Z; Heinecke, L; Jiang, H; Kruse, S; Kang, W; Li, K; Liu, S; Liu, X; Liu, Y; Ni, J; Schwalb, A; Stoof-Leichsenring, K. R; Shen, W; Herzschuh, U. (2022). Sedimentary ancient DNA reveals past ecosystem and biodiversity changes on the Tibetan Plateau: Overview and prospects. *Quaternary Science Reviews*, *293*, 107703. https://doi.org/10.1016/j.quascirev.2022.107703

Kapp, J. D; Green, R. E; Shapiro, B. (2021). A Fast and Efficient Single-stranded Genomic Library Preparation Method Optimized for Ancient DNA. *Journal of Heredity*, *112*(3), 241–249. https://doi.org/10.1093/jhered/esab012

Kehlmaier, C; Barlow, A; Hastings, A. K; Vamberger, M; Paijmans, J. L. A; Steadman, D. W; Albury, N. A; Franz, R; Hofreiter, M; Fritz, U. (2017). Tropical ancient DNA reveals relationships of the extinct Bahamian giant tortoise *Chelonoidis alburyorum*. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1846), 20162235. https://doi.org/10.1098/rspb.2016.2235

Khomutova, T. E; Kashirskaya, N. N; Demkina, T. S; Kuznetsova, T. V; Fornasier, F; Shishlina, N. I; Borisov, A. V. (2019). Precipitation pattern during warm and cold periods in the Bronze Age (around 4.5-3.8 ka BP) in the desert steppes of Russia: Soil-microbiological approach for palaeoenvironmental reconstruction. *Quaternary International*, *507*, 84–94. https://doi.org/10.1016/j.quaint.2019.02.013

Knight, R. (2017). Expanding our Understanding of the Role of the Microbiome in Health and Disease. *Archives of Medical Research*, *48*(8), 663–665. https://doi.org/10.1016/j.arcmed.2018.02.002

Koerner, W; Dupouey, J. L; Dambrine, E; Benoit, M. (1997). Influence of Past Land Use on the Vegetation and Soils of Present Day Forest in the Vosges Mountains, France. *The Journal of Ecology*, *85*(3), 351. https://doi.org/10.2307/2960507

Kohn, E. (2013). *How Forests Think: Toward an Anthropology Beyond the Human* (First). University of California Press.

Lacerda-Júnior, G. V; Noronha, M. F; Cabral, L; Delforno, T. P; De Sousa, S. T. P; Fernandes-Júnior, P. I; Melo, I. S; Oliveira, V. M. (2019). Land Use and Seasonal Effects on the Soil Microbiome of a Brazilian Dry Forest. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.00648

Lan, T; Lindqvist, C. (2018). Paleogenomics: Genome-Scale Analysis of Ancient DNA and Population and Evolutionary Genomic Inferences. *Population Genomics*, 323–360. https://doi.org/10.1007/13836_2017_7

Lewis, D. E; Chauhan, A; White, J. R; Overholt, W; Green, S. J; Jasrotia, P; Wafula, D; Jagoe, C. (2012). Microbial and Geochemical Assessment of Bauxitic Un-mined and Post-mined Chronosequence Soils from Mocho Mountains, Jamaica. *Microbial Ecology*, *64*(3), 738–749. https://doi.org/10.1007/s00248-012-0020-3

Li, J; Zhang, X; Xiao, L; Liu, K; Li, Y; Zhang, Z; Chen, Q; Ao, X; Liao, D; Gu, Y; Ma, M; Yu, X; Xiang, Q; Chen, J; Zhang, X; Yang, T; Penttinen, P; Zhao, K. (2020). Changes in soil microbial communities at Jinsha earthen site are associated with earthen site deterioration. *BMC Microbiology*, *20*(1). https://doi.org/10.1186/s12866-020-01836-1

Li, K; Stoof-Leichsenring, K. R; Liu, S; Jia, W; Liao, M; Liu, X; Ni, J; Herzschuh, U. (2021). Plant sedimentary DNA as a proxy for vegetation reconstruction in eastern and northern Asia. *Ecological Indicators*, *132*, 108303. https://doi.org/10.1016/j.ecolind.2021.108303

Liu, Q; Zhang, H; Chang, F; Xie, P; Zhang, Y; Wu, H; Zhang, X; Peng, W; Liu, F. (2022). eDNA revealed in situ microbial community changes in response to Trapa japonica in Lake Qionghai and Lake Erhai, southwestern China. *Chemosphere*, *288*, 1-12. https://doi.org/10.1016/j.chemosphere.2021.132605

Lyons, K. M. (2020). *Vital Decomposition: Soil Practitioners and Life Politics*. Duke University Press Books.

Macnaughton, S. J; Jenkins, T. L; Wimpee, M. H; Cormiér, M. R; White, D. C. (1997). Rapid extraction of lipid biomarkers from pure culture and environmental samples using pressurized accelerated hot solvent extraction. *Journal of Microbiological Methods*, *31*(1–2), 19–27. https://doi.org/10.1016/s0167-7012(97)00081-x

Martin-Laurent, F; Philippot, L; Hallet, S; Chaussod, R; Germon, J. C; Soulas, G; Catroux, G. (2001). DNA Extraction from Soils: Old Bias for New Microbial Diversity Analysis Methods. *Applied and Environmental Microbiology*, *67*(5), 2354–2359. https://doi.org/10.1128/aem.67.5.2354-2359.2001

Martiny, J. B. H; Bohannan, B. J; Brown, J. H; Colwell, R. K; Fuhrman, J. A; Green, J. L; Horner-Devine, M. C; Kane, M; Krumins, J. A; Kuske, C. R; Morin, P. J; Naeem, S; Øvreås, L; Reysenbach, A. L; Smith, V. H; Staley, J. T. (2006). Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology*, *4*(2), 102–112. https://doi.org/10.1038/nrmicro1341

Massilani, D; Morley, M. W; Mentzer, S. M; Aldeias, V; Vernot, B; Miller, C; Stahlschmidt, M; Kozlikin, M. B; Shunkov, M. V; Derevianko, A. P; Conard, N. J; Wurz, S; Henshilwood, C. S; Vasquez, J; Essel, E; Nagel, S; Richter, J; Nickel, B; Roberts, R. G; Meyer, M. (2021).

Microstratigraphic preservation of ancient faunal and hominin DNA in Pleistocene cave sediments. *Proceedings of the National Academy of Sciences*, *119*(1). https://doi.org/10.1073/pnas.2113666118

Matisoo-Smith, E. 2008. Using DNA in Landscape Archaeology. In *Handbook of Landscape Archaeology*, edited by David B. Thomas J., pp. 521–529. Routledge Member of the Taylor and Francis Group, New York, NY.

Mejbel, H. S; Dodsworth, W; Baud, A; Gregory-Eaves, I; Pick, F. R. (2021). Comparing Quantitative Methods for Analyzing Sediment DNA Records of Cyanobacteria in Experimental and Reference Lakes. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.669910

Mendes, L. W; Tsai, S. M; Navarrete, A. A; De Hollander, M; Veen, J. A; Kuramae, E. E. (2015). Soil-Borne Microbiome: Linking Diversity to Function. *Microbial Ecology*, *70*(1), 255–265. https://doi.org/10.1007/s00248-014-0559-2

Mendoza-Revilla, J; Chacón-Duque, J. C; Fuentes-Guajardo, M; Ormond, L; Wang, K; Hurtado, M; Villegas, V; Granja, V; Acuña-Alonzo, V; Jaramillo, C; Arias, W; Barquera, R; Gómez-Valdés, J; Villamil-Ramírez, H; Silva de Cerqueira, C. C; Badillo Rivera, K. M.' Nieves-Colón, M. A; Gignoux, C. R; Wojcik, G. L; Hellenthal, G. (2022). Disentangling Signatures of Selection Before and After European Colonization in Latin Americans. *Molecular Biology and Evolution*, *39*(4). https://doi.org/10.1093/molbev/msac076

Mergeay, J; Vanoverbeke, J; Verschuren, D; De Meester, L. (2007). Ecology, recolonization, and dispersal through time in a planktonic crustacean. *Ecology, 88(12),* 3032-3043. https://doi.org/10.1890/06-1538.1

Mhete, M; Eze, P. N; Rahube, T. O; Akinyemi, F. O. (2020). Soil properties influence bacterial abundance and diversity under different land-use regimes in semi-arid environments. *Scientific African*, *7*, e00246. https://doi.org/10.1016/j.sciaf.2019.e00246

Miller, D. N; Bryant, J. E; Madsen, E. L; Ghiorse, W. C. (1999). Evaluation and Optimization of DNA Extraction and Purification Procedures for Soil and Sediment Samples. *Applied and Environmental Microbiology*, *65*(11), 4715–4724. https://doi.org/10.1128/aem.65.11.4715-4724.1999

Mitchell, K. J; Rawlence, N. J. (2021). Examining Natural History through the Lens of Palaeogenomics. *Trends in Ecology &Amp; Evolution*, *36*(3), 258–267. https://doi.org/10.1016/j.tree.2020.10.005

Moguel, B; Pérez, L; Alcaraz, L. D; Blaz, J; Caballero, M; Muñoz-Velasco, I; Becerra, A; Laclette, J. P; Ortega-Guerrero, B; Romero-Oliva, C. S; Herrera-Estrella, L; Lozano-García, S. (2021). Holocene life and microbiome profiling in ancient tropical Lake Chalco, Mexico. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-92981-8

Mohammed, R. S; Turner, G; Fowler, K; Pateman, M; Nieves-Colón, M. A; Fanovich, L; Cooke, S. B; Dávalos, L. M; Fitzpatrick, S. M; Giovas, C. M; Stokowski, M; Wrean, A. A; Kemp, M; LeFebvre, M. J; Mychajliw, A. M. (2022). Colonial Legacies Influence Biodiversity Lessons: How Past Trade Routes and Power Dynamics Shape Present-Day Scientific Research and Professional Opportunities for Caribbean Scientists. *The American Naturalist*, *200*(1), 140–155. https://doi.org/10.1086/720154

Montenegro, A; Araujo, A; Eby, M; Ferreira, L; Hetherington, R; Weaver, A. (2006). Parasites, Paleoclimate, and the Peopling of the Americas. *Current Anthropology*, *47*(1), 193–200. https://doi.org/10.1086/499553

Mummey, D. L; Stahl, P. D; Buyer, J. S. (2002). Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. *Applied Soil Ecology*, *21*(3), 251–259. https://doi.org/10.1016/s0929-1393(02)00090-2
Mumy, K. L; Findlay, R. H. (2004). Convenient determination of DNA extraction efficiency using an external DNA recovery standard and quantitative-competitive PCR. *Journal of Microbiological Methods*, *57*(2), 259–268. https://doi.org/10.1016/j.mimet.2004.01.013

Muñoz Guevara, L. V. (2020). *Informe Bioarqueológico Final de Campo - Yacimiento La Gallera - Ceiba, Puerto Rico*.

Naylor, D; Sadler, N; Bhattacharjee, A; Graham, E. B; Anderton, C. R; McClure, R; Lipton, M; Hofmockel, K. S; Jansson, J. K. (2020). Soil Microbiomes Under Climate Change and Implications for Carbon Cycling. *Annual Review of Environment and Resources*, *45*(1), 29–59. https://doi.org/10.1146/annurev-environ-012320-082720

Nägele, K; Posth, C; Iraeta Orbegozo, M; Chinique de Armas, Y; Hernández Godoy, S. T; González Herrera, U. M; Nieves-Colón, M. A; Sandoval-Velasco, M; Mylopotamitaki, D; Radzeviciute, R; Laffoon, J; Pestle, W. J; Ramos-Madrigal, J; Lamnidis, T. C; Schaffer, W. C; Carr, R. S; Day, J. S; Arredondo-Antúnez, C; Rangel-Rivero, A; Schroeder, H. (2020). Genomic insights into the early peopling of the Caribbean. *Science*, *369*(6502), 456–460. https://doi.org/10.1126/science.aba8697

Nerlich, A. G; Lösch, S. (2009). Paleopathology of Human Tuberculosis and the Potential Role of Climate. *Interdisciplinary Perspectives on Infectious Diseases*, *2009*, 1–9. https://doi.org/10.1155/2009/437187

Newsom, L. A. (2014). Environmental Archaeology. *Encyclopedia of Caribbean Archaeology*, 143–147. https://doi.org/10.2307/j.ctvx1hst1.70

Nieves-Colón, M. A. (2022). Anthropological genetic insights on Caribbean population history. *Evolutionary Anthropology: Issues, News, and Reviews*, *31*(3), 118–137. https://doi.org/10.1002/evan.21935

Nieves-Colón, M. A; Pestle, W. J; Reynolds, A. W; Llamas, B; De la Fuente, C; Fowler, K; Skerry, K. M; Crespo-Torres, E; Bustamante, C. D; Stone, A. C. (2019). Ancient DNA Reconstructs the Genetic Legacies of Precontact Puerto Rico Communities. *Molecular Biology and Evolution*, *37*(3), 611–626. https://doi.org/10.1093/molbev/msz267
Nieves-Colón, M. A; Ozga, A. T; Pestle, W. J; Cucina, A; Tiesler, V; Stanton, T. W; Stone, A. C. (2018). Comparison of two ancient DNA extraction protocols for skeletal remains from tropical environments. *American Journal of Physical Anthropology*, *166*(4), 824–836. https://doi.org/10.1002/ajpa.23472

Oliver, J. R; Rivera-Collazo, I. (2012a). *A Reassessment of María de la Cruz Cave Site, Puerto Rico: The 2012 Excavations*.

Oliver, J. R; Rivera-Collazo, I. (2012b). A reassessment of María de la Cruz Site, Puerto Rico: The 2012 excavations. In *25th International Congress of the Association of Caribbean Archaeology (IACA)*.

Orlando, L; Allaby, R; Skoglund, P; Der Sarkissian, C; Stockhammer, P. W; Ávila-Arcos, M. C; Fu, Q; Krause, J; Willerslev, E; Stone, A. C; Warinner, C. (2021). Ancient DNA analysis. *Nature Reviews Methods Primers*, *1*(1). https://doi.org/10.1038/s43586-020-00011-0

Orr, C. H; Williams, R; Halldórsdóttir, H. H; Birley, A; Greene, E; Nelson, A; Ralebitso-Senior, T. K; Taylor, G. (2021). Unique chemical parameters and microbial activity lead to increased archaeological preservation at the Roman frontier site of Vindolanda, UK. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-94853-7

Orsi, W. D; Coolen, M. J. L; Wuchter, C; He, L; More, K. D; Irigoien, X; Chust, G; Johnson, C; Hemingway, J. D; Lee, M; Galy, V; Giosan, L. (2017). Climate oscillations reflected within the microbiome of Arabian Sea sediments. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-05590-9

Pagán-Jiménez, J. R; Ali, Z; Santiago-Marrero, C. G; Hofman, C. L. (2020). Plantscapes of dwelling: Precolonial household mounds, phytocultural dynamics and the ensuing human ecosystems at El Flaco and El Carril (cal. AD 990–1450), northern Dominican Republic. *Review of Palaeobotany and Palynology*, *274*, 104160. https://doi.org/10.1016/j.revpalbo.2020.104160

Pascher, K; Švara, V; Jungmeier, M. (2022). Environmental DNA-Based Methods in Biodiversity Monitoring of Protected Areas: Application Range, Limitations, and Needs. *Diversity*, *14*(6), 463. https://doi.org/10.3390/d14060463

Pascual, J; Garcia, C; Hernandez, T; Moreno, J; Ros, M. (2000). Soil microbial activity as a biomarker of degradation and remediation processes. *Soil Biology and Biochemistry*, *32*(13), 1877–1883. https://doi.org/10.1016/s0038-0717(00)00161-9

Pearman, J. K; Thomson-Laing, G; Howarth, J. D; Vandergoes, M. J; Thompson, L; Rees, A; Wood, S. A. (2021). Investigating variability in microbial community composition in replicate environmental DNA samples down lake sediment cores. *PLOS ONE*, *16*(5), e0250783. https://doi.org/10.1371/journal.pone.0250783

Pérez, V; Liu, Y; Hengst, M. B; Weyrich, L. S. (2022). A Case Study for the Recovery of Authentic Microbial Ancient DNA from Soil Samples. *Microorganisms*, *10*(8), 1623. https://doi.org/10.3390/microorganisms10081623

Peters, S; Borisov, A. V; Reinhold, S; Korobov, D. S; Thiemeyer, H. (2014). Microbial characteristics of soils depending on the human impact on archaeological sites in the Northern Caucasus. *Quaternary International*, *324*, 162–171. https://doi.org/10.1016/j.quaint.2013.11.020

Pilliod, D. S; Goldberg, C. S; Laramie, M. B; Waits, L. P. (2013). Application of environmental DNA for inventory and monitoring of aquatic species. *Fact Sheet*. https://doi.org/10.3133/fs20123146

Premdas, R. R. (2011). Identity, ethnicity, and the Caribbean homeland in an era of globalization. *Social Identities*, *17*(6), 811–832. https://doi.org/10.1080/13504630.2011.606676

Ratnasingham, S. (2019) mBrave: The multiplex barcode research and visualization environment. *Biodiversity Information Science and Standards, 3:e37986.* https://doi.org/10.3897/biss.3.37986

Rijal, D. P; Heintzman, P. D; Lammers, Y; Yoccoz, N. G; Lorberau, K. E; Pitelkova, I; Goslar, T; Murguzur, F. J. A; Salonen, J. S; Helmens, K. F; Bakke, J; Edwards, M. E; Alm, T; Bråthen, K. A; Brown, A. G; Alsos, I. G. (2021). Sedimentary ancient DNA shows terrestrial plant richness continuously increased over the Holocene in northern Fennoscandia. *Science Advances*, *7*(31). https://doi.org/10.1126/sciadv.abf9557

Rivera-Collazo, I. C. (2015). Por el camino verde: Long-term tropical socioecosystem dynamics and the Anthropocene as seen from Puerto Rico. *The Holocene* 25(10):1604–1611.

Rivera-Collazo, I. C; Rodríguez-Franco, C; Garay-Vázquez, J.J. (2018). A Deep-Time Socio Ecosystem Framework to Understand Social Vulnerability on a Tropical Island. *Environmental Archaeology* 23(1):97–108.

Rivera-Collazo, I; Oliver, J; Declet-Perez, M; Garay-Vázquez, J. J; Burton, M; Cantu, K; Lozano, H; Morris, M; Rodríguez-Delgado, E. (In Progress). *Human Paleoecology in Pre Columbian Puerto Rico 2019 Tierras Nuevas Archaeological Project Field Report*. Reporte arqueológico para el Instituto de Cultura Puertorriqueña (ICP).

Rivera-Collazo, I. (2022). Environment, climate and people: exploring human responses to climate change. *Journal of Anthropological Archaeology* 68. https://doi.org/10.1016/j.jaa.2022.101460.

Rizzi, E., Lari, M., Gigli, E., De Bellis, G., & Caramelli, D. (2012). Ancient DNA studies: new perspectives on old samples. *Genetics Selection Evolution*, *44*(1). https://doi.org/10.1186/1297-9686-44-21

Rodríguez Ramos, R. (2009). What is the Caribbean?: An archaeological perspective. *Journal of Caribbean Archaeology*, *3*, 3.

Santiago-Rodriguez, T. M; Narganes-Storde, Y. M; Chanlatte, L; Crespo-Torres, E; Toranzos, G. A; Jimenez-Flores, R; Hamrick, A; Cano, R. J. (2013). Microbial Communities in Pre-Columbian Coprolites. *PLoS ONE*, *8*(6), e65191. https://doi.org/10.1371/journal.pone.0065191

Seeber, P. A; Epp, L. S. (2022). Environmental DNA and metagenomics of terrestrial mammals as keystone taxa of recent and past ecosystems. *Mammal Review*, *52*(4), 538–553. https://doi.org/10.1111/mam.12302

Shapiro, B; Barlow A; Heintzman,P. D; Hofreiter, M; Paijmans, J. L. A; Soares André, E. R. (2019). *Ancient DNA - methods and protocols second edition*. Humana Press, New York, NY.

Siles, J. A; Öhlinger, B; Cajthaml, T; Kistler, E; Margesin, R. (2018). Characterization of soil bacterial, archaeal and fungal communities inhabiting archaeological human-impacted layers at Monte Iato settlement (Sicily, Italy). *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-20347-8

Sipilä, T. P; Yrjälä, K; Alakukku, L; Palojärvi, A. (2012). Cross-Site Soil Microbial Communities under Tillage Regimes: Fungistasis and Microbial Biomarkers. *Applied and Environmental Microbiology*, *78*(23), 8191–8201. https://doi.org/10.1128/aem.02005-12

Sistiaga, A; Husain, F; Uribelarrea, D; Martín-Perea, D. M; Ferland, T; Freeman, K. H; Diez-Martín, F; Baquedano, E; Mabulla, A; Domínguez-Rodrigo, M; Summons, R. E. (2020). Microbial biomarkers reveal a hydrothermally active landscape at Olduvai Gorge at the dawn of the

Acheulean, 1.7 Ma. *Proceedings of the National Academy of Sciences*, *117*(40), 24720–24728. https://doi.org/10.1073/pnas.2004532117

Sméralda, J; Castro Romero, M. (2020). Deconstructing the concept of decolonisation. *International Review of Psychiatry*, *32*(4), 374–380. https://doi.org/10.1080/09540261.2020.1772585

Smith, B. D. (2011). General patterns of niche construction and the management of 'wild' plant and animal resources by small-scale pre-industrial societies. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *366*(1566), 836–848. https://doi.org/10.1098/rstb.2010.0253

Staley, J. T. (1997). Biodiversity: are microbial species threatened? *Current Opinion in Biotechnology*, *8*(3), 340–345. https://doi.org/10.1016/s0958-1669(97)80014-6

Stilling, R. M; Bordenstein, S. R; Dinan, T. G; Cryan, J. F. (2014). Friends with social benefits: host-microbe interactions as a driver of brain evolution and development? *Frontiers in Cellular and Infection Microbiology*, *4*. https://doi.org/10.3389/fcimb.2014.00147

Stoof-Leichsenring, K. R; Epp, L. S; Trauth, M. H; Tiedemann, R. (2012). Hidden diversity in diatoms of Kenyan lake Naivasha: A genetic approach detects temporal variation. *Molecular Ecology, 21,* 1918-1930. https://doi.org/10.1111/j.1365-294X.2011.05412.x

Straube, D; Juen, A. (2013). Storage and shipping of tissue samples for DNA analyses: A case study on earthworms. *European Journal of Soil Biology*, *57*, 13–18. https://doi.org/10.1016/j.ejsobi.2013.04.001

Suleymanov, R. R; Ovsyannikov, V. V; Kolonskih, A. G; Abakumov, E. V; Kungurtsev, A. Y; Suleymanov, A. R. (2020). Soil-Archaeological Study of the Votikeevo Medieval Archeological Site in the Northern Forest-Steppe Zone of the Southern Cis-Ural Region. *Eurasian Soil Science*, *53*(3), 283–293. https://doi.org/10.1134/s1064229320030084

Sun, Y; Luo, C; Jiang, L; Song, M; Zhang, D; Li, J; Li, Y; Ostle, N. J; Zhang, G. (2020). Land-use changes alter soil bacterial composition and diversity in tropical forest soil in China. *Science of the Total Environment*, *712*, 136526. https://doi.org/10.1016/j.scitotenv.2020.136526

Taberlet, P. (2018). *Environmental DNA: For Biodiversity Research and Monitoring* (Illustrated). Oxford University Press, USA.

Talas, L; Stivrins, N; Veski, S; Tedersoo, L; Kisand, V. (2021). Sedimentary Ancient DNA (sedaDNA) Reveals Fungal Diversity and Environmental Drivers of Community Changes throughout the Holocene in the Present Boreal Lake Lielais Svētiņu (Eastern Latvia). *Microorganisms*, *9*(4), 719. https://doi.org/10.3390/microorganisms9040719

Vernot, B; Zavala, E. I; Gómez-Olivencia, A; Jacobs, Z; Slon, V; Mafessoni, F; Romagné, F; Pearson, A; Petr, M; Sala, N; Pablos, A; Aranburu, A; De Castro, J. M. B; Carbonell, E; Li, B; Krajcarz, M. T; Krivoshapkin, A. I; Kolobova, K. A; Kozlikin, M. B; Meyer, M. (2021). Unearthing Neanderthal population history using nuclear and mitochondrial DNA from cave sediments. *Science*, *372*(6542). https://doi.org/10.1126/science.abf1667

Warinner, C; Speller, C; Collins, M. J; Lewis, C. M. (2015). Ancient human microbiomes. *Journal of Human Evolution*, *79*, 125–136. https://doi.org/10.1016/j.jhevol.2014.10.016

Warinner, C; Herbig, A; Mann, A; Fellows Yates, J. A; Weiß, C. L; Burbano, H. A; Orlando, L; Krause, J. (2017). A Robust Framework for Microbial Archaeology. *Annual Review of Genomics and Human Genetics*, *18*(1), 321–356. https://doi.org/10.1146/annurev-genom-091416-035526
Watzinger, A. (2015). Microbial phospholipid biomarkers and stable isotope methods help reveal soil functions. *Soil Biology and Biochemistry*, *86*, 98–107. https://doi.org/10.1016/j.soilbio.2015.03.019

Wibowo, M. C; Yang, Z; Borry, M; Hübner, A; Huang, K. D; Tierney, B. T; Zimmerman, S; Barajas-Olmos, F; Contreras-Cubas, C; García-Ortiz, H; Martínez-Hernández, A; Luber, J. M; Kirstahler, P; Blohm, T; Smiley, F. E; Arnold, R; Ballal, S. A; Pamp, S. J; Russ, J; Kostic, A. D. (2021). Reconstruction of ancient microbial genomes from the human gut. *Nature*, *594*(7862), 234–239. https://doi.org/10.1038/s41586-021-03532-0

Wingate, L; Ogée, J; Cuntz, M; Genty, B; Reiter, I; Seibt, U; Yakir, D; Maseyk, K; Pendall, E. G; Barbour, M. M; Mortazavi, B; Burlett, R; Peylin, P; Miller, J; Mencuccini, M; Shim, J. H; Hunt, J; Grace, J. (2009). The impact of soil microorganisms on the global budget of $\delta^{18}O$ in atmospheric CO^{2+} *Proceedings of the National Academy of Sciences*, *106*(52), 22411–22415. https://doi.org/10.1073/pnas.0905210106

Wiscovitch-Russo, R; Rivera-Perez, J; Narganes-Storde, Y. M; García-Roldán, E; Bunkley-Williams, L; Cano, R; Toranzos, G. A. (2020). Pre-Columbian zoonotic enteric parasites: An insight into Puerto Rican indigenous culture diets and life styles. *PLOS ONE*, *15*(1), e0227810. https://doi.org/10.1371/journal.pone.0227810

Zhang, L; Lv, J. (2020). Metagenomic analysis of microbial community and function reveals the response of soil respiration to the conversion of cropland to plantations in the Loess Plateau of China. *Global Ecology and Conservation*, *23*, e01067. https://doi.org/10.1016/j.gecco.2020.e01067

Zhang, M; Cao, P; Dai, Q. Y; Wang, Y. Q; Feng, X. T; Wang, H. R; Wu, H; Min-Shan Ko, A; Mao, X. W; Liu, Y. C; Yu, L; Roos, C; Nadler, T; Xiao, W; Andrew Bennett, E; Fu, Q. M. (2021). Comparative analysis of DNA extraction protocols for ancient soft tissue museum samples. *Zoological Research*, *42*(3), 280–286. https://doi.org/10.24272/j.issn.2095-8137.2020.377