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Mexico City, September 5th 2017

Lic. Jaime Maussan Flota
PRESENTE

Re: Results of old DNA.massive sequencing process.

Dear Lic. Maussan,

I am informing you hereby about the history and status of the ongoing genomic analysis process on tissue samples from two different mummies.

1.- On June 15th, 6 samples from 2 mummies, dated approximately 1500 years ago, from the Nazca region of Peru were received. The samples received are described in Table 1.

2.- On June 24th, the 6 samples DNA was purified. DNA was obtained under sterile conditions, minimizing the risk of contamination, in accordance with the standards recommended for archaeological samples (Shapiro B, Heslington M. 2012). Integrity and total amount of recovered DNA were determined. The DNA had a range of 8,000 base pairs (bp) to 700 bp, obtaining from 8 µg to 0.5 µg..

Table 2 shows the recovered amount of each sample and in Figure 1, the evaluation of the size and amount of the old DNA obtained.

3.- On June 27th, samples 1, 3, 5 and 6 were selected because they had the right DNA amountf to process and were handed over to Dr. Alfredo Mendoza Vargas, Unit Manager Sequencing and identification of the polymorphisms of the National Institute of Genomic Medicine, so that the samples were processed to be read in a massive sequencer (Illumina Myseq). In order to improve the success of sequencing, it has been proposed to repair the DNA of the chemical modifications undergone because of its age. This repair was performed with the New England Biolabs M0309S PreCR® Repair Mix Kit. This one was imported and was used on August 11th.

After the repair and purification, the DNA was quantified and we obtained:

Mummy 1 0.896 ng / ul with approx. 13ul
Mummy 3 0.132 ng / ul with approx. 13ul
Mummy 5 0.144 ng / ul with approx. 13ul
Mummy 6 0.196 ng / ul with approx. 13ul

The realization of massive sequencing was prepared with these DNA libraries. The mummy 1 sample was made with 10 ng and the rest of the samples between 1 and 2 ng with all the volume counted.

4.- On August 29th, sample sequencing was obtained, reaching approximately 600,000 readings per sample. In the following link, you can find the genomic readings obtained.
https://www.dropbox.com/sh/yd9gitxd09ecpux/AAAHtROmZ10yp6u2gEYCsg53a?dl=0

The DNA readings of each sample were evaluated in the Illumina BaseSpace Sequence Hub platform, using an application to align the sequences with the human genome. These results are shown in Figures 2 to 5. In general, it has been found that DNA reads contain about 30% of DNA similar to that of humans. The other sequences are most likely bacterial origin sequences, which is common in this type of samples.

5.- Old DNA readings are currently in a bioinformatic study, to find out what genetic information can be retrieved, to analyze its meaning and to determine genomic sequencing of greater depth is possible to obtain, in order to have more information and to define the ancestral origins of the samples.

While waiting for your comments, I send you my cordial greetings.

Dr. Rogelio A. Alonso Morales.

References: Shapiro B, Heslington M. 2012. Ancient DNA - Methods and Protocols. Humana Press

Table 1.- List of old samples received on June 15th 2017

# ID	Legend on the tube	Weight in g
1	HAND 00-1	2.38
2	BRAIN 00-10	1.57
3	MARIA B0 HOM	0.56
4	NECK BONE - VERTEBRAE 00-12-VICTORIA 540 MG	0.53
5	HIP BONE02-12 VICTORIA 0.8325 MG	0.33
6	NECK BONE VICTORIA 00-17 PIEL 187 MG	0.17

Table 2.- Amount of DNA obtained on the old samples.

Sample No	Concentration in ng/ul	Volume in ul	Total quantity ug
1	110	80	8.80
2	19	60	1.14
3	50	60	3.00
4	11	50	0.50
5	25	85	2.12
6	35	35	1.22

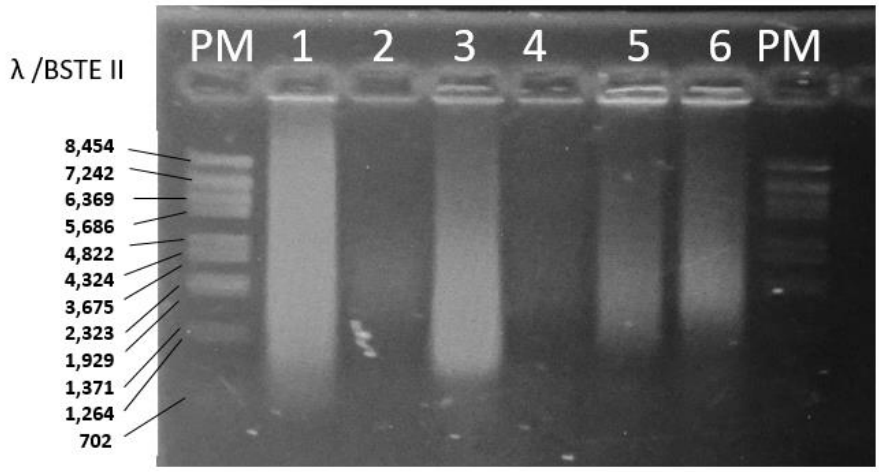

Figure 1.- Evaluation of the DNA quality and uantity. Electroforesis in Agarosa 1%. PM - Marcador molecular weight (DNA lambda / BsteII) .. On the right, list of the molecular weight in pb. At the top, number of each sample. In each track, 5 µl of the

Figures 3-5. - We present the results obtained from old DNA sequences by aligning them on the human genome.

RESULTS FOR INPUT SAMPLE MOMIA3

ALIGNMENT STATISTICS

	Reads	Percentage
Total PF	658,294	100.00%
Paired	658,074	99.97%
Read 1	329,037	49.98%
Read 2	329,037	49.98%
Aligned	221,187	33.60%
Properly Paired	217,914	98.52%
Singletons	2,207	1.00%
Secondary Alignments	220	0.10%
Supplementary Alignments	0	0.00%
Duplicates	12,056	5.45%

Please note that "Paired", "Read 1", "Read 2" and "Aligned" percentages are calculated based on the "Total PF" value. All other percentages are calculated based on the "Aligned" value.

Secondary Alignments: Alignments for reads mapping to multiple locations that are not considered the primary alignment.
Supplementary Alignments: Partial alignments for reads split across multiple genomic locations. The first part of the alignment is the primary alignment and the other parts are called supplementary alignments.
Duplicates: Aligned reads marked as possible PCR duplicates by MarkDuplicates.
For more details, please see the SAM specification.

RESULTS FOR INPUT SAMPLE MOMIA1

ALIGNMENT STATISTICS

	Reads	Percentage
Total PF	684,995	100.00%
Paired	682,622	99.65%
Read 1	341,311	49.83%
Read 2	341,311	49.83%
Aligned	135,774	19.82%
Properly Paired	126,544	93.20%
Singletons	3,917	2.88%
Secondary Alignments	2,373	1.75%
Supplementary Alignments	0	0.00%
Duplicates	2,167	1.60%

Please note that "Paired", "Read 1", "Read 2" and "Aligned" percentages are calculated based on the "Total PF" value. All other percentages are calculated based on the "Aligned" value.

Secondary Alignments: Alignments for reads mapping to multiple locations that are not considered the primary alignment.
Supplementary Alignments: Partial alignments for reads split across multiple genomic locations. The first part of the alignment is the primary alignment and the other parts are called supplementary alignments.
Duplicates: Aligned reads marked as possible PCR duplicates by MarkDuplicates.
For more details, please see the SAM specification.

RESULTS FOR INPUT SAMPLE MOMIA5

ALIGNMENT STATISTICS

	Reads	Percentage
Total PF	675,694	100.00%
Paired	675,302	99.94%
Read 1	337,651	49.97%
Read 2	337,651	49.97%
Aligned	196,731	29.12%
Properly Paired	194,598	98.92%
Singletons	983	0.50%
Secondary Alignments	392	0.20%
Supplementary Alignments	0	0.00%
Duplicates	2,805	1.43%

Please note that "Paired", "Read 1", "Read 2" and "Aligned" percentages are calculated based on the "Total PF" value. All other percentages are calculated based on the "Aligned" value.

Secondary Alignments: Alignments for reads mapping to multiple locations that are not considered the primary alignment.
Supplementary Alignments: Partial alignments for reads split across multiple genomic locations. The first part of the alignment is the primary alignment and the other parts are called supplementary alignments.
Duplicates: Aligned reads marked as possible PCR duplicates by MarkDuplicates.
For more details, please see the SAM specification.

RESULTS FOR INPUT SAMPLE MOMIA6

ALIGNMENT STATISTICS

	Reads	Percentage
Total PF	180,026	100.00%
Paired	179,922	99.94%
Read 1	89,961	49.97%
Read 2	89,961	49.97%
Aligned	65,296	36.27%
Properly Paired	64,232	98.37%
Singletons	498	0.76%
Secondary Alignments	104	0.16%
Supplementary Alignments	0	0.00%
Duplicates	809	1.24%

Please note that "Paired", "Read 1", "Read 2" and "Aligned" percentages are calculated based on the "Total PF" value. All other percentages are calculated based on the "Aligned" value.

Secondary Alignments: Alignments for reads mapping to multiple locations that are not considered the primary alignment.
Supplementary Alignments: Partial alignments for reads split across multiple genomic locations. The first part of the alignment is the primary alignment and the other parts are called supplementary alignments.
Duplicates: Aligned reads marked as possible PCR duplicates by MarkDuplicates.
For more details, please see the SAM specification.