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Illumina library construction for Extraction Method A, B, C (For FMS samples) and D, E (PRP and RM)

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Faezah Mohd Salleh¹, Jazmin Ramos-Madrigal¹, Fernando Penaloza¹, Shanlin Liu¹, Mikkel-Holger S Sinding¹, Riddhi P Patel¹, Renata Martins¹, Dorina Lenz¹, Jorns Fickel¹, Christian Roos¹, Mohd Shahir Shamsir¹, Mohammad Shahfiz Azman¹, Burton K Lim¹, Stephen J Rossiter¹, Andreas Wilting¹, M Thomas P Gilbert¹

¹Natural History Museum of Denmark, Copenhagen, Denmark

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Faezah Mohd Salleh

ABSTRACT

Library building protocol for archival samples with single 8-nt adapters for Illumina platforms.

MATERIALS

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⊗ Buffer EB **Qiagen Catalog #19086**

⊗ 0.5 μM of Illumina P5 and P7 adapter mix **Contributed by users**

⊗ MinElute **Qiagen**

⊗ Primer IS4 and individual Indexing Primers **Contributed by users**

BEFORE START INSTRUCTIONS

Calculate the DNA input and prepare the library adapters.

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End-repair

- 1 End-repair step following Fortes & Paijmans 2015, with T4 Plynuclease kinase and T4 Polymerase, and heat inactivation of the enzymes (to remove column clean-up step).

Note

Fortes, G.G. & Paijmans, J.L.A. (2015) Analysis of whole mitogenomes from ancient samples. in Whole genome amplification, Humana Press, USA.

Ligation

- 2 Ligation step following Fortes & Paijmans, 2015 with 0.5 μM of Illumina P5 and P7 adapter mix (20 μM each).

Note

Fortes, G.G. & Paijmans, J.L.A. (2015) Analysis of whole mitogenomes from ancient samples. in Whole genome amplification, Humana Press, USA.

Library purification

- 3 Purify Adapter-ligated libraries with MinElute (Qiagen, Hilden, DE).

- 4 Elute libraries in 20 μ L elution Buffer EB.

🧴 20 μ L Buffer EB

Adapater Fill-in

- 5 Fill-in step of the adapters before library indexing, following Fortes & Paijmans, 2015.

Note

Fortes, G.G. & Paijmans, J.L.A. (2015) Analysis of whole mitogenomes from ancient samples. in Whole genome amplification, Humana Press, USA.

Indexing PCR

- 6 Perform indexing PCR reactions in a total volume of 15 μ L with AmpliTaq Gold (Applied Biosystems) with Primer IS4 and individual Indexing Primers, following Fortes & Paijmans, 2015.

Note

The total number of cycles did not exceed 10 for the first round of amplification.

Note

Fortes, G.G. & Paijmans, J.L.A. (2015) Analysis of whole mitogenomes from ancient samples. in Whole genome amplification, Humana Press, USA.

- 7 Verify indexed libraries with Agilent Tapestation HS (Agilent) for fragment size estimation and molar concentrations.
- 8 Perform parallel amplifications for each sample, with corrected number of PCR cycles, in order to avoid over-amplification and increased read clonality.
- 9 Purify indexed libraries with MinElute (Qiagen, Hilden, DE).

10 Elute libraries in 20 μ L elution Buffer EB.

20 μ L Buffer EB