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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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Keywords: illumina library construction, library building protocol for archival sample, nt adapters for illumina platform, archival sample, illumina platform, fms sample, library building protocol, nt adapter, sample

Abstract

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

Materials

MATERIALS

⊗ Buffer EB **Qiagen Catalog #19086**

⊗ 0.5 µM of Illumina P5 and P7 adapter mix

⊗ MinElute **Qiagen**

⊗ Primer IS4 and individual Indexing Primers

Troubleshooting

Before start

Calculate the DNA input and prepare the library adapters.

End-repair

- 1 End-repair step following Fortes & Paijmans 2015, with T4 Polynuclease 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 μ M of Illumina P5 and P7 adapter mix (20 μ M 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

Adapter 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