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

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Abstract

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

Citation: Faezah Mohd Salleh,Jazmin Ramos-Madrigal,Fernando Penaloza,Shanlin Liu,Mikkel-Holger S Sinding,Riddhi P Patel,Renata Martins,Dorina Lenz,Jorns Fickel,Christian Roos,Mohd Shahir Shamsir,Mohammad Shahfiz Azman,Burton K Lim,Stephen J Rossiter,Andreas Wilting,M Thomas P Gilbert Illumina library construction for Extraction Method A, B, C (For FMS samples) and D, E (PRP and RM). **protocols.io**

dx.doi.org/10.17504/protocols.io.ingcdbw

Published: 05 Jul 2017

Before start

Calculate the DNA input and prepare the library adapters.

Materials

Buffer EB 19086 by Qiagen

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

MinElute by Qiagen

✓ Primer IS4 and individual Indexing Primers by Contributed by users

Protocol

End-repair

Step 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).

NOTES

GigaScience Database 26 Jun 2017

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

Ligation

Step 2.

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

NOTES

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

Library purification

Step 3.

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

Library purification

Step 4.

Elute libraries in 20 µL elution Buffer EB.

AMOUNT

20 µl Additional info: Buffer EB

Adapater Fill-in

Step 5.

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

NOTES

GigaScience Database 26 Jun 2017

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

Indexing PCR

Step 6.

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

NOTES

GigaScience Database 26 Jun 2017

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

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

Indexing PCR

Step 7.

Verify indexed libraries with Agilent Tapestation HS (Agilent) for fragment size estimation and molar concentrations.

Indexing PCR

Step 8.

Perform parallel amplifications for each sample, with corrected number of PCR cycles, in order to avoid over-amplification and increased read clonality.

Indexing PCR

Step 9.

Purify indexed libraries with MinElute (Qiagen, Hilden, DE).

Indexing PCR

Step 10.

Elute libraries in 20 µL elution Buffer EB.

AMOUNT

20 µl Additional info: Buffer EB