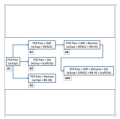

Mar 25, 2020

Amplification Free Paired End Library Construction Protocol

In 1 collection

Graham J Etherington[1], Darren Heavens[1], David Baker[1], Ashleigh Lister[1], Rose McNelly[1], Gonzalo Garcia[1], Bernardo Clavijo[1], Iain Macaulay[1], Wilfried Haerty[1], Federica Di Palma[1]

[1]The Earlham Institute, Norwich Research Park, Norwich, NR4 7UZ, United Kingdom

1 Works for me dx.doi.org/10.17504/protocols.io.bd3ti8nn

Graham Etherington

ABSTRACT

Amplification Free Paired End Library Construction Protocol.

1 A total of **600 ng** of DNA was sheared in a **60 µl** volume on a Covaris S2 (Covaris, Massachusetts, USA) for 1 cycle of **00:00:40** with a duty cycle of 5%, cycles per burst of 200 and intensity of 3.

2 The fragmented molecules were then end repaired in **100 µl** volume using the NEB End Repair Module (NEB, Hitchin, UK) incubating the reaction at **22 °C** for **00:30:00** .

3 Post incubation **58 µl** beads of CleanPCR beads (GC Biotech, Alphen aan den Rijn, The Netherlands) were added using a positive displacement pipette to ensure accuracy and the DNA precipitated onto the beads.

4 This is then washed twice with 70% ethanol and the end repaired molecules eluted in **25 µl** Nuclease free water (Qiagen, Manchester, UK).

5 End repaired molecules were then A tailed in **30 µl** volume using in the NEB A tailing module (NEB) incubating the reaction at **37 °C** for **00:30:00** .

6 To the A tailed library molecules **1 µl** of an appropriate Illumina TruSeq Index adapter (Illumina, San Diego, USA) is added and mixed, then **31 µl** of Blunt/ TA ligase (NEB) is added and incubated at **22 °C** for **00:10:00** .

7 Post incubation **5 µl** of stop ligation is added and the reaction incubated at **Room temperature** for **00:05:00** .

Citation: Graham J Etherington, Darren Heavens, David Baker, Ashleigh Lister, Rose McNelly, Gonzalo Garcia, Bernardo Clavijo, Iain Macaulay, Wilfried Haerty, Federica Di Palma (03/25/2020). Amplification Free Paired End Library Construction Protocol. https://dx.doi.org/10.17504/protocols.io.bd3ti8nn

8 Following this incubation **67 µl** beads of CleanPCR beads (GC Biotech, Alphen aan den Rijn, The Netherlands) were added and the DNA precipitated onto the beads.

9 The samples are then washed twice with 70% ethanol and the end repaired molecules eluted in **100 µl** nuclease free water.

10 Two further CleanPCR bead based purifications were undertaken to remove any adapter dimer molecules that may have formed during the adapter ligation step. The first with 0.9x volume beads, the second with 0.6x and the final library eluted in **25 µl** Resuspension Buffer (Illumina).

11 Library QC was performed by running a **1 µl** aliquot on a High Sensitivity BioAnalyser chip (Agilent, Stockport, UK) and the DNA concentration measured using the High Sensitivity Qubit (Thermo Fisher, Cambridge, UK).

12 To determine the number of viable library molecules the library was subjected to quantification by the Kappa qPCR Illumina quantification kit (Kapa Biosystems, London, UK) and a test lane run at 10pM on a MiSeq (Illumina) with 2x300bp reads to allow the library to be characterised prior to generation of the 60x coverage required on the Hiseq2500s (Illumina) with a 2x250bp read metric.

Citation: Graham J Etherington, Darren Heavens, David Baker, Ashleigh Lister, Rose McNelly, Gonzalo Garcia, Bernardo Clavijo, Iain Macaulay, Wilfried Haerty, Federica Di Palma (03/25/2020). Amplification Free Paired End Library Construction Protocol. https://dx.doi.org/10.17504/protocols.io.bd3ti8nn