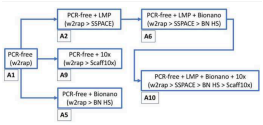
Mar 25, 2020

Amplification Free Paired End Library Construction Protocol

GigaScience

In 1 collection



DOI
dx.doi.org/10.17504/protocols.io.bd3ti8nn

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

GigaScience Press

Graham Etherington
The Earlham Institute

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.bd3ti8nn

External link: https://doi.org/10.1093/gigascience/giaa045

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

Manuscript citation:
Graham J Etherington, Darren Heavens, David Baker, Ashleigh Lister, Rose McNelly, Gonzalo Garcia, Bernardo Clavijo, Iain Macaulay, Wilfried Haerty, Federica Di Palma, Sequencing smart: De novo sequencing and assembly approaches for a non-model mammal, GigaScience, Volume 9, Issue 5, May 2020, giaa045, https://doi.org/10.1093/gigascience/giaa045

Protocol status: Working
We use this protocol and it's working

Created: March 23, 2020

Last Modified: March 26, 2020

Protocol Integer ID: 34643

Keywords: polecat, vertebrate, non-model organism, Illumina, chromium, Bionano, assembly, sequencing,

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 .

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 2×300bp reads to allow the library to be characterised prior to generation of the 60x coverage required on the Hiseq2500s (Illumina) with a 2×250bp read metric.