MAR 25, 2020

Long Mate Pair Library Construction Protocol

In 1 collection

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GigaScience Press

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bd3ui8nw

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ABSTRACT

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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. Long Mate Pair Library Construction Protocol. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bd3ui8nw>

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

Created: Mar 23, 2020

Last Modified: Mar 26, 2020

PROTOCOL integer ID:
 34644

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

- 1 For the Tagmentation reactions 3 µg and 3 µg of Genomic DNA was prepared in 308 µL and then 80 µL 5x Tagment Buffer Mate Pair (Illumina) added followed by 12 µL Mate Pair Tagmentation Enzyme (Illumina) and the reaction gently vortexed to mix.
- 2 This was then incubated for 00:30:00 at 55 °C, 100µl of Neutralize Tagment Buffer (Illumina) added and then incubated at Room temperature for 00:05:00.
- 3 A 1x volume bead clean-up was performed with CleanPCR beads and the DNA eluted in 165 µL of Nuclease free Water.
- 4 A 1 µL aliquot was run on a BioAnalyser 1200 chip and DNA concentration determined using a Qubit HS Assay.
- 5 Strand Displacement was performed by combining 162 µL of tagmented DNA, 20 µL 10x Strand Displacement Buffer (Illumina), 8 µL dNTPs (Illumina) and 10 µL Strand Displacement Polymerase (Illumina).
- 6 This was then incubated at Room temperature for 00:30:00.
- 7 A 0.75x volume bead clean-up was performed with CleanPCR beads and the DNA eluted in 16 µL of Nuclease free Water and the eluted DNA from the 3 µg and 3 µg reactions pooled.
- 8 A 1 µL aliquot was diluted 1:6 and run on a BioAnalyser 1200 chip and DNA concentration determined using a Qubit HS Assay.

- 9 Size selection was performed on a Sage Science ELF (Sage Science, Beverly, USA). The 30 µL in each of collection wells was replaced with fresh buffer and the collection and elution current checked prior to loading the sample. To 30 µL of the pooled Strand Displaced reaction 10 µL of loading solution was added and then loaded onto a 0.75% Cassette which was configured to separate the sample for 03:30:00 and then each fraction eluted for 00:35:00.
- 10 Post size selection, the 30 µL from each of the 12 collection wells was recovered and the DNA concentration determined using a Qubit HS Assay.
- 11 Circularisation was performed by combining 30 µL of size fractionated DNA, 12.5 µL of 10x circularisation buffer (Illumina), 3 µL Circularisation Enzyme (Illumina) and 85 µL nuclease free water.
- 12 These were then incubated at 30 °C overnight. Linear DNA was digested by adding 3.75 µL Exonuclease (Illumina) and incubation at 37 °C for 00:30:00 was followed by 70 °C for 00:30:00 to denature the enzyme and 5 µL of stop ligation (Illumina) added.
- 13 During exonuclease treatment 240 µL of M280 Dynabeads (Thermo Fisher) were prepared by washing twice with 600 µL Bead Bind Buffer (Illumina) before resuspending in 1560 µL Bead Bind Buffer.
- 14 Circularised DNA was then sheared in a 130 µL volume on a Covaris S2 for 2 cycles of 00:00:37 with a duty cycle of 10%, cycles per burst of 200 and intensity of 4.
- 15 To 130 µL fragmented DNA 130 µL of washed M280 beads was added, mixed and then placed on a lab rotator at Room temperature for 00:20:00.

- 16 Library molecules bound to M280 beads were then washed four times with 200 μL Bead Washer Buffer (Illumina) and twice with 200 μL Resuspension Buffer (Illumina).
- 17 A master mix containing 1105 μL nuclease free water, 130 μL 10x End Repair Reaction Buffer (NEB, Hitchin, UK) and 65 μL end repair enzyme mix (NEB) was prepared and 100 μL added to each tube, mixed with the beads and incubated at Room temperature for 00:30:00 .
- 18 End repaired library molecules bound to M280 beads were then washed four times with 200 μL Bead Washer Buffer and twice using 200 μL Resuspension Buffer.
- 19 A master mix containing 325 μL nuclease free water, 39 μL A Tailing 10x Reaction Buffer (NEB) and 26 μL A tailing enzyme mix (NEB) was prepared and 30 μL added to each tube, mixed with the beads and incubated at 37 $^{\circ}\text{C}$ for 00:30:00 .
- 20 To the A tailed library molecules 1 μL of the appropriate Illumina Index adapter (Illumina) was added and mixed then 31 μL of Blunt/ TA ligase (NEB) added and incubated at Room temperature for 00:10:00 .
- 21 Post incubation 5 μL of stop ligation added and then the adapter ligated library molecules bound to M280 beads were then washed four times with 200 μL Bead Washer Buffer and twice with 200 μL Resuspension Buffer.
- 22 A master mix containing 240 μL nuclease free water, 300 μL 2x Kappa HiFi (Kappa Biosystems) and 60 μL Illumina Primer Cocktail (Illumina) was prepared and 50 μL added to each tube, mixed with the beads and the contents, including beads, transferred to a 200 μL PCR tube.
- 23 Each sample was then subjected to amplification on a Veriti Thermal Cycler (Thermo Fisher) with the following conditions: 98 $^{\circ}\text{C}$ for 00:03:00 , 8, 10 or 12 cycles of PCR depending upon copy number entering circularisation of 98 $^{\circ}\text{C}$ for 00:00:10 , 60 $^{\circ}\text{C}$ for

00:00:30, 72 °C for 00:00:30 followed by 72 °C for 00:05:00 and Hold at 4 °C.

- 24 Post amplification the PCR tubes were placed on a magnetic plate, the beads allowed to pellet and then 45 µL of the PCR transferred to a 2 mL Lobind Eppendorf Tube. To this 31.5 µL beads of CleanPCR beads were added to precipitate the DNA, the beads washed twice with 70% ethanol and the final library eluted in 20 µL resuspension buffer.
- 25 Library QC was performed by running a 1 µL aliquot on a High Sensitivity BioAnalyser chip and the DNA concentration measured using the High Sensitivity Qubit.
- 26 Libraries to be sequence were then pooled based on DNA concentration and the quantification of the pool was determined by the Kappa qPCR Illumina quantification kit with the pool run at 10pM on a HiSeq with a 2x250bp reads read metric.
- 27 Reads generated were then processed through NextClip which takes LMP FASTA reads and looks to categorise them into four groups based on the presence of the Nextera adapter junction sequence. Category A pairs contain the adaptor in both reads, Category B pairs contain the adaptor in only read 2, Category C pairs contain the adaptor in only read 1, Category D pairs do not contain the adaptor in either read. NextClip also uses a k-mer-based approach to estimate the PCR duplication rate while reads are examined.