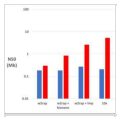

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Long Mate Pair Library Construction Protocol

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.bd3ui8nw

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ABSTRACT

Long Mate Pair Library Construction Protocol

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.

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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** .

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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 °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 °C** for **00:03:00** , 8, 10 or 12 cycles of PCR depending upon copy number entering circularisation of **98 °C** for **00:00:10** , **60 °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.

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