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 We use this protocol and it's working

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🌐 Amplification Free Paired End Library Construction Protocol

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ABSTRACT

Amplification Free Paired End Library Construction Protocol.

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sequencing

- 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 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.