Low-Pass WGS Sequencing Library Preparation

Christopher T T Boniface[1]

[1]Oregon Health Sciences University

Jul 19, 2021

1 Works for me Share dx.doi.org/10.17504/protocols.io.bwqdpds6

Christopher T Boniface

ABSTRACT

Simple protocol for generating WGS libraries using KAPA HyperPrep library preparation reagents.

DOI

dx.doi.org/10.17504/protocols.io.bwqdpds6

PROTOCOL CITATION

Christopher T T Boniface 2021. Low-Pass WGS Sequencing Library Preparation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bwqdpds6

KEYWORDS

low-pass wgs; lp-wgs

CREATED

Jul 19, 2021

LAST MODIFIED

Jul 19, 2021

PROTOCOL INTEGER ID

51685

MATERIALS TEXT

QIAamp DNA FFPE Tissue Kit
https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/qiaamp-dna-ffpe-tissue-kit/

AgenCourt AMPure XP Beads (Beckman-Coulter, Cat. #A63880)

Digital Electrophoresis Chips and Kits for Bio-Analyzer, High Sensitivity DNA Kit (Agilent, Cat. #5067-4626)

KAPA Hyper-Prep Kit, Illumina Platforms (Roche, KR8500)

KAPA HiFi Hotstart PCR master mix (Roche)

TSHTDM adaptor oligos (n=fixed multiplexing index base):
Sense:
5'-AATGATACGGCGACCACCGAGATCTACACnnnnnnnnACACTCTTTCCCTACACGACGCTCTTCCGATC*T-3'
Antisense:
5'-/5Phos/GATCGGAAGAGCACACGTCTGAACTCCAGTCACnnnnnnnnATCTCGTATGCCGTCTTCTGCTTG-3'

Illumina Library Amplification primers, Standard Desalting purification:
Forward (p5):
5'-AATGATACGGCGACCACCGA-3'
Reverse (p7):
5'-CAAGCAGAAGACGGCATACGA-3'

DNA extraction 3h 30m

1 Extract 10-50ng of tumor DNA from FFPE needle-core biopsy or resection using QIAamp DNA FFPE Tissue Kit 2h

2 Quantify extracted DNA and then sonicate 10-50ng of DNA to fragment to ~150bp fragments. 30m

3 Check sonicated DNA size distribution using Agilent Bioanalyzer 2100 system or similar electrophoresis 1h

Library Preparation 3h

4 Using 10-50 ng of cfDNA from step 2 and KAPA HyperPrep Kit, prepare and carry out end-repair and A-tailing reaction per manufacturer's protocol (https://rochesequencingstore.com/wp-content/uploads/2017/10/KAPA-HyperPrep-Kit_KR0961-%E2%80%93-v7.19.pdf) 1h

5 During step 4 incubations, anneal TSHTDM adaptor oligos to an appropriate stock concentration (100uM) by combining, in equal parts, i5 (sense) and i7 (antisense) adaptor oligonucleotides being mindful of the index sequences used. Anneal the single-stranded oligos by heating the mixture to 95C for 5 min and letting cool to RT for 30-60 minutes. 1h

6 On ice, dilute above annealed adaptors such that 5 uL gives a 200:1, [adaptor]:[cfDNA], molar excess and combine with above end-repair and A-tailing reaction, ligase, and ligation buffer per manufacturer's protocol. Be careful not to allow above reagents and/or ligation reaction to exceed 4-16C prior to next incubation step. 5m

7 Ligate 15 minutes at 20C in a thermocycler (lid heat "off"). 20m

Citation: Christopher T T Boniface (07/19/2021). Low-Pass WGS Sequencing Library Preparation. https://dx.doi.org/10.17504/protocols.io.bwqdpds6

8 Bead-purify ligation reaction: Add 88uL (0.8X vol.) well-mixed, room-temperature SPRI Ampure XP Bead slurry (Beckman-Coulter) and mix thoroughly by pipetting or vortexing. Incubate at room temperature for 10 minutes, separate supernatant from beads using magnet and discard supernatant. Immediately add 200 uL of 80% EtOH to wash, remove EtOH and repeat wash. After removing second EtOH wash, very briefly spin-down and quickly remove excess EtOH.* Immediately resuspend beads in 20 ul of nH2O. 30m

***Note:** It's very important not to let beads dry after removal of second EtOH wash. Proceed immediately to spin step, quickly remove remaining EtOH by pipet and resuspend in 20 uL nH2O. It is not critical that every last bit of EtOH is removed - the risk of reduced yield from over-drying greatly outweighs any issues associated with a small amount of EtOH carry-over.

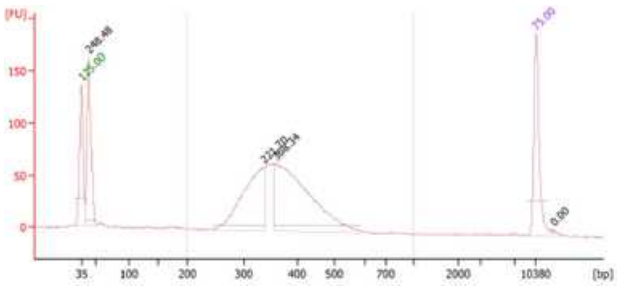
Library Amplification 2h 25m

9 Prepare 50 ul PCR reaction using 25ul KAPA HiFi Hotstart 2x MasterMix and Illumina Library Amplification Primers (10 uM [final]), 20 ul of above post-ligation reaction (from step 7), and 5 uL of nH2O + primers. 10m

10 Amplify library using KAPA HiFi Hotstart temperature protocol for 8-12 cycles (60C annealing temperature). 45m

11 Bead purify using 50ul bead solution (1X vol.) washing and eluting as above (step 8). 30m

12 Quantify using BioAnalyzer 2100 (more accurate quantification can be done with qPCR via KAPA Library Quantification Kit but DNA electrophoresis is necessary to assess molecular size distribution). 1h



Representative BioAnalyzer 2100 trace of an amplified library using the Agilent High Sensitivity DNA Kit.

13 Amplified libraries are now ready for dilution and sequencing using an Illumina NGS platform. 40-80M PE reads is sufficient for 0.5-1X mean WGS coverage for analysis.

Citation: Christopher T T Boniface (07/19/2021). Low-Pass WGS Sequencing Library Preparation. https://dx.doi.org/10.17504/protocols.io.bwqdpds6