Illumina Whole Genome Sequencing V.1

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This protocol is used to produce Ilumina libraries for whole genome sequencing. DNA input is 100 ng. User provide at least 300 ng of gDNA if it's possible to get enough materials for 2 attempts of library prep and sample QC. The enzymatic fragmentation reaction (for both Kapa and IDT kit) is very sensitive to the presence of EDTA, which must be removed or neutralized prior to fragmentation. EDTA in DNA preparations is usually introduced via elution buffers used in the final stages of the DNA extraction or purification process. The fragmentation condition of this protocol is for complex genome. Fragment size may reduce if the input DNA is from amplicon, plasmid or virus DNA.

Rita Kuo 2021. Illumina Whole Genome Sequencing. **protocols.io**
https://protocols.io/view/illumina-whole-genome-sequencing-bz9dp926

Whole Genome Library Prep, Whole Genome Sequencing, Illumina

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Reagents
- KAPA Hyperplus kit
- Illumina Y adapter
- KAPA DNA quantification kit
- 200 proof Ethanol
- Nuclease free water
- 10 mM Tris-HCl (pH 8.0 – 8.5)
- Ultra pure agarose

Disposables
- PCR 8 stripe tubes
- Tips
- 1.5 ml Tubes

Equipment
- Thermocycler
- Magnetic stand
- Pipettes
- Dano Drop
- Qubit
- Bioanalyzer

Sample QC

1 Use Dano Drop and Gel electrophoresis to quantify and qualify samples. If the sample is contaminated with RNA, inhibitors or degraded, do not proceed. A 260/280 ratio of ~1.8 is generally accepted as pure for DNA. If samples contain EDTA, proceed with bead clean up.

Bead clean up 15m

2 Bring the sample volume to 20 ul in PCR 8 stripe tubes

3 Add 18 ul of beads into the tube. Gently mix, incubate at room temperature for 5 minutes. 5m

4 Place the tube on a magnetic stand. Remove supernatant when it is clear.

5 Wash twice with freshly made 75 % Etoh and remove 75 % Etoh from the tube. Try to remove all residual ethanol without disturbing the beads.

6 Open the lid to let the bead dry at room temperature for 3 – 5 min, or until all of the ethanol has evaporated. 5m

7 Add 22 ul of 50 C nuclease free water. Quick vortex. Incubate samples in room temperature for 5 minutes. 5m

8 Repeat step 3-7 one more time.

9 Add 37 ul of 50 C nuclease free water. Quick vortex. Incubate samples in room temperature for 5 minutes.

10 Transfer 36 ul of eluted DNA to new 8 strip tubes.

11 Take 1 ul of the sample for Nanodrop measurement. If EDTA still presents in the sample, perform additional bead cleanup step using 1:1 sample bead ratio.

Enzymatic Fragmentation

12 NOTE: If the DNA preparation does contain EDTA, dilute in the EDTA-containing buffer in which samples are currently suspended, in a total of 30 µL. To each reaction with 30 µL of EDTA-containing DNA, add 5 µL of diluted Conditioning Solution.
Assemble each fragmentation reaction on ice by adding the components in this order:

A	B	C
Component	Volume (ul)	
Double-stranded DNA (with 5ul of conditioning solution if needed)	35	
KAPA Frag Buffer (10X)*	5	
KAPA Frag Enzyme*	10	
Total volume	50	

13 Vortex gently by flicking the tubes and spin down briefly. Return the tube(s) to ice. Proceed immediately to the next step.

14 Incubate in a thermocycler at 37 °C for 3 mins and hold at 4 °C. Set the temperature of the **heated lid to 50°C**.

15 Transfer reactions to ice, and proceed immediately to End Repair and A-tailing.

End Repair and A-Tailing

16 IMPORTANT! The KAPA HyperPrep/Plus End Repair & A-tailing Buffer may contain white precipitates when thawed. Ensure the buffer is thoroughly vortexed until the precipitate has been resuspended. Heat at 37°C for 5 – 10 min, if indicated.

Pre-mix end repair & A-tailing buffer and enzyme based on the sample number. Add 10 ul of End Repair and A-tailing reaction premix to the fragmented DNA.

Component	Volume(ul)
Fragemented DNA	50
End Repair & A-Tailing Buffer*	7
HyperPrep/HyperPlus ERAT Enzyme Mix**	3
Total volume	60

17 Vortex gently and spin down briefly. Return the reaction tubes to ice. Proceed immediately to the next step.

18 Incubate in a thermocycler at 65 °C for **30 mins** and hold at 4 °C. Set the temperature of the **heated lid to 85°C.**

19 Proceed immediately to Adapter Ligation. **Keep 1 ul of the DNA for library QC.**

Adaptor Ligation

20 IMPORTANT! The KAPA HyperPrep Ligation Buffer contains a high concentration of PEG 6000 and is very viscous. Small PEG 6000 droplets may be visible when thawed and require special attention during pipetting. Ensure the buffer is thoroughly vortexed until the droplets have been resuspended. Heat at 37°C for 5-10 min, if indicated.

Add 2.5 µl of 18 µM adapter to each reaction, quick vortex and spin. Document which index was used in which library.

Sample ID	Index ID	Sample ID	Index ID	Sample ID	Index ID
1		7		13	
2		8		14	
3		9		15	
4		10		16	
5		11			
6		12			

21 Pre-mix water, ligation buffer and DNA ligase based on the sample number. Add 47.5 ul of ligation premix to each reaction. quick vortex and spin.

Component	Volume (ul)
DNA with adaptor	62.5
Water	7.5
Ligation Buffer	30
Ligase	10
Total volume	110

Incubate at 20 °C for 15- 60 minutes.

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Library clean up

23 Add 88 ul of beads into the tube. Gently mix, incubate at room temperature for 5 minutes.

24 Place the tube on a magnetic stand. Remove supernatant when it is clear.

25 Wash twice with freshly made 75 % Etoh and remove 75 % Etoh from the tube. Try to remove all residual ethanol without disturbing the beads.

26 Open the lid to let the bead dry at room temperature for 3 – 5 min, or until all of the ethanol has evaporated.

27 Add 50 ul of nuclease free water. Quick vortex. Incubate samples in room temperature for 5 minutes.

28 Add 25ul of nuclease free water. Quick vortex. Incubate samples in room temperature for 5 minutes.

29 Transfer the 23 ul of the clear supernatant to a new tube.

Library QC

30 Use 1 ul of the library and fragmented DNA to run on Bioanalyzer using DNA HS kit to determine the average size and concentration of the final library.

IMPORTANT: The final library size should be shifted up by ~150 bp compared to the insert size distribution. If there are no size shifts, it may indicate failure of adapter ligation. Consider failing the libraries with no size shifts.