GenNature Library Preparation Kit Quick Manual

(Cat. No.: NGS01203) For Illumina Sequencing Platform

Components in the Kit	Storage
5× TA Buffer	-20℃
TAT	-20℃
2× PCR Master Mix	-20℃
PB (Purification Beads)	4℃

Remain stable for 12 months. Avoid repeated freeze-thaw cycles.

Please follow the work flow: ① Tagment DNA & Purification (50 min); ② PCR Enrichment & Purification (50 min); ③ Quantification & Qualification.

Step 1.
Tagment DNA & Purification

DNA Tagment (20 min)

1. Thaw and keep the 5× TA Buffer on ice, and then gently vortex and centrifuge briefly.
2. Thaw and keep the TAT on ice, and then mix well by flicking.
3. We recommend thawing the PB at room temperature in this step.
4. Prepare the *TAG Mix* in each well of a plate or tube on ice as listed below.

Components	5 x TA Buffer	TAT	DNA	Nuclease-Free Water	Total
Volume per Reaction	6 μL	1 μL	X μL (25 ng total)	(23 - X) μL	**30 μL**

5. Gently pipet the mixture up and down for 15~20 times or vortex to mix thoroughly, then centrifuge briefly.
6. Place the plate or tube(s) on a thermal cycler and run the TAG Program (Lid Set at 65℃).

Temperature	55℃	4℃
Duration	10 min	Hold

7. **Important!** After the temperature drops down to 4℃, immediately perform the next purification procedure, otherwise the DNA will be over-fragmented.

Purification (30 min)

1. Thaw the PB at room temperature for at least 30 min, prepare 450 µL fresh 80% (v/v) ethanol per sample.
2. Vortex 15 sec to mix the PB thoroughly.
3. After the temperature of thermal cycler drops down to 4℃, remove the samples, and then add 45 µL PB into each sample.
4. Thoroughly pipet the mixture up and down for 15~20 times, incubate at room temperature for 5 min.
5. Place the plate or tube(s) on a magnet for at least 2 min until the liquid is clear.
6. Carefully discard the supernatant. Do not discard the beads.
7. While keeping the plate or tube(s) on the magnet, add 200 µL fresh 80% (v/v) ethanol. Incubate for 30 sec, and then remove the ethanol.
8. Repeat one ethanol wash by performing Step 7.
9. While keeping the plate or tube(s) on the magnet, allow the beads to air dry for 1~3 min. Do not over dry the beads.
10. Remove the sample plate or tube(s) from the magnet and elute in 25 µL Nuclease-Free Water or equivalent buffer.
11. Thoroughly pipet the mixture up and down for 15~20 times, incubate for 5 min at room temperature.
12. Place the plate tube(s) on a magnet for at least 2 min until the liquid is clear.
13. Transfer 23 µL elution to a new plate or tube(s). Make sure that no beads are carried over.

At this point, you can seal the sample plate or tube(s) and store at 4℃ overnight or -20℃ for up to 1 week before performing the next step.

Step 2.
PCR Enrichment & Purification

PCR Enrichment (20 min)

1. Thaw and keep the GS and GN (Supplied in the GenNature Adapter Set, Cat. No.: NGS09605a/ NGS09605b/ NGS09605c/ NGS09605d) on ice, gently vortex and centrifuge briefly.
2. Thaw and keep the 2× PCR Master Mix on ice, gently vortex and centrifuge briefly.
3. Prepare the *PCR Enrichment Mix* in each well of a plate or tube on ice as listed below.

Components	Purified DNA	GS*	GN*	2× PCR Master Mix	Total
Volume per Reaction	23 µL	1 µL	1 µL	25 µL	**50 µL**

* Note: Use different GN & GS adapter pairs (Index 1 & 2) from GenNature Adapter Set a/ b/ c/ d for each sample that will be pooled together for sequencing. The Set provides at most 384 different Index 1 & 2 (GN & GS) pairs from 24 different GN (Index 1) and 16 different GS (Index 2).

4. Gently pipet the mixture up and down for 15~20 times or vortex to mix thoroughly, then centrifuge briefly.
5. Place the plate or tube(s) on a thermal cycler and run the PCR Enrichment Program (Lid Set at 105℃).

Temperature	72℃	98℃	98℃	62℃	72℃	72℃	4℃
Duration	3 min	2 min	20 sec	30 sec	30 sec	5 min	Hold
Cycles	1	1	12			1	1

Purification (30 min)

1. Thaw the PB at room temperature for at least 30 min, prepare 450 µL fresh 80% (v/v) ethanol per sample.
2. Vortex 15 sec to mix the PB thoroughly.
3. After the PCR Enrichment Program ends, remove the samples, and then add 40 µL PB into each sample.
4. Thoroughly pipet the mixture up and down for 15~20 times, incubate at room temperature for 5 min.
5. Place the plate or tube(s) on a magnet for at least 2 min until the liquid is clear.
6. Carefully discard the supernatant. Do not discard the beads.
7. While keeping the plate or tube(s) on the magnet, add 200 µL fresh 80% (v/v) ethanol. Incubate for 30 sec, and then remove the ethanol.
8. Repeat one ethanol wash by performing Step 7.

9. While keeping the plate or tube(s) on the magnet, allow the beads to air dry for 1~3 min. Do not over dry the beads.
10. Remove the sample plate or tube(s) from the magnet and elute in 22 μL of Nuclease-Free Water or equivalent buffer.
11. Thoroughly pipet the mixture up and down for 15~20 times, incubate for 5 min at room temperature.
12. Place the plate tube(s) on a magnet for at least 2 min until the liquid is clear.
13. Transfer 20 μL elution to a new plate or tube(s). Make sure that no beads are carried over.
14. Store the DNA library at -20℃ for the subsequent quantification, qualification and sequencing.

Step 3.
Quantification & Qualification

Quantification

Use 1 μL of each DNA sample with Qubit HS dsDNA Assay Kit for sample quantification. Real-time PCR is required for more accurate quantification.

Qualification

Run the library on an Agilent Technology 2100 Bioanalyzer using a High-Sensitivity DNA Chip to check the size distribution. A distribution of DNA libraries fragments with a size range from about 200 bp to 2000 bp will be expected.

Welcome to download detailed user guide from GenScript official website.

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