GenTrack Library Preparation Kit Quick Manual

(Cat. No.: NGS01201) For Illumina Sequence Platform

Components	Storage
End Prep Buffer	-20℃
End Prep Enzyme	-20℃
Ligation Prep Buffer	-20℃
Ligation Prep Enzyme	-20℃
2× PCR Master Mix	-20℃
Primer Mix	-20℃
PB (Purification Beads)	4℃

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

Please follow the work flow: ① Fragment Preparation; ② End-Repair and A-Tailing (60 min); ③ Adapter Ligation (45 min); ④ Amplification (40-70 min); ⑤ Quantification & Qualification.

Step 1. Fragment Preparation

Fragment DNA (Optional)

We recommend fragmenting at least 1 μg genomic DNA to an expected size range using the following methods a or b. **This step is not supplied in the GenTrack DNA Library Preparation Kit.**

a. Ultrasonicator instruments (and compatible tubes if necessary), such as Covaris® E220 Focused-ultrasonicator, Covaris® S220 Focused-ultrasonicator etc.

b. Fragmentase, such as DNase I, NEB NEBNext® dsDNA Fragmentase etc.

Size-Selection (Optional)

We recommend inputting at least 50 ng fragmented DNA to select 200~600 bp fragments using the following methods a or b. **This step is not supplied in the GenTrack DNA Library Preparation Kit.**

a. Size-selection beads, such as Beckman Agencourt® AMPure XP Beads, Yeasen Hieff NGS® DNA Selection Beads etc.

b. Normal agarose gel electrophoresis of DNA: cut the gel slice to do gel extraction using gel extraction kit such as Genscript QuickClean II Gel Extraction Kits, Qiagen QIAquick® Gel Extraction Kit, NEB Monarch® DNA Gel Extraction Kit etc. Gel size selection system, such as ThermoFisher E-Gel SizeSelect II, ThermoFisher E-Gel NGS etc.

Run the DNA on an Agilent Technologies 2100 Bioanalyzer using a High-Sensitivity DNA chip to check the size distribution.

Step 2. End-Repair and A-Tailing

End-Repair and A-Tailing (60 min)

1. Thaw the End Prep Buffer at room temperature, then keep it on ice, gently vortex and centrifuge briefly.
2. Thaw and keep the End Prep Enzyme on ice, then mix well by flicking.
3. Prepare the *End Prep Mix* in each well of a plate or tube as listed below.

Components	End Prep Buffer	End Prep Enzyme	Size-Selected DNA	Nuclease-Free Water	Total
Volume per reaction	17.8 µL	2.2 µL	x µL (1~1000 ng)	(30.0 - x) µL	**50.0 µL**

4. Gently pipet the mixture up and down for 15~20 times to mix thoroughly.
5. Place the plate or tube(s) on a thermal cycler and run the End Prep Program (Lid Set at 100℃).

Temperature	37 ℃	75 ℃	4 ℃
Duration	30 min	30 min	Hold

Step 3. Adapter Ligation

Adapter Ligation (15 min)

1. Thaw the Ligation Prep Buffer and the Adapters at room temperature, then keep them on ice. Gently vortex and centrifuge briefly before use.
2. Thaw and keep the Ligation Prep Enzyme on ice, then mix well by flicking.
3. Prepare the *Adapter Ligation Mix* in each well of a plate or tube containing *End Prep Mix* in the order listed below on ice.

Component	*End Prep Mix* after Reaction	GenTrack Adapter*	Ligation Prep Buffer#	Ligation Prep Enzyme	Total
Volume per Reaction	50.0 µL	2.5 µL	26.5 µL	1.0 µL	**80.0 µL**

* Note: Use different indexes from GenTrack Adapter Set (Cat. No.: NGS09602) for each sample that will be pooled together for sequencing. The Set provides at most 96 different index-adapters.

After the buffer is added, mix well before add the Ligation Prep Enzyme.

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 Adapter Ligation Program (without a Heated Lid).

Temperature	22℃	4℃
Duration	15 min	Hold

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. Vertex 15 sec to mix PB thoroughly.
3. After the Adapter Ligation 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 the 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, 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 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.

Step 4. Amplification

Amplification (10-40 min)

1. Thaw the Primer Mix at room temperature, then gently vortex and centrifuge briefly.
2. Thaw and keep the 2× PCR Master Mix on ice, then gently vortex and centrifuge briefly.
3. Prepare the *PCR Amplification Mix* in each well of a plate or tube(s) containing the samples on ice as listed below.

Components	Adapter Ligated DNA	2× PCR Master Mix	Primer Mix	Total
Volume per Reaction	20 µL	25 µL	5 µL	**50 µL**

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

Temperature	98℃	98℃	62℃	72℃	72℃	4℃
Duration	2 min	30 sec	30 sec	30 sec	3 min	Hold
Cycles	1	2~17			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. Mix the PB thoroughly by vortexing for 15 sec.
3. Add 50 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 the 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, 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 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 (DNA libraries) to a new plate or tube(s). Make sure that no beads are carried over.
14. Store the DNA libraries at -20℃ for the subsequent quantification, qualification and sequencing.

Step 5. 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 300 bp to 800 bp will be expected.

Welcome to download detailed user guide from GenScript official website.

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