Tapestri® DNA Library Reamplification Protocol

IMPORTANT

- *Vortex all reagents unless directed otherwise.*
- *Thaw -20 °C reagents on ice.*
- *Always use a PCR skirt.*

Targeted PCR Clean Up

NOTE *Equilibrate AMPure XP reagent to room temperature. Prepare 1 mL fresh 80% ethanol using nuclease-free water.*

1. Collect the remaining 1st Targeted PCR product and add **nuclease-free water** to the tube to reach 100 µL total volume.
2. Thoroughly vortex AMPure XP reagent at high speed immediately prior to usage.
3. Add **76 µL (0.76X) of AMPure XP reagent** to the tube. Vortex for **5 seconds** and quick-spin to collect the contents.
4. Incubate tube at room temperature for **5 minutes**.
5. Place the tube onto the magnet and wait **2 minutes** for the beads to separate from solution.
6. Without removing the tube from the magnet, remove the clear liquid and discard.
7. Carefully add **200 µL** of freshly prepared **80% ethanol**, wait **30 seconds**, and remove the ethanol without disturbing the AMPure XP beads.
8. Repeat **Step 7** once, for a total of two washes.
9. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the beads.
10. Dry AMPure XP bead pellets in the tube on the magnet by incubating at room temperature for **2 minutes**. Avoid overdrying the beads.
11. Remove the tube from the magnet and add **40 µL of nuclease-free water**. Vortex and quick-spin to collect the contents.
12. Incubate the tube at room temperature for **2 minutes**.
13. Place the tube onto the magnet and wait for at least **2 minutes** or until the solution is clear.
14. Transfer **35 µL of purified PCR product** to a new 0.2 mL PCR tube.
15. Store the concentrated PCR product on ice and proceed to the next step, or store at -20 °C long-term.

Library PCR Amplification

1. In **two new 0.2 mL PCR tubes**, prepare **2x Library PCR reaction** (50 µL per tube, each using the same Library Index):

Reagent	Volume (uL)
Library Mix (●)	25
Library Index (●)	10
Targeted DNA PCR product	15
Total Volume	**50**

2. Vortex and quick-spin tubes to collect the contents.
3. Transfer the PCR tube to the thermocycler, and run the Library PCR protocol **with 14 cycles**:

Step	Temperature	Time	Cycle
1	95 °C	3 min	
2	98 °C	20 sec	**14**
3	62 °C	20 sec	
4	72 °C	45 sec	
5	72 °C	2 min	
6	4 °C	HOLD	

4. Store at room temperature and continue to the next step.

Library PCR Clean Up

NOTE *Equilibrate AMPure XP reagent to room temperature. Always use freshly prepared 80% ethanol.*

1. **Combine the two 50 µL reactions** (total volume = 100 µL).
2. Thoroughly vortex AMPure XP reagent at high speed immediately prior to usage.
3. Add **69 µL (0.69X) of AMPure XP reagent** to the tube. Vortex for **5 seconds** and quick-spin to collect the contents.
4. Incubate the tube at room temperature for **5 minutes**.
5. Place the tube onto the magnet and wait **2 minutes** for the beads to separate from solution.
6. Without removing the tube from the magnet, remove the clear liquid and discard.
7. Carefully add **200 µL** of freshly prepared **80% ethanol**, wait **30 seconds**, and remove the ethanol without disturbing the AMPure XP beads.
8. Repeat **Step 7** once, for a total of two washes.
9. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the beads.
10. Dry AMPure XP bead pellets in the tube on the magnet by incubating at room temperature for **2 minutes**. Avoid overdrying the beads.
11. Remove the tube from the magnet and add **110 µL of nuclease-free water**. Vortex and quick-spin to collect the contents.
12. Incubate the tube at room temperature for **2 minutes**.
13. Place the tube onto the magnet and wait for at least **2 minutes** or until the solution is clear.
14. Transfer **100 µL of purified PCR product** to a new 0.2 mL PCR tube.
15. Add **72 µL (0.72X) of AMPure XP reagent** to the tube with eluted PCR product. Vortex for **5 seconds** and quick-spin to collect the contents.
16. Incubate the tube at room temperature for **5 minutes**.
17. Place the tube onto the magnet and wait **2 minutes** for the beads to separate from solution.
18. Without removing the tube from the magnet, remove the clear liquid and discard.
19. Carefully add **200 µL** of freshly prepared **80% ethanol**, wait **30 seconds**, and remove the ethanol without disturbing the AMPure XP beads.
20. Repeat **Step 19** once, for a total of two washes.
21. Keeping the tube on the magnet, remove all residual ethanol from the tube without disturbing the beads.
22. Dry AMPure XP bead pellets in the tube on the magnet by incubating at room temperature for **2 minutes**. Avoid overdrying the beads.
23. Remove the tube from the magnet and add **12 µL of nuclease-free water**. Vortex and quick-spin to collect the contents.
24. Incubate the tube at room temperature for **2 minutes**.
25. Place the tube onto the magnet and wait for at least **2 minutes** or until the solution is clear.
26. Transfer **10 µL** of purified PCR product to a new 0.2 mL PCR tube or 1.5 mL DNA LoBind Eppendorf tube.
27. Store the purified PCR product on ice and proceed to the next step, or store at -20 °C long term.

Quantify and Pool Library

1. Dilute the sample 10x and run 1 µL of the diluted sample on a High-Sensitivity Bioanalyzer chip or equivalent. Quantify the sample using Qubit or equivalent.