Tapestri® Targeted Bulk DNA Sequencing

IMPORTANT

- *Genomic DNA may be extracted using any DNA extraction kit according to the manufacturer's instructions (for example, Quick-DNA™ Miniprep Kit, Zymo Research, D3024).*
- *This protocol requires the Forward and Reverse Primer Pools from any Tapestri Single-Cell DNA Panel (not included in the Tapestri Bulk NGS Ancillary Kit).*
- *Vortex all reagents unless directed otherwise.*
- *Thaw -20 °C reagents on ice.*
- *Always use a PCR skirt.*

Targeted PCR Amplification

1. Dilute genomic DNA to a concentration of **20 ng/µL** (range 5–50 ng/µL).
2. In two separate 1.5 mL DNA LoBind tubes, dilute the **Forward** (●) **and Reverse** (●) **Primer Pools** 10x by adding **5 µL of primer to 45 µL of nuclease-free water**.
3. Vortex and quick-spin to collect the contents. *Store diluted primers at -20 °C for future use.*
4. In a new 0.2 mL PCR tube, prepare the targeted PCR reaction:

Reagent	Volume (µL)
Barcoding Mix	12.5
Forward Primer Pool (1/10 dilution)	2
Reverse Primer Pool (1/10 dilution)	2
Bulk Primer (●)	4
Genomic DNA (5–50 ng/µL)	4.5
Total Volume	**25**

5. Vortex and quick-spin to collect the contents.
6. Transfer the PCR tube to the thermocycler, and run the Targeted PCR protocol:

			20-100	101-200	201-300	>300	
Amplicon Number							
Step	Ramp	Temp.	Time	Time	Time	Time	Cycle
1	4 °C/s	98 °C	6 min	6 min	6 min	6 min	
2	1 °C/s	95 °C	30 sec	30 sec	30 sec	30 sec	10
3		72 °C	10 sec	10 sec	10 sec	10 sec	
4		61 °C	3 min	4.5 min	6 min	9 min	
5		72 °C	20 sec	20 sec	20 sec	20 sec	
6	1 °C/s	95 °C	30 sec	30 sec	30 sec	30 sec	10
7		72 °C	10 sec	10 sec	10 sec	10 sec	
8		48 °C	3 min	4.5 min	6 min	9 min	
9		72 °C	20 sec	20 sec	20 sec	20 sec	
10	4 °C/s	72 °C	2 min	2 min	2 min	2 min	
11		4 °C	HOLD	HOLD	HOLD	HOLD	

IMPORTANT *Make sure to set up two separate 10 cycle programs each with a ramp rate of 1 °C/s.*

Digest Targeted PCR Product

1. Add 17 µL of nuclease-free water to the tube.
2. Vortex and quick-spin to collect the contents.
3. Add **5 µL DNA Clean Up Buffer** (●) and **3 µL DNA Clean Up Enzyme** (●) to the sample tube for a total volume of 50 µL.
4. Vortex and quick-spin to collect the contents.
5. Transfer tube to a thermocycler, thermo mixer or heat block and incubate at **37 °C** for **60 minutes**.
6. Store at room temperature and continue to the next step.

Clean Up Targeted PCR Product

NOTE *Equilibrate AMPure XP reagent to room temperature. Prepare 2 mL fresh 80% ethanol using nuclease-free water.*

1. Add **50 µL of nuclease-free water** to the tube (total volume = 100 µL).
2. Thoroughly vortex AMPure XP reagent at high speed immediately prior to usage.
3. Add **70 µL (0.70X) 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 **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 **76 µL (0.76X) of AMPure XP reagent** to the tube with eluted PCR product. Vortex for **5 seconds** and quick-spin to collect the contents.
16. Incubate 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 **55 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 **50 µL of purified PCR product** to a new 0.2 mL PCR tube.
27. Store the purified PCR product on ice and proceed to the next step, or store at -20 °C long-term.

Library PCR Amplification

1. In a new 0.2 mL PCR tube, prepare the library PCR reaction:

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

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

Step	Temperature	Time	Cycle
1	95 °C	3 min	
2	98 °C	20 sec	10
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.

Clean Up Library PCR Product

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

1. Add **50 µL of nuclease-free water** to the tube (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. Normalize libraries to 5 nM and pool in equal volumes.
   Contact support@missionbio.com if final libraries are < 2.0 ng/µL.

Sequence Tapestri Bulk DNA Libraries

IMPORTANT

- *The final DNA library consists of target-specific amplicons ranging from 350–550 bp with a peak at ~450 bp (see example below).*
- *If primer dimers (200–250 bp) make up more than 10% of the final library by molarity, contact support@missionbio.com.*
- *High molecular weight products (> 600 bp) do not need to be removed, as they will not impact the sequencing data.*
- *It is recommended to sequence 80 read pairs per amplicon for each sample (2 x 150 bp).*
- *For example, for a DNA panel with 321 amplicons, each bulk DNA library requires ~25,000 read pairs. Libraries will likely require at least 100x dilution for most sequencing configurations.*