*VARIANT*Plex™—HS/HGC workflow overview

Follow the instructions and incubations for each step. All mixing steps should be performed on ice. Pipette up and down 8 times or vortex to mix after re-suspending each lyosphere and spin down before incubations and transfers. For incubations, use a lid heated ≥100°C except where specified otherwise.

DNA Fragmentation

Add 10-250 ng DNA to DNA Fragmentation reagent for a total volume of 50 µL.

Incubate as indicated.

Step	Incubation temperature	Incubation time
1	4°C	1 min
2	37°C	12 min
3	72°C	20 min
4	4°C	Hold

End Repair

Transfer 50 µL DNA Fragmentation mixture to End Repair reagent.

Incubate as indicated with unheated lid.

Step	Incubation temperature	Incubation time
1	25°C	30 min
2	4°C	Hold
AMPure™ XP clean-up (125 µL). Elute in 20 µL.		

Ligation Step 1

Transfer 20 µL End Repair mixture to Ligation Step 1 reagent.

Incubate as indicated.

Step	Incubation temperature	Incubation time
1	37°C	15 min
2	4°C	Hold
AMPure XP clean-up (50 µL). Elute in 42 µL.		

MBC Adapters

Transfer 40 µL Ligation Step 1 mixture to the MBC adapters.

Ligation Step 2

Transfer the entire volume of the MBC adapters to Ligation Step 2 reagent.

Incubate as indicated with unheated lid.

Optional stopping point before purification. Store at –10°C to –30°C.

Step	Incubation temperature	Incubation time
1	22°C	5 min
2	4°C	Hold
Ligation clean-up beads (50 µL). Elution volume varies*, 5 mM NaOH, 75°C 10 min.		

First PCR

Add GSP1 primers (volume varies*) to the First PCR HS/HGC reagent. Then add the entire volume of purified Adapter Ligation mixture and mix.

Incubate as indicated.

Optional stopping point after this step. Store purified product (eluted from beads) at –10°C to –30°C.

Incubation temperature	Incubation time	# of cycles
95°C	3 min	1
95°C	30 sec	Varies*
Varies*	10 sec	
Varies*	Varies*	
72°C	3 min	1
4°C	Hold	1
AMPure XP clean-up (32 µL). Elution varies*		

*Refer to Product Insert for panel-specific parameters

Second PCR

Transfer 40 µL of purified First PCR mixture and mix.

Incubate as indicated.

Optional stopping point after this step. Store purified product (eluted from beads) at –10°C to –30°C.

Incubation temperature	Incubation time	# of cycles
95°C	3 min	1
95°C	30 sec	Varies*
Varies*	10 sec	
Varies*	Varies*	
72°C	3 min	1
4°C	Hold	1
AMPure XP clean-up (32 µL). Elute in 20 µL.		

*Refer to Product Insert for panel-specific parameters

Proceed with protocol: Quantify, Normalize, and Sequence Protocol for Illumina®

Appendix: AMPure® XP bead purification†

1. Add AMPure XP beads to reaction.
2. Mix to homogeneous solution.
3. Incubate at RT for 5 min.
4. Briefly spin down.
5. Place on magnet for 4 min.
6. Discard supernatant carefully.
7. Wash beads 2x with 200 µL fresh 70% EtOH.
8. Remove residual fluid with 20 µL pipette.
9. Air dry 3–5 min at RT.
10. Elute DNA with volume* of 10 mM Tris-HCl pH 8.0.
11. Place on magnet for 2 min.
12. Remove purified product.

* Refer to Product Insert for panel-specific parameters

† Work at room temperature for bead purifications

Appendix: Ligation cleanup beads purification†

1. Perform buffer exchange with 50 µL of fresh Ligation cleanup buffer.
2. Combine 50 µL Ligation cleanup beads with the entire volume from the Ligation Step 2 reaction.
3. Mix solution by vortexing and incubate 5 min (2x).
4. Briefly spin down.
5. Place tubes on magnet for 1 min.
6. Discard supernatant carefully.
7. Wash beads 2x with 200 µL Ligation cleanup buffer.
8. Wash beads with 200 µL ultrapure H_2O.
9. Resuspend beads in volume* 5 mM NaOH.
10. Incubate reactions 10 min at 75°C.
11. Cool reactions to 4°C.
12. Briefly spin down.
13. Place on magnet for 2 min.
14. Remove purified product.

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