

VariantPlex® 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 prior to incubations and transfers. For incubations, use a lid heated $\geq 100^{\circ}$ 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 =

Step	Incubation Temperature	Incubation Time
1	25°C	30 min
2	4°C	Hold
AMPure TM 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 TM XP clean-up (50 μL). Elute in 42 μL		

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

Incubate as indicated with unheated lid =

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

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 along up bonds (FO ut) Flution		

Ligation clean-up beads (50 μL). Elution volume varies*, 5 mM NaOH, 75°C 10 min

Ligation Step 2



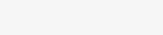
First PCR

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 at -10°C to -30°C.

Incubation Temperature	Incubation Time	# of cycles
95°C	3 min	1
95°C	30 sec	
Varies* (ramp rate 100%)		Varies*
72°C	3 min	1
4°C	Hold	1

AMPure[™] XP clean-up (32 μL). Elution varies³

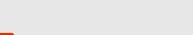


Add GSP2 primers (volume varies*) to the Second PCR HS/HGC reagent. Then add the entire volume of purified Second PCR reagent and mix.

Incubate as indicated 🦈







Optional stopping point after this step. Store at -10°C to -30°C.

Incubation Temperature	Incubation Time	# of cycles
95°C	3 min	1
95°C	30 sec	
Varies * (ramp rate 100%)		Varies*
72°C	3 min	1
4°C	Hold	1
AMPure TM 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[†]

- Add AMPure XP beads to reaction
- Mix to homogeneous solution
- Incubate at RT for 5 min
- Briefly spin down
- Place on magnet for 4 min
- Discard supernatant carefully
- Wash beads 2x with 200 µL fresh 70% EtOH
- Remove residual fluid with 20 µL pipette
- Air dry 3-5 min at RT
- Elute DNA with volume* of 10 mM Tris-HCl pH 8.0
- Place on magnet for 2 min
- >purified product
- *Refer to Product Insert for panel-specific parameters
- [†]Work at room temperature for bead purifications

Appendix: Ligation cleanup beads purification[†]

- Perform buffer exchange with 50 μL of fresh Ligation cleanup buffer
- Combine 50 μL Ligation cleanup beads with 50 μL Ligation Step 2 reaction
- Mix solution by vortexing and incubate 5 min (2x)
- Briefly spin down
- Place tubes on magnet for 1 min
- Place on magnet for 4 min
- Discard supernatant carefully
- Wash beads 2x with 200 μL Ligation cleanup buffer
- Wash beads with 200 µL ultrapure H₂O
- Resuspend beads in volume* 5 mM NaOH
- Incubate reactions 10 min at 75°C
- Cool reactions to 4°C
- Briefly spin down
- Place on magnet for 2 min
- >purified product