

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 before incubations and transfers. For incubations, use a lid heated $\geq 100^{\circ}$ C except where specified otherwise.

		Step	Incubation temperature	Incubation time
DNA Fragmentation	Add 10-250 ng DNA to DNA Fragmentation reagent for a total volume of 50 μ L.	1	4°C	1 min
		2	37°C	12 min
		3	72°C	20 min
		4	4°C	Hold
			1 1 2	Incubation
End Repair	Transfer 50 μL DNA Fragmentation mixture to End Repair reagent. Incubate as indicated with <u>unheated lid</u> .	Step	Incubation temperature	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	Incubatior 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.			
MBC Adapters		Step	Incubation temperature	
MBC Adapters	Transfer 40 µL Ligation Step 1 mixture to the MBC adapters. Transfer the entire volume of the MBC adapters to Ligation Step 2 reagent.		temperature	time
MBC Adapters	Transfer the entire volume of the MBC adapters to Ligation	1	temperature 22°C	time 5 min
	Transfer the entire volume of the MBC adapters to Ligation Step 2 reagent.	1 2 Lig	temperature	time 5 min Hold μL).
	Transfer the entire volume of the MBC adapters to Ligation Step 2 reagent. Incubate as indicated with <u>unheated lid</u> . Optional stopping point before purification.	1 2 Lig	temperature 22°C 4°C ation clean-up beads (50	time 5 min Hold μL).
	Transfer the entire volume of the MBC adapters to Ligation Step 2 reagent. Incubate as indicated with <u>unheated lid</u> . Optional stopping point before purification.	1 2 Lig	temperature 22°C 4°C ation clean-up beads (50	time 5 min Hold μL). 75°C 10 min.
	Transfer the entire volume of the MBC adapters to Ligation Step 2 reagent. Incubate as indicated with <u>unheated lid</u> .	1 2 Lig Elution volum	temperature 22°C 4°C ation clean-up beads (50 me varies*, 5 mM NaOH,	time 5 min Hold μL). 75°C 10 min.
	Transfer the entire volume of the MBC adapters to Ligation Step 2 reagent. Incubate as indicated with <u>unheated lid</u> . Optional stopping point before purification. Store at -10°C to -30°C.	1 2 Lig Elution volue	temperature 22°C 4°C ation clean-up beads (50 me varies*, 5 mM NaOH, Incubation time	time 5 min Hold μL). 75°C 10 min. # of cycles
	Transfer the entire volume of the MBC adapters to Ligation Step 2 reagent. Incubate as indicated with <u>unheated lid</u> . Optional stopping point before purification. Store at -10°C to -30°C. Add GSP1 primers (volume varies*) to the First PCR HS/HGC reagent. Then add the entire	1 2 Lig Elution volue Incubation temperature 95°C	temperature 22°C 4°C ation clean-up beads (50 me varies*, 5 mM NaOH, Incubation time 3 min	time 5 min Hold μL). 75°C 10 min. # of cycles
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. Add GSP1 primers (volume varies*) to the First PCR HS/HGC reagent. Then add the entire volume of purified Adapter Ligation mixture and mix.	1 2 Lig Elution volum Incubation temperature 95°C 95°C	temperature 22°C 4°C ation clean-up beads (50 me varies*, 5 mM NaOH, Incubation time 3 min 30 sec	time 5 min Hold μL). 75°C 10 min. # of cycles
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. 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) 	1 2 Lig Elution volue Incubation temperature 95°C 95°C Varies*	temperature 22°C 4°C ation clean-up beads (50 me varies*, 5 mM NaOH, Incubation time 3 min 30 sec 10 sec	time 5 min Hold μL). 75°C 10 min. # of cycles
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. 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.	1 2 Lig Elution volue Incubation temperature 95°C 95°C Varies* Varies*	temperature 22°C 4°C ation clean-up beads (50 me varies*, 5 mM NaOH, Incubation time 3 min 30 sec 10 sec Varies*	5 min Hold μL). 75°C 10 min. # of cycles 1 Varies*



		Incubation temperature	Incubation time	# of cycles
	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.	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 μ L 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 μ L Ligation cleanup buffer.
- **8.** Wash beads with 200 μ L ultrapure H₂O.
- 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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