LiquidPlex™ 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 ≥100°C, except where specified otherwise.

Complete End Repair

Add 5-300 ng cfDNA to the Complete End Repair reagent for a total volume of 50 µL.

Incubate as indicated

Step	Incubation Temperature	Incubation Time
1	25°C	30 min
2	4°C	Hold

AMPure™ XP clean-up (150 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 (60 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 <u>unheated lid</u>

STOP **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

STOP **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*
Varies* (ramp rate 100%)		
72°C	3 min	1
4°C	Hold	1

AMPure™ XP clean-up (48 µL). Elution varies*

*Refer to Product Insert for panel-specific parameters

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Second PCR

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 👉

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

AMPure™ XP clean-up (48 µL). Elute in 24 µL

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

*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 50µL 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 H_2O
- 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