FusionPlex® 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.

Random Priming

Add 20-250 ng RNA to Random Priming reagent for a total volume of 20 µL.

Incubate as indicated 👉

Step	Incubation Temperature	Incubation Time
1	65°C	5 min
2	4°C	Hold

First Strand cDNA Synthesis

Transfer 20 µL Random Priming mixture to First Strand cDNA Synthesis reagent.

Incubate as indicated 👉

Transfer 1 µL First Strand cDNA Synthesis mixture to 9 µL water for PreSeq RNA QC Assay.

Step	Incubation Temperature	Incubation Time
1	25°C	10 min
2	42°C	30 min
3	80°C	20 min
4	4°C	Hold

Second Strand cDNA Synthesis

Add 21 µL ultrapure water to 19 µL First Strand cDNA Synthesis mixture and then transfer to Second Strand cDNA Synthesis reagent.

Incubate as indicated 👉

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

Step	Incubation Temperature	Incubation Time
1	16°C	60 min
2	75°C	20 min
3	4°C	Hold

PreSeq RNA QC Assay

Build qPCR reaction and incubate as per your master mix-specific instructions.

iTaq SYBR Green Supermix (Bio-Rad) – 5 µL
10X VCP Primer Mix – 1 µL
Diluted cDNA sample or NTC – 4 µL

Incubate as indicated 👉

Incubation Temperature	Incubation Time	# of cycles
95°C	20 [20#] sec	1
95°C	3 [15#] sec	35
60°C	30 [60#] sec	
60-95°C	0.5°C/sec increment	1

Times in [] are for standard cycling

End Repair

Transfer 40 µL Second Strand cDNA Synthesis mixture to End Repair reagent.

Incubate as indicated with <u>unheated lid</u> 👉

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

AMPure™ XP clean-up (100 µL). Elute in 20 µL

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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 👉

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). Elute in 18 µL
5 mM NaOH, 75°C 10 min

First PCR

Add 2 µL GSP1 primers to the First PCR reagent. Then add 18 µL 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	15*
65°C *	5 min* (ramp rate 100%)	
72°C	3 min	1
4°C	Hold	1

AMPure™ XP clean-up (24 µL). Elute in 20 µL

Second PCR

Add 2 µL GSP2 primers to Second PCR reagent. Then add 18 µL of purified First PCR 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	20*
65°C *	5 min* (ramp rate 100%)	
72°C	3 min	1
4°C	Hold	1

AMPure™ XP clean-up (24 µL). Elute in 20 µL

* Refer to Product Insert for panel-specific parameters

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