LOOP GENOMICS

LoopSeq™ Long Amplicon 3x8-Plex Quick Start Guide

Version 1.0

Sample QC and Dilution

- Quantify template DNA by Qubit
- Dilute DNA stock to **2 ng/ul** using Buffer EB

End-repair, A-tailing, Barcode Ligation

- Combine the following, mix thoroughly, and dispense **25ul** per reaction:

Reagent	1x vol	8x vol	24x vol
BC End Prep Mix	23.5	206.8	620.4
BC End Prep Enzyme	1.5	13.2	39.6
Total	25	220	660

- Add **5ul of diluted DNA sample** to the ERAT mastermix, mix thoroughly
- Incubate at 20°C for 10 minutes, then 65°C for 30 minutes, then HOLD at 4°C

- Combine the following, mix thoroughly, and dispense **15.5ul** per reaction:

Reagent	1x vol	8x vol	24x vol
BC Ligation Mix	15	132	396
BC Ligation Additive	0.5	4.4	13.2
Total	15.5	136.4	409.2

- Add **4.5ul unique BC adaptor** per sample well , mix thoroughly
- Incubate at 20°C for 15 minutes, then 65°C for 10 minutes, then HOLD at 4°C

- Add **3ul** of **Inactivation Enzyme M**, mix thoroughly
- Incubate at 37°C for 10 minutes, then 80°C for 5 minutes, then HOLD at 4°C

- Perform a cleanup reaction using **30ul** SPRI reagent
- Wash twice in 200ul 80% ethanol
- Elute in **20ul** Buffer EB

Barcode Distribution

- Dilute **2ul BC DNA** from previous step with **18ul** of Buffer EB, mix thoroughly
- Using new PCR tubes, combine **15ul** of **Amplification Mix S** and **5ul** of **Diluted BC DNA**, mix thoroughly

- Initiate the following PCR program:

Temperature	Duration	STEP
95°C	5 min	1 cycle
98°C	20 seconds	
60°C	20 seconds	28 cycles
72°C	4 min	
4°C	HOLD	

Set the heated lid at 100°C
Ramp rate = 2°C/second

- Add **30ul** of Buffer EB or Nuclease-free water to each PCR reaction
- Perform a cleanup reaction using **30ul** SPRI reagent per pool
- Wash twice in 200ul 80% ethanol
- Elute in **15ul** Buffer EB per pool

- Add **5ul** of **Distribution Mix** and **2ul** of **Distribution Enzyme**, mix thoroughly
- Incubate at 20°C for 15 minutes, then 75°C for 5 minutes, then HOLD at 4°C

Barcode Distribution

- Add **75.5ul** of **Activation Mix** and **2.5ul** of **Activation Enzyme**, mix thoroughly
- Incubate at 20°C for 2 hours, then 65°C for 10 minutes, then HOLD at 4°C

- Pool **15ul** from each of the 8 reactions with unique indices, for up to 3 pools from 24 samples
- Per pool, add **7.2ul** of **Neutralization Enzyme**, mix thoroughly
- Incubate at 37°C for 15 minutes, then HOLD at 4°C

- Per pool, perform a cleanup reaction using **102ul** SPRI reagent
- Wash twice in 200ul 80% ethanol
- Elute in **20ul** Buffer EB

Library Preparation

- Per pool, using a new PCR tube combine **on ice** the following:

Fragmentation Mix	**20ul**
Fragmentation Enzyme	**10ul**
DNA from previous step	**20ul**

- Mix thoroughly, briefly spin down and **return to ice**

- Set up the following PCR program, pause at 4°C

Temperature	Duration	STEP
4°C	1 min	Pause until ready
32°C	12 min	1 cycle
65°C	30 min	1 cycle
4°C	HOLD	

Set the heated lid at 100°C

- Place the samples in the pre-chilled PCR block and resume the program

- Per pool, add **40ul** of **Ligation Mix** and **10ul** of **Ligation Enzyme**, mix thoroughly
- Incubate at 20°C for 15 minutes (heated lid off), then HOLD at 4°C

- Per pool, perform a cleanup reaction using **80ul** SPRI reagent
- Wash twice in 200ul 80% ethanol
- Elute in **20ul** Buffer EB

- Per pool, using a new PCR tube combine the following and mix thoroughly:

Index Master Mix	**25ul**
Index Primer P1 to P7	**5ul (choose one Index Primer)**
DNA from previous step	**20ul**

- Initiate the following PCR program:

Temperature	Duration	STEP
98°C	45 seconds	1 cycle
98°C	15 seconds	
60°C	30 seconds	11 cycles
72°C	30 seconds	
4°C	HOLD	

Set the heated lid at 100°C

- Perform a cleanup reaction using **40ul** SPRI reagent
- Wash twice in 200ul 80% ethanol
- Elute in **20ul** Buffer EB