ForenSeq MainstAY Product Line with ForenSeq Enhanced PCR1 Buffer System Checklist

Amplify and Tag Targets

Purified DNA

- [ ] **1.** Using nuclease-free water, dilute 1 ng purified DNA input to 0.125 ng/µl.
- [ ] **2.** In the Master Mix tube, combine the following volumes per sample:
  - ePCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPMC or DPMD (2 µl)
- [ ] **3.** Pipette and centrifuge briefly.
- [ ] **4.** [Optional] Distribute master mix among an 8-tube strip.
- [ ] **5.** Add 7 µl master mix to the FSP.
- [ ] **6.** In a 1.7 ml tube, combine:
  - 2800M (2 µl)
  - Nuclease-free water (158 µl)
- [ ] **7.** Invert three times and centrifuge briefly.
- [ ] **8.** Add 8 µl diluted 2800M to the FSP.
- [ ] **9.** Add 8 µl nuclease-free water to the FSP.
- [ ] **10.** Add 8 µl 0.125 ng/µl DNA to the FSP and pipette.
- [ ] **11.** Centrifuge at 1000 × g for 30 seconds.
- [ ] **12.** Place on the thermal cycler and run the PCR1 program.

SAFE STOPPING POINT

If you are stopping, store the plate at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Enrich Targets

- [ ] **1.** Centrifuge the FSP at 1000 × g for 30 seconds.
- [ ] **2.** Piece the foil covering the UDI plate and transfer 8 µl UDI adapter to each sample well. For details on the UDI Adapter Plate Layout, Refer to Table 1.
- [ ] **3.** Briefly centrifuge PCR2 and pipette to mix.
- [ ] **4.** [Optional] Distribute PCR2 among an 8-tube strip.
- [ ] **5.** Add 27 µl PCR2.
- [ ] **6.** Pipette to mix.
- [ ] **7.** Centrifuge at 1000 × g for 30 seconds.
- [ ] **8.** Place on the thermal cycler and run PCR2m.

Table 1 UDI Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI00 01	UDI00 02	UDI00 03	UDI00 04	UDI00 05	UDI00 06	UDI00 07	UDI00 08	UDI00 09	UDI00 10	UDI00 11	UDI00 12
B	UDI00 13	UDI00 14	UDI00 15	UDI00 16	UDI00 17	UDI00 18	UDI00 19	UDI00 20	UDI00 21	UDI00 22	UDI00 23	UDI00 24
C	UDI00 25	UDI00 26	UDI00 27	UDI00 28	UDI00 29	UDI00 30	UDI00 31	UDI00 32	UDI00 33	UDI00 34	UDI00 35	UDI00 36
D	UDI00 37	UDI00 38	UDI00 39	UDI00 40	UDI00 41	UDI00 42	UDI00 43	UDI00 44	UDI00 45	UDI00 46	UDI00 47	UDI00 48
E	UDI00 49	UDI00 50	UDI00 51	UDI00 52	UDI00 53	UDI00 54	UDI00 55	UDI00 56	UDI00 57	UDI00 58	UDI00 59	UDI00 60
F	UDI00 61	UDI00 62	UDI00 63	UDI00 64	UDI00 65	UDI00 66	UDI00 67	UDI00 68	UDI00 69	UDI00 70	UDI00 71	UDI00 72
G	UDI00 73	UDI00 74	UDI00 75	UDI00 76	UDI00 77	UDI00 78	UDI00 79	UDI00 80	UDI00 81	UDI00 82	UDI00 83	UDI00 84
H	UDI00 85	UDI00 86	UDI00 87	UDI00 88	UDI00 89	UDI00 90	UDI00 91	UDI00 92	UDI00 93	UDI00 94	UDI00 95	UDI00 96

SAFE STOPPING POINT

If you are stopping, store the plate at 2°C to 8°C for up to 7 days. Alternatively, leave on the thermal cycler overnight.

Purify Libraries

- [ ] **1.** Add 45 µl SPB2 to each well of the PBP.
- [ ] **2.** Centrifuge the sealed FSP at 1000 × g for 30 seconds.
- [ ] **3.** Transfer 45 µl reaction from each well of the FSP to the corresponding well of the PBP.
- [ ] **4.** Discard the FSP plate.
- [ ] **5.** Seal the PBP and shake at 1800 rpm for 2 minutes.
- [ ] **6.** Incubate at room temperature for 5 minutes.
- [ ] **7.** Place on the magnetic stand and wait until the liquid is transparent (~5 minutes).
- [ ] **8.** Remove and discard all supernatant.
- [ ] **9.** Keep on the magnetic stand and wash as follows.
  - [ ] **a.** Add 200 µl fresh 80% EtOH to each well.
  - [ ] **b.** Incubate for 30 seconds.
  - [ ] **c.** Remove and discard all supernatant.
- [ ] **10.** Wash a second time.
- [ ] **11.** With a 20 µl pipette, remove residual EtOH from each well.
- [ ] **12.** Remove from the magnetic stand.
- [ ] **13.** Add 52.5 µl RSB to each well.
- [ ] **14.** Seal and shake at 1800 rpm for 2 minutes.

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15. If the beads are not fully resuspended, pipette to mix or reshake at 1800 rpm for 2 minutes.
16. Incubate at room temperature for 2 minutes.
17. Place on the magnetic stand and wait until the liquid is clear (~2 minutes).
18. Transfer 50 µl supernatant from each well of the PBP to the corresponding well of the PLP.
19. Seal and centrifuge at 1000 × g for 30 seconds.

Normalize Libraries

1. In the LNA1/LNB1 Master Mix tube, combine the following volumes per sample:
   - LNA1 (46.8 µl)
   - LNB1 (8.5 µl)
2. Vortex and invert several times.
3. Transfer to a reagent reservoir.
4. Add 45 µl LNA1/LNB1 Master Mix to the NWP.
5. To clear any aspirated beads, place the PLP on the magnetic stand until clear.
6. Transfer 20 µl supernatant from the PLP to the NWP.
7. Shake at 1800 rpm for 30 minutes.
8. While shaking, perform steps 9–11.
9. In the 0.1 N HP3 tube, combine the following volumes per sample:
   - Nuclease-free water (33.3 µl)
   - HP3 (1.8 µl)
10. Invert several times and set aside.
11. Add 30 µl LNS2 to the NLP.
12. Immediately after shaking, place the NWP on the magnetic stand until clear.
13. Remove and discard all supernatant.
14. Remove from the magnetic stand.
15. Wash as follows.
    a. Add 45 µl LNW1 to each well.
    b. Shake at 1800 rpm for 5 minutes.
    c. Place on the magnetic stand until clear.
    d. Remove and discard all supernatant.
    e. Remove from the magnetic stand.
16. Wash a **second** time.
17. Centrifuge at 1000 × g for 30 seconds.
18. Place on the magnetic stand until clear.
19. Remove residual LNW1.
20. Remove from the magnetic stand.
21. Add 32 µl 0.1 N HP3.
22. Shake at 1800 rpm for 5 minutes.
23. Place on the magnetic stand until clear.
24. Transfer 30 µl supernatant from the NWP to the NLP.
25. Pipette to mix.
26. Centrifuge at 1000 × g for 30 seconds.

SAFE STOPPING POINT
If you are stopping, store the plate at -25°C to -15°C for up to 30 days.

Pool Libraries

1. Transfer 5 µl of each library to an 8-tube strip.
2. Store the NLP at -25°C to -15°C for up to 30 days.
3. Transfer libraries from the 8-tube strip to the PNL tube.
4. Vortex and centrifuge briefly.

SAFE STOPPING POINT
If you are stopping, store the tube at -25°C to -15°C for up to 30 days.

Denature and Dilute Libraries

1. In the Denatured HSC tube, combine:
   - HSC (2 µl)
   - HP3 (2 µl)
   - Nuclease-free water (36 µl)
2. Pipette gently to mix. Cap and centrifuge briefly to mix.
3. Incubate at room temperature for 5 minutes.
4. Add 600 µl HT1 to the DNL tube.
5. Place the PNL tube in the microheating system for 2 minutes.
6. Transfer 12 µl library from the PNL tube to the DNL tube.
7. Pipette to mix.
8. Store the PNL tube at -25°C to -15°C for ≤ 30 days.
9. Add 4 µl denatured HSC to the DNL tube.
10. Pipette to mix.
11. Vortex and centrifuge briefly.
12. Transfer entire volume to the reagent cartridge.

Acronyms

Acronym	Definition
2800M	Control DNA 2800M
A50X	Index 2 (i5) Index Adapter
DNL	Diluted Normalized Libraries
DPMC	DNA Primer Mix C
DPMD	DNA Primer Mix D (To be used only with MainstAY SE Kit)
ePCR1	Enhanced PCR1 Reaction Mix
FEM	Enzyme Mix
FSP	ForenSeq Sample Plate
HP3	2 N NaOH

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Acronym	Definition
HSC	Human Sequencing Control
HT1	Hybridization Buffer
LNA1	Library Normalization Additives 1
LNB1	Library Normalization Beads 1
LNS2	Library Normalization Storage Buffer 2
LNW1	Library Normalization Wash 1
NLP	Normalized Library Plate
NWP	Normalization Working Plate
PBP	Purification Bead Plate
PCR2	PCR2 Reaction Mix
PLP	Purified Library Plate
PNL	Pooled Normalized Libraries
R7XX	Index 1 (i7) Index Adapter
RSB	Resuspension Buffer
SPB2	Sample Purification Beads 2
ProK	Proteinase K