

ForenSeq MainstAY Kit 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 (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	UD 100 01	UD 100 02	UD 100 03	UD 100 04	UD 100 05	UD 100 06	UD 100 07	UD 100 08	UD 100 09	UD 100 10	UD 100 11	UD 100 12
B	UD 100 13	UD 100 14	UD 100 15	UD 100 16	UD 100 17	UD 100 18	UD 100 19	UD 100 20	UD 100 21	UD 100 22	UD 100 23	UD 100 24
C	UD 100 25	UD 100 26	UD 100 27	UD 100 28	UD 100 29	UD 100 30	UD 100 31	UD 100 32	UD 100 33	UD 100 34	UD 100 35	UD 100 36
D	UD 100 37	UD 100 38	UD 100 39	UD 100 40	UD 100 41	UD 100 42	UD 100 43	UD 100 44	UD 100 45	UD 100 46	UD 100 47	UD 100 48
E	UD 100 49	UD 100 50	UD 100 51	UD 100 52	UD 100 53	UD 100 54	UD 100 55	UD 100 56	UD 100 57	UD 100 58	UD 100 59	UD 100 60
F	UD 100 61	UD 100 62	UD 100 63	UD 100 64	UD 100 65	UD 100 66	UD 100 67	UD 100 68	UD 100 69	UD 100 70	UD 100 71	UD 100 72

G	UD 100 73	UD 100 74	UD 100 75	UD 100 76	UD 100 77	UD 100 78	UD 100 79	UD 100 80	UD 100 81	UD 100 82	UD 100 83	UD 100 84
H	UD 100 85	UD 100 86	UD 100 87	UD 100 88	UD 100 89	UD 100 90	UD 100 91	UD 100 92	UD 100 93	UD 100 94	UD 100 95	UD 100 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 μ l supernatant from each well of the PBP to the corresponding well of the PLP.
- 19. Seal and centrifuge at 1000 \times g for 30 seconds.

Normalize Libraries

- 1. In the LNA1/LNB1 Master Mix tube, combine the following volumes per sample:
 - LNA1 (46.8 μ l)
 - LNB1 (8.5 μ l)
- 2. Vortex and invert several times.
- 3. Transfer to a reagent reservoir.
- 4. Add 45 μ l 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 μ l 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 μ l)
 - HP3 (1.8 μ l)
- 10. Invert several times and set aside.
- 11. Add 30 μ l 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 μ l 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 \times 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 μ l 0.1 N HP3.
- 22. Shake at 1800 rpm for 5 minutes.
- 23. Place on the magnetic stand until clear.
- 24. Transfer 30 μ l supernatant from the NWP to the NLP.
- 25. Pipette to mix.
- 26. Centrifuge at 1000 \times 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 μ l 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 μ l)
 - HP3 (2 μ l)

- Nuclease-free water (36 μ l)
- 2. Vortex and centrifuge briefly.
- 3. Incubate at room temperature for 5 minutes.
- 4. Add 600 μ l HT1 to the DNL tube.
- 5. Place the PNL tube in the microheating system for 2 minutes.
- 6. Transfer 12 μ l 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 \leq 30 days.
- 9. Add 4 μ l 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
ePCR1	Enhanced PCR1 Reaction Mix
FEM	Enzyme Mix
FSP	ForenSeq Sample Plate
HP3	2 N NaOH
HSC	Human Sequencing Control
HT1	Hybridization Buffer

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Acronym	Definition
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