ForenSeq MainstAY Product Line Checklist

VEROGEN

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:
  - PCR1 (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:
  - NA24385 (2 µl)
  - Nuclease-free water (158 µl)
- [ ] 7. Invert three times and centrifuge briefly.
- [ ] 8. Add 8 µl diluted NA24385 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.

Crude Lysate

- [ ] 1. In the Master Mix tube, combine the following volumes per sample:
  - PCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPMC or DPMD (2 µl)
  - Nuclease-free water (6 µl)
- [ ] 2. Pipette and centrifuge briefly.
- [ ] 3. [Optional] Distribute master mix among an 8-tube strip.
- [ ] 4. Add 13 µl master mix the FSP.
- [ ] 5. In a 1.7 ml tube, combine:
  - NA24385 (2 µl)
  - Nuclease-free water (38 µl)
- [ ] 6. Invert three times and centrifuge briefly.
- [ ] 7. Add 2 µl diluted NA24385 to the FSP.
- [ ] 8. Add 2 µl nuclease-free water to the FSP.
- [ ] 9. Add 2 µl diluted crude lysate to the FSP.
- [ ] 10. Centrifuge at 1000 × g for 30 seconds.
- [ ] 11. 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.

FTA Card

- [ ] 1. Place a 1.2 mm FTA card punch into each FSP well.
- [ ] 2. Add 100 µl 1X TBE Buffer.
- [ ] 3. Place on a PCR tube storage rack.
- [ ] 4. Shake at 1800 rpm for 2 minutes.
- [ ] 5. Centrifuge at 1000 × g for 30 seconds.
- [ ] 6. Remove and discard all supernatant.
- [ ] 7. Add the following volumes to each control well:
  - PCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPMC or DPMD (2 µl)
- [ ] 8. In a 1.7 ml tube, combine:
  - NA24385 (2 µl)
  - Nuclease-free water (158 µl)
- [ ] 9. Invert three times and centrifuge briefly.
- [ ] 10. Add 8 µl diluted NA24385 to the positive control wells.
- [ ] 11. Pipette to mix.
- [ ] 12. Add 8 µl nuclease-free water to the negative control wells.
- [ ] 13. Pipette to mix.
- [ ] 14. In the FTA Master Mix tube, combine the following volumes per sample:
  - PCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPMC or DPMD (2 µl)
  - Nuclease-free water (8 µl)
- [ ] 15. Pipette, and then centrifuge briefly.
- [ ] 16. [Optional] Distribute FTA Master Mix among an 8-tube strip.
- [ ] 17. Add 15 µl FTA Master Mix to the FSP.
- [ ] 18. Centrifuge at 1000 × g for 30 seconds.
- [ ] 19. 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. Centrifuge the UDI plate at 1000 x g for 30 seconds. 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 PCR2.

Table 1 UDI Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI00101	UDI00102	UDI00103	UDI00104	UDI00105	UDI00106	UDI00107	UDI00108	UDI00109	UDI00110	UDI00111	UDI00112
B	UDI00113	UDI00114	UDI00115	UDI00116	UDI00117	UDI00118	UDI00119	UDI00120	UDI00121	UDI00122	UDI00123	UDI00124
C	UDI00125	UDI00126	UDI00127	UDI00128	UDI00129	UDI00130	UDI00131	UDI00132	UDI00133	UDI00134	UDI00135	UDI00136
D	UDI00137	UDI00138	UDI00139	UDI00140	UDI00141	UDI00142	UDI00143	UDI00144	UDI00145	UDI00146	UDI00147	UDI00148
E	UDI00149	UDI00150	UDI00151	UDI00152	UDI00153	UDI00154	UDI00155	UDI00156	UDI00157	UDI00158	UDI00159	UDI00160
F	UDI00161	UDI00162	UDI00163	UDI00164	UDI00165	UDI00166	UDI00167	UDI00168	UDI00169	UDI00170	UDI00171	UDI00172
G	UDI00173	UDI00174	UDI00175	UDI00176	UDI00177	UDI00178	UDI00179	UDI00180	UDI00181	UDI00182	UDI00183	UDI00184
H	UDI00185	UDI00186	UDI00187	UDI00188	UDI00189	UDI00190	UDI00191	UDI00192	UDI00193	UDI00194	UDI00195	UDI00196

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.
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
NA24385	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)
FEM	Enzyme Mix
FSP	ForenSeq Sample Plate
HP3	2 N NaOH
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
PCR1	ForenSeq Enhanced PCR1 Reaction Mix
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