ForenSeq MainstAY Kit Checklist

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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.

Crude Lysate

- ☐ 1. In the Master Mix tube, combine the following volumes per sample:
  - ePCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPMC (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:
  - 2800M (2 µl)
  - Nuclease-free water (38 µl)
- ☐ 6. Invert three times and centrifuge briefly.
- ☐ 7. Add 2 µl diluted 2800M 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:
  - ePCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPMC (2 µl)
- ☐ 8. In a 1.7 ml tube, combine:
  - 2800M (2 µl)
  - Nuclease-free water (158 µl)
- ☐ 9. Invert three times and centrifuge briefly.
- ☐ 10. Add 8 µl diluted 2800M 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:
  - ePCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPMC (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. 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 2.
- ☐ 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.

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Table 1 UDI Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI001	UDI002	UDI003	UDI004	UDI005	UDI006	UDI007	UDI008	UDI009	UDI010	UDI011	UDI012
B	UDI013	UDI014	UDI015	UDI016	UDI017	UDI018	UDI019	UDI020	UDI021	UDI022	UDI023	UDI024
C	UDI025	UDI026	UDI027	UDI028	UDI029	UDI030	UDI031	UDI032	UDI033	UDI034	UDI035	UDI036
D	UDI037	UDI038	UDI039	UDI040	UDI041	UDI042	UDI043	UDI044	UDI045	UDI046	UDI047	UDI048
E	UDI049	UDI050	UDI051	UDI052	UDI053	UDI054	UDI055	UDI056	UDI057	UDI058	UDI059	UDI060
F	UDI061	UDI062	UDI063	UDI064	UDI065	UDI066	UDI067	UDI068	UDI069	UDI070	UDI071	UDI072
G	UDI073	UDI074	UDI075	UDI076	UDI077	UDI078	UDI079	UDI080	UDI081	UDI082	UDI083	UDI084
H	UDI085	UDI086	UDI087	UDI088	UDI089	UDI090	UDI091	UDI092	UDI093	UDI094	UDI095	UDI096

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. 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 2.
- ☐ 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 2 UDI Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	UDI001	UDI002	UDI003	UDI004	UDI005	UDI006	UDI007	UDI008	UDI009	UDI010	UDI011	UDI012
B	UDI013	UDI014	UDI015	UDI016	UDI017	UDI018	UDI019	UDI020	UDI021	UDI022	UDI023	UDI024
C	UDI025	UDI026	UDI027	UDI028	UDI029	UDI030	UDI031	UDI032	UDI033	UDI034	UDI035	UDI036
D	UDI037	UDI038	UDI039	UDI040	UDI041	UDI042	UDI043	UDI044	UDI045	UDI046	UDI047	UDI048
E	UDI049	UDI050	UDI051	UDI052	UDI053	UDI054	UDI055	UDI056	UDI057	UDI058	UDI059	UDI060
F	UDI061	UDI062	UDI063	UDI064	UDI065	UDI066	UDI067	UDI068	UDI069	UDI070	UDI071	UDI072
G	UDI073	UDI074	UDI075	UDI076	UDI077	UDI078	UDI079	UDI080	UDI081	UDI082	UDI083	UDI084
H	UDI085	UDI086	UDI087	UDI088	UDI089	UDI090	UDI091	UDI092	UDI093	UDI094	UDI095	UDI096

SAFE STOPPING POINT

If you are stopping, store the plate at -25°C to -15°C for up to 1 year.

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.

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- ☐ 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. Vortex and centrifuge briefly.
- ☐ 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
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
ePCR1	ForenSeq Enhanced PCR1 Reaction Mix
PCR2	PCR2 Reaction Mix
PLP	Purified Library Plate

ForenSeq MainstAY Kit Checklist

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Acronym	Definition
PNL	Pooled Normalized Libraries
R7XX	Index 1 (i7) Index Adapter
RSB	Resuspension Buffer
SPB2	Sample Purification Beads 2