Checklist : ForenSeq DNA Signature Prep Kit with ForenSeq Enhanced PCR1 Buffer System

Amplify and Tag Targets

Purified DNA

1. Using nuclease-free water, dilute 1 ng purified DNA input to 0.2 ng/µl.
2. In the Master Mix tube, combine the following volumes per sample:
   - ePCR1 (4.7 µl)
   - FEM (0.3 µl)
   - DPMA or DPMB (5 µl)
3. Pipette and centrifuge briefly.
4. [Optional] Distribute master mix among an 8-tube strip.
5. Add 10 µl master mix to the FSP.
6. In a 1.7 ml tube, combine:
   - 2800M (2 µl)
   - Nuclease-free water (98 µl)
7. Invert three times and centrifuge briefly.
8. Add 5 µl diluted 2800M to the FSP.
9. Add 5 µl nuclease-free water to the FSP.
10. Add 5 µl 0.2 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. Place the Index 1 Adapter tubes in the ForenSeq Index Plate Fixture.
3. Place the Index 2 Adapter tubes in the ForenSeq Index Plate Fixture.
4. Place the FSP on the ForenSeq Index Plate Fixture.
5. Add index adapters:
   a. Add 4 µl R7XX down each column.
   b. Replace the cap on each Index 1 Adapter tube.
   c. Add 4 µl A50X across each row.
   d. Replace the cap on each Index 2 Adapter tube.
6. Vortex PCR2, and then centrifuge briefly.
7. [Optional] Distribute PCR2 among an 8-tube strip.
8. Add 27 µl PCR2.
9. Centrifuge at 1000 × g for 30 seconds.
10. Place on the thermal cycler and run the PCR2 program.

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

Calculate the appropriate volume for the SPB Master Mix:

SPB Beads Master Mix	SPB	ProK
for 96 samples	5.50 ml	25.0 ul
for 64 samples	3.50 ml	16.4 ul
for 48 samples	2.70 ml	12.5 ul
for 32 samples	1.75 ml	8.2 ul
for 24 samples	1.30 ml	6.2 ul

1. Add 45 µl SPB Master Mix to the PBP.
2. Centrifuge the FSP at 1000 × g for 30 seconds.
3. Transfer 45 µl reaction from the FSP to the PBP.
4. Shake at 1800 rpm for 2 minutes.
5. Incubate at room temperature for 5 minutes.
6. Place on the magnetic stand until clear.
7. Remove and discard all supernatant.
8. Wash as follows.
   a. Add 200 µl fresh 80% EtOH.
   b. Incubate for 30 seconds.
   c. Remove and discard all supernatant.
9. Wash a **second** time.
10. Centrifuge at 1000 × g for 30 seconds.
11. Place on the magnetic stand.
12. Remove residual EtOH.
13. Remove from the magnetic stand.
14. Add 52.5 µl RSB.
15. Shake at 1800 rpm for 2 minutes.
16. If necessary, pipette or reshake.
17. Incubate at room temperature for 2 minutes.
18. Place on the magnetic stand until clear.
19. Transfer 50 µl supernatant from the PBP to the PLP.
20. 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 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.

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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 591 µl HT1 to the DNL tube.
5. Transfer 7 µl library from the PNL tube to the DNL tube.
6. Pipette to mix.
7. Store the PNL tube at -25°C to -15°C for ≤ 30 days.
8. Add 4 µl denatured HSC to the DNL tube.
9. Pipette to mix.
10. Vortex and centrifuge briefly.
11. Place on the microheating system for 2 minutes.
12. Invert several times to mix.
13. Immediately cool for 5 minutes.
14. Transfer entire volume to the reagent cartridge.

Acronyms

Acronym	Definition
2800M	Control DNA 2800M
A50X	Index 2 (i5) Index Adapter
DNL	Diluted Normalized Libraries
DPMA	DNA Primer Mix A
DPMB	DNA Primer Mix B
ePCR1	Enhanced PCR1 Reaction Mix
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
PCR2	PCR2 Reaction Mix
PLP	Purified Library Plate

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Acronym	Definition
PNL	Pooled Normalized Libraries
R7XX	Index 1 (i7) Index Adapter
RSB	Resuspension Buffer
SPB	Sample Purification Beads
ProK	Proteinase K