Amplify and Tag Targets

☐1 **For purified DNA or crude lysate**—Perform the following steps:

- ☐a Dilute input material as follows.
  - [Purified DNA] Dilute 1 ng to 0.2 ng/µl with nuclease-free water.
  - [Crude lysate] Dilute 2 µl with 3 µl nuclease-free water.
- ☐b Create a master mix in the Master Mix tube.
  - PCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPMA or DPMB (5.0 µl)
- ☐c Pipette to mix and then centrifuge briefly.
- ☐d Add 10 µl master mix to FSP plate.
- ☐e Dilute 2 µl 2800M with 98 µl nuclease-free water in a 1.5 ml microcentrifuge tube. Gently flick and then centrifuge briefly.
- ☐f Add 5 µl diluted 2800M to the appropriate well.
- ☐g Add 5 µl nuclease-free water to the appropriate well.
- ☐h Add samples. Pipette to mix.
  - [Purified DNA] Add 5 µl diluted DNA.
  - [Crude lysate] Add 5 µl diluted crude lysate.

☐2 **For FTA card input material**—Perform the following steps:

- ☐a Place a 1.2 mm FTA card punch into the FSP plate.
- ☐b Add 100 µl 1X TBE buffer.
- ☐c Place on a PCR tube storage rack.
- ☐d Shake at 1800 rpm for 2 minutes.
- ☐e Centrifuge at 1000 × g for 30 seconds.
- ☐f Remove and discard all supernatant.
- ☐g Add the following to the FTA Master Mix tube.
  - PCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPMA or DMPB (5.0 µl)
  - Nuclease-free water (5.0 µl)
- ☐h Add 15 µl FTA master mix to an FTA punch.
- ☐i Add 15 µl FTA master mix to an empty well.
- ☐j Dilute 2 µl 2800M with 98 µl nuclease-free water in a 1.5 ml microcentrifuge tube. Gently flick and then centrifuge briefly.
- ☐k Add 5 µl diluted 2800M to the appropriate well.

☐3 Centrifuge at 1000 × g for 30 seconds.

☐4 Transport to the post-PCR area.

☐5 Place on the thermal cycler and run the PCR1 program.

SAFE STOPPING POINT

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

Enrich Targets

☐1 Centrifuge at 1000 × g for 30 seconds.

☐2 Arrange Index 1 (i7) adapters in columns 1–12.

☐3 Arrange Index 2 (i5) adapters in rows A–H.

☐4 Place the plate on the ForenSeq Index Plate Fixture.

☐5 Using a multichannel pipette, add 4 µl of each Index 1 (i7) adapter to each column.

☐6 Using a multichannel pipette, add 4 µl of each Index 2 (i5) adapter to each row.

☐7 Vortex PCR2 and then centrifuge briefly.

☐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, seal the plate and store at 2°C to 8°C for up to 7 days. Alternatively, leave on the thermal cycler overnight.

Purify Libraries

☐1 Do the following depending on the number of libraries:

Number of Libraries	Procedure
< 16	Add 50 µl SPB × the number of libraries to a 1.7 ml microcentrifuge tube.
16–96	Add [50 µl SPB × (the number of libraries/8)] + 5 µl SPB to each well of a column of a new midi plate.
> 96	Add (50 µl SPB × the number of libraries) + 200 µl SPB to a multichannel reagent reservoir.

☐2 Add 45 µl SPB to the PBP plate.
☐3 Centrifuge the FSP plate at 1000 × g for 30 seconds.
☐4 Transfer 45 µl to the PBP plate.
☐5 Shake at 1800 rpm for 2 minutes.
☐6 Incubate at room temperature for 5 minutes.
☐7 Place on the magnetic stand until liquid is clear.
☐8 Remove and discard all supernatant.
☐9 Wash 2 times with 200 µl 80% EtOH.
☐10 Centrifuge at 1000 × g for 30 seconds.
☐11 Place on the magnetic stand.
☐12 Use a 20 µl pipette to remove residual EtOH.
☐13 Add 52.5 µl RSB.
☐14 Shake at 1800 rpm for 2 minutes.
☐15 Incubate at room temperature for 2 minutes.
☐16 Place on the magnetic stand until liquid is clear.
☐17 Transfer 50 µl to the PLP plate.
☐18 Centrifuge at 1000 × g for 30 seconds.

SAFE STOPPING POINT

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

Normalize Libraries

☐1 Add 46.8 µl LNA1 and 8.5 µl LNB1 to the LNA1/LNB1 Master Mix tube.
☐2 Vortex and then invert several times to mix.
☐3 Pour into a reagent reservoir.
☐4 Transfer 45 µl to the NWP plate.
☐5 Place the PLP plate on the magnetic stand until liquid is clear.
☐6 Transfer 20 µl from the PLP plate to the NWP plate.
☐7 Shake at 1800 rpm for 30 minutes.
☐8 Prepare 0.1 N HP3 in a new 1.5 ml microcentrifuge tube.
- Nuclease-free water (33.3 µl)
- HP3 (1.8 µl)

☐9 Invert several times to mix.
☐10 Add 30 µl LNS2 to the NLP plate
☐11 Place the NWP plate on the magnetic stand until liquid is clear.
☐12 Remove and discard all supernatant.
☐13 Remove from the magnetic stand.
☐14 Add 45 µl LNW1.
☐15 Shake at 1800 rpm for 5 minutes.
☐16 Place on the magnetic stand until liquid is clear.
☐17 Remove and discard all supernatant.
☐18 Repeat steps 14–17 for a total of 2 washes.
☐19 Remove from the magnetic stand.
☐20 Centrifuge at 1000 × g for 30 seconds.
☐21 Place on the magnetic stand until liquid is clear.
☐22 Use a 20 µl pipette to remove residual supernatant.
☐23 Remove from the magnetic stand.
☐24 Add 32 µl freshly prepared 0.1 N HP3.
☐25 Shake at 1800 rpm for 5 minutes.
☐26 Place on the magnetic stand until liquid is clear.
☐27 Transfer 30 µl to the NLP plate. Pipette to mix.
☐28 Centrifuge at 1000 × g for 30 seconds.

SAFE STOPPING POINT

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

Pool Libraries

☐1 Transfer 5 µl to a new 8-tube strip.
☐2 Transfer the contents to the PNL tube.
☐3 Vortex and then centrifuge briefly.

SAFE STOPPING POINT

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

Denature and Dilute Libraries

☐1 Create an HSC denaturation reaction in the HSC mixture tube.
- HSC (2 µl)
- HP3 (2 µl)
- Nuclease-free water (36 µl)

☐2 Vortex and then centrifuge briefly.
☐3 Incubate at room temperature for 5 minutes.
☐4 Add 591 µl HT1 to the DNL tube.
☐5 Transfer 7 µl from the PNL tube to the DNL tube. Pipette to mix.
☐6 Transfer 2 µl HSC mixture to the DNL tube. Pipette to mix.
☐7 Vortex and then centrifuge briefly.
☐8 Place on the 96°C microheating system for 2 minutes.
☐9 Invert several times to mix.
☐10 Immediately place in the ice-water bath or on the benchtop cooler for 5 minutes.
☐11 Immediately load the entire contents onto the reagent cartridge.

Acronyms

Acronym	Definition
2800M	Control DNA 2800M
DNL	Diluted Normalized Libraries
DPMA	DNA Primer Mix A
DPMB	DNA Primer Mix B
FEM	Enzyme Mix
FSP	ForenSeq Sample Plate
HP3	2N 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	PCR1 Reaction Mix
PCR2	PCR2 Reaction Mix

Acronym	Definition
PLP	Purified Library Plate
PNL	Pooled Normalized Libraries
RSB	Resuspension Buffer
SPB	Sample Purification Beads