ForenSeq Imagen Kit 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)
  - DPME or DPMF (Geo) (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.
- [ ] **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.

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)
  - DPME or DPMF (Geo) (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.** Add 8 µl nuclease-free water to the negative control wells.
- [ ] **12.** In the FTA Master Mix tube, combine the following volumes per sample:
  - PCR1 (4.7 µl)
  - FEM (0.3 µl)
  - DPME or DPMF (Geo) (2 µl)
  - Nuclease-free water (8 µl)
- [ ] **13.** Pipette, and then centrifuge briefly.
- [ ] **14.** [Optional] Distribute FTA Master Mix among an 8-tube strip.
- [ ] **15.** Add 15 µl FTA Master Mix to the FSP.
- [ ] **16.** Centrifuge at 1000 × g for 30 seconds.
- [ ] **17.** 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.** Pierce the foil covering of the UDI plate and transfer 8 µl UDI adapter to each sample well. See Table 1 for the UDI Adapter Plate layout.
- [ ] **3.** Briefly centrifuge PCR2 and pipette to mix.
- [ ] **4.** [Optional] Distribute PCR2 among an 8-tube strip.
- [ ] **5.** Add 27 µl PCR2.
- [ ] **6.** Centrifuge at 1000 × g for 30 seconds.
- [ ] **7.** 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	UDI0001	UDI0002	UDI0003	UDI0004	UDI0005	UDI0006	UDI0007	UDI0008	UDI0009	UDI0010	UDI0011	UDI0012
B	UDI0013	UDI0014	UDI0015	UDI0016	UDI0017	UDI0018	UDI0019	UDI0020	UDI0021	UDI0022	UDI0023	UDI0024
C	UDI0025	UDI0026	UDI0027	UDI0028	UDI0029	UDI0030	UDI0031	UDI0032	UDI0033	UDI0034	UDI0035	UDI0036
D	UDI0037	UDI0038	UDI0039	UDI0040	UDI0041	UDI0042	UDI0043	UDI0044	UDI0045	UDI0046	UDI0047	UDI0048
E	UDI0049	UDI0050	UDI0051	UDI0052	UDI0053	UDI0054	UDI0055	UDI0056	UDI0057	UDI0058	UDI0059	UDI0060
F	UDI0061	UDI0062	UDI0063	UDI0064	UDI0065	UDI0066	UDI0067	UDI0068	UDI0069	UDI0070	UDI0071	UDI0072
G	UDI0073	UDI0074	UDI0075	UDI0076	UDI0077	UDI0078	UDI0079	UDI0080	UDI0081	UDI0082	UDI0083	UDI0084
H	UDI0085	UDI0086	UDI0087	UDI0088	UDI0089	UDI0090	UDI0091	UDI0092	UDI0093	UDI0094	UDI0095	UDI0096

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.

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Purify Libraries

- [ ] **1.** Add 45 µl SPB2 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.** Discard the FSP plate.
- [ ] **5.** Shake at 1800 rpm for 2 minutes.
- [ ] **6.** Incubate at room temperature for 5 minutes.
- [ ] **7.** Place on the magnetic stand until clear.
- [ ] **8.** Remove and discard all supernatant.
- [ ] **9.** Wash as follows.
    - [ ] **a.** Add 200 µl fresh 80% EtOH.
    - [ ] **b.** Incubate for 30 seconds.
    - [ ] **c.** Remove and discard all supernatant.
- [ ] **10.** Wash a **second** time.
- [ ] **11.** Remove residual EtOH.
- [ ] **12.** Remove from the magnetic stand.
- [ ] **13.** Add 52.5 µl RSB.
- [ ] **14.** Shake at 1800 rpm for 2 minutes.
- [ ] **15.** If necessary, pipette or reshake.
- [ ] **16.** Incubate at room temperature for 2 minutes.
- [ ] **17.** Place on the magnetic stand until clear.
- [ ] **18.** Transfer 50 µl supernatant from the PBP to the PLP and discard the PBP plate appropriately.
- [ ] **19.** 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.
    - Store the remaining buffer at the appropriate temperature.
- [ ] **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.** 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.** Using a multichannel pipette, 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 8 µl library from the PNL tube to the DNL tube.
- [ ] **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.** Vortex and centrifuge briefly.
- [ ] **10.** Transfer entire volume to the reagent cartridge.

Acronyms

Acronym	Definition
DNL	Diluted Normalized Libraries
DPME	DNA Primer Mix E
DPMF (Geo)	DNA Primer Mix F
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
NA24385	NA24385 Positive Amplification Control DNA
NLP	Normalized Library Plate
NWP	Normalization Working Plate
PBP	Purification Bead Plate
PCR1	PCR1 Reaction Mix
PCR2	PCR2 Reaction Mix
PLP	Purified Library Plate
PNL	Pooled Normalized Libraries
RSB	Resuspension Buffer
SPB2	Sample Purification Beads 2
UDI	Unique Dual Indexes