

# ForenSeq Imagen Kit with ForenSeq Enhanced PCR1 Buffer System Checklist

## 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)
  - 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.

## 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	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
	100	100	100	100	100	100	100	100	100	100	100	100
	01	02	03	04	05	06	07	08	09	10	11	12
B	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
	100	100	100	100	100	100	100	100	100	100	100	100
	13	14	15	16	17	18	19	20	21	22	23	24
C	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
	100	100	100	100	100	100	100	100	100	100	100	100
	25	26	27	28	29	30	31	32	33	34	35	36
D	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
	100	100	100	100	100	100	100	100	100	100	100	100
	37	38	39	40	41	42	43	44	45	46	47	48
E	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
	100	100	100	100	100	100	100	100	100	100	100	100
	49	50	51	52	53	54	55	56	57	58	59	60
F	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
	100	100	100	100	100	100	100	100	100	100	100	100
	61	62	63	64	65	66	67	68	69	70	71	72
G	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
	100	100	100	100	100	100	100	100	100	100	100	100
	73	74	75	76	77	78	79	80	81	82	83	84
H	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD	UD
	100	100	100	100	100	100	100	100	100	100	100	100
	85	86	87	88	89	90	91	92	93	94	95	96

### 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 μl
for 64 samples	3.50 ml	16.4 μl
for 48 samples	2.70 ml	12.5 μl
for 32 samples	1.75 ml	8.2 μl
for 24 samples	1.30 ml	6.2 μl

1. Add 45 μl SPB2 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. 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.

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## 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
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
NA24385	NA24385 Positive Amplification Control DNA
NLP	Normalized Library Plate
NWP	Normalization Working Plate
PBP	Purification Bead Plate
PCR2	PCR2 Reaction Mix

# ForenSeq Imagen Kit with ForenSeq Enhanced PCR1 Buffer System Checklist

Acronym	Definition
PLP	Purified Library Plate
PNL	Pooled Normalized Libraries
ProK	Proteinase K
RSB	Resuspension Buffer
SPB2	Sample Purification Beads 2
UDI	Unique Dual Indexes