TruSeq Custom Amplicon v1.5 Checklist

Hybridize Oligo Pool

- ☐1 Add 5 µl ACD1 and 5 µl TE or water to 1 well of the HYP plate.
- ☐2 Add 10 µl gDNA to each remaining well.
- ☐3 Add 5 µl ACP1 to the well containing ACD1.
- ☐4 Add 5 µl CAT to each well containing gDNA.
- ☐5 Centrifuge at 1000 × g for 1 minute.
- ☐6 Add 35 µl OHS2. Pipette to mix.
- ☐7 Centrifuge at 1000 × g for 1 minute.
- ☐8 Place on the preheated heat block and incubate for 1 minute.
- ☐9 Reset the temperature to 40°C and incubate for 80 minutes.

Remove Unbound Oligos

- ☐1 Make sure that the heat block has cooled to 40°C.
- ☐2 Remove from the heat block.
- ☐3 Centrifuge at 1000 × g for 1 minute.
- ☐4 Transfer each sample to the FPU plate.
- ☐5 Cover and centrifuge at 2400 × g for 2 minutes.
- ☐6 Wash 2 times with 45 µl SW1.
- ☐7 Reassemble the FPU plate.
- ☐8 Add 45 µl UB1.
- ☐9 Cover and centrifuge at 2400 × g for 2 minutes.

Extend and Ligate Bound Oligos

- ☐1 Add 45 µl ELM4 to the FPU plate.
- ☐2 Incubate at 37°C for 45 minutes.

Amplify Libraries

- ☐1 Arrange the Index 1 (i7) adapters in columns 1–12.
- ☐2 Arrange the Index 2 (i5) adapters in rows A–H.
- ☐3 Place the plate on a TruSeq Index Plate Fixture.
- ☐4 Add 4 µl of each Index 1 (i7) adapter down each column.
- ☐5 Add 4 µl of each Index 2 (i5) adapter across each row.
- ☐6 Remove the FPU plate from the incubator and do the following.
  - ☐a Replace the aluminum foil seal with the filter plate lid.
  - ☐b Centrifuge at 2400 × g for 2 minutes.
  - ☐c Add 25 µl 50 mM NaOH. Pipette to mix.
  - ☐d Incubate at room temperature for 5 minutes.
- ☐7 Add 56 µl TDP1 to a full tube (2.8 ml) of PMM2. Invert to mix.
- ☐8 Transfer 22 µl PMM2/TDP1 mixture to the IAP plate.
- ☐9 Transfer eluted samples from the FPU plate to the IAP plate.
- ☐10 Centrifuge at 1000 × g for 1 minute.
- ☐11 Transfer the IAP plate to the post-amplification area.
- ☐12 Place on the preprogrammed thermal cycler and run the PCR 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.

Clean Up Libraries

- ☐1 Centrifuge the IAP plate at 1000 × g for 1 minute.
- ☐2 Run an aliquot of library and control on 4% agarose gel (5 µl) or Bioanalyzer (1 µl).
- ☐3 Add the appropriate volume of AMPure XP beads to the CLP plate.
- ☐4 Transfer all the supernatant from the IAP plate to the CLP plate.
- ☐5 Shake at 1800 rpm for 2 minutes.
- ☐6 Incubate at room temperature for 10 minutes.
- ☐7 Place on a magnetic stand until liquid is clear.
- ☐8 Remove and discard all supernatant.
- ☐9 Wash 2 times with 200 µl 80% EtOH.
- ☐10 Use a 20 µl pipette to remove residual EtOH.
- ☐11 Remove from the magnetic stand and air-dry for 10 minutes.
- ☐12 Add 30 µl EBT.
- ☐13 Shake at 1800 rpm for 2 minutes.
- ☐14 Incubate at room temperature for 2 minutes.
- ☐15 Place on a magnetic stand until liquid is clear.
- ☐16 Transfer 20 µl supernatant from the CLP plate to the LNP plate.
- ☐17 Centrifuge at 1000 × g for 1 minute.

Normalize Libraries

- ☐1 For 96 samples, add 4.4 ml LNA1 to a new 15 ml conical tube.
- ☐2 Use a P1000 pipette to resuspend LNB1.
- ☐3 Transfer 800 µl LNB1 to the tube of LNA1.
- ☐4 Add 45 µl LNA1/LNB1 to the LNP plate.
- ☐5 Shake at 1800 rpm for 30 minutes.
- ☐6 Place on a magnetic stand until liquid is clear.
- ☐7 Remove and discard all supernatant.
- ☐8 Remove from the magnetic stand.
- ☐9 Wash 2 times with 45 µl LNW1.
- ☐10 Use a 20 µl pipette to remove residual LNW1.
- ☐11 Remove from the magnetic stand.
- ☐12 Add 30 µl fresh 0.1 N NaOH.
- ☐13 Shake at 1800 rpm for 5 minutes.
- ☐14 Place the LNP plate on a magnetic stand until liquid is clear.
- ☐15 Add 30 µl LNS2 to the SGP plate.
- ☐16 Transfer 30 µl supernatant from the LNP plate to the SGP plate.
- ☐17 Centrifuge at 1000 × g for 1 minute.

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 Centrifuge at 1000 × g for 1 minute.
- ☐2 Transfer 5 µl of each library to an 8-tube strip.
- ☐3 Transfer the contents of the 8-tube strip to the PAL tube. Pipette to mix.
- ☐4 Denature and dilute pooled libraries to the loading concentration for the sequencing instrument you are using. See the denature and dilute libraries guide for your instrument.

Acronyms

Acronym	Definition
ACD1	Amplicon Control DNA 1
ACP1	Amplicon Control Oligo Pool 1
CAT	Custom Amplicon Oligo Tube
CLP	Clean-up Plate
EBT	Elution Buffer with Tris
ELM4	Extension Ligation Mix 4
FPU	Filter Plate Unit
HT1	Hybridization Buffer
HYP	Hybridization Plate
IAP	Indexed Amplification Plate
LNA1	Library Normalization Additives 1
LNB1	Library Normalization Beads 1
LNP	Library Normalization Plate
LNS2	Library Normalization Storage Buffer 2
LNW1	Library Normalization Wash 1
OHS2	Oligo Hybridization for Sequencing Reagent 2
PAL	Pooled Amplicon Library
PMM2	PCR Master Mix 2

Acronym	Definition
SGP	Storage Plate
SW1	Stringent Wash 1
TDP1	TruSeq DNA Polymerase 1
UB1	Universal Buffer 1