TruSeq Methyl Capture EPIC Library Prep Kit Checklist

Fragment DNA

- ☐1 Normalize gDNA to 10 ng/µl. Pipette or vortex to mix.
- ☐2 Centrifuge briefly.
- ☐3 Transfer 50 µl DNA to Covaris tubes.
- ☐4 Centrifuge at 280 × g for 5 seconds.
- ☐5 Fragment the DNA.
- ☐6 Centrifuge at 280 × g for 5 seconds.
- ☐7 Transfer 50 µl sample volume to a new eight-tube strip.
- ☐8 [Recommended] Run 1 µl using a High Sensitivity DNA chip.

SAFE STOPPING POINT

If you are stopping, cap the tubes and store at 2°C to 8°C overnight.

Clean Up Fragmented DNA

- ☐1 Add 80 µl SPB. Pipette or vortex to mix.
- ☐2 Incubate at room temperature for 3 minutes.
- ☐3 Place on a magnetic stand until liquid is clear.
- ☐4 Remove and discard all supernatant.
- ☐5 Wash two times with 200 µl 80% EtOH.
- ☐6 Centrifuge briefly, and then place on a magnetic stand.
- ☐7 Remove and discard residual EtOH.
- ☐8 Air-dry for 5 minutes.
- ☐9 Add 60 µl RSB.
- ☐10 Remove from the magnetic stand, and then pipette or vortex to mix.
- ☐11 Incubate at room temperature for 1 minute.

Repair Ends

- ☐1 Invert ERP3 to mix, and then tap.
- ☐2 Add 40 µl ERP3. Pipette or vortex to mix.
- ☐3 Place on the thermal cycler and run the ERP program. Remove when samples reach 4°C.
- ☐4 Briefly centrifuge at 280 × g.
- ☐5 Add 120 µl SPM.
- ☐6 Pipette to mix.
- ☐7 Incubate at room temperature for 3 minutes.
- ☐8 Place on a magnetic stand until liquid is clear. Keep on the magnetic stand until step 11.
- ☐9 Remove and discard supernatant.
- ☐10 Wash two times with 200 µl 80% EtOH.
- ☐11 Centrifuge briefly.
- ☐12 Place on a magnetic stand. Remove and discard residual EtOH.
- ☐13 Air-dry for 5 minutes.
- ☐14 Remove from the magnetic stand.
- ☐15 Add 17.5 µl RSB. Pipette or vortex to mix.
- ☐16 Incubate at room temperature for 1 minute.

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Adenylate 3′ Ends

- ☐1 Add 12.5 µl ATL2.
- ☐2 Pipette or vortex to mix.
- ☐3 Place on the thermal cycler and run the ATAIL70 program.

Ligate Adapters

- ☐1 Briefly centrifuge the DNA adapter tubes.
- ☐2 Remove LIG2 from storage.
- ☐3 Add the following to the 8-tube strip containing sample.
  - RSB (1.5 µl)
  - LIG2 (2.5 µl)
  - DNA adapters (4 µl)
- ☐4 Pipette or vortex to mix, and then centrifuge briefly.
- ☐5 Place on the thermal cycler and run the LIG program. Remove when samples reach 4°C.
- ☐6 Centrifuge briefly.
- ☐7 Vortex STL to mix.
- ☐8 Add 5 µl STL. Pipette or vortex to mix.
- ☐9 Add 43 µl SPM. Pipette or vortex to mix.
- ☐10 Incubate at room temperature for 3 minutes.
- ☐11 Place on a magnetic stand until liquid is clear. Keep on the magnetic stand until step .
- ☐12 Remove and discard supernatant.
- ☐13 Wash two times with 200 µl 80% EtOH.
- ☐14 Centrifuge briefly, and then place on a magnetic stand.
- ☐15 Remove and discard residual EtOH.
- ☐16 Air-dry for 5 minutes.
- ☐17 Elute and combine sets of four samples with 40 µl RSB.

Hybridize Probes

- ☐1 Add the following reagents in the order listed to the tube.
  - Blocker (10 µl)
  - EPIC Oligos (10 µl)
  - SPM (150 µl)
- ☐2 Pipette carefully to mix, and make sure that the mixture is homogenous.
- ☐3 Incubate at room temperature for 10 minutes.
- ☐4 Place on a magnetic stand until liquid is clear.
- ☐5 Remove and discard supernatant.
- ☐6 Wash two times with 200 µl 80% EtOH.
- ☐7 Centrifuge briefly.
- ☐8 Place on a magnetic stand. Remove and discard residual EtOH.
- ☐9 Air-dry for 5 minutes.
- ☐10 Add 7.7 µl CT4. Pipette or vortex to mix.
- ☐11 Incubate at room temperature for 2 minutes.
- ☐12 Place on a magnetic stand until liquid is clear.
- ☐13 Add 2.5 µl EHB2 to a new tube.
- ☐14 Transfer 7.5 µl to the eight-tube strip containing 2.5 µl EHB2.
- ☐15 Pipette or vortex to mix.
- ☐16 Place on the thermal cycler and run the MC HYB1 program.
- ☐17 Keep at the 58°C holding temperature for 35 minutes–2 hours.

Capture Hybridized Probes

- ☐1 Centrifuge tubes at 280 × g for 1 minute.
- ☐2 Vortex SMB to mix.
- ☐3 Add 250 µl SMB to a new tube.
- ☐4 Transfer each pool to the tubes of SMB.
- ☐5 Rinse out hybridization tube with SMB.
- ☐6 Vortex to mix.
- ☐7 Incubate at room temperature for 25 minutes.
- ☐8 Centrifuge briefly.
- ☐9 Place on a magnetic stand until liquid is clear.
- ☐10 Remove and discard all supernatant.
- ☐11 Remove from the magnetic stand.
- ☐12 Vortex EWS.
- ☐13 Add 200 µl EWS.
- ☐14 Pulse vortex to mix until SMB pellet is resuspended.
- ☐15 Place on the 50°C heat block for 20 minutes.
- ☐16 Place on a magnetic stand until liquid is clear.
- ☐17 Remove and discard all supernatant.
- ☐18 Remove from the magnetic stand.
- ☐19 Repeat steps 13–18.
- ☐20 Centrifuge briefly.
- ☐21 Place on a magnetic stand and remove the remaining supernatant.
- ☐22 Create elution premix in a 1.7 ml microcentrifuge tube.
  - ET1 (31.4 µl)
  - HP3 (1.7 µl)
- ☐23 Add 30 µl elution premix. Pipette or vortex to mix.
- ☐24 Incubate at room temperature for 5 minutes, and then centrifuge briefly.
- ☐25 Place on a magnetic stand until liquid is clear.
- ☐26 Add 5 µl ET2 to a new eight-tube strip.
- ☐27 Transfer 29 µl supernatant to ET2. Pipette or vortex to mix.

Perform Second Hybridization

- ☐1 Add 6 µl water, 50 µl CT3, and 10 µl EPIC Oligos to the tube from the previous step.
- ☐2 Pipette or vortex to mix, and then centrifuge briefly.
- ☐3 Place on the thermal cycler and run the MC HYB2 program.

SAFE STOPPING POINT

Keep at the 58°C holding temperature for at least 14.5 hours.

Perform Second Capture

- ☐1 Centrifuge tubes at 280 × g for 1 minute.
- ☐2 Vortex SMB to mix.
- ☐3 Add 250 µl SMB to a new tube.
- ☐4 Transfer each pool to the tubes.
- ☐5 Vortex to mix.
- ☐6 Incubate at room temperature for 25 minutes.
- ☐7 Centrifuge briefly.
- ☐8 Place on a magnetic stand until liquid is clear.
- ☐9 Remove and discard all supernatant.
- ☐10 Remove from the magnetic stand.
- ☐11 Vortex EWS.
- ☐12 Add 200 µl EWS.
- ☐13 Pulse vortex to mix.
- ☐14 Place on the 50°C heat block for 30 minutes.
- ☐15 Place on a magnetic stand until liquid is clear.
- ☐16 Remove and discard all supernatant.
- ☐17 Remove from the magnetic stand.
- ☐18 Repeat steps 12–17.
- ☐19 Centrifuge briefly.
- ☐20 Place on a magnetic stand and remove the remaining supernatant.
- ☐21 Create elution premix in a 1.7 ml microcentrifuge tube.
  - ET1 (18.8 µl)
  - HP3 (1 µl)
- ☐22 Add 18 µl elution premix.
- ☐23 Pipette or vortex to mix.
- ☐24 Incubate at room temperature for 5 minutes, and then centrifuge briefly.
- ☐25 Place on a magnetic stand until liquid is clear.
- ☐26 Add 2.9 µl ET2 to a new eight-tube strip.
- ☐27 Transfer 17.1 µl supernatant to the tube.
- ☐28 Pipette or vortex to mix. Proceed ***immediately***.

Bisulfite Conversion

- ☐1 Add 130 µl Lightning Conversion Reagent to sample.
- ☐2 Pipette or vortex to mix, and then centrifuge briefly.
- ☐3 Place on the thermal cycler and run the BSF CON program.
- ☐4 Add 600 µl M-Binding Buffer and 10 µl MagBinding Beads to a new tube.
- ☐5 Transfer entire sample to the tube of M-Binding Buffer and MagBinding Beads, and then vortex to mix.
- ☐6 Incubate at room temperature for 5 minutes.
- ☐7 Centrifuge briefly.
- ☐8 Place on a magnetic stand until liquid is clear.
- ☐9 Remove and discard all supernatant.
- ☐10 Wash one time with 400 µl M-Wash Buffer.
- ☐11 Add 200 µl L-Desulphonation Buffer, and then vortex to mix.
- ☐12 Incubate at room temperature for 15 minutes.
- ☐13 Place on a magnetic stand until liquid is clear.
- ☐14 Remove and discard all supernatant.
- ☐15 Wash two times with 400 µl M-Wash Buffer.
- ☐16 Centrifuge briefly, and then place on a magnetic stand.
- ☐17 Remove and discard supernatant.
- ☐18 Place on the 50°C heat block until dry.
- ☐19 Add 23 µl RSB. Pipette or vortex thoroughly to mix.
- ☐20 Place on the 50°C heat block for 4 minutes.
- ☐21 Place on a magnetic stand until liquid is clear.
- ☐22 Transfer 20 µl DNA to a new eight-tube strip.

Amplify Enriched Library

- ☐1 Add the following reagents to 20 µl DNA from the previous step.
  - PPC (5 µl)
  - Kapa HiFi Uracil+ (25 µl)
- ☐2 Pipette or vortex to mix.
- ☐3 Place on the thermal cycler and run the AMP MC program.

SAFE STOPPING POINT

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

Clean Up Amplified Enriched Library

- ☐1 Add 50 µl SPB to the tube, and then pipette or vortex to mix.
- ☐2 Incubate at room temperature for 3 minutes.
- ☐3 Place on a magnetic stand until liquid is clear.
- ☐4 Remove and discard supernatant.
- ☐5 Wash two times with 200 µl 80% EtOH.
- ☐6 Centrifuge briefly.
- ☐7 Place on a magnetic stand and remove residual EtOH.
- ☐8 Air-dry until dry.
- ☐9 Add 20 µl RSB, and then pipette or vortex to mix.
- ☐10 Incubate at room temperature for 1 minute.
- ☐11 Place on a magnetic stand until liquid is clear.
- ☐12 Transfer 19 µl supernatant to a new tube.

SAFE STOPPING POINT

If you are stopping, cap the tube and store at -25°C to -15°C for up to 6 months.

Check Enriched Libraries

- ☐1 Quantify the postenriched library using the Qubit dsDNA HS Assay Kit.
  - Use 2 µl as the loading volume.
  - Use the dsDNA and high sensitivity settings.
  - Record STD1 and STD2 readings.
  - Measure the library concentration.
- ☐2 Run 1 µl using a High Sensitivity DNA chip.

Acronyms

Acronym	Definition
ATL2	A-Tailing Mix
BLR	Blocker
CT3	Capture Target Buffer 3
CT4	Capture Target Buffer 4
EDTA	Ethylenediaminetetraacetic Acid
EPIC	Epigenetic Oligo Pool
ERP2	End Repair Mix 2
ET1	Elute Target Buffer 1
ET2	Elute Target Buffer 2
EWS	Enrichment Wash Solution
HP3	2 N NaOH
LIG2	Ligation Mix 2
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SPB	Sample Purification Beads
SPM	Sample Purification Mix
STL	Stop Ligation Buffer