Fragment DNA

- ☐1 Mix 5 ml RSB and 10 µl EDTA.
- ☐2 Normalize 100 ng gDNA with premix to 50 µl and mix.
- ☐3 Centrifuge.
- ☐4 Transfer 50 µl DNA to Covaris tubes or plate wells.
- ☐5 Centrifuge.
- ☐6 Fragment the DNA using the following settings.

Setting	M220	S2	S220	E220	LE220
Duty Factor	20	10	10	10	30
Intensity	—	5	—	—	—
Peak Power	50	—	175	175	450
Cycles/Burst	200	200	200	200	200
Duration	375	280	280	280	360/rack 420/tube
Temp.	20	7	7	7	7
Water Level	—	12	12	6	6
Intensifier	—	—	—	Yes	—

- ☐7 Centrifuge.
- ☐8 Transfer 50 µl supernatant.
- ☐9 Add 100 µl SPB and mix.
- ☐10 Incubate at room temperature for 5 minutes.
- ☐11 Centrifuge.
- ☐12 Place on a magnetic stand until liquid is clear.
- ☐13 Remove and discard all supernatant.
- ☐14 Wash 2 times with 200 µl 80% EtOH.
- ☐15 Centrifuge.
- ☐16 Incubate on the magnetic stand for 30 seconds.
- ☐17 Use a 20 µl pipette to remove residual EtOH.
- ☐18 Air-dry until dry.
- ☐19 Add 62.5 µl RSB.
- ☐20 Remove from the magnetic stand and mix.
- ☐21 Incubate at room temperature for 2 minutes.
- ☐22 Centrifuge.
- ☐23 Place on a magnetic stand until liquid is clear.
- ☐24 Transfer 60 µl supernatant.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Repair Ends and Select Library Size

- ☐1 Add 40 µl ERP3 and mix.
- ☐2 Centrifuge.
- ☐3 Incubate as follows.
  - [Plate] Place on the 30°C microheating system for 30 minutes, and then place on ice.
  - [Tube] Place on the thermal cycler and run the ERP program.
- ☐4 Centrifuge.
- ☐5 Add 90 µl SPB and mix.
- ☐6 Incubate at room temperature for 5 minutes.
- ☐7 Centrifuge.
- ☐8 Place on a magnetic stand until liquid is clear.
- ☐9 Transfer 185 µl supernatant.
- ☐10 Add 125 µl SPB and mix.
- ☐11 Incubate at room temperature for 5 minutes.
- ☐12 Centrifuge.
- ☐13 Place on a magnetic stand until liquid is clear.
- ☐14 Remove and discard all supernatant.
- ☐15 Wash 2 times with 200 µl 80% EtOH.
- ☐16 Centrifuge.
- ☐17 Incubate on the magnetic stand for 30 seconds.
- ☐18 Use a 20 µl pipette to remove residual EtOH.
- ☐19 Air-dry until dry.
- ☐20 Add 20 µl RSB.
- ☐21 Remove from the magnetic stand and mix.
- ☐22 Incubate at room temperature for 2 minutes.
- ☐23 Centrifuge.
- ☐24 Place on a magnetic stand until liquid is clear.
- ☐25 Transfer 17.5 µl supernatant.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Adenylate 3′ Ends

- ☐1 Add 12.5 µl ATL2 and mix.
- ☐2 Centrifuge.
- ☐3 [Plate] Incubate as follows.
  - ☐a Place on the 37°C microheating system for 30 minutes.
  - ☐b Move to the 70°C microheating system for 5 minutes.
  - ☐c Place on ice for 5 minutes.
- ☐4 [Tube] Place on the thermal cycler and run the ATAIL70 program.
- ☐5 Centrifuge.

Ligate Adapters

- ☐1 Add the following.
  - RSB (2.5 µl)
  - LIG2 (2.5 µl)
  - DNA adapters (2.5 µl)
- ☐2 Mix thoroughly.
- ☐3 Centrifuge.
- ☐4 Incubate as follows.
  - [Plate] Place on the 30°C microheating system for 10 minutes, and then place on ice.
  - [Tube] Place on the thermal cycler and run the LIG program.
- ☐5 Centrifuge.
- ☐6 Add 5 µl STL and mix.
- ☐7 Centrifuge.
- ☐8 Perform steps 9 through 24 using the **Round 1** volumes.
- ☐9 Add SPB.

	Round 1	Round 2
SPB	42.5 µl	50 µl

- ☐10 Mix thoroughly.
- ☐11 Incubate at room temperature for 5 minutes.
- ☐12 Centrifuge.
- ☐13 Place on a magnetic stand until liquid is clear.
- ☐14 Remove and discard all supernatant.
- ☐15 Wash 2 times with 200 µl 80% EtOH.
- ☐16 Centrifuge.
- ☐17 Incubate on the magnetic stand for 30 seconds.
- ☐18 Use a 20 µl pipette to remove residual EtOH.
- ☐19 Air-dry until dry.
- ☐20 Add RSB.

	Round 1	Round 2
RSB	52.5 µl	27.5 µl

- ☐21 Mix thoroughly.
- ☐22 Incubate at room temperature for 2 minutes.
- ☐23 Centrifuge.
- ☐24 Place on a magnetic stand until liquid is clear.
- ☐25 Transfer 50 µl supernatant to a new plate or to a new tube.
- ☐26 Repeat steps 9 through 24 with the new plate or tube using the **Round 2** volumes.
- ☐27 Transfer 25 µl supernatant.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Enrich DNA Fragments

- ☐1 Place on ice and add 5 µl PPC.
- ☐2 Add 20 µl EPM and mix.
- ☐3 Centrifuge.
- ☐4 Place on the thermal cycler and run the PCRNano program.
- ☐5 Centrifuge.
- ☐6 Add 35 µl SPB.
- ☐7 Mix thoroughly.
- ☐8 Incubate at room temperature for 5 minutes.
- ☐9 Centrifuge.
- ☐10 Place on a magnetic stand until liquid is clear.
- ☐11 Transfer 82 µl supernatant.
- ☐12 Add 82 µl SPB and mix.
- ☐13 Incubate at room temperature for 5 minutes.
- ☐14 Place on a magnetic stand until liquid is clear.
- ☐15 Remove and discard all supernatant.
- ☐16 Wash 2 times with 200 µl 80% EtOH.
- ☐17 Centrifuge.
- ☐18 Incubate on the magnetic stand for 30 seconds.
- ☐19 Use a 20 µl pipette to remove residual EtOH.
- ☐20 Air-dry until dry.
- ☐21 Add 17.5 µl RSB and mix.
- ☐22 Incubate at room temperature for 2 minutes.
- ☐23 Centrifuge.
- ☐24 Place on a magnetic stand until liquid is clear.
- ☐25 Transfer 15 µl supernatant.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Validate Libraries

- ☐1 Quantify the libraries using the Qubit dsDNA HS Assay Kit.
  - ☐a Use 1 µl as the loading volume.
  - ☐b Use the dsDNA and high sensitivity settings.
  - ☐c Record STD1 and STD2 readings.
  - ☐d Measure the library concentration in duplicate and use the average.
- ☐2 Check the library size distribution:
  - If using a High Sensitivity DNA chip:
    - Dilute the DNA library 1:10 with RSB.
    - Run 1 µl diluted DNA library.
  - If using a DNA 1000 chip, run 1 µl undiluted DNA library.

Hybridize Probes

- ☐1 Combine the following amount of each DNA library, making sure that each library has a unique index.

Plexity	Each Library	Total Pool
3-plex	250 ng	750 ng
6-plex	200 ng	1200 ng
9-plex	150 ng	1350 ng
12-plex	100 ng	1200 ng

  - If the total volume is > 40 µl, concentrate the pooled sample to 40 µl.
  - If the total volume is < 40 µl, increase the volume to 40 µl with RSB.
- ☐2 Add the following to a new tube. Pipette to mix.
  - DNA library pool (40 µl)
  - CT3 (50 µl)
  - CEX (10 µl)
- ☐3 Centrifuge.
- ☐4 Place on the thermal cycler and run the TE HYB program.
- ☐5 Keep at the 58°C holding temperature for at least 90 minutes and up to 24 hours.

Capture Hybridized Probes

- ☐1 Add 250 µl SMB to a new tube.
- ☐2 Immediately transfer the sample to the tube containing SMB. Pipette to mix.
- ☐3 Incubate at room temperature for 25 minutes.
- ☐4 Centrifuge.
- ☐5 Place on a magnetic stand until liquid is clear.
- ☐6 Remove and discard all supernatant.
- ☐7 Remove from the magnetic stand.
- ☐8 Add 200 µl SWS. Pipette to mix.
- ☐9 Place on the 50°C heat block for 30 minutes.
- ☐10 Place on a magnetic stand until liquid is clear.
- ☐11 Remove and discard all supernatant.
- ☐12 Remove from the magnetic stand.
- ☐13 Repeat steps 8–12 for a total of 2 washes.
- ☐14 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex.
- ☐15 Add 23 µl elution premix. Pipette to mix.
- ☐16 Incubate at room temperature for 2 minutes.
- ☐17 Centrifuge.
- ☐18 Place on a magnetic stand until liquid is clear.
- ☐19 Transfer 21 µl supernatant.
- ☐20 Add 4 µl ET2. Pipette to mix.
- ☐21 Centrifuge.

Perform Second Hybridization

- ☐1 Add the following to the tube. Pipette to mix.
  - DNA library pool (25 µl)
  - RSB (15 µl)
  - CT3 (50 µl)
  - CEX (10 µl)
- ☐2 Centrifuge.
- ☐3 Place on the thermal cycler and run the TE HYB program.
- ☐4 Keep at the 58°C holding temperature for at least 14.5 hours and up to 24 hours.

Perform Second Capture

- ☐1 Add 250 µl SMB to a new tube.
- ☐2 Immediately transfer the sample to the tube containing SMB. Pipette to mix.
- ☐3 Incubate at room temperature for 25 minutes.
- ☐4 Centrifuge.
- ☐5 Place on a magnetic stand until liquid is clear.
- ☐6 Remove and discard all supernatant.
- ☐7 Remove from the magnetic stand.
- ☐8 Add 200 µl SWS. Pipette to mix.
- ☐9 Place on the 50°C heat block for 30 minutes.
- ☐10 Place on a magnetic stand until liquid is clear.
- ☐11 Remove and discard all supernatant.
- ☐12 Remove from the magnetic stand.
- ☐13 Repeat steps 8–12 for a total of 2 washes.
- ☐14 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex.
- ☐15 Add 23 µl elution premix. Pipette to mix.
- ☐16 Incubate at room temperature for 2 minutes.
- ☐17 Centrifuge.
- ☐18 Place on a magnetic stand until liquid is clear.
- ☐19 Transfer 21 µl supernatant.
- ☐20 Add 4 µl ET2 and mix.
- ☐21 Centrifuge.

Clean Up Captured Library

- ☐1 Add 45 µl SPB. Pipette to mix.
- ☐2 Incubate at room temperature for 5 minutes.
- ☐3 Centrifuge.
- ☐4 Place on a magnetic stand until liquid is clear.
- ☐5 Remove and discard all supernatant.
- ☐6 Wash 2 times with 200 µl 80% EtOH.
- ☐7 Centrifuge.
- ☐8 Incubate on the magnetic stand for 30 seconds.
- ☐9 Use a 20 µl pipette to remove residual EtOH.
- ☐10 Air-dry until dry.
- ☐11 Add 27.5 µl RSB. Pipette to mix.
- ☐12 Incubate at room temperature for 2 minutes.
- ☐13 Centrifuge.
- ☐14 Place on a magnetic stand until liquid is clear.
- ☐15 Transfer 25 µl supernatant.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Amplify Enriched Library

- ☐1 Add 5 µl PPC.
- ☐2 Add 20 µl NEM. Pipette to mix.
- ☐3 Centrifuge.
- ☐4 Place on the thermal cycler and run the AMP8 program.

SAFE STOPPING POINT

If you are stopping, seal the plate or 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 Centrifuge.
- ☐2 Add 45 µl SPB. Pipette to mix.
- ☐3 Incubate at room temperature for 5 minutes.
- ☐4 Centrifuge.
- ☐5 Place on a magnetic stand until liquid is clear.
- ☐6 Remove and discard all supernatant.
- ☐7 Wash 2 times with 200 µl 80% EtOH.
- ☐8 Centrifuge.
- ☐9 Incubate on the magnetic stand for 30 seconds.
- ☐10 Use a 20 µl pipette to remove residual EtOH.
- ☐11 Air-dry until dry.
- ☐12 Add 22 µl RSB. Pipette to mix.
- ☐13 Incubate at room temperature for 2 minutes.
- ☐14 Centrifuge.
- ☐15 Place on a magnetic stand until liquid is clear.
- ☐16 Transfer 20 µl supernatant.

SAFE STOPPING POINT

If you are stopping, seal the plate or cap the tube and store at -25°C to -15°C for up to 7 days.

Validate Enriched Libraries

☐1 Quantify using the Qubit dsDNA HS Assay Kit.
- ☐a Use 1 µl as the loading volume.
- ☐b Use the dsDNA and high sensitivity settings.
- ☐c Record STD1 and STD2 readings.
- ☐d Measure the library concentration

☐2 Run 1 µl using a High Sensitivity DNA chip.

Acronyms

Acronym	Definition
ATL2	A Tailing Mix
CEX	Coding Exome Oligos
CT3	Capture Target Buffer 3
DAP	DNA Adapter Plate
EE1	Enrichment Elution Buffer 1
EPM	Enhanced PCR Mix
ERP	End Repair Mix
ET2	Elute Target Buffer 2
HP3	2N NaOH
LIG	Ligation Mix
NEM	Enrichment Amplification Mix
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SPB	Sample Purification Beads
STL	Stop Ligation Buffer
SWS	Streptavidin Wash Solution