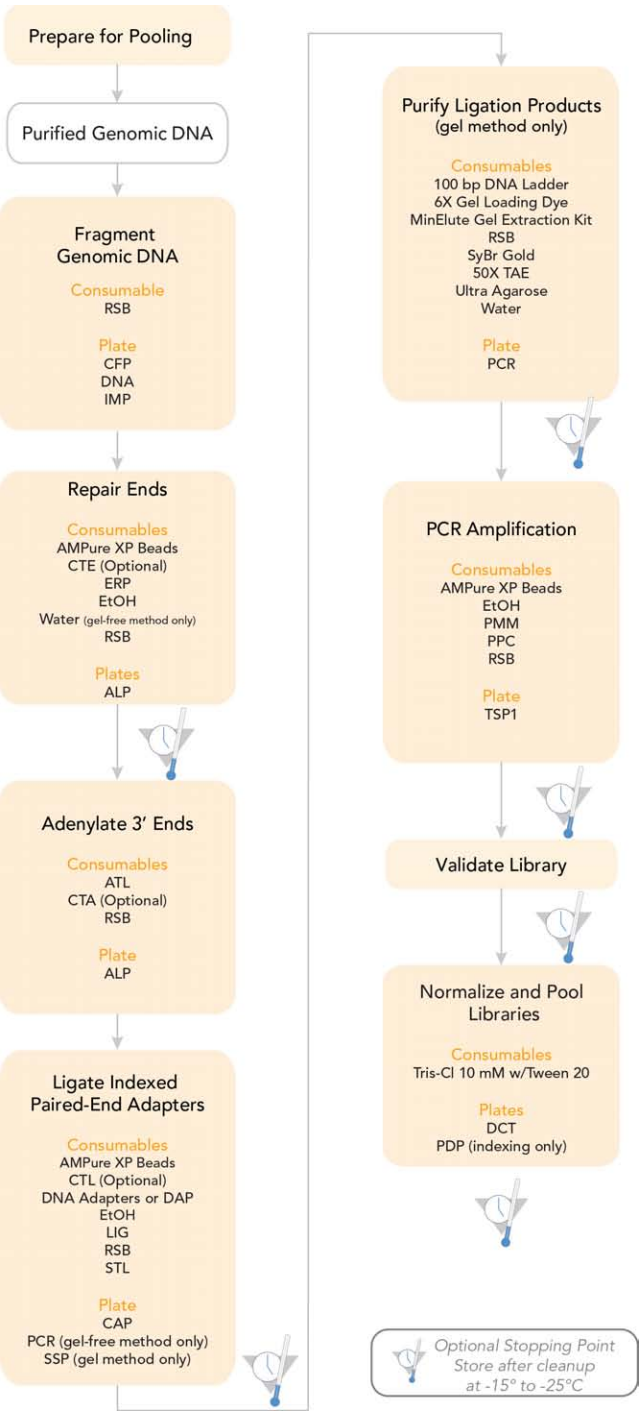
TruSeq DNA Sample Prep LS Protocol

Experienced User Card

FOR RESEARCH USE ONLY



NOTE

- Unless familiar with the protocol in the latest version of the TruSeq DNA Sample Preparation Guide, new or less experienced users are strongly advised to follow the protocol in the guide before using this Experienced User Card.
- For optimal sample tracking and quality control, fill out the TruSeq DNA Sample Preparation LS Lab Tracking Form as you perform TruSeq DNA Sample Preparation.

Fragment DNA

This process describes how to optimally fragment the gDNA depending on the downstream application. Covaris shearing generates dsDNA fragments with 3' or 5' overhangs. The fragmentation process described below was optimized to obtain final libraries with the following differences:

Table 1 Fragmentation Method Options

	Whole-genome Resequencing	TruSeq Enrichment	
	Gel Method	Gel-free Method	Gel Method
Covaris Shearing Duration	40 seconds	120 seconds	
Insert Size	300–400 bp	100–900 bp	200–300 bp

Calculate the amount of DNA to be fragmented based on 1 µg input DNA for each sample.

Illumina-Supplied Consumables

- Resuspension Buffer (RSB) (1 tube)
- CFP (Covaris Fragmentation Plate) barcode label
- DNA (DNA Plate) barcode label
- IMP (Insert Modification Plate) barcode label

User-Supplied Consumables

- 96-well 0.3 ml PCR plates (2)
- Covaris Tubes
- DNA samples

Make CFP

[_] 1 Illumina recommends to quantify gDNA samples using a fluorometric-based method such as Qubit or PicoGreen.

[_] 2 Illumina recommends to normalize the gDNA samples to a final volume of 55 µl at 20 ng/µl into each well of the new 0.3 ml PCR plate labeled with the DNA barcode.

Fragment DNA

[_] 1 Shear 1 µg of gDNA sample by transferring 52.5 µl of each DNA sample from the DNA plate to each Covaris tube in the new 0.3 ml PCR plate labeled with CFP barcode.

[_] 2 Fragment the DNA using the following settings:

Table 2 Covaris S220 or Covaris E220 Settings

Setting	Whole-genome Resequencing	TruSeq Enrichment
Duty factor	10%	10%
Peak Incident Power	175	175
Cycles per burst	200	200
Duration	40 seconds	2 x 60 seconds (120 seconds total)
Mode	Frequency sweeping	Frequency sweeping
Temperature	5.5° to 6°C	5.5° to 6°C

Table 3 Covaris S2 or E210 Settings

Setting	Whole-genome Resequencing	TruSeq Enrichment
Duty cycle	10%	10%
Intensity	5.0	5.0
Cycles per burst	200	200
Duration	40 seconds	2 x 60 seconds (120 seconds total)
Mode	Frequency sweeping	Frequency sweeping
Displayed Power	Covaris S2 - 23W Covaris E210 - 18W	Covaris S2 - 23W Covaris E210 - 18W
Temperature	5.5° to 6°C	5.5° to 6°C

[_] 3 Seal the Covaris tubes and centrifuge to 600 xg for 1 minute.

[_] 4 Transfer 50 µl of fragmented DNA from each Covaris tube in the CFP plate to the corresponding well of the new 0.3 ml PCR plate labeled with the IMP barcode using a single channel pipette.

Perform End Repair

This process converts the overhangs resulting from fragmentation into blunt ends using an End Repair Mix. The 3' to 5' exonuclease activity of this mix removes the 3' overhangs and the polymerase activity fills in the 5' overhangs.

Illumina-Supplied Consumables

- (Optional) End Repair Control (CTE) (1 tube per 48 reactions)
- End Repair Mix (ERP) (1 tube per 48 reactions)
- Resuspension Buffer (RSB) (1 tube)
- ALP (Adapter Ligation Plate) barcode label

User-Supplied Consumables

- 96-well 0.3 ml PCR plate
- AMPure XP Beads
- Freshly Prepared 80% Ethanol (EtOH)
- Microseal 'B' Adhesive Seal
- PCR Grade Water (for gel-free method for enrichment only)
- RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)
- RNase/DNase-free Strip Tubes and Caps (if using multichannel pipettes)

Make IMP

[_] 1 Do one of the following:
- If using the in-line control reagent:
  - Centrifuge the thawed End Repair Control tube to 600 xg for 5 seconds.
  - Add 10 µl of thawed End Repair Control to each well of the IMP plate that contains 50 µl of fragmented DNA.
- If not using the in-line control reagent, add 10 µl of Resuspension Buffer to each well of the IMP plate that contains 50 µl of fragmented DNA.

[_] 2 Add 40 µl of End Repair Mix to each well of the IMP plate containing the fragmented DNA. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 3 Seal the IMP plate with a Microseal 'B' adhesive seal.

Incubate 1 IMP

[_] 1 Place the sealed IMP plate on the pre-heated thermal cycler. Close the lid and incubate at 30°C for 30 minutes.

[_] 2 Remove the IMP plate from the thermal cycler.

Clean Up IMP

[_] 1 Remove the adhesive seal from the IMP plate.

[_] 2 Vortex the AMPure XP Beads until they are well dispersed.

[_] 3 Do one of the following:

- If using the gel-free method:
  - Determine the amount of AMPure XP beads and PCR grade water needed to combine to prepare a diluted bead mixture:
    AMPure XP beads: # of samples X 160 µl x 0.85 = µl AMPure XP beads.
    PCR grade water: # of samples X 160 µl x 0.15 = µl PCR grade water.
  - Add 160 µl of the diluted bead mixture to each well of the IMP plate containing 100 µl of End Repair Mix. Gently pipette the entire volume up and down 10 times to mix thoroughly.
- If using the gel method, add 160 µl well-mixed AMPure XP Beads to each well of the IMP plate containing 100 µl of End Repair Mix. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 4 Incubate the IMP plate at room temperature for 15 minutes.

[_] 5 Place the IMP plate on the magnetic stand at room temperature for 15 minutes or until the liquid appears clear.

[_] 6 Using a 200 µl single channel or multichannel pipette set to 127.5 µl, remove and discard 127.5 µl of the supernatant from each well of the IMP plate.

[_] 7 Repeat step 6 once.

[_] 8 With the IMP plate on the magnetic stand, add 200 µl of freshly prepared 80% EtOH to each well with a sample without disturbing the beads.

[_] 9 Incubate the IMP plate at room temperature for 30 seconds, then remove and discard all of the supernatant from each well. Take care not to disturb the beads.

[_] 10 Repeat steps 8 and 9 once for a total of two 80% EtOH washes.

[_] 11 Let the IMP plate stand at room temperature for 15 minutes to dry, then remove the plate from the magnetic stand.

[_] 12 Resuspend the dried pellet in each well with 17.5 µl Resuspension Buffer. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 13 Incubate the IMP plate at room temperature for 2 minutes.

[_] 14 Place the IMP plate on the magnetic stand at room temperature for 5 minutes or until the liquid appears clear.

[_] 15 Transfer 15 µl of the clear supernatant from each well of the IMP plate to the corresponding well of the new 0.3 ml PCR plate labeled with the ALP barcode.

SAFE STOPPING POINT
If you do not plan to proceed to *Adenylate 3' Ends* on page 7 immediately, the protocol can be safely stopped here. If you are stopping, seal the ALP plate with a Microseal 'B' adhesive seal and store at -15° to -25°C for up to seven days.

Adenylate 3' Ends

A single 'A' nucleotide is added to the 3' ends of the blunt fragments to prevent them from ligating to one another during the adapter ligation reaction. A corresponding single 'T' nucleotide on the 3' end of the adapter provides a complementary overhang for ligating the adapter to the fragment. This strategy ensures a low rate of chimera (concatenated template) formation.

Illumina-Supplied Consumables

- (Optional) A-Tailing Control (CTA) (1 tube per 48 reactions)
- A-Tailing Mix (ATL) (1 tube per 48 reactions)
- Resuspension Buffer (RSB) (1 tube)

User-Supplied Consumables

- Microseal 'B' Adhesive Seal
- RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)
- RNase/DNase-free Strip Tubes and Caps (if using multichannel pipettes)

Add ATL

[_] 1 Do one of the following:
- If using the in-line control reagent, add 2.5 µl of thawed A-Tailing Control to each well of the ALP plate.
- If not using the in-line control reagent, add 2.5 µl of Resuspension Buffer to each well of the ALP plate.

[_] 2 Add 12.5 µl of thawed A-Tailing Mix to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 3 Seal the ALP plate with a Microseal 'B' adhesive seal.

Incubate 1 ALP

[_] 1 Place the sealed ALP plate on the pre-heated thermal cycler. Close the lid and incubate at 37°C for 30 minutes.

[_] 2 Immediately remove the ALP plate from the thermal cycler, then proceed immediately to *Ligate Adapters* on page 9.

Adenylate 3' Ends

Ligate Adapters

This process ligates multiple indexing adapters to the ends of the DNA fragments, preparing them for hybridization onto a flow cell.

Illumina-Supplied Consumables

- Ligation Mix (LIG) (1 tube per 48 reactions)
- Choose from the following depending on the kit you are using:
  - TruSeq DNA LT Sample Prep Kit contents:
    - DNA Adapter Indices (AD001–AD016, AD018–AD023, AD025, AD027) (1 tube per column of 8 reactions, depending on the DNA Adapter Indices being used)
  - TruSeq DNA HT Sample Prep Kit contents:
    - DAP (DNA Adapter Plate)
- (Optional) Ligation Control (CTL) (1 tube per 48 reactions)
- Resuspension Buffer (RSB) (1 tube)
- Stop Ligation Buffer (STL) (1 tube per 48 reactions)
- CAP (Clean Up ALP Plate) barcode label
- DAP (DNA Adapter Plate) barcode label (if using the HT kit)
- PCR (Polymerase Chain Reaction) barcode label (for gel-free method only)
- SSP (Size Separate Plate) barcode label (for gel method only)

User-Supplied Consumables

- 96-well 0.3 ml PCR plates (2)
- AMPure XP Beads
- Freshly Prepared 80% Ethanol (EtOH)
- Microseal 'B' Adhesive Seals
- RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)
- RNase/DNase-free Strip Tubes and Caps (if using multichannel pipettes)

Add LIG

[_] 1 Do one of the following:

- If using DNA Adapter tubes, centrifuge the appropriate/desired thawed tubes to 600 xg for 5 seconds.
- If using a DAP:
  - Thaw the plate for 10 minutes at room temperature on the benchtop. Visually inspect the wells to ensure that they all are completely thawed.
  - Remove the adapter plate tape seal.
  - Centrifuge the plate at 280 xg for 1 minute to collect all of the adapter to the bottom of the well.
  - Remove the plastic cover and save the cover if you are not processing the entire plate at once.
  - If this is the first time using this DAP, apply the DAP barcode label to the plate.

NOTE
- The DAP is single-use for each well..
- Illumina recommends that the DAP does not undergo more than 4 freeze/thaw cycles.

[_] 2 Centrifuge the Ligation Control (if using Ligation Control) and Stop Ligation Buffer tubes to 600 xg for 5 seconds.

[_] 3 Immediately before use, remove the Ligation Mix tube from -15° to -25°C storage.

[_] 4 Remove the adhesive seal from the ALP plate.

[_] 5 Do one of the following:
- If using the in-line control reagent, add 2.5 µl of thawed Ligation Control to each well of the ALP plate.
- If not using the in-line control reagent, add 2.5 µl of Resuspension Buffer to each well of the ALP plate.

[_] 6 Add 2.5 µl of Ligation Mix to each well of the ALP plate.

[_] 7 Return the Ligation Mix tube back to -15° to -25°C storage immediately after use.

[_] 8 Do one of the following:
- If using DNA Adapter tubes, add 2.5 µl of the appropriate/desired thawed DNA Adapter Index to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.
- If using a DAP:
  - Place the DAP on the benchtop so that the part number barcode on the long side of the plate is facing you and the clipped corner is located on the lower left.
  - Do one of the following to pierce the foil seal:
    - If using the entire plate at once, use the bottom of a clean 96-well semi-skirted PCR plate to pierce a hole in all of the well seals simultaneously by gently but firmly pressing the clean plate over the foil seal.
    - If using only part of the plate, use the bottom of a clean eight-tube strip, with caps attached, to pierce holes in the desired columns that will be used for ligation. Repeat with a new, clean eight-tube strip, with caps attached, for each column of adapters that will be used for ligation.
  - Using an 8-tip multichannel pipette, transfer 2.5 µl of the appropriate/desired thawed DNA Adapter from the DAP well to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 9 Seal the ALP plate with a Microseal 'B' adhesive seal.

[_] 10 Centrifuge the ALP plate to 280 xg for 1 minute.

Incubate 2 ALP

[_] 1 Incubate the ALP plate on the pre-heated thermal cycler, with the lid closed, at 30°C for 10 minutes.

[_] 2 Remove the ALP plate from the thermal cycler.

Add STL

[_] 1 Remove the adhesive seal from the ALP plate.

[_] 2 Add 5 µl of Stop Ligation Buffer to each well of the ALP plate to inactivate the ligation. Gently pipette the entire volume up and down 10 times to mix thoroughly.

Clean Up ALP

[_] 1 Vortex the AMPure XP Beads until they are well dispersed, then add 42.5 µl of mixed AMPure XP Beads to each well of the ALP plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 2 Incubate the ALP plate at room temperature for 15 minutes.

[_] 3 Place the ALP plate on the magnetic stand at room temperature for 5 minutes or until the liquid appears clear.

[_] 4 Remove and discard 80 µl of the supernatant from each well of the ALP plate.

[_] 5 With the ALP plate remaining on the magnetic stand, add 200 µl of freshly prepared 80% EtOH to each well without disturbing the beads.

[_] 6 Incubate the ALP plate at room temperature for 30 seconds, then remove and discard all of the supernatant from each well.

[_] 7 Repeat steps 5 and 6 once for a total of two 80% EtOH washes.

[_] 8 While keeping the ALP plate on the magnetic stand, let the samples air dry at room temperature for 15 minutes and then remove the plate from the magnetic stand.

[_] 9 Resuspend the dried pellet in each well with 52.5 µl Resuspension Buffer. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 10 Incubate the ALP plate at room temperature for 2 minutes.

[_] 11 Place the ALP plate on the magnetic stand at room temperature for 5 minutes or until the liquid appears clear.

[_] 12 Transfer 50 µl of the clear supernatant from each well of the ALP plate to the corresponding well of the new 0.3 ml PCR plate labeled with the CAP barcode.

[_] 13 Vortex the AMPure XP Beads until they are well dispersed, then add 50 µl of mixed AMPure XP Beads to each well of the CAP plate for a second clean up. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 14 Incubate the CAP plate at room temperature for 15 minutes.

[_] 15 Place the CAP plate on the magnetic stand at room temperature for 5 minutes or until the liquid appears clear.

[_] 16 Remove and discard 95 µl of the supernatant from each well of the CAP plate.

[_] 17 With the CAP plate remaining on the magnetic stand, add 200 µl of freshly prepared 80% EtOH to each well without disturbing the beads.

[_] 18 Incubate the CAP plate at room temperature for 30 seconds, then remove and discard all of the supernatant from each well.

[_] 19 Repeat steps 17 and 18 once for a total of two 80% EtOH washes.

[_] 20 While keeping the CAP plate on the magnetic stand, let the samples air dry at room temperature for 15 minutes and then remove the plate from the magnetic stand.

[_] 21 Resuspend the dried pellet in each well with 22.5 µl Resuspension Buffer. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 22 Incubate the CAP plate at room temperature for 2 minutes.

[_] 23 Place the CAP plate on the magnetic stand at room temperature for 5 minutes or until the liquid appears clear.

[_] 24 Do one of the following:

- If using the gel-free method:
  - Transfer 20 µl of the clear supernatant from each well of the CAP plate to the corresponding well of the new 0.3 ml PCR plate labeled with the PCR barcode.
  - Proceed to *Enrich DNA Fragments* on page 15.
- If using the gel method:
  - Transfer 20 µl of the clear supernatant from each well of the CAP plate to the corresponding well of the new 0.3 ml PCR plate labeled with the SSP barcode.
  - Proceed to *Purify Ligation Products (gel method only)* on page 13.

SAFE STOPPING POINT

If you do not plan to proceed to *Enrich DNA Fragments* on page 15 or *Purify Ligation Products (gel method only)* on page 13 immediately, the protocol can be safely stopped here. If you are stopping, seal the PCR or SSP plate with a Microseal 'B' adhesive seal and store at -15° to -25°C for up to seven days.

Purify Ligation Products (gel method only)

This process is only performed when using the gel method. If you are running the gel-free method in preparation for the TruSeq Enrichment protocol, proceed to *Enrich DNA Fragments* on page 15.

This process purifies the products of the ligation reaction on a gel and removes unligated adapters, as well as any adapters that might have ligated to one another, and selects a size-range of sequencing library appropriate for cluster generation.

Table 4 Size Selection Options

	Whole-genome Resequencing	TruSeq Enrichment
Insert Size Target	300–400 bp[a]	200–300 bp
3 mm Slice Location	400–500 bp	300–400 bp

a. +/- 1 standard deviation of 20 bp, i.e, a < 20% variance for read lengths of 2 × 75 bp or shorter

Illumina-Supplied Consumables

- PCR (Polymerase Chain Reaction Plate) barcode label
- Resuspension Buffer (RSB) (1 tube)

User-Supplied Consumables

- 50 X TAE Buffer
- 96-well 0.3 ml PCR plate
- BenchTop 100 bp DNA Ladder
- Clean Scalpels
- Certified Low-range Ultra Agarose
- Distilled Water
- 6X Gel Loading Dye
- MinElute Gel Extraction Kit
- SyBr Gold Nucleic Acid Gel Stain

Size Separate SSP

[_] 1 Prepare a 150 ml, 2% agarose with SyBr Gold gel using 1 X TAE Buffer as follows:

- [_] a Add 3 g of agarose powder in 150 ml of 1X TAE buffer.
- [_] b Microwave the gel buffer until the agarose powder is completely dissolved.
- [_] c Cool the gel buffer on the bench for 5 minutes, and then add 15 µl of SyBr Gold. Swirl to mix.
- [_] d Pour the entire gel buffer to the gel tray.

[_] 2 Remove the adhesive seal from the thawed SSP plate.

[_] 3 Add 4 µl of 6X Gel Loading Dye to each well of the SSP plate.

[_] 4 Add 17 µl Resuspension Buffer and 4 µl of 6X Gel Loading Dye to 3 µl of DNA ladder.

[_] 5 When the agarose gel is set, put it in the gel electrophoresis unit and fill the tank with 1X TAE Buffer to the maximum fill mark.

[_] 6 Load all of the ladder solution onto one lane of the gel.

[_] 7 Load the samples from each well of the SSP plate onto the other lanes of the gel, leaving a gap of at least one empty lane between samples and ladders.

[_] 8 Run the gel at 120 V constant voltage for 120 minutes.

[_] 9 View the gel on a Dark Reader transilluminator.

[_] 10 Do one of the following:

- For whole-genome resequencing, excise a band from the gel spanning the width of the lane and ranging in size from 400-500 bp using a clean scalpel. Use the DNA ladder as a guide.
- For enrichment, excise a band from the gel spanning the width of the lane and ranging in size from 300-400 bp using a clean scalpel. Use the DNA ladder as a guide.

Size Separate Gel

[_] 1 Follow the instructions in the MinElute Gel Extraction Kit to purify each sample. Incubate the gel slices in the QG solution at room temperature (not at 50°C as instructed) until the gel slices have completely dissolved, while vortexing every 2 minutes.

[_] 2 Follow the instructions in the MinElute Gel Extraction Kit to purify on one MinElute spin column, eluting in 25 µl of QIAGEN EB.

[_] 3 Transfer 20 µl of each sample from the MinElute collection tube to the new 0.3 ml PCR plate labeled with the PCR barcode using a single channel pipette.

SAFE STOPPING POINT

If you do not plan to proceed to *Enrich DNA Fragments* on page 15 immediately, the protocol can be safely stopped here. If you are stopping, seal the PCR plate with a Microseal 'B' adhesive seal and store at -15° to -25°C for up to seven days.

Enrich DNA Fragments

This process uses PCR to selectively enrich those DNA fragments that have adapter molecules on both ends and to amplify the amount of DNA in the library. The PCR is performed with a PCR primer cocktail that anneals to the ends of the adapters. The number of PCR cycles should be minimized to avoid skewing the representation of the library.

Illumina-Supplied Consumables

- PCR Master Mix (PMM) (1 tube per 48 reactions)
- PCR Primer Cocktail (PPC) (1 tube per 48 reactions)
- Resuspension Buffer (RSB) (1 tube)
- TSP1 (Target Sample Plate) barcode label

User-Supplied Consumables

- 96-well 0.3 ml PCR plate
- AMPure XP Beads
- Freshly Prepared 80% Ethanol (EtOH)
- Microseal 'B' Adhesive Seals
- RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)
- RNase/DNase-free Strip Tubes and Caps (if using multichannel pipettes)

Make PCR

The following procedure assumes 1 µg of input DNA to library preparation and is designed to result in high library yields.

[_] 1 Add 5 µl of thawed PCR Primer Cocktail to each well of the PCR plate.

[_] 2 Add 25 µl of thawed PCR Master Mix to each well of the PCR plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 3 Seal the PCR plate with a Microseal 'B' adhesive seal.

Amp PCR

[_] 1 Place the sealed PCR plate on the pre-programmed thermal cycler. Close the lid and select **PCR** to amplify the plate.

Clean Up PCR

[_] 1 Remove the adhesive seal from the PCR plate.

[_] 2 Vortex the AMPure XP Beads until they are well dispersed, then do one of the following:

- If using the DNA Adapter tubes, add 50 µl of the mixed AMPure XP Beads to each well of the PCR plate containing 50 µl of the PCR amplified library. Gently pipette the entire volume up and down 10 times to mix thoroughly.
- If using the DAP, add 47.5 µl of the mixed AMPure XP Beads to each well of the PCR plate containing 50 µl of the PCR amplified library. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 3 Incubate the PCR plate at room temperature for 15 minutes.

[_] 4 Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid appears clear.

[_] 5 Remove and discard 95 µl of the supernatant from each well of the PCR plate.

[_] 6 With the PCR plate remaining on the magnetic stand, add 200 µl of freshly prepared 80% EtOH to each well without disturbing the beads.

[_] 7 Incubate the PCR plate at room temperature for 30 seconds, then remove and discard all of the supernatant from each well.

[_] 8 Repeat steps 6 and 7 once for a total of two 80% EtOH washes.

[_] 9 While keeping the PCR plate on the magnetic stand, let the samples air dry at room temperature for 15 minutes and then remove the plate from the magnetic stand.

[_] 10 Resuspend the dried pellet in each well with 32.5 µl Resuspension Buffer. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 11 Incubate the PCR plate at room temperature for 2 minutes.

[_] 12 Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid appears clear.

[_] 13 Transfer 30 µl of the clear supernatant from each well of the PCR plate to the corresponding well of the new 0.3 ml PCR plate labeled with the TSP1 barcode.

[_] 14 Do one of the following:

- If performing whole-genome resequencing, proceed to *Validate Library* on page 17.
- If performing enrichment, proceed to the *TruSeq Enrichment Guide* for instructions on how to quantify and qualify your library.

SAFE STOPPING POINT

If you do not plan to proceed to *Validate Library* on page 17 or TruSeq Enrichment immediately, the protocol can be safely stopped here. If you are stopping, seal the TSP1 plate with a Microseal 'B' adhesive seal and store at -15° to -25°C for up to seven days.

Validate Library

Illumina recommends performing the following procedures for quality control analysis on your whole-genome resequencing sample library and quantification of the DNA library templates. If performing enrichment, proceed directly to the *TruSeq Enrichment Guide* for instructions on how to quantify and qualify your library.

Quantify Libraries

In order to achieve the highest quality of data on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of every flow cell. This requires accurate quantitation of DNA library templates. Quantify your libraries using qPCR according to the Illumina *Sequencing Library qPCR Quantification Guide*.

Quality Control (Optional)

To verify the size of your PCR enriched fragments, check the template size distribution by running an aliquot of the DNA library on a gel or on a Agilent Technologies 2100 Bioanalyzer using a High Sensitivity DNA chip or DNA 1000 chip. Running samples on a Bioanalyzer should be used for qualitative purposes only.

- If validating by gel, load 10% of the volume of the library on a gel and check that the size range is as expected: a narrow smear similar in size to the DNA excised from the gel after the ligation.
- If using the Agilent Bioanalyzer with a High Sensitivity DNA chip, make a 1:50 dilution of the library using water and load 1 µl of the diluted library on the Agilent High Sensitivity DNA chip.
- If using the Agilent Bioanalyzer with a DNA 1000 chip, load 1 µl of the library on the Agilent DNA 1000 chip.

Validate Library

Normalize and Pool Libraries

This process describes how to prepare DNA templates that will be applied to cluster generation. Indexed DNA libraries are normalized to 10 nM in the DCT plate and then pooled in equal volumes in the PDP plate. DNA libraries not intended for indexing are normalized to 10 nM in the DCT plate without pooling.

Illumina-Supplied Consumables

- DCT (Diluted Cluster Template) barcode label
- PDP (Pooled DCT Plate) barcode label (for indexing only)

User-Supplied Consumables

- 96-well 0.3 ml PCR plate (for indexing only)
- 96-well MIDI plate
- Microseal 'B' Adhesive seals
- Tris-Cl 10 mM, pH8.5 with 0.1% Tween 20

Make DCT

[_] 1 Transfer 10 µl of sample library from each well of the TSP1 plate to the corresponding well of the new MIDI plate labeled with the DCT barcode.

[_] 2 Normalize the concentration of sample library in each well of DCT plate to 10 nM using Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20.

[_] 3 Gently pipette the entire normalized sample library volume up and down 10 times to mix thoroughly.

[_] 4 Depending on the type of library you want to generate, do one of the following:

- For non-indexed libraries, the protocol stops here. Do one of the following:
  - Proceed to cluster generation.
  - Seal the DCT plate with a Microseal 'B' adhesive seal and store at -15° to -25°C.
- For indexed libraries, proceed to Make PDP.

Make PDP (for indexing only)

[_] 1 Determine the number of samples to be combined together for each pool.

[_] 2 Do one of the following:

- If pooling 2–24 samples:
  - Transfer 10 µl of each normalized sample library to be pooled from the DCT plate to one well of the new 0.3 ml PCR plate labeled with the PDP barcode.

  The total volume in each well of the PDP plate should be 10X the number of combined sample libraries and will be 20–240 µl (2–24 libraries).
- If pooling 25–96 samples:
  - Using a multichannel pipette, transfer 5 µl of each normalized sample library in column 1 from the DCT plate to column 1 of the new MIDI plate labeled with the PDP barcode.

- Transfer 5 µl of each normalized sample library in column 2 from the DCT plate to column 1 of the PDP plate.
- Repeat the transfer for as many times as there are remaining columns in the DCT plate. The result will be a PDP plate with pooled samples in column 1. Gently pipette the entire volume of each well of column 1 up and down 10 times to mix thoroughly.
- Combine the contents of each well of column 1 into well A2 of the PDP plate, for the final pool.

[_] 3 Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 4 Do one of the following:

- Proceed to cluster generation.
- Seal the PDP plate with a Microseal 'B' adhesive seal and store at -15° to -25°C.