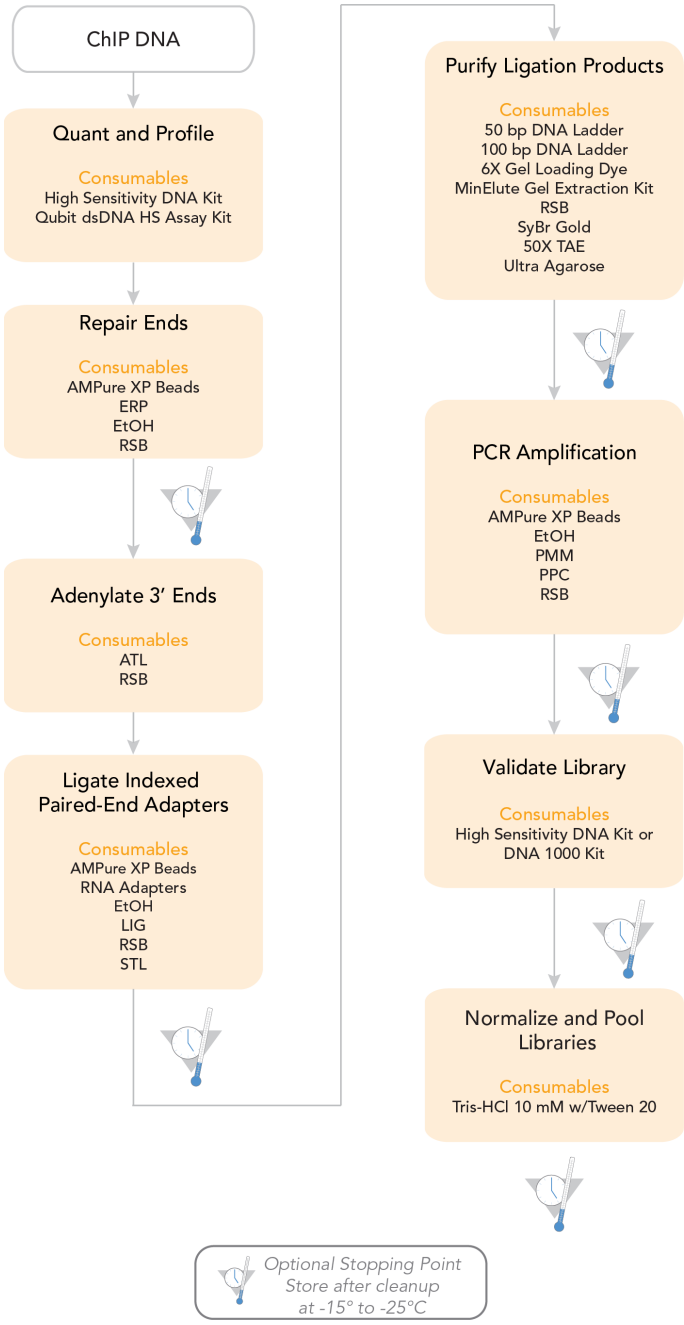
TruSeq ChIP Sample Preparation

Experienced User Card and Lab Tracking Form

FOR RESEARCH USE ONLY

Date: _____ Description: _____

Illumina Kit Lot #: _____ _____



NOTE

Unless familiar with the protocol in the latest version of the TruSeq ChIP Sample Preparation *Guide (part # 15023092)*, new or less experienced users are advised to follow the protocol in the guide before using this Experienced User Card and Lab Tracking Form.

Experienced User Card and Lab Tracking Form

Date/Time: _____ Operator: _____

Consumables

Item	Lot Number
A-Tailing Mix (ATL)	Lot #: _____
End Repair Mix (ERP)	Lot #: _____
Ligation Mix (LIG)	Lot #: _____
PCR Master Mix (PMM)	Lot #: _____
PCR Primer Cocktail (PPC)	Lot #: _____
Resuspension Buffer (RSB)	Lot #: _____
Stop Ligation Buffer (STL)	Lot #: _____
80% Ethanol	Date Prepared: _____

Adapter Indices	Lot Number
RNA Adapter Index 1 (AR001)	Lot #: _____
RNA Adapter Index 2 (AR002)	Lot #: _____
RNA Adapter Index 3 (AR003)	Lot #: _____
RNA Adapter Index 4 (AR004)	Lot #: _____
RNA Adapter Index 5 (AR005)	Lot #: _____
RNA Adapter Index 6 (AR006)	Lot #: _____
RNA Adapter Index 7 (AR007)	Lot #: _____
RNA Adapter Index 8 (AR008)	Lot #: _____
RNA Adapter Index 9 (AR009)	Lot #: _____
RNA Adapter Index 10 (AR010)	Lot #: _____
RNA Adapter Index 11 (AR011)	Lot #: _____
RNA Adapter Index 12 (AR012)	Lot #: _____
RNA Adapter Index 13 (AR013)	Lot #: _____
RNA Adapter Index 14 (AR014)	Lot #: _____
RNA Adapter Index 15 (AR015)	Lot #: _____
RNA Adapter Index 16 (AR016)	Lot #: _____
RNA Adapter Index 18 (AR018)	Lot #: _____
RNA Adapter Index 19 (AR019)	Lot #: _____

Experienced User Card and Lab Tracking Form

Date/Time: _____ Operator: _____

Consumables

Adapter Indices	Lot Number
RNA Adapter Index 20 (AR020)	Lot #: _____
RNA Adapter Index 21 (AR021)	Lot #: _____
RNA Adapter Index 22 (AR022)	Lot #: _____
RNA Adapter Index 23 (AR023)	Lot #: _____
RNA Adapter Index 24 (AR024)	Lot #: _____
RNA Adapter Index 25 (AR025)	Lot #: _____
RNA Adapter Index 27 (AR027)	Lot #: _____

Date/Time: _____ Operator: _____

Quant and Profile

The protocol is optimized for 5–10 ng input ChIP DNA. This procedure describes how to assess your input ChIP DNA quantity and quality following your ChIP experiment before starting library preparation. Illumina recommends a DNA insert size range of 200–800 bp.

Consumables

Item	Quantity	Storage	Supplied By
96-well 0.3 ml PCR plate	1 per 96 samples	15°C to 30°C	User
Agilent High Sensitivity DNA Kit	1 per 12 samples	As indicated by manufacturer	User
ChIP DNA	5–10 ng	-15°C to -25°C	User
Qubit assay tubes or Axygen PCR-05-C tubes	1 per sample	15°C to 30°C	User
Qubit dsDNA HS Assay Kit	1	As indicated by manufacturer	User

Procedure

[_] 1 Verify the size distribution of each ChIP DNA sample by running a 1 µl aliquot on Agilent High Sensitivity DNA chip using an Agilent Technologies 2100 Bioanalyzer.

[_] 2 Quantify 1 µl of each ChIP DNA sample using a Qubit dsDNA HS Assay Kit.

[_] 3 Illumina recommends normalizing the ChIP DNA samples to a final volume of 50 µl at 100–200 pg/µl into each well of a new 96-well 0.3 ml PCR plate.

Comments

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Quant and Profile

Date/Time: _____ Operator: _____

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.

Consumables

Item	Quantity	Storage	Supplied By
End Repair Mix (ERP)	1 tube per 48 reactions	-15°C to -25°C	Illumina
Resuspension Buffer (RSB)	1 tube	-15°C to -25°C (2°C to 8°C after initial thaw)	Illumina
96-well 0.3 ml PCR plate	1	15°C to 30°C	User
AMPure XP beads	160 µl per sample	2°C to 8°C	User
Freshly Prepared 80% Ethanol (EtOH)	400 µl per sample	15°C to 30°C	User
Microseal 'B' Adhesive Seal	1	15°C to 30°C	User
RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)	2	15°C to 30°C	User
RNase/DNase-free Eight-Tube Strips and Caps (if using multichannel pipettes)	2	15°C to 30°C	User

Procedure

[_] 1 Add 10 µl Resuspension Buffer to each well of the 96-well 0.3 ml PCR plate that contains 50 µl ChIP DNA.

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

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

[_] 4 Place the sealed PCR plate on the pre-heated thermal cycler. Close the lid and incubate at 30°C for 30 minutes.

Start time: _____ Stop time: _____

[_] 5 Remove the PCR plate from the thermal cycler.

[_] 6 Remove the adhesive seal from the PCR plate.

[_] 7 Vortex the AMPure XP Beads until they are well dispersed.

[_] 8 Add 160 µl well-mixed AMPure XP Beads to each well of the PCR plate containing 100 µl End Repair Mix. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 9 Incubate the PCR plate at room temperature for 15 minutes.

Start time: _____ Stop time: _____

Experienced User Card and Lab Tracking Form

Date/Time: _____ Operator: _____

[_] 10 Place the PCR plate on the magnetic stand at room temperature for 15 minutes or until the liquid is clear.

Start time: _____ Stop time: _____

Start time: _____ Stop time: _____

[_] 11 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 PCR plate.

[_] 12 Repeat step 11 one time.

[_] 13 With the PCR plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads.

[_] 14 Incubate the PCR plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well. Take care not to disturb the beads.

[_] 15 Repeat steps 13 and 14 one time for a total of two 80% EtOH washes.

[_] 16 Let the PCR plate stand at room temperature for 15 minutes to dry, and then remove the plate from the magnetic stand.

Start time: _____ Stop time: _____

[_] 17 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.

[_] 18 Incubate the PCR plate at room temperature for 2 minutes.

Start time: _____ Stop time: _____

[_] 19 Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

Start time: _____ Stop time: _____

[_] 20 Transfer 15 µl of the clear supernatant from each well of the PCR plate to the corresponding well of a new 96-well 0.3 ml PCR plate.

SAFE STOPPING POINT

If you do not plan to proceed immediately to *Adenylate 3' Ends* on page 9, you can safely stop the protocol here. If you are stopping, seal the PCR plate with a Microseal 'B' adhesive seal and store at -15°C to -25°C for up to 7 days.

Comments

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Date/Time: _____ Operator: _____

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.

Consumables

Item	Quantity	Storage	Supplied By
A-Tailing Mix (ATL)	1 tube per 48 reactions	-15°C to -25°C	Illumina
Resuspension Buffer (RSB)	1 tube	2°C to 8°C	Illumina
Microseal 'B' Adhesive Seal	1	15°C to 30°C	User
RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)	2	15°C to 30°C	User
RNase/DNase-free Eight-Tube Strips and Caps (if using multichannel pipettes)	2	15°C to 30°C	User

Procedure

[_] 1 Add 2.5 µl Resuspension Buffer to each well of the PCR plate.

[_] 2 Add 12.5 µl thawed A-Tailing 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.

[_] 4 Place the sealed PCR plate on the pre-programmed thermal cycler. Close the lid and select **ATAIL70**.

- [_] a Choose the pre-heat lid option and set to 100°C
- [_] b 37°C for 30 minutes
- [_] c 70°C for 5 minutes
- [_] d Hold at 4°C

[_] 5 When the thermal cycler temperature is 4°C, remove the PCR plate from the thermal cycler, and then proceed immediately to *Ligate Adapters* on page 11.

Comments

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_____

Adenylate 3' Ends

Date/Time: _____ Operator: _____

Ligate Adapters

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

Consumables

Item	Quantity	Storage	Supplied By
Ligation Mix (LIG)	1 tube per 48 reactions	-15°C to -25°C	Illumina
Resuspension Buffer (RSB)	1 tube	2°C to 8°C	Illumina
RNA Adapter Indices (AR001–AR016, AR018–AR023, AR025, AR027) (depending on the RNA Adapter Indices being used)	1 tube per column of 8 reactions	-15°C to -25°C	Illumina
Stop Ligation Buffer (STL)	1 tube per 48 reactions	-15°C to -25°C	Illumina
96-well 0.3 ml PCR plates	2		User
AMPure XP beads	42.5 µl per sample	2°C to 8°C	User
Freshly Prepared 80% Ethanol (EtOH)	800 µl per sample	15°C to 30°C	User
Microseal 'B' Adhesive Seal	1	15°C to 30°C	User
RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)	6	15°C to 30°C	User
RNase/DNase-free Eight-Tube Strips and Caps (if using multichannel pipettes)	6	15°C to 30°C	User

Procedure

[_] 1 Centrifuge the Stop Ligation Buffer and appropriate/desired thawed RNA Adapter tubes to 600 × g for 5 seconds.

[_] 2 Immediately before use, remove the Ligation Mix tube from -15°C to -25°C storage.

[_] 3 Remove the adhesive seal from the PCR plate.

[_] 4 Add 2.5 µl Resuspension Buffer to each well of the PCR plate.

[_] 5 Add 2.5 µl Ligation Mix to each well of the PCR plate.

[_] 6 Return the Ligation Mix tube back to -15°C to -25°C storage immediately after use.

[_] 7 Add 2.5 µl thawed RNA Adapter Index to each well of the PCR plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 8 Seal the PCR plate with a Microseal 'B' adhesive seal.

[_] 9 Centrifuge the PCR plate to 280 × g for 1 minute.

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Date/Time: _____ Operator: _____

[_] 10 Incubate the PCR plate on the pre-heated thermal cycler, with the lid closed, at 30°C for 10 minutes.
Start time: _____ Stop time: _____

[_] 11 Remove the PCR plate from the thermal cycler.

[_] 12 Remove the adhesive seal from the PCR plate.

[_] 13 Add 5 µl Stop Ligation Buffer to each well of the PCR plate to inactivate the ligation. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 14 Vortex the AMPure XP Beads until they are well dispersed.

[_] 15 Add 42.5 µl mixed AMPure XP Beads to each well of the PCR plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 16 Incubate the PCR plate at room temperature for 15 minutes.
Start time: _____ Stop time: _____

[_] 17 Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.
Start time: _____ Stop time: _____

[_] 18 Remove and discard 80 µl of the supernatant from each well of the PCR plate.

[_] 19 With the PCR plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads.

[_] 20 Incubate the PCR plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well.

[_] 21 Repeat steps 19 and 20 one time for a total of two 80% EtOH washes.

[_] 22 With 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.
Start time: _____ Stop time: _____

[_] 23 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.

[_] 24 Incubate the PCR plate at room temperature for 2 minutes.
Start time: _____ Stop time: _____

[_] 25 Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.
Start time: _____ Stop time: _____

[_] 26 Transfer 50 µl of the supernatant from each well of the PCR plate to the corresponding well of a new 96-well 0.3 ml PCR plate.

[_] 27 Vortex the AMPure XP Beads until they are well dispersed, and then add 50 µl mixed AMPure XP Beads to each well of the PCR plate for a second cleanup. Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 28 Incubate the PCR plate at room temperature for 15 minutes.
Start time: _____ Stop time: _____

Experienced User Card and Lab Tracking Form

Date/Time: _____ Operator: _____

[_] 29 Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

Start time: _____ Stop time: _____

[_] 30 Remove and discard 95 µl of the supernatant from each well of the PCR plate.

[_] 31 With the PCR plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads.

[_] 32 Incubate the PCR plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well.

[_] 33 Repeat steps 31 and 32 one time for a total of two 80% EtOH washes.

[_] 34 With 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.

Start time: _____ Stop time: _____

[_] 35 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.

[_] 36 Incubate the PCR plate at room temperature for 2 minutes.

Start time: _____ Stop time: _____

[_] 37 Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

Start time: _____ Stop time: _____

[_] 38 Transfer 20 µl of the clear supernatant from each well of the PCR plate to the corresponding well of a new 96-well 0.3 ml PCR plate.

SAFE STOPPING POINT

If you do not plan to proceed immediately to *Purify Ligation Products* on page 15, you can safely stop the protocol here. If you are stopping, seal the PCR plate with a Microseal 'B' adhesive seal and store at -15°C to -25°C for up to 7 days.

Comments

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_____

Ligate Adapters

Date/Time: _____ Operator: _____

Purify Ligation Products

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. A narrow 250–300 bp size-range of DNA fragments is selected for ChIP library construction appropriate for cluster generation.

Perform gel electrophoresis and band excision after adapter ligation to remove excess adapter and adapter dimers and to tighten the range of fragment sizes. Ligation reaction products are separated on an agarose gel and a ~2 mm wide gel slice containing DNA of the desired size is excised.

Consumables

Item	Quantity	Storage	Supplied By
Resuspension Buffer (RSB)	1 tube	2°C to 8°C	Illumina
6X Gel Loading Dye	8 µl + 4 µl per sample	15°C to 30°C	User
50 X TAE Buffer	150 ml	15°C to 30°C	User
96-well 0.3 ml PCR plate	1	15°C to 30°C	User
50 bp DNA Ladder	1	-15°C to -25°C	User
100 bp DNA Ladder	1	-15°C to -25°C	User
Certified Low-range Ultra Agarose	3 g	2° to 8°C	User
GeneCatchers or Clean Scalpels	2	15°C to 30°C	User
MinElute Gel Extraction Kit	1	15°C to 30°C	User
SYBR Gold Nucleic Acid Gel Stain	15 µl	-15°C to -25°C	User

Procedure

[_] 1 Prepare a 150 ml, 2% agarose with SYBR Gold gel using 1X 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 dissolved.
- [_] c Cool the gel buffer on the bench for 5 minutes, and then add 15 µl SYBR Gold. Swirl to mix.

  Start time: _____ Stop time: _____

- [_] d Pour the entire gel buffer to the gel tray.

[_] 2 Add 4 µl of 6X Gel Loading Dye to each well of the PCR plate.

[_] 3 Add 17 µl Resuspension Buffer and 4 µl of 6X Gel Loading Dye to 1 µl of the 50 bp DNA ladder.

[_] 4 Add 17 µl Resuspension Buffer and 4 µl of 6X Gel Loading Dye to 1 µl of the 100 bp DNA ladder.

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Date/Time: _____ Operator: _____

[_] 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 50 bp ladder solution onto one lane of the gel.

[_] 7 Load all of the 100 bp ladder solution onto another lane of the gel.

[_] 8 Load the samples from each well of the PCR plate onto the other lanes of the gel, leaving a gap of at least one empty lane between samples and ladders.

[_] 9 Run gel at 120 V for 10 minutes, then 60 V for 180 minutes (6 V/cm).

Start time: _____ Stop time: _____

Start time: _____ Stop time: _____

[_] 10 View the gel on a Dark Reader transilluminator or a UV transilluminator.

[_] 11 Photograph the gel before a slice is excised.

[_] 12 Place a GeneCatcher or a clean scalpel vertically above the sample in the gel at the desired size of the template.

[_] 13 Excise a gel slice of the sample lane at exactly 250–300 bp using the markers as a guide. Use two Gene Catchers for this band range if needed.

[_] 14 Place the gel slice in a new 2.0 ml DNA LoBind tube.

[_] 15 Photograph the gel after the slice was excised.

[_] 16 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 dissolved, while vortexing every 2 minutes.

Start time: _____ Stop time: _____

[_] 17 Follow the instructions in the MinElute Gel Extraction Kit to purify on one MinElute column, eluting in 25 µl QIAGEN Buffer EB.

[_] 18 Transfer 20 µl of each sample from the MinElute collection tube to a new 96-well 0.3 ml PCR plate, using a single channel pipette.

SAFE STOPPING POINT

If you do not plan to proceed immediately to *Enrich DNA Fragments* on page 17, you can safely stop the protocol 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 7 days.

Comments

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Date/Time: _____ Operator: _____

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 the PCR Primer Cocktail that anneals to the ends of the adapters. Minimize the number of PCR cycles to retain library representation.

Consumables

Item	Quantity	Storage	Supplied By
PCR Master Mix (PMM)	1 tube per 48 reactions	-15°C to -25°C	Illumina
PCR Primer Cocktail (PPC)	1 tube per 48 reactions	-15°C to -25°C	Illumina
Resuspension Buffer (RSB)	1 tube	2°C to 8°C	Illumina
96-well 0.3 ml PCR plate	1	15°C to 30°C	User
AMPure XP beads	50 µl per sample	2°C to 8°C	User
Freshly Prepared 80% Ethanol (EtOH)	400 µl per sample	15°C to 30°C	User
Microseal 'B' Adhesive Seal	1	15°C to 30°C	User
RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)	4	15°C to 30°C	User
RNase/DNase-free Eight-Tube Strips and Caps (if using multichannel pipettes)	4	15°C to 30°C	User

Procedure

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

[_] 2 Add 25 µl 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.

[_] 4 Place the sealed PCR plate on the pre-programmed thermal cycler. Close the lid and select **PCR** to amplify the plate.

- [_] a Choose the pre-heat lid option and set to 100°C
- [_] b 98°C for 30 seconds
- [_] c 18 cycles of:
  - 98°C for 10 seconds
  - 60°C for 30 seconds
  - 72°C for 30 seconds
- [_] d 72°C for 5 minutes
- [_] e Hold at 4°C

[_] 5 Remove the adhesive seal from the PCR plate.

Date/Time: _____ Operator: _____

[_] 6 Vortex the AMPure XP Beads until they are well dispersed.

[_] 7 Add 50 µl 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.

[_] 8 Incubate the PCR plate at room temperature for 15 minutes.

Start time: _____ Stop time: _____

[_] 9 Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

Start time: _____ Stop time: _____

[_] 10 Remove and discard 95 µl of the supernatant from each well of the PCR plate.

[_] 11 With the PCR plate on the magnetic stand, add 200 µl freshly prepared 80% EtOH to each well without disturbing the beads.

[_] 12 Incubate the PCR plate at room temperature for 30 seconds, and then remove and discard all of the supernatant from each well.

[_] 13 Repeat steps 11 and 12 one time for a total of two 80% EtOH washes.

[_] 14 With 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.

Start time: _____ Stop time: _____

[_] 15 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.

[_] 16 Incubate the PCR plate at room temperature for 2 minutes.

Start time: _____ Stop time: _____

[_] 17 Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

Start time: _____ Stop time: _____

[_] 18 Transfer 15 µl of the clear supernatant from each well of the PCR plate to the corresponding well of a new 96-well 0.3 ml PCR plate.

SAFE STOPPING POINT

If you do not plan to proceed immediately to *Validate Library* on page 19 or TruSeq Enrichment, you can safely stop the protocol here. If you are stopping, seal the PCR plate with a Microseal 'B' adhesive seal and store at -15°C to -25°C for up to 7 days.

Comments

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Date/Time: _____ Operator: _____

Validate Library

Perform the following procedures for quality control analysis on your sample library and quantification of the ChIP DNA library templates.

Quantify Libraries

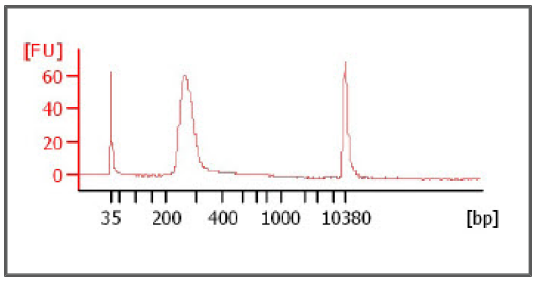
To achieve the highest quality data on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of the flow cell. Optimizing cluster densities requires accurate quantitation of DNA library templates. Quantify your libraries using qPCR according to the Illumina *Sequencing Library qPCR Quantification Guide (part # 11322363).*

Quality Control

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

- If using the Agilent Bioanalyzer with a High Sensitivity DNA chip:
  - Make a 1:20 dilution of the library using water
  - 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.

Figure 1 Example of DNA Library Distribution for TruSeq ChIP Sample Prep Libraries



Comments

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Validate Library

Date/Time: _____ Operator: _____

Normalize and Pool Libraries

This process describes how to prepare DNA templates for cluster generation. Indexed DNA libraries are normalized to 10 nM, and then pooled in equal volumes. DNA libraries not intended for pooling are normalized to 10 nM without pooling.

Consumables

Item	Quantity	Storage	Supplied By
96-well 0.3 ml PCR plate (for pooling only)	1	15°C to 30°C	User
96-well MIDI plate	1	15°C to 30°C	User
Microseal 'B' Adhesive Seal	1	15°C to 30°C	User
Tris-HCl 10 mM, pH8.5 with 0.1% Tween 20	Enough to normalize each sample to 10 nM	15°C to 30°C	User

Procedure

[_] 1 Transfer 10 µl of sample library from each well of the PCR plate to the corresponding well of a new 96-well MIDI plate.

[_] 2 Normalize the concentration of sample library in each well of the MIDI plate to 10 nM using Tris-HCl 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-pooled libraries, the protocol stops here. Do one of the following:
  - Proceed to cluster generation.
  - Seal the MIDI plate with a Microseal 'B' adhesive seal and store at -15°C to -25°C.
- For pooled libraries, proceed to step 5.

[_] 5 Determine the number of samples to be combined together for each pool.

[_] 6 Transfer 10 µl of each normalized sample library to be pooled from the MIDI plate to one well of a new 96-well 0.3 ml PCR plate.

[_] 7 Gently pipette the entire volume up and down 10 times to mix thoroughly.

[_] 8 Do one of the following:

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

Comments

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

Illumina Website	www.illumina.com
Email	techsupport@illumina.com

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at www.illumina.com/msds.

Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to www.illumina.com/support, select a product, then click **Documentation & Literature**.

Part # 15036179 Rev. B

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