TruSeq® ChIP Sample Preparation Guide

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ILLUMINA PROPRIETARY
Part # 15023092 Rev. B
Catalog # IP-202-9001DOC
October 2013

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Introduction

This protocol explains how to prepare up to 24 pooled paired-end indexed chromatin-immunoprecipitation (ChIP) DNA libraries, using the reagents provided in the Illumina® TruSeq® ChIP Sample Preparation Kit, for subsequent cluster generation and DNA sequencing. The goal of this protocol is to add adapter sequences onto the ends of ChIP DNA to generate indexed single read or paired-end sequencing libraries.

Input ChIP DNA (5–10 ng) is blunt-ended and phosphorylated. A single 'A' nucleotide is added to the 3' ends of the fragments in preparation for ligation to an adapter that has a single-base 'T' overhang. The ligation products are purified and accurately size-selected by agarose gel electrophoresis. Size-selected DNA is purified and PCR-amplified to enrich for fragments that have adapters on both ends. The final purified product is then quantitated before cluster generation.

The sample preparation protocol offers:

Streamlined Workflow

- Master-mixed reagents to reduce reagent containers and pipetting
- Universal adapter for preparation of single read, paired-end, and indexing

Index Adapter Tags All Samples

- Contains adapter index tubes recommended for preparing and pooling 24 or fewer samples for sequencing
- Additional adapters and primers not necessary
- Enables indexing earlier in the process

The protocol is compatible with no indexing or a lower indexing pooling level. The libraries generated do not require PCR amplification to enable cluster generation, although PCR is recommended in the standard protocol to robustly meet the yield requirements of most standard applications.

NOTE
The protocols described in this guide assume that you have confirmed your kit contents and obtained all of the requisite consumables and equipment.

What's New

The following changes were made in this guide revision:

- New *Additional Resources* section which contains references to:
  - Training
  - Best Practices content on the Illumina website.
  - Experienced User Card and Lab Tracking form combined document.
  - Pooling guidelines documented in the TruSeq Sample Preparation Pooling Guide.
  - Illumina Experiment Manager (IEM)
- Moved Acronyms, Kit Contents, Consumables and Equipment, and Indexed Adapter Sequences to the end of the guide.
- Changed Resuspension Buffer (RSB) storage to 2°C to 8°C after the initial thaw.

DNA Input Recommendations

It is important to quantitate the input DNA and assess the DNA quality before performing TruSeq ChIP Sample Preparation.

Input DNA Quantitation

Follow these DNA input recommendations:

- Correct quantification of ChIP DNA is essential.
- 5–10 ng ChIP-enriched, fragmented input DNA is recommended.
- The ultimate success or failure of library preparation strongly depends on using an accurately quantified amount of input DNA.
- It is difficult to measure the ChIP DNA starting amount accurately, because the yield is low (< 10 ng).
- Methods for ChIP pulldown and fragmentation are dependent upon individual antibodies and procedures. Reference literature or other sources for recommendations.
- Illumina recommends using fluorometric based methods for quantification including Qubit or PicoGreen to provide accurate quantification of ChIP DNA. UV spectrophotometric-based methods, such as the Nanodrop, measure any nucleotides present in the sample including RNA, dsDNA, ssDNA, and free nucleotides, which can give an inaccurate measurement of ChIP DNA.
- It is important that the concentration of the DNA solution falls within the detection range of the Qubit dsDNA HS Assay.
- Use multiple methods of quantification to verify results.
- DNA quantification methods that rely on intercalating fluorescent dyes measure only double-stranded DNA and are less subject to the presence of excess nucleic acids.
  - These methods require the preparation of calibration curves and are highly sensitive to pipetting error.
  - Make sure that pipettes are correctly calibrated and are not used at the volume extremes of their performance specifications.

Assessing DNA Quality

- Absorbance measurements at 260 nm are commonly used to assess DNA quality:
  - The ratio of absorbance at 260 nm to absorbance at 280 nm is used as an indication of sample purity, and values of 1.8–2.0 are considered indicative of relatively pure DNA.
  - The presence of RNA or small nucleic acid fragments, such as nucleotides, can compromise both absorbance measurements.
  - Carefully collect ChIP DNA samples to make sure that they are free of contaminants.
- A further validation step can be performed with the Agilent Bioanalyzer with a High Sensitivity Chip for the correct ChIP DNA size distribution, presence of contaminants, etc.

Additional Resources

The following resources are available for TruSeq ChIP Sample Preparation protocol guidance and sample tracking. Access these and other resources on the Illumina website at support.illumina.com/sequencing/kits.ilmn. Then, select **TruSeq ChIP Sample Prep Kit Support**.

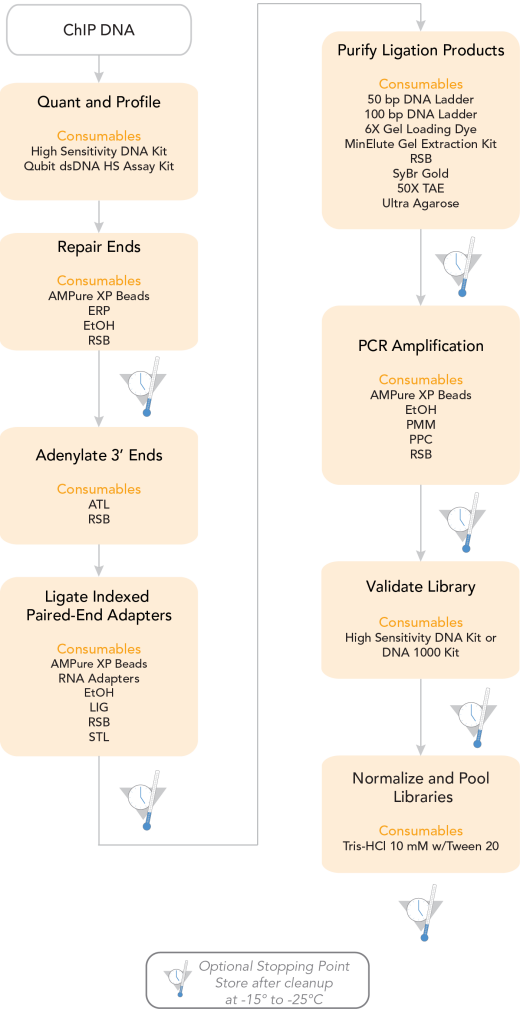
Resource	Description
Training	Illustrates elements of the TruSeq ChIP Sample Preparation process. Viewing these videos is recommended for new and less experienced users before starting sample preparation. Click **Training** on **TruSeq ChIP Sample Prep Kit Support**
Best Practices	Provides best practices specific to this protocol. Review these best practices before starting sample preparation. Topics include: • Handling Liquids • Handling Master Mix Reagents • Handling Magnetic Beads • Avoiding Cross-Contamination • Potential DNA Contaminants • Temperature Considerations • Equipment Click **Best Practices** on **TruSeq ChIP Sample Prep Kit Support**
TruSeq ChIP Sample Preparation Experienced User Card and Lab Tracking Form (part # 15036179)	Provides protocol instructions, but with less detail than what is provided in this user guide. **New or less experienced users are advised to follow this user guide and not the EUC and LTF.** Click **Documentation & Literature** on **TruSeq ChIP Sample Prep Kit Support**

Resource	Description
TruSeq Sample Preparation Pooling Guide (part # 15042173)	Provides TruSeq pooling guidelines for sample preparation. Review this guide before beginning library preparation. Click **Documentation & Literature** on **TruSeq ChIP Sample Prep Kit Support**
Illumina Experiment Manager (IEM)	Enables you to create and edit appropriate sample sheets for Illumina sequencers and analysis software and record parameters for your sample plate. To download the software, click **Downloads** on **TruSeq ChIP Sample Prep Kit Support** or To download the documentation, click **Documentation & Literature** on **TruSeq ChIP Sample Prep Kit Support**

Sample Prep Workflow

The following figure illustrates the steps in the TruSeq ChIP Sample Prep protocol.

Figure 1 TruSeq ChIP Sample Preparation Workflow



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.

NOTE
This protocol requires 5–10 ng ChIP DNA as starting material, usually the result of pooling three independent ChIP experiments. If the pooled volume is larger than 50 µl, use a SpeedVac without heat to concentrate your ChIP DNA to approximately 50 µl.

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.

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

Preparation

- Remove the following from -15°C to -25°C storage and thaw them at room temperature:
  - End Repair Mix

- Resuspension Buffer

NOTE
The Resuspension Buffer can be stored at 2°C to 8°C after the initial thaw.

- Review Best Practices for *Handling Magnetic Beads*. See *Additional Resources* on page 7 for information on how to access TruSeq ChIP Sample Preparation Best Practices on the Illumina website.
- Remove the AMPure XP beads from storage and let stand for at least 30 minutes to bring them to room temperature.
- Pre-heat the thermal cycler to 30°C.
- Choose the thermal cycler pre-heat lid option and set to 100°C

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.
5. Remove the PCR plate from the thermal cycler.

NOTE
Review Best Practices for *Handling Magnetic Beads*. See *Additional Resources* on page 7 for information on how to access TruSeq ChIP Sample Preparation Best Practices on the Illumina website.

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

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.

NOTE

Leave the PCR plate on the magnetic stand while performing the following 80% EtOH wash steps (13–15).

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.

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.

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

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 14, 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.

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

Preparation

- Remove the A-Tailing Mix from -15°C to -25°C storage and thaw at room temperature.
- Remove the Resuspension Buffer from 2°C to 8°C storage and bring it to room temperature.
- Remove the PCR plate from -15°C to -25°C storage, if it was stored at the conclusion of *Perform End Repair* on page 11.
  - Let the plate stand to thaw at room temperature.
  - Centrifuge the thawed plate to 280 × g for 1 minute.
  - Remove the adhesive seal from the plate.

- Pre-program the thermal cycler with the following program and save as **ATAIL70**:
  - Choose the pre-heat lid option and set to 100°C
  - 37°C for 30 minutes
  - 70°C for 5 minutes
  - Hold at 4°C

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 16.

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

Preparation

- Remove the following from -15°C to -25°C storage and thaw them at room temperature:
  - Appropriate RNA Adapter tubes
  - Stop Ligation Buffer

  NOTE
  Do not remove the Ligation Mix tube from -15°C to -25°C storage until instructed to do so in the procedures.
- Review Best Practices for *Handling Magnetic Beads*. See *Additional Resources* on page 7 for information on how to access TruSeq ChIP Sample Preparation Best Practices on the Illumina website.
- Remove the AMPure XP beads from storage and let stand for at least 30 minutes to bring them to room temperature.
- Remove the Resuspension Buffer from 2°C to 8°C storage and bring it to room temperature.
- Pre-heat the thermal cycler to 30°C.
- Choose the thermal cycler pre-heat lid option and set to 100°C

NOTE
Illumina recommends arranging samples that are going to be combined into a common pool in the same row and including a common index in each column. This arrangement facilitates pipetting operations when dispensing indexed adapters and pooling indexed libraries later in the protocol.

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.

10 Incubate the PCR plate on the pre-heated thermal cycler, with the lid closed, at 30°C for 10 minutes.

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.

NOTE

Review Best Practices for *Handling Magnetic Beads*. See *Additional Resources* on page 7 for information on how to access TruSeq ChIP Sample Preparation Best Practices on the Illumina website.

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.

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

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

NOTE

Leave the PCR plate on the magnetic stand while performing the following 80% EtOH wash steps (19–21).

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.

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.

25 Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.

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.

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

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

NOTE

Leave the PCR plate on the magnetic stand while performing the following 80% EtOH wash steps (31–33)

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.

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.

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

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 21, 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.

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.

NOTE

Test your electrophoresis unit in advance to make sure that you can readily resolve DNA in the range below 1000 base pairs. The DNA smear should be sufficiently resolved to enable you to excise a narrow band of a chosen size with a standard deviation as low as 5% of the median (i.e., a gel slice at 300 bp, where +/- one standard deviation is equivalent to a size range of 280–320 bp). The conditions described are typical and validated gel electrophoresis conditions.

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.

NOTE

Cutting a band of 250–300 bp on a 2% agarose gel results in an insert size of approximately 150–200 bp and accounts for the influence of the adapters on the gel mobility.

NOTE

These procedures have only been verified using the consumables specified in this guide and by performing the gel method specified. Any deviation from these materials and procedures may result in incorrect size excision or require additional user optimization.

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

Item	Quantity	Storage	Supplied By
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

Preparation

- Prepare 1X TAE buffer (> 1 L)
- Remove the PCR plate from -15°C to -25°C storage, if it was stored at the conclusion of *Ligate Adapters* on page 16
  - Let the plate stand to thaw at room temperature.
  - Centrifuge the thawed PCR plate to 280 × g for 1 minute.
  - Remove the adhesive seal from the thawed PCR plate.
- Make sure that the Resuspension Buffer is at room temperature.
- Clean the tray, the comb, and the gel tank with ethanol and rinse them thoroughly with deionized water to avoid cross-contamination.

NOTE
Use the 12-well comb included with the recommended gel system.

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.
   d Pour the entire gel buffer to the gel tray.

NOTE
The final concentration of SYBR Gold should be 1X in the agarose gel buffer.

WARNING
It is important to pre-stain your gel with SYBR Gold. When using other staining dyes or staining the gel after running, the DNA will migrate more slowly than the ladder. This will result in cutting out the wrong size fragments.

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.

WARNING
Do not overload the DNA ladder. Without clear and distinct bands, it is difficult to excise the correct fragment size. Also, an overloaded ladder might run faster than the DNA sample library.

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.
Dimensions recommended for the electrophoresis unit:
12 cm x 14 cm (W x L), 800 ml buffer volume

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.

NOTE
Flanking the library on both sides with ladders can make the library excision easier.

NOTE
When handling multiple samples, to avoid the risk of cross-contamination between libraries, leave a gap of at least one empty lane between samples. Use ladders on the first and last well of the gel to help locate the gel area to be excised.

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

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.

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 25, 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.

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.

NOTE

PCR enriches for fragments that have adapters ligated on both ends. Fragments with only one or no adapters on their ends are by-products of inefficiencies in the ligation reaction. Neither species can be used to make clusters. Fragments without any adapters cannot hybridize to surface-bound primers in the flow cell. Fragments with an adapter on only one end can hybridize to surface bound primers, but cannot form clusters.

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

Preparation

- Remove the PCR Master Mix and PCR Primer Cocktail from -15°C to -25°C storage and thaw them at room temperature. When thawed, keep the tubes on ice.
- Centrifuge the thawed PCR Master Mix and PCR Primer Cocktail tubes to 600 × g for 5 seconds.
- Review Best Practices for *Handling Magnetic Beads*. See *Additional Resources* on page 7 for information on how to access TruSeq ChIP Sample Preparation Best Practices on the Illumina website.
- Remove the AMPure XP beads from storage and let stand for at least 30 minutes to bring them to room temperature.
- Remove the Resuspension Buffer from 2°C to 8°C storage and bring it to room temperature.
- Remove the PCR plate from -15°C to -25°C storage, if it was stored at the conclusion of *Purify Ligation Products* on page 21.
  - Let the plate stand to thaw at room temperature.
  - Centrifuge the thawed plate to 280 × g for 1 minute.
  - Remove the adhesive seal from the thawed plate.
- Pre-program the thermal cycler with the following program and save as **PCR**:
  - Choose the pre-heat lid option and set to 100°C
  - 98°C for 30 seconds
  - 18 cycles of:
    - 98°C for 10 seconds
    - 60°C for 30 seconds
    - 72°C for 30 seconds
  - 72°C for 5 minutes
  - Hold at 4°C

NOTE

Illumina recommends 18 cycles of PCR for robust protocol performance. However, to optimize yield versus cycle number, a titration of PCR cycles can also be performed.

Procedure

CAUTION
To avoid sample cross-contamination, set up PCR reactions (all components except the template DNA) in a designated clean area, preferably a PCR hood with UV sterilization and positive air flow.

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

NOTE
Review Best Practices for *Handling Magnetic Beads*. See *Additional Resources* on page 7 for information on how to access TruSeq ChIP Sample Preparation Best Practices on the Illumina website.

5. Remove the adhesive seal from the PCR plate.
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.
9. Place the PCR plate on the magnetic stand at room temperature for 5 minutes or until the liquid is clear.
10. Remove and discard 95 µl of the supernatant from each well of the PCR plate.

NOTE

Leave the PCR plate on the magnetic stand while performing the following 80% EtOH wash steps (11–13).

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.

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.

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

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 29 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.

Validate Library

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

Quantify Libraries

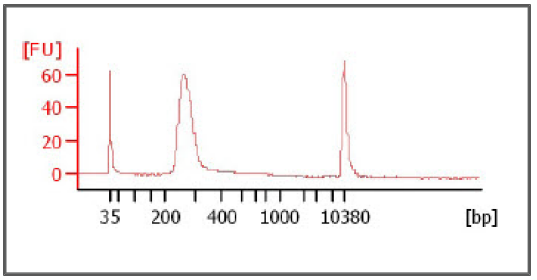
To achieve the highest quality data on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of the flow cell. Optimizing cluster densities requires accurate quantitation of DNA library templates. Quantify your libraries using qPCR according to the Illumina *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 2 Example of DNA Library Distribution for TruSeq ChIP Sample Prep Libraries



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

Preparation

- Remove the PCR plate from -15°C to -25°C storage, if it was stored at the conclusion of *Enrich DNA Fragments* on page 25.
  - Let the plate stand to thaw at room temperature.
  - Centrifuge the thawed plate to 280 × g for 1 minute.
  - Remove the adhesive seal from the thawed plate.

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.

NOTE
Depending on the yield quantification data of each sample library, the final volume in the MIDI plate can vary from 10–400 µl.

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. For more information, see the cluster generation section of the user guide for your Illumina platform.
  - 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.

NOTE
Note the sample that is in each well, to avoid pooling two samples with the same index.

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.
The total volume in each well of the PCR plate is 10X the number of combined sample libraries and 20–240 µl (2–24 libraries). For example, the volume for 2 samples is 20 µl, the volume for 12 samples is 120 µl, or the volume for 24 samples is 240 µl.

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

8 Do one of the following:
- Proceed to cluster generation. For more information, see the cluster generation section of the user guide for your Illumina platform.
- Seal the PCR plate with a Microseal 'B' adhesive seal and store at -15°C to -25°C.

Acronyms

Table 1 Acronyms

Acronym	Definition
ATL	A-Tailing Mix
ChIP	Chromatin immunoprecipitation
dsDNA	double-stranded DNA
ERP	End Repair Mix
EUC	Experienced User Card
IEM	Illumina Experiment Manager
LIG	Ligation Mix
LTF	Lab Tracking Form
PCR	Polymerase Chain Reaction
PMM	PCR Master Mix
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
STL	Stop Ligation Buffer

Kit Contents

Check to make sure that you have all of the TruSeq ChIP Sample Prep reagents identified in this section before starting the TruSeq ChIP Sample Preparation protocol.

48 Samples - Set A Box or Set B Box

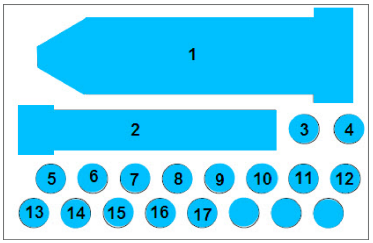
You will receive either box A or B with the kit depending on the set ordered.

Store at -15° to -25°C

These boxes are shipped on dry ice. As soon as you receive them, store the following components at -15° to -25°C.

Set A

Figure 3 TruSeq ChIP Sample Prep Kit, 48 Samples-Set A Box, part # 15034288

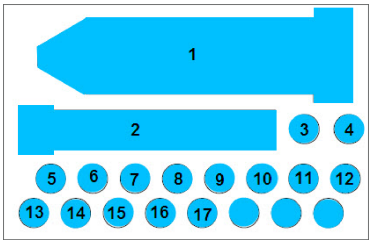


Slot	Reagent	Part #	Description
1	RSB	15026770	Resuspension Buffer
2	ERP	15012494	End Repair Mix
3	ATL	15012495	A-Tailing Mix
4	LIG	15026773	Ligation Mix
5	STL	15012546	Stop Ligation Buffer
6	AR002	15026634	RNA Adapter Index 2
7	AR004	15026636	RNA Adapter Index 4
8	AR005	15026637	RNA Adapter Index 5
9	AR006	15026638	RNA Adapter Index 6
10	AR007	15026640	RNA Adapter Index 7
11	AR012	15026645	RNA Adapter Index 12

Slot	Reagent	Part #	Description
12	AR013	15024655	RNA Adapter Index 13
13	AR014	15024656	RNA Adapter Index 14
14	AR015	15024657	RNA Adapter Index 15
15	AR016	15024658	RNA Adapter Index 16
16	AR018	15024660	RNA Adapter Index 18
17	AR019	15024661	RNA Adapter Index 19
18			Empty
19			Empty
20			Empty

Set B

Figure 4 TruSeq ChIP Sample Prep Kit, 48 Samples-Set B Box, part # 15034289



Slot	Reagent	Part #	Description
1	RSB	15026770	Resuspension Buffer
2	ERP	15012494	End Repair Mix
3	ATL	15012495	A-Tailing Mix
4	LIG	15026773	Ligation Mix
5	STL	15012546	Stop Ligation Buffer
6	AR001	15026633	RNA Adapter Index 1
7	AR003	15026635	RNA Adapter Index 3
8	AR008	15026641	RNA Adapter Index 8
9	AR009	15026642	RNA Adapter Index 9
10	AR010	15026643	RNA Adapter Index 10
11	AR011	15026644	RNA Adapter Index 11
12	AR020	15024662	RNA Adapter Index 20
13	AR021	15024663	RNA Adapter Index 21
14	AR022	15024664	RNA Adapter Index 22

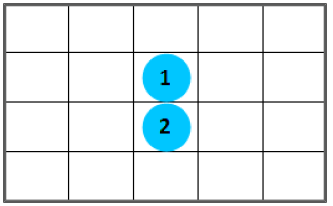
Slot	Reagent	Part #	Description
15	AR023	15024665	RNA Adapter Index 23
16	AR025	15024667	RNA Adapter Index 25
17	AR027	15024668	RNA Adapter Index 27
18			Empty
19			Empty
20			Empty

48 Samples - PCR Box

Store at -15°C to -25°C

This box is shipped on dry ice. As soon as you receive it, store the following components at -15°C to -25°C.

Figure 5 TruSeq ChIP Sample Prep Kit, 48 Samples-PCR Box, part # 15027084



Slot	Reagent	Part #	Description
1	PMM	15026785	PCR Master Mix
2	PPC	15026786	PCR Primer Cocktail

Consumables and Equipment

Check to make sure that you have all of the necessary user-supplied consumables and equipment before starting the TruSeq ChIP Sample Preparation protocol.

Table 2 User-Supplied Consumables

Consumable	Supplier
2.0 ml DNA LoBind tubes	Eppendorf, catalog # 022431048
6X gel loading dye	BioLabs, catalog # B7021S
10 µl barrier pipette tips	General lab supplier
10 µl multichannel pipettes	General lab supplier
10 µl single channel pipettes	General lab supplier
20 µl barrier pipette tips	General lab supplier
20 µl multichannel pipettes	General lab supplier
20 µl single channel pipettes	General lab supplier
50 bp DNA ladder	NEB, catalog # N3236L
50 X TAE buffer	Bio-Rad, part # 161-0743
96-well storage plates, round well, 0.8 ml ("MIDI" plate)	Fisher Scientific, part # AB-0859
100 bp DNA ladder	NEB, catalog # N3231L
200 µl barrier pipette tips	General lab supplier
200 µl multichannel pipettes	General lab supplier
200 µl single channel pipettes	General lab supplier
1000 µl barrier pipette tips	General lab supplier
1000 µl multichannel pipettes	General lab supplier

Consumable	Supplier
1000 µl single channel pipettes	General lab supplier
Agencourt AMPure XP 60 ml kit	Beckman Coulter Genomics, part # A63881
Certified low-range ultra agarose	Bio-Rad, part # 161-3107
ChIP DNA (5–10 ng)	User experimental samples
Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, part # E7023
GeneCatchers or Clean Scalpel	Gel Company, catalog # PKB4.0 or PKB6.5 General lab supplier
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
MinElute Gel Extraction Kit	QIAGEN, part # 28604
PCR grade water	General lab supplier
Qubit assay tubes or Axygen PCR-05-C tubes	Life Technologies, catalog # Q32856 or VWR, part # 10011-830
Qubit dsDNA HS Assay Kit	Life Technologies, 100 assays - catalog # Q32851 500 assays - catalog # Q32854
RNaseZap (to decontaminate surfaces)	General lab supplier
RNase/DNase-free eight-tube strips and caps	General lab supplier
RNase/DNase-free multichannel reagent reservoirs, disposable	VWR, part # 89094-658
SYBR Gold Nucleic acid gel stain	Invitrogen, part # S11494

Consumable	Supplier
Tris-HCl 10 mM, pH8.5 with 0.1% Tween 20	General lab supplier
Tween 20	Sigma-Aldrich, part # P7949
Ultra pure water	General lab supplier

Table 3 User-Supplied Equipment

Equipment	Supplier
2100 Bioanalyzer Desktop System	Agilent, part # G2940CA
[Optional] Agilent DNA 1000 Kit	Agilent, part # 5067-1504
Agilent High Sensitivity DNA Kit	Agilent, part # 5067-4626
96-well thermal cycler (with heated lid)	General lab supplier
Dark Reader transilluminator or a UV transilluminator	Clare Chemical Research, catalog # DR195M
Electrophoresis power supply	General lab supplier
Magnetic stand-96	Life Technologies, catalog # AM10027
Microplate centrifuge	General lab supplier
Qubit 2.0 Fluorometer	Life Technologies, catalog # Q32866 products.invitrogen.com/ivgn/product/Q32866
Thermo Scientific Owl B2 EasyCast Mini Gel System	(US) Thermo Scientific, part # B2, or Fisher Scientific, part # 09-528-110B (Other Regions) Fisher Scientific, part # OWL-130-101J B
Vortexer	General lab supplier

Indexed Adapter Sequences

The TruSeq ChIP Sample Prep Kit contains the following indexed adapter sequences.

NOTE

- The index numbering is not contiguous. There is no Index 17, 24, or 26.
- The base in parentheses () indicates the base for the seventh cycle and is not considered as part of the index sequence. Record the index in the sample sheet as only six bases. For indices 13 and above, the seventh base (in parentheses) might not be A, which is seen in the seventh cycle of the index read.
- For more information on the number of cycles used to sequence the index read, reference your instrument user guide.

Table 4 TruSeq ChIP Sample Prep Kit Set A Indexed Adapter Sequences

Adapter	Sequence	Adapter	Sequence
AR002	CGATGT(A)	AR013	AGTCAA(C)
AR004	TGACCA(A)	AR014	AGTTCC(G)
AR005	ACAGTG(A)	AR015	ATGTCA(G)
AR006	GCCAAT(A)	AR016	CCGTCC(C)
AR007	CAGATC(A)	AR018	GTCCGC(A)
AR012	CTTGTA(A)	AR019	GTGAAA(C)

Table 5 TruSeq ChIP Sample Prep Kit Set B Indexed Adapter Sequences

Adapter	Sequence	Adapter	Sequence
AR001	ATCACG(A)	AR020	GTGGCC(T)
AR003	TTAGGC(A)	AR021	GTTTCG(G)
AR008	ACTTGA(A)	AR022	CGTACG(T)
AR009	GATCAG(A)	AR023	GAGTGG(A)
AR010	TAGCTT(A)	AR025	ACTGAT(A)
AR011	GGCTAC(A)	AR027	ATTCCT(T)

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 6 Illumina General Contact Information

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

Table 7 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 # 15023092 Rev. B

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