TruSeq® ChIP Sample Preparation Guide

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Introduction

This protocol explains how to prepare up to 24 pooled paired-end indexed chromatin-immunoprecipitation (ChIP) DNA libraries for subsequent cluster generation and DNA sequencing using the reagents provided in the Illumina® TruSeq® ChIP Sample Preparation Kit. 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, and 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 prior to 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

- 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.

Getting Started

This section explains standard operating procedures and precautions for performing the TruSeq ChIP Sample Preparation. You will also find the kit contents and lists of standard equipment and consumables.

The TruSeq ChIP Sample Prep protocol described in this guide assumes that you are familiar with the contents of this section, have implemented all the recommendations, and have obtained all of the requisite equipment and consumables.

Acronyms

Table 1 TruSeq ChIP Sample Preparation 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

Best Practices

When preparing ChIP libraries for sequencing, you should always adhere to good molecular biology practices. Read through the entire protocol prior to starting, to ensure all of the required materials are available and your equipment is programmed and ready to use.

NOTE

For more information, see the *TruSeq Sample Preparation Best Practices and Troubleshooting Guide* which you can download from the Illumina website at http://www.illumina.com. Go to the TruSeq ChIP Sample Preparation support page and click the **Documentation & Literature** tab. A MyIllumina account is required.

Handling Liquids

Good liquid handling measures are essential, particularly when quantifying libraries or diluting concentrated libraries for making clusters.

- Small differences in volumes (±0.5 µl) can sometimes give rise to very large differences in cluster numbers (~100,000).
- Small volume pipetting can be a source of potential error in protocols that require generation of standard curves, such as qPCR, or those that require small but precise volumes, such as the Agilent Bioanalyzer.
- If small volumes are unavoidable, then due diligence should be taken to make sure that pipettes are correctly calibrated.
- Make sure that pipettes are not used at the volume extremes of their performance specifications.
- To minimize pipetting errors, especially with small volume enzyme additions, prepare the reagents for multiple samples simultaneously. As a result, pipette once from the reagent tubes with a larger volume, rather than many times with small volumes. This will allow you to aliquot in a single pipetting movement to individual samples and standardize across multiple samples.

Handling Master Mix Reagents

When handling the master mix reagents:

- Minimize freeze-thaw cycles. If you do not intend to consume the reagents in one use, dispense the reagent into aliquots after the initial thaw and refreeze the

aliquots in order to avoid excessive freeze-thaw cycles. However, if you aliquot, you might not have enough reagents for the full number of reactions over multiple uses.

- Add reagents in the order indicated and avoid making master-mixes containing the in-line controls.
- Take care while adding the A-Tailing Mix (ATL) and Ligation Mix (LIG) due to the viscosity of the reagents.

Handling Magnetic Beads

Follow appropriate handling methods when working AMPure XP Beads:

NOTE
Cleanup procedures have only been validated using the 96-well plates and the magnetic stand specified in the Consumables and Equipment list. Comparable performance is not guaranteed when using a microcentrifuge tube or other formats, or other magnets.

- Prior to use, allow the beads to come to room temperature.
- Do not reuse beads. Always add fresh beads when performing these procedures.
- Immediately prior to use, vortex the beads until they are well dispersed. The color of the liquid should appear homogeneous.
- After adding the beads to the reaction, mix the solution gently and thoroughly by pipetting up and down 10 times, making sure the liquid comes in contact with the beads and that the beads are resuspended homogeneously.
- Take care to minimize bead loss which can impact final yields.
- Change the tips for each sample.
- Let the mixed samples incubate for 15 minutes at room temperature for maximum recovery.
- When aspirating the cleared solution from the reaction plate and wash step, it is important to keep the plate on the magnetic stand and to not disturb the separated magnetic beads. Aspirate slowly to prevent the beads from sliding down the sides of the wells and into the pipette tips.
- To prevent the carryover of beads after elution, approximately 2.5 µl of supernatant are left when the eluates are removed from the bead pellet.
- Prepare fresh 80% ethanol. Ethanol tends to absorb water from the air, therefore, fresh 80% ethanol should be prepared for optimal results.

- Be sure to remove all of the ethanol from the bottom of the wells, as it can contain residual contaminants.
- Keep the reaction plate on the magnetic stand and let it air-dry at room temperature to prevent potential bead loss due to electrostatic forces. Allow for the complete evaporation of residual ethanol, as the presence of ethanol will impact the performance of the subsequent reactions. Illumina recommends at least 15 minutes drying time, but a longer drying time might be required. Remaining ethanol can be removed with a 20 µl pipette.
- Use the Resuspension Buffer (RSB) for DNA elution.
- Avoid over drying the beads, which can impact final yields.
- Resuspend the dried pellets using a single channel or multichannel pipette.
- When removing and discarding supernatant from the wells, use a single channel or multichannel pipette and take care not to disturb the beads.
- To maximize sample recovery during elution, incubate the sample/bead mix for 2 minutes at room temperature before placing the samples onto the magnet.

Avoiding Cross-Contamination

Practice the following to avoid cross-contamination:

- Open only one adapter tube at a time.
- Pipette carefully to avoid spillage.
- Clean pipettes and change gloves between handling different adapter stocks.
- Clean work surfaces thoroughly before and after the procedure.

Potential DNA Contaminants

Avoid potential DNA contaminants:

- Incorrect DNA quantitation can result from DNA contamination, for example, interference from superfluous nucleic acids in a sample (e.g., RNA, small nucleic acid fragments, nucleotides, single-stranded DNA), excess proteins, or other contaminating materials.
- DNA quality can also affect the quantity of usable DNA in a sample. For example, if the DNA is damaged (e.g., heavily nicked or containing extensive apurinic/apyrimidinic sites), many of the fragments generated may fail during library preparation.
- High molecular weight dsDNA derived from host genomes can also interfere with accurate quantitation. For example, bacterial artificial chromosomes (BACs)

and other bacterially-derived plasmids usually contain a small percentage of the chromosomal DNA from the host cells, despite the best purification efforts. These sequences might ultimately give rise to unwanted clusters on a flow cell lane. However, this contamination can be accurately quantified by analyzing aligned reads generated during sequencing against known bacterial sequences and subtracting these out. High molecular weight contamination can also be estimated prior to library preparation using qPCR assays designed to target unique chromosomal markers.

Temperature Considerations

Temperature is an important consideration for making ChIP DNA libraries:

- Keep libraries at temperatures ≤37°C, except where specifically noted.
- Place reagents on ice after thawing at room temperature.
- Avoid elevated temperatures, particularly in the steps preceding the adapter ligation.
- When processing more than 48 samples manually, Illumina recommends processing the plate on a bed of ice whenever possible, especially during the enzymatic steps (when using the End Repair Mix, A-Tailing Mix, and Ligation Mix). A large number of samples processed at room temperature may result in uneven catalytic activity, which can lead to reduced quality of the end product.
- DNA fragments that have a high AT content are more likely to denature into single strands than GC-rich fragments, which can result in an increased probability of creating a bias in the sequencing coverage.
- Take care not to denature the library prior to the agarose gel electrophoresis process, because single-stranded DNA has a different migration rate.

Usage Guidelines

Illumina recommends these guidelines as the most efficient lab setup and pipetting process when performing the procedures specified in the protocol.

NOTE

The TruSeq ChIP Sample Prep Kit contains enough of each reagent to prepare 48 samples at one time. If an alternate lab setup and pipetting process is used, Illumina cannot guarantee that there will be enough of every reagent for the full number of samples.

NOTE
When using multichannel pipettes, take care to pipette accurately into the wells, as variations in volume will affect the sample preparation. Change tips after each sample.

Reference the following table to determine the required reagent volume per sample for these guidelines.

Table 2 TruSeq ChIP Sample Prep Reagent Volumes

Reagent	Description	Volume per Sample (µl)
AR0XX	RNA Adapter tube	2.5
ATL	A-Tailing Mix	12.5
ERP	End Repair Mix	40
LIG	Ligation Mix	2.5
PMM	PCR Master Mix	25
PPC	PCR Primer Cocktail	5
STL	Stop Ligation Buffer	5

Preparing More Than 24 Samples

When preparing more than 24 samples, follow these guidelines as you perform each procedure in the protocol. Use a multichannel pipette with eight tips to perform all transfers from the reagent vessel to the sample plate.

Sample Distribution

Distribute each sample into a separate column of the 96-well 0.3 ml PCR plate.

Reagents in Reservoirs

When each of the following reagents is required in the protocol, distribute each into a separate multichannel reagent reservoir as follows:

- 80% Ethanol
- AMPure XP Beads
- Resuspension Buffer

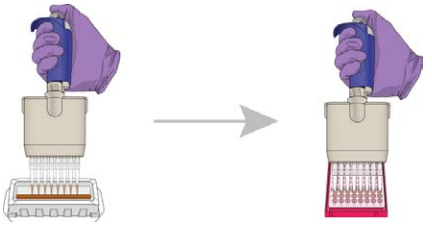
1 Determine the volume needed for each of the above reagents using the equation (# of samples x volume per sample) + 600 µl dead volume. Reference the protocol for the required reagent volume per sample.

2 Fill a separate multichannel reagent reservoir with the determined amount of each reagent.

When each of the above reagents is required in the protocol, distribute each to the sample plate as follows:

1 Using an eight tip multichannel pipette, transfer the reagent in the reservoir to the samples in the plate as follows, while holding the pipette vertically. Reference the protocol for the required reagent volume per sample.

Figure 1 Transfer Reagent from Reservoir to Sample Plate with 24 or More Samples



a Pipette the required reagent volume per sample from the reservoir.
b Add the reagent to column 1 of the sample plate. Change the tips.
c Pipette the required reagent volume per sample from the reservoir.
d Add the reagent to column 2 of the sample plate. Change the tips.
e Repeat as needed for each column containing a sample.

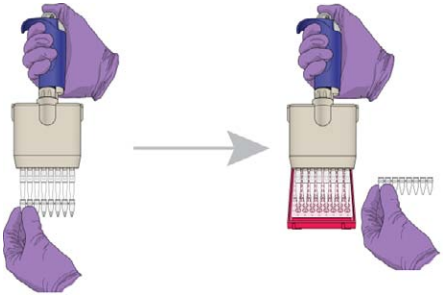
Reagents in Strip Tubes

When the reagents listed in Table 2, except the adapters, are required in the protocol, distribute each evenly across eight wells of an eight-tube strip. Add an allowance of 5 µl for dead volume per well.

When each reagent in an eight-tube strip is required in the protocol, distribute each to the sample plate as follows:

1 Using an eight tip multichannel pipette, transfer the reagent in the eight-tube strip to the samples in the plate as follows, while holding the pipette vertically. Reference Table 2 for the required reagent volume per sample.

Figure 2 Transfer Reagent from Strip Tube to Sample Plate with 24 or More Samples



a Pipette the reagent from the eight strip wells.
b Add the reagent to column 1 of the sample plate. Change the tips.
c Pipette the reagent from the eight strip wells.
d Add the reagent to column 2 of the sample plate. Change the tips.
e Repeat as needed for each column containing a sample.

Index Adapters

When using RNA index adapter tubes, do one of the following:

- Add 2.5 µl of the appropriate/desired adapter index individually to each well of the plate containing a sample, using a single channel pipette.
- Using an eight-tube strip:
  - Distribute the index adapters into the wells of an eight-tube strip, with a different adapter in each well.
  - Add 2.5 µl of the appropriate/desired adapter index from the well of the eight-tube strip to each well of the plate containing a sample, using a multichannel pipette.

Preparing 12–24 Samples

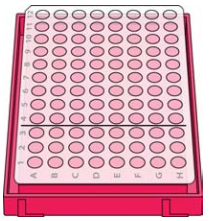
When preparing 12–24 samples, follow these guidelines as you perform each procedure in the protocol. Use a multichannel pipette with three tips to perform all transfers from the reagent vessel to the sample plate.

Sample Distribution

Distribute the 12–24 samples into three columns and four to eight rows (e.g., four rows per 12 samples) of the plate. Draw a line on the plate to visually separate the

three columns.

Figure 3 Draw Line on Plate



Reagents in Reservoirs

When each of the following reagents is required in the protocol, distribute each into a separate multichannel reagent reservoir as follows:

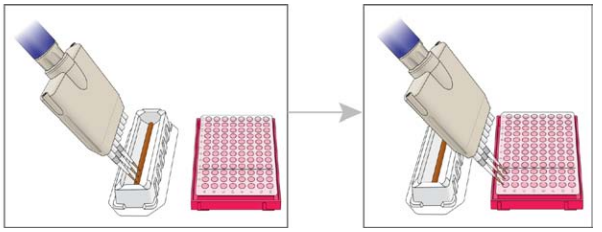
- 80% Ethanol
- AMPure XP Beads
- Resuspension Buffer

1. Determine the volume needed using the equation (# of samples x volume per sample) + 600 µl dead volume. Reference the protocol for the required reagent volume per sample.
2. Fill a separate multichannel reagent reservoir with the determined amount of each reagent.

When each of the above reagents is required in the protocol, distribute each to the sample plate as follows:

1. Using a multichannel pipette with three tips, transfer the reagent in the reservoir to the samples in the plate as follows, while holding the pipette vertically. Reference the protocol for the required reagent volume per sample.

Figure 4 Transfer Reagent from Reservoir to Sample Plate with 12–24 Samples



a Pipette the required reagent volume per sample from the reservoir.
b Add the reagent to row 1 of the sample plate. Change the tips.
c Pipette the required reagent volume per sample from the reservoir.
d Add the reagent to row 2 of the sample plate. Change the tips.
e Repeat as needed for each row containing a sample.

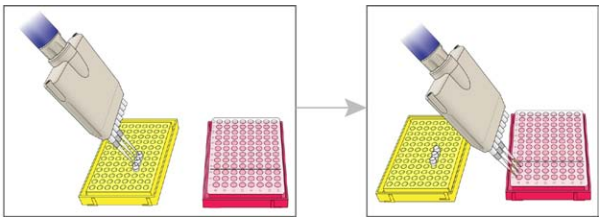
Reagents in Strip Tubes

When the reagents listed in Table 2, except the adapters, are required in the protocol, distribute each evenly across the three wells of an eight-tube strip. Add an allowance of 5 µl for dead volume per well.

When each reagent in an eight-tube strip is required in the protocol, distribute each to the sample plate as follows:

1 Using a multichannel pipette with three tips, transfer the reagent in the eight-tube strip to the samples in the plate as follows, while holding the pipette vertically. Reference Table 2 for the required reagent volume per sample.

Figure 5 Transfer Reagent from Strip Tube to Sample Plate with 12–24 Samples



a Pipette the reagent from the three strip wells.
b Add the reagent to row 1 of the sample plate. Change the tips.
c Pipette the reagent from the three strip wells.
d Add the reagent to row 2 of the sample plate. Change the tips.
e Repeat as needed for each row containing a sample.

Index Adapter Tubes

When RNA index adapter tubes are used, add 2.5 µl of the appropriate/desired adapter index individually to each well of the plate containing a sample, using a single channel pipette.

Preparing Less Than 12 Samples

When preparing less than 12 samples, follow these guidelines as you perform each procedure in the protocol:

- Add each reagent individually to the samples using a single channel pipette.
- If planning more than three freeze-thaw cycles, aliquot the reagents equally into six separate vessels.

Equipment

Review the programming instructions for your thermal cycler user guide to ensure that it is programmed appropriately using the heated lid function.

DNA Input Recommendations

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

Input DNA Quantitation

Follow these DNA input recommendations:

- Correct quantification of ChIP DNA is essential.
- 5–10 ng 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 accurately measure the ChIP DNA starting amount, 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-spec based methods, such as the Nanodrop, will 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 validate results.
- DNA quantification methods that rely on intercalating fluorescent dyes measure only double-stranded DNA and are less subject to 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.
  - Both absorbance measurements can be compromised by the presence of RNA or small nucleic acid fragments such as nucleotides.
  - ChIP DNA samples should be carefully collected to make sure that they are free of contaminants.
- A further validation step can be performed using the Agilent Bioanalyzer with a High Sensitivity Chip for the correct ChIP DNA size distribution, presence of contaminants, etc.

Tracking Tools

Illumina provides the following tools for sample tracking and guidance in the lab:

- **Experienced User Card (EUC)** to guide you through the protocol, but with less detail than provided in this user guide. **New or less experienced users are strongly advised to follow this user guide and not the EUC.**
- **Lab Tracking Form (LTF)** to record information about library preparation such as operator name, sample and index information, start and stop times, reagent lot numbers, and barcodes.
  - Create a copy of the lab tracking form for each time you perform this protocol to prepare a library for sequencing.
  - Use it online and save it electronically or print it and fill it out manually.

NOTE

You can download the above TruSeq ChIP Sample Preparation

documents from the Illumina website at http://www.illumina.com. Go to the TruSeq ChIP Sample Preparation support page and click the **Documentation & Literature** tab. A MyIllumina account is required.

- **Illumina Experiment Manager (IEM)** to create your sample sheet using a wizard-based application. The sample sheet is used to record information about your samples for later use in data analysis. The IEM guides you through the steps to create your sample sheet based on the analysis workflow for your run. The IEM provides a feature for recording parameters for your sample plate, such as sample ID, indices, and other parameters applicable to your 96-well plate.
  - When prompted to select a Sample Prep Kit in IEM, choose **TruSeq LT**.

NOTE
IEM can be run on any Windows platform. You can download it from the Illumina website at http://www.illumina.com. A MyIllumina account is required.

Kit Contents

Check to ensure that you have all of the TruSeq ChIP Sample Prep reagents identified in this section before proceeding.

48 Samples - Set A Box or Set B Box

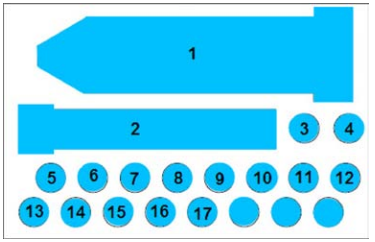
You will receive either box A or B with the kit depending on the set ordered.

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

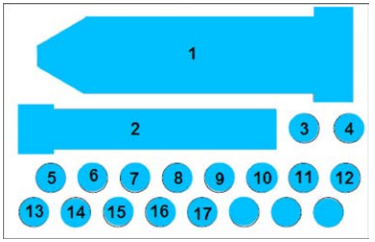
Figure 6 TruSeq ChIP Sample Prep Kit, 48 Samples-Set A Box, part # 15034288



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



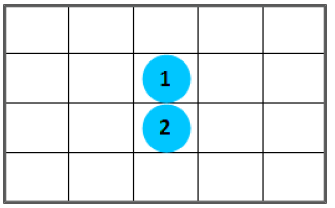
Slot	Reagent	Part #	Description
1	RSB	15026770	Resuspension Buffer
2	ERP	15012494	End Repair Mix
3	ATL	15012495	A-Tailing Mix
4	LIG	15026773	Ligation Mix
5	STL	15012546	Stop Ligation Buffer
6	AR001	15026633	RNA Adapter Index 1
7	AR003	15026635	RNA Adapter Index 3
8	AR008	15026641	RNA Adapter Index 8
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
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° to -25°C

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

Figure 8 TruSeq ChIP Sample Prep Kit, 48 Samples-PCR Box, part # 15027084



Slot	Reagent	Part #	Description
1	PMM	15026785	PCR Master Mix
2	PPC	15031748	PCR Primer Cocktail

Consumables and Equipment

Check to make sure that you have all of the necessary user-supplied consumables and equipment before proceeding to sample preparation.

Table 3 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

Consumable	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
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

Consumable	Supplier
Microseal 'B' adhesive seals	BioRad, 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
RNase/DNase zapper (to decontaminate surfaces)	General lab supplier
RNase/DNase-free 8-well PCR strip tubes 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
Tris-Cl 10 mM, pH8.5 with 0.1% Tween 20	General lab supplier
Tween 20	Sigma, part # P7949
Ultra pure water	General lab supplier

Table 4 User-Supplied Equipment

Equipment	Supplier
[Optional] 2100 Bioanalyzer Desktop System	Agilent, part # G2940CA
[Optional] Agilent DNA 1000 Kit	Agilent, part # 5067-1504
[Optional] 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 http://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 the indexed adapter sequences. The set (A or B) containing the adapter is also specified.

NOTE

- The index numbering is not contiguous. Index 17, 24, and 26 are skipped.
- The base in parentheses () indicates the base for the seventh cycle and is not considered as part of the index sequence. The index should be recorded in the sample sheet as only six bases. For indexes 13 and above, the seventh base (in parentheses) might not be A, and this will be seen in the seventh cycle of the index read.

Table 5 TruSeq ChIP Sample Prep Kit Indexed Adapter Sequences

Adapter	Sequence	Set		Adapter	Sequence	Set
AR001	ATCACG(A)	B		AR013	AGTCAA(C)	A
AR002	CGATGT(A)	A		AR014	AGTTCC(G)	A
AR003	TTAGGC(A)	B		AR015	ATGTCA(G)	A
AR004	TGACCA(A)	A		AR016	CCGTCC(C)	A
AR005	ACAGTG(A)	A		AR018	GTCCGC(A)	A
AR006	GCCAAT(A)	A		AR019	GTGAAA(C)	A
AR007	CAGATC(A)	A		AR020	GTGGCC(T)	B
AR008	ACTTGA(A)	B		AR021	GTTTCG(G)	B
AR009	GATCAG(A)	B		AR022	CGTACG(T)	B
AR010	TAGCTT(A)	B		AR023	GAGTGG(A)	B
AR011	GGCTAC(A)	B		AR025	ACTGAT(A)	B
AR012	CTTGTA(A)	A		AR027	ATTCCT(T)	B

Adapter Tube Pooling Guidelines

When using the index adapter tubes from the TruSeq ChIP Sample Prep Kit, follow these pooling guidelines for single-indexed sequencing. The TruSeq ChIP Sample Prep Kit Set A and B, each contain 12 unique index adapter tubes. When designing low-plexity index pools for single-indexed sequencing, always use at least two unique and compatible barcodes for each index sequenced. The following table describes possible pooling strategies for 2–4 samples generated with the adapter index tubes in each set.

- For 5–11plex pools, use 4-plex options with any other available adapters
- Not all color-balanced pools are listed. Check the color balance of such user-designed pools using the Illumina Experiment Manager's sample sheet generator.

Table 6 Single-Indexed Pooling Strategies for 2–4 Samples

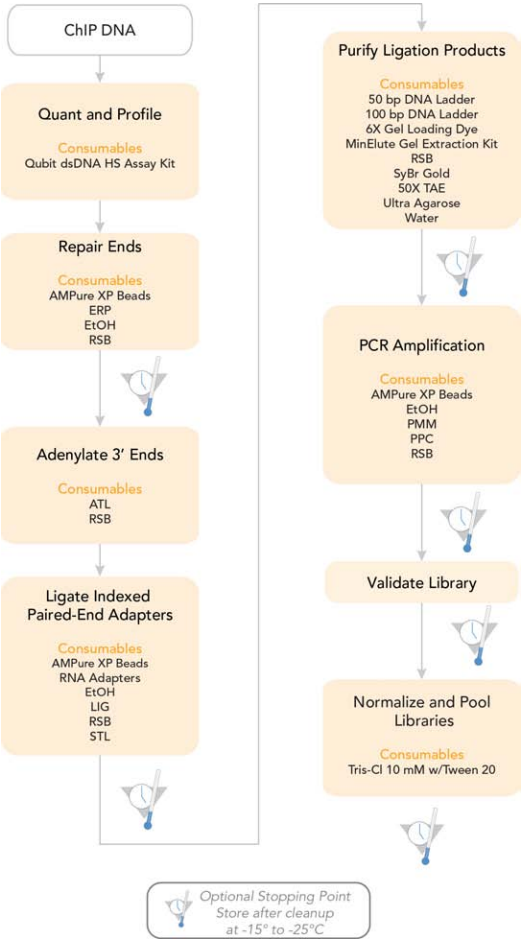
Plexity	Option	Set A Only	Set B Only
2	1	AR006 and AR012	Not recommended
	2	AR005 and AR019	
3	1	AR002 and AR007 and AR019	AR001 and AR010 and AR020
	2	AR005 and AR006 and AR015	AR003 and AR009 and AR025
	3	2-plex options with any other adapter	AR008 and AR011 and AR022
4	1	AR005 and AR006 and AR012 and AR019	AR001 and AR008 and AR010 and AR011
	2	AR002 and AR004 and AR007 and AR016	AR003 and AR009 and AR022 and AR027
	3	3-plex options with any other adapter	3-plex options with any other adapter

For more information on the Single-Indexed Sequencing workflow, see the Illumina HiSeq®, HiScan®, and Genome Analyzer™ user guides.

Sample Prep Workflow

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

Figure 9 TruSeq ChIP Sample Prep 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 prior to 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		User
Agilent High Sensitivity DNA chip	1 per 12 samples		User
ChIP DNA	5–10 ng		User
Qubit assay tubes or Axygen PCR-05-C tubes	1 per sample		User
Qubit dsDNA HS Assay Kit	1		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 to normalize 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° to -25°C	Illumina
Resuspension Buffer (RSB)	1 tube	-15° to -25°C	Illumina
96-well 0.3 ml PCR plate	1		User
AMPure XP beads	160 µl per sample	2° to 8°C	User
Freshly Prepared 80% Ethanol (EtOH)	400 µl per sample	Room temperature	User
Microseal 'B' Adhesive Seal	1		User
RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)	2		User
RNase/DNase-free Strip Tubes and Caps (if using multichannel pipettes)	2		User

Preparation

- Remove the following from -15° to -25°C storage and thaw them at room temperature:
  - End Repair Mix
  - Resuspension Buffer
- Review *Handling Magnetic Beads* on page 6.

- 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 of Resuspension Buffer to each well of the 96-well 0.3 ml PCR plate that contains 50 µl of ChIP DNA.

2 Add 40 µl of 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
Before performing clean up, review *Handling Magnetic Beads* on page 6 when working with AMPure XP Beads.

6 Remove the adhesive seal from the PCR plate.

7 Vortex the AMPure XP Beads until they are well dispersed, then add 160 µl well-mixed AMPure XP Beads to each well of the PCR plate containing 100 µl of End Repair Mix. 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 15 minutes or until the liquid appears clear.

10 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.

11 Repeat step 10 once.

NOTE
Leave the PCR plate on the magnetic stand while performing the following 80% EtOH wash steps (12–14).

12 With the PCR plate on the magnetic stand, add 200 µl of freshly prepared 80% EtOH to each well with a sample without disturbing the beads.

13 Incubate the PCR 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.

14 Repeat steps 12 and 13 once for a total of two 80% EtOH washes.

15 Let the PCR plate stand at room temperature for 15 minutes to dry, then remove the plate from the magnetic stand.

16 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.

17 Incubate the PCR plate at room temperature for 2 minutes.

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

19 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 to *Adenylate 3' Ends* on page 30 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.

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° to -25°C	Illumina
Resuspension Buffer (RSB)	1 tube	-15° to -25°C	Illumina
Microseal 'B' Adhesive Seal	1		User
RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)	2		User
RNase/DNase-free Strip Tubes and Caps (if using multichannel pipettes)	2		User

Preparation

- Remove the A-Tailing Mix from -15° to -25°C storage and thaw at room temperature.
- Remove the PCR plate from -15° to -25°C storage, if it was stored at the conclusion of *Perform End Repair* on page 27 and let stand to thaw at room temperature.
  - Centrifuge the thawed PCR plate to 280 xg for 1 minute.
  - Remove the adhesive seal from the PCR 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 of Resuspension Buffer to each well of the PCR plate.
2. Add 12.5 µl of 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**.
5. When the thermal cycler temperature is 4°C, remove the PCR plate from the thermal cycler, then proceed immediately to *Ligate Adapters* on page 32.

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° to -25°C	Illumina
Resuspension Buffer (RSB)	1 tube	-15° to -25°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° to -25°C	Illumina
Stop Ligation Buffer (STL)	1 tube per 48 reactions	-15° to -25°C	Illumina
96-well 0.3 ml PCR plates	2		User
AMPure XP beads	42.5 µl per sample	2° to 8°C	User
Freshly Prepared 80% Ethanol (EtOH)	800 µl per sample	Room temperature	User
Microseal 'B' Adhesive Seal	1		User
RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)	6		User
RNase/DNase-free Strip Tubes and Caps (if using multichannel pipettes)	6		User

Preparation

- Remove the following from -15° 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° to -25°C storage until instructed to do so in the procedures.

- Review *Handling Magnetic Beads* on page 6.
- 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

NOTE
When indexing libraries using adapter index tubes, Illumina recommends arranging samples that will be combined into a common pool in the same row. Each column should contain a common index. This will facilitate 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 xg for 5 seconds.
2. Immediately before use, remove the Ligation Mix tube from -15° to -25°C storage.
3. Remove the adhesive seal from the PCR plate.
4. Add 2.5 µl of Resuspension Buffer to each well of the PCR plate.
5. Add 2.5 µl of Ligation Mix to each well of the PCR plate.
6. Return the Ligation Mix tube back to -15° to -25°C storage immediately after use.
7. Add 2.5 µl of the appropriate/desired 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 xg 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 of 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
Before performing clean up, review *Handling Magnetic Beads* on page 6 when working with AMPure XP Beads.

14 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 PCR plate. Gently pipette the entire volume up and down 10 times to mix thoroughly.

15 Incubate the PCR plate at room temperature for 15 minutes.

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

17 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 (18–20).

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

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

20 Repeat steps 18 and 19 once for a total of two 80% EtOH washes.

21 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.

22 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.

23 Incubate the PCR plate at room temperature for 2 minutes.

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

25 Transfer 50 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.

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

27 Incubate the PCR plate at room temperature for 15 minutes.

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

29 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 (30–32)

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

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

32 Repeat steps 30 and 31 once for a total of two 80% EtOH washes.

33 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.

34 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.

35 Incubate the PCR plate at room temperature for 2 minutes.

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

37 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 to *Purify Ligation Products* on page 37 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.

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, and selects a narrow 250–300 bp size-range of DNA fragments 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 will result in an insert size of approximately 150–200 bp and account 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 below. 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	-15° to -25°C	Illumina
6X Gel Loading Dye	8 µl + 4 µl per sample		User

Item	Quantity	Storage	Supplied By
50 X TAE Buffer	150 ml		User
96-well 0.3 ml PCR plate	1		User
50 bp DNA Ladder	1		User
100 bp DNA Ladder	1		User
Certified Low-range Ultra Agarose	3 g	2° to 8°C	User
GeneCatchers or Clean Scalpels	2		User
MinElute Gel Extraction Kit	1		User
SyBr Gold Nucleic Acid Gel Stain	15 µl		User

Preparation

- Prepare 1X TAE buffer (> 1 L)
- Remove the PCR plate from -15° to -25°C storage, if it was stored at the conclusion of *Ligate Adapters* on page 32 and let stand to thaw at room temperature.
  - Centrifuge the thawed PCR plate to 280 xg for 1 minute.
  - Remove the adhesive seal from the thawed PCR plate.
- 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 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.

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

WARNING
It is very 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 to 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 ladder solution onto one lane of the gel.

7 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 and use ladders on the first and last well of the gel to help locate the gel area to be excised.

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

9 View the gel on a Dark Reader transilluminator or a UV transilluminator.

10 Photograph the gel before a slice is excised.

11 Place a GeneCatcher or a clean scalpel vertically above the sample in the gel at the desired size of the template.

12 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.

13 Place the gel slice in a new 2.0 ml DNA LoBind tube.

14 Photograph the gel after the slice was excised.

15 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.

16 Follow the instructions in the MinElute Gel Extraction Kit to purify on one MinElute column, eluting in 25 µl of QIAGEN EB.

17 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 to *Enrich DNA Fragments* on page 41 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.

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, as fragments without any adapters cannot hybridize to surface-bound primers in the flow cell, and 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° to -25°C	Illumina
PCR Primer Cocktail (PPC)	1 tube per 48 reactions	-15° to -25°C	Illumina
Resuspension Buffer (RSB)	1 tube	-15° to -25°C	Illumina
96-well 0.3 ml PCR plate	1		User
AMPure XP beads	50 µl per sample	2° to 8°C	User
Freshly Prepared 80% Ethanol (EtOH)	400 µl per sample	Room temperature	User
Microseal 'B' Adhesive Seal	1		User
RNase/DNase-free Reagent Reservoirs (if using multichannel pipettes)	4		User

Item	Quantity	Storage	Supplied By
RNase/DNase-free Strip Tubes and Caps (if using multichannel pipettes)	4		User

Preparation

- Remove the PCR Master Mix and PCR Primer Cocktail from -15° 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 xg for 5 seconds.
- Review *Handling Magnetic Beads* on page 6.
- Remove the AMPure XP beads from storage and let stand for at least 30 minutes to bring them to room temperature.
- Remove the PCR plate from -15° to -25°C storage, if it was stored at the conclusion of *Purify Ligation Products* on page 37 and let stand to thaw at room temperature.
  - Centrifuge the thawed PCR plate to 280 xg for 1 minute.
  - Remove the adhesive seal from the thawed PCR 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 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.

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

NOTE

Before performing clean up, review *Handling Magnetic Beads* on page 6 when working with AMPure XP Beads.

5 Remove the adhesive seal from the PCR plate.

6 Vortex the AMPure XP Beads until they are well dispersed, then 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.

7 Incubate the PCR plate at room temperature for 15 minutes.

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

9 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 (10–12).

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

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

12 Repeat steps 10 and 11 once for a total of two 80% EtOH washes.

13 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.

14 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.

15 Incubate the PCR plate at room temperature for 2 minutes.

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

17 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 to *Validate Library* on page 45 or TruSeq Enrichment 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.

Validate Library

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

Quantify Libraries

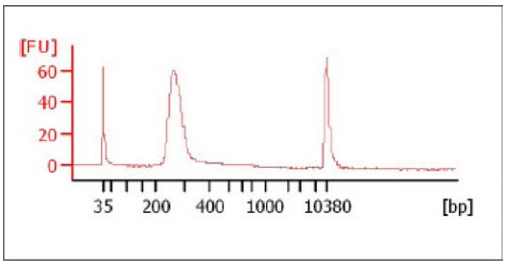
In order to achieve the highest quality of data on Illumina sequencing platforms, it is important to create optimum cluster densities across every lane of every flow cell. This requires accurate quantitation of DNA library templates. Quantify your libraries using qPCR according to the Illumina *Sequencing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

Quality Control

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 using the Agilent Bioanalyzer with a High Sensitivity DNA chip, make a 1:20 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.

Figure 10 Example of DNA Library Distribution for TruSeq ChIP Sample Prep



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, then pooled in equal volumes. DNA libraries not intended for indexing are normalized to 10 nM without pooling.

Consumables

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

Preparation

- Remove the PCR plate from -15° to -25°C storage, if it was stored at the conclusion of *Enrich DNA Fragments* on page 41, and let stand to thaw at room temperature.
  - Centrifuge the thawed PCR plate to 280 xg for 1 minute.
  - Remove the adhesive seal from the thawed PCR 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-Cl 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-indexed libraries, the protocol stops here. Do one of the following:
  - Proceed to cluster generation. For more information, see the *Illumina Cluster Generation User Guide*.
  - Seal the MIDI plate with a Microseal 'B' adhesive seal and store at -15° to -25°C.
- For indexed libraries, proceed to step 5.

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

NOTE
Keep track of which sample goes into which 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 should be 10X the number of combined sample libraries and will be 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 *Illumina Cluster Generation User Guide*.
- Seal the PCR plate with a Microseal 'B' adhesive seal and store at -15° to -25°C.

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 7 Illumina General Contact Information

Illumina Website	http://www.illumina.com
Email	techsupport@illumina.com

Table 8 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 http://www.illumina.com/msds.

Product Documentation

You can obtain PDFs of additional product documentation from the Illumina website. Go to http://www.illumina.com/support and select a product. To download documentation, you will be asked to log in to MyIllumina. After you log in, you can view or save the PDF. To register for a MyIllumina account, please visit https://my.illumina.com/Account/Register.

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