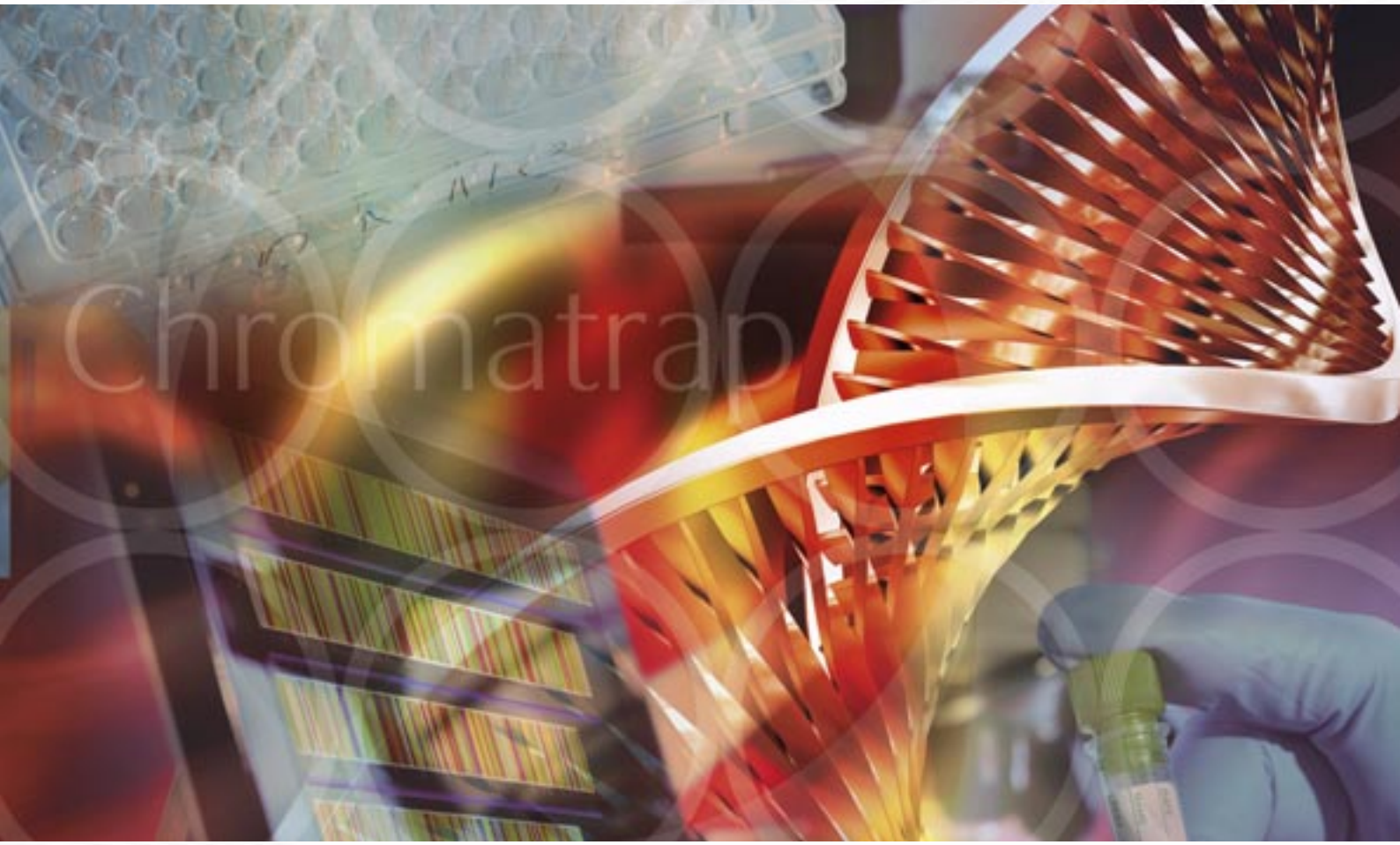
Chromatrap

V 2.1

# Chromatrap® ChIP-seq

## A bead-free chromatin immunoprecipitation assay for next generation sequencing

*Catalogue no 500189, 500190, 500191, 500192*




ADVANCEMENTS IN EPIGENETICS

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# Introduction

Chromatin Immunoprecipitation (ChIP) is a powerful tool in the study of epigenetics. The technique is used to study the association of specific proteins, or their modified isoforms, with defined genomic regions. In a ChIP assay, DNA-protein complexes (chromatin) are selectively immunoprecipitated using matching antibodies and the resulting fractions treated to separate the DNA and protein components. Polymerase Chain Reaction (PCR), Real Time PCR (qPCR), hybridization on microarrays (ChIP-on-chip), or Next Generation Sequencing (NGS) are typically used to identify DNA fragments of defined sequence.

Genome-wide mapping of protein-DNA interactions is essential for a complete understanding of gene regulation. A detailed map of epigenetic marks and transcription factor binding is necessary for deducing the regulatory networks that underpin gene expression in a variety of biological systems. The most widely used tool for examining these interactions is ChIP followed by massively parallel sequencing (ChIP-seq). NGS of ChIP enriched DNA enables identification of target DNA sites that were in direct physical contact with regulatory mechanisms in vivo. Mapping of these sequenced fragments to whole genome sequence databases allows quick and efficient analysis of the DNA interaction pattern of any transcription factor or epigenetic modification.

ChIP-seq is an attractive alternative to ChIP-on-Chip. With the ability to sequence millions of DNA fragments in a single run, generating single-base pair resolution, fewer artefacts and greater coverage, ChIP-seq offers significantly improved data compared with previous technology. Many examples of ChIP-seq yielding mechanistic understanding of cellular regulatory processes can be found in the literature, including transcriptional regulation (Lee 2002, Chen et al 2008, Nielsen 2008) epigenetic regulation (Barski 2007) and nucleosome organisation (Heintzman 2009, Tolstorukov 2009).

The short reads generated by next-generation sequencing (NGS) platforms are ideal for ChIP-seq and allow precise mapping of protein binding sites as well as improved identification of sequence motifs. Importantly, ChIP-seq allows the spatial resolution for profiling post translational modifications of chromatin and histone variants as well as nucleosome positioning. With the increasing performance of sequencing platforms, ChIP-seq is the leading technology for genome-scale analysis of protein-DNA interactions.

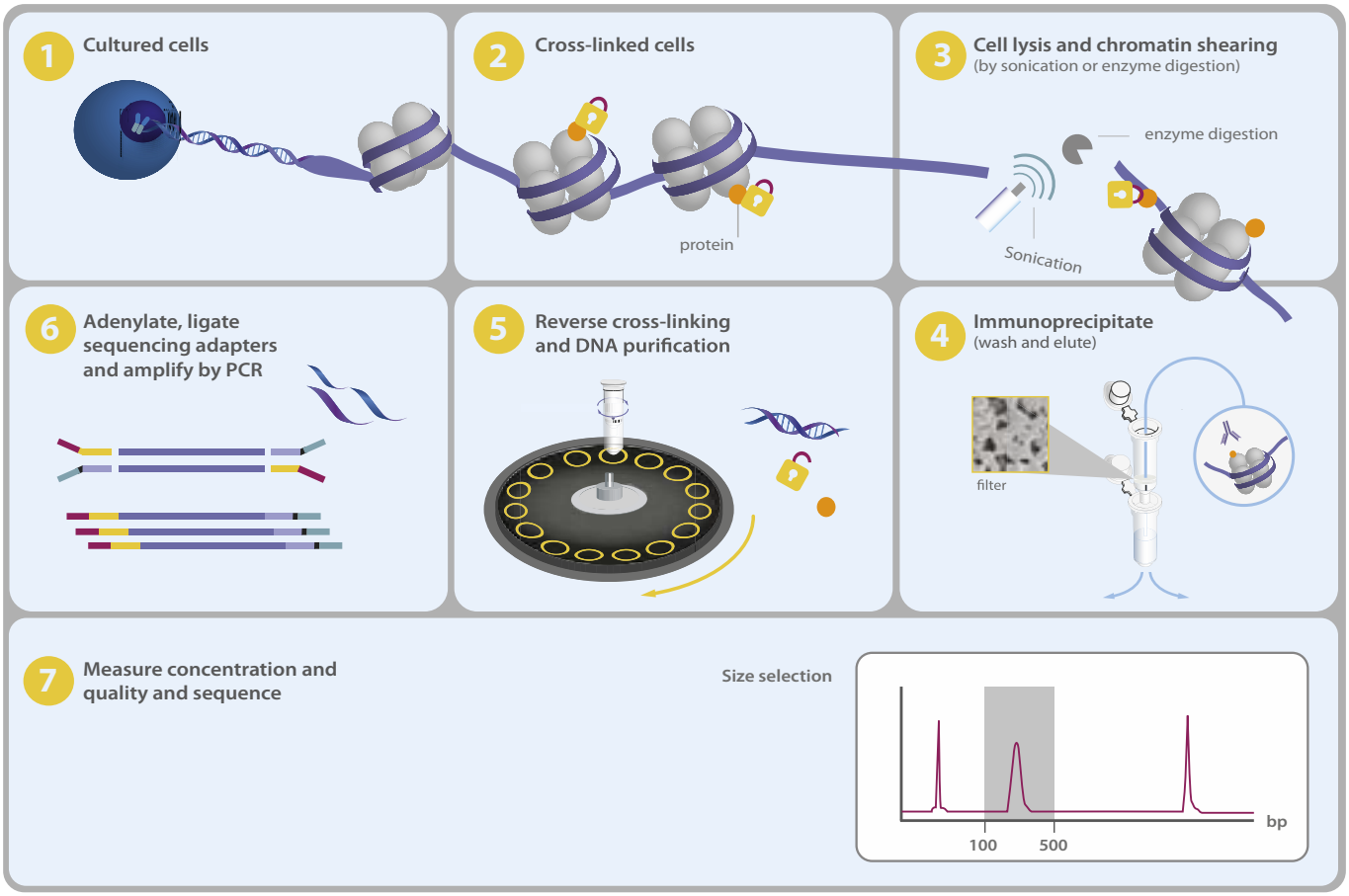


**Figure 1:** *Overview of ChIP-seq process*

# Chromatrap® ChIP-seq

Chromatrap®'s unique patented* technology provides a quicker, easier and more sensitive way of performing. In this revolutionary system, filter discs of an inert porous plastic, Vyon®, replace magnetic or agarose beads. Protein A or Protein G has been attached in the correct orientation throughout the filter to maximise the capture efficiency of the target chromatin/antibody complex.

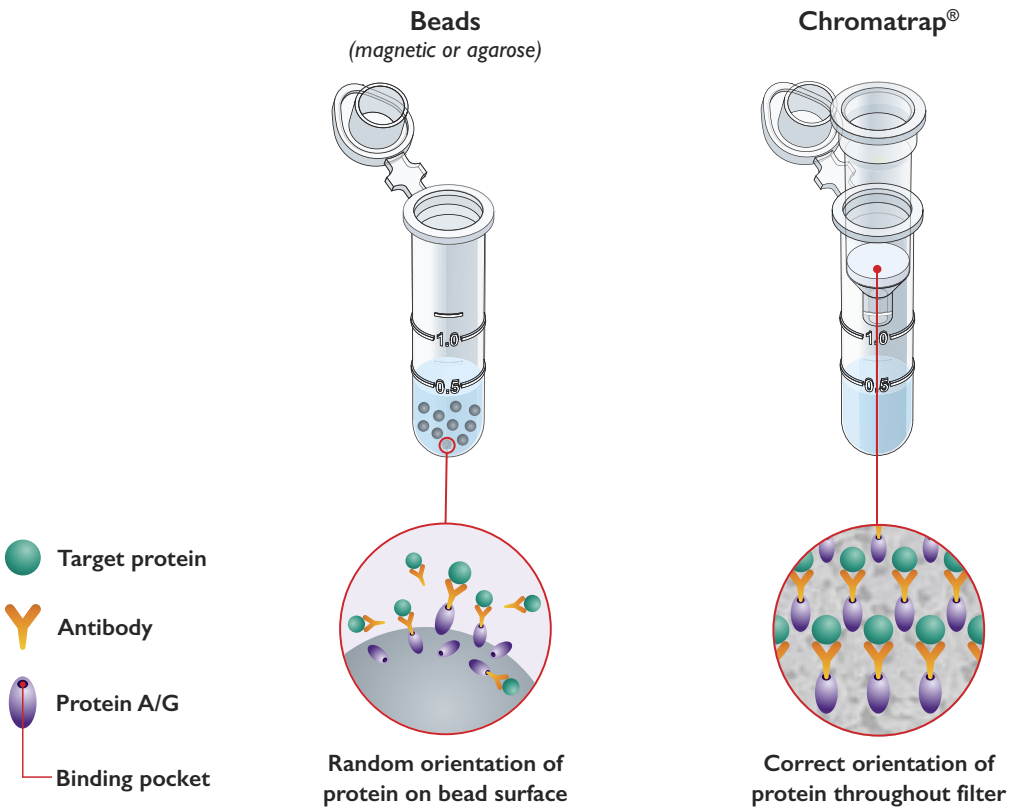


**Figure 2:** *Chromatrap® ChIP technology*

*A Chromatrap® ChIP-seq assay using cell lines or tissue consists of five key steps:*

1. *Preparation of high quality chromatin using the reagents provided*
2. *Immunoprecipitation of chromatin using ChIP-validated antibody of interest specific to the target protein*
3. *Capture of the antibody-chromatin complex using the Chromatrap® spin column technology*
4. *Recovery of the enriched DNA using kit supplied reverse cross-linking, proteinase digestion and DNA clean-up reagents*
5. *DNA analysis*

Chromatrap® utilises this solid state technology in parallel with Next Generation Sequencing (NGS) to deliver a precise ChIP-seq protocol from small cell numbers and low chromatin concentrations.

**Advantages of Chromatrap® ChIP-seq:**

- *ChIP-seq with as little as 1000 cells or 5 mg tissue (5 mg - 300 mg)*
- *ChIP-seq from as low as 50 ng up to 50 µg of chromatin*
- *Low background due to the inert filter technology*
- *Fast protocol – no blocking steps or overnight incubations*
- *NGS quality DNA from a single IP without the need to pool samples*
- *Compatible with qPCR, sequencing and mass spectrometry as downstream processes*

**UK Patent No. GB2482209, & GB2527623, US Patent No. 9523681 & 10,435,480, Chinese Patent No. ZL 2011 8 0067254.X, Japan Patent No. JP 6088434 and Australian Patent No. AU 2011340263.*

# Kit overview and timetable

The Chromatrap® ChIP-seq kit allows the user to perform up to 24 ChIP assays from cell collection through to immunoprecipitation, including up to 10 chromatin sample preparations. The kit provides all of the major components required for performing ChIP assays to obtain adequate high quality DNA for NGS library preparation. This protocol provides supporting information and tips for library synthesis, cluster generation and sequencing analysis using the Illumina® platforms.

| Step | Process | Time required | Day |
|---|---|---|---|
| 1 | Cell fixation and collection | 0.5 hour | 1 |
| 2 | Cell lysis and chromatin shearing | 0.5 hour | 1 |
| 3 | Immunoprecipitation | 1 hour | 1 |
| 4 | Reverse cross-linking and DNA purification | 3.5 hours | 1 |
| 5 | Quantitative PCR analysis | 1 hour | 1 |
| 6 | *Chromatrap® NGS library synthesis | 5 hours | 2 |
| 7 | Library quantification and quality analysis | 2 hours | 2 |

**Table 1:** *Chromatrap® ChIP-seq protocol overview.*
**When using the Chromatrap® UniqSeq library preparation kit (Cat no 500264, 500265, 500266 and 500267). Library preparation time with other kits will vary and may take up to 2 days.*

# Kit components

Chromatrap® ChIP-seq kits (500189, 500190, 500191 and 500192) allow the user to perform up to 24 ChIP assays from cell collection through to immunoprecipitation. Upon receipt, please ensure the components are stored at the temperatures listed in Table 2.

| Kit Component | Quantity | Storage |
|---|---|---|
| Chromatrap® ChIP-seq Spin Columns | 24 | 4°C |
| Column Conditioning Buffer | 60 ml | 4°C |
| Wash Buffer 1 | 50 ml | 4°C |
| Wash Buffer 2 | 50 ml | 4°C |
| Wash Buffer 3 | 50 ml | 4°C |
| 1.3 M Glycine | 20 ml | 4°C |
| Lysis Buffer | 10 ml | 4°C |
| Digestion Buffer (only in 500191 and 500192) | 10 ml | 4°C |
| ChIP-seq Enzymatic Stop Solution (only in 500191 and 500192) | 200 µl | 4°C |
| ChIP-seq Elution Buffer | 3 ml | 4°C |
| 5 M NaCl | 500 µl | 4°C |
| 1 M $NaHCO_3$ | 500 µl | 4°C |
| Hypotonic Buffer | 10 ml | 4°C |
| ChIP-seq Positive Antibody | 20 µl (0.2 µg/µl) | -20°C |
| ChIP-seq Negative Antibody | 20 µl (0.2 µg/µl) | -20°C |
| Shearing Cocktail* (only in 500191 and 500192) | 100 µl | -20°C |
| Protease Inhibitor Cocktail (PIC) | 50 µl | -20°C |
| Proteinase K stop solution | 100 µl | -20°C |
| Proteinase K | 50 µl | -20°C |
| GAPDH Forward primer | 50 µl (8 µM) | -20°C |
| GAPDH Reverse primer | 50 µl (8 µM) | -20°C |
| 1.5 ml Collection tube | 50 | RT |
| Chromatrap® DNA purification columns | 24 | RT |
| DNA Binding Buffer | 15 ml | RT |
| DNA Wash Buffer | 15 ml | RT |
| DNA Elution Buffer | 2 ml | RT |

**Table 2:** *Chromatrap® ChIP-seq reagents and materials*

*It is recommended that you aliquot Shearing Cocktail on receipt of the kit to minimise the number of freeze thaw cycles and maintain the activity of the cocktail.

The kits are manufactured DNase free and when stored as directed are stable for up to 6 months. Sufficient material is supplied for 24 ChIP assays and up to 10 chromatin sample preparations.

# Additional materials required

## Reagents and consumables

- *PBS*
- *37% formaldehyde, molecular biology grade*
- *Nuclease-free water*
- *100 bp ladder*
- *Cell-scrapers*
- *Microcentrifuge tubes (0.5 ml and 1.5 ml)*
- *PCR plates*
- *Pipettes and tips (filter tips are recommended)*
- *ChIP validated antibody*
- *qPCR primer pairs for gene of interest*
- *For enzymatic shearing 0.1% SDS solution*
- *3 M Sodium Acetate pH 5.2*
- *Scalpel blade (for tissue)*

## Equipment

- *Microcentrifuge (4°C)*
- *Agarose gel electrophoresis equipment*
- *Rocking platform for culture plates/flasks*
- *Spectrophotometer/fluorometer for DNA quantification*
- *Sonicator*
- *End to end rotator*

## Additional materials required for sequencing using Illumina® platforms

- *Thermocycler*
- *Qubit® 2.0 fluorometer with dsDNA high sensitivity kit (or equivalent fluorometric quantification method)*
- *Agilent Technologies 2100 Bioanalyzer with high sensitivity DNA kit*
- *Library sample preparation kit*
- *Library quantification kit*
- *DNA purification kit with size selection, such as the Chromatrap® Size Selection kit (Cat. no. 500262)*

## Optional materials

- *Phenol Chloroform*
- *100% Ethanol*
- *70% Ethanol*
- *Linear Polyacrylamide (LPA)*
- *DNA LoBind tubes (optional)*

# ChIP-seq considerations

## Antibody quality

The success and value of any ChIP-seq experiment is dependent on the quality of the antibody used. A highly-specific antibody will increase the relative enrichment of the target compared with the background, making it easier to detect binding events during data analysis. Many commercially available antibodies are listed as ChIP-seq grade and, wherever possible, should be used for your experiments. However, lot to lot variations and variability in quality does occur and the antibodies of choice should be validated before use.

## Sample quantity

The amount of starting material required to obtain sufficient yields of enriched DNA to prepare a ChIP-seq library will depend on the quality of both chromatin and antibody, the abundance of the target protein and gene and the sensitivity and efficiency of the library preparation kit used. The Chromatrap® ChIP-seq kit has been optimised for library preparation with the Chomatrap® UniqSeq kit (cat. no. 500264/500265). High quality ChIP-seq data can be obtained from as little as 1000 cells using this workflow (see Troubleshooting for guidance on buffer volumes from small cell numbers). A minimum of 500 pg of DNA is required for library preparation using Chomatrap® UniqSeq. The input requirements of other library preparation kits will depend on the manufacturers' limits and will need to be optimised by the user. However, if sequence duplication becomes an issue we would recommend increasing the quantity of starting material for library amplification to help minimise duplication levels. Additionally, the number of PCR cycles used during the enrichment step can also be reduced if duplication remains an issue.

## Shearing

The experimental and processing steps in ChIP can introduce potential sources of artefacts. For example, chromatin shearing does not result in uniform fragmentation whether sheared mechanically through sonication or by enzymatic digestion. Open chromatin tends to shear more easily than closed regions, creating an uneven distribution of sequence fragments. Equally, nucleases used during enzymatic digestion exhibit a more pronounced sequence bias during cleavage. We find that once optimised, both sonication and enzymatic shearing generate fragment sizes ideal for ChIP-seq.

## Control experiment

Peaks identified during sequencing analysis must be compared to the same region in a matched control sample in order to verify their significance. For example, a random region of repetitive sequences may appear enriched due to the number of copies of the region, creating a false-positive result. There are three commonly used controls: input DNA (DNA that has not been immunoprecipitated); mock IP (DNA treated the same but without antibody during the IP); and non-specific IP (IP with an antibody targeting a protein not known to be involved in DNA binding such as IgG). There is no consensus as to which control is most appropriate to use, however, input DNA and IgG controls are commonly used as they account for bias related to the shearing of DNA and amplification. We recommend using input as a control.

## Reaction Conditions

ChIP-seq requires careful optimisation of numerous reaction conditions from the number of cells used in culture to the number of fragment clusters for optimal sequencing analysis. Chromatrap® ChIP-seq reduces the number of optimisation steps required and has been tailored for use with Illumina® sequencing platforms. The Chromatrap® UniqSeq Kit (Cat. no. 500264/500265) contains adapters and indices compatible with Illumina sequencing instruments only, such as the MiSeq, HiSeq and NextSeq. For all other sequencing platforms such as Ion Torrent, the SOLiD® system and Roche 454, the appropriate library kit will need to be provided by the user.

# Experimental planning

## 1. Cell culture

This protocol has been optimised for use with cell lines, tissue and primary cells, and provides enough reagents for up to 10 chromatin preparations ($15x10^6$ cells) and up to 24 ChIP assays. Lower cell numbers can be used, however, volumes of buffers will need to be adjusted accordingly (Table 3).

## 2. Shearing optimisation

The success of a ChIP assay is highly dependent on the quality of chromatin prepared. The shearing conditions described within the protocol are suitable for a variety of cell types and may be taken as a guide. However, given the variations between cell types, we recommend optimising shearing conditions before progressing with ChIP (see Troubleshooting for more information).

## 3. Slurry volume

A key advantage of the Chromatrap® technique compared to conventional bead based assay is the flexibility in chromatin loading. The fundamental requirement for optimal antibody binding is to load 50 ng-50 µg chromatin in a total volume of 1 ml ensuring that the chromatin does not exceed more than 10% (100 µl) of the total 1 ml slurry volume.

## 4. Quantification

For library synthesis, it is critical to determine the concentration of IPd DNA using a high sensitivity fluorescence based quantification method as UV-based spectrophotometers such as the NanoDrop are unreliable for quantification of low quantities of DNA (see Troubleshooting). The concentration of DNA will be influenced by a variety of factors including cell type, target abundance and antibody affinity.

## 5. Positive and negative IP controls

In addition to the ChIP validated antibody, we recommend the use of a positive and negative control antibody. We suggest including one negative IgG control antibody corresponding to the host species in which the antibody of interest was raised for each series of ChIP reactions. We provide a positive ChIP-seq grade control antibody, H3K4me3, and recommend using 1 µg chromatin and 2 µg H3K4me3 to validate successful IP. See Step 3a of the protocol for more information.

## 6. Quantitative PCR validation

Before beginning library synthesis for sequencing, we recommend analysing the IP'd DNA in qPCR using at least one positive and one negative control target of your choice. In order to have sufficient DNA for library preparation, it is recommended that not more than 10% of the total IPd DNA be used for qPCR. If necessary, DNA can be diluted 1:10 to provide an adequate volume for triplicate PCR reactions. Control targets for the antibody of choice should be analysed by the user as appropriate.

## 7. Quantitative PCR interpretation

The efficiency of immunoprecipitation provides an indicator of the relative success of a ChIP assay and requires the interpretation of qPCR data to determine which DNA fragments have been enriched. This can be expressed as the recovery of the locus calculated as a percentage of input as follows:

$$\% \text{ recovery} = 2^{(Ct_{input} - Ct_{sample})} * \text{dilution} * 100$$

# Protocol

Wherever this 'pause point' symbol appears, it signifies that if required, the sample can be stored at -80°C.

## Step 1 – Chromatin preparation; fixation and collection

The following section describes fixation for adherent cells (step 1a), suspension cells (step 1b), and fresh/frozen tissue (step1c). Chromatin extraction from other sources will require optimisation by the user. Remember to prepare enough chromatin for any biological IP controls.

**Step 1a: For adherent cells**

1. Culture between 1000-1.5x$10^7$ cells.
2. Remove media and wash with **pre-warmed PBS** at room temperature (RT).
3. Remove the PBS and add basic cell culture media (this should not contain any serum or large molecular weight proteins) containing 1% formaldehyde, ensure all cells are covered in order to fix the cells and cross link the DNA/protein complexes.
4. Incubate for 10 minutes at RT with gentle agitation on a rocking platform.
5. Remove the fixation solution and add 0.65 M glycine solution to quench the reaction

**N.B. Glycine is supplied as a 1.3 M solution and should be diluted 50:50 with PBS before use, refer to Table 3 for optimum volume for starting cell number).**

6. Incubate for 5 minutes at RT with gentle agitation on a rocking platform.
7. Remove the glycine solution and collect the cells by scraping **in ice cold PBS** (ensure sufficient PBS to cover the surface of the cells). Collect cells by centrifugation at 3500 x g for 5 minutes at 4°C.
8. Discard the supernatant. Proceed to Step 2.

*At this point the protocol can be continued or the pellet can be frozen and stored at -80°C, if freezing the pellet add 1 µl Protease Inhibitor Cocktail (PIC).*

**Step 1b: For suspension cells**

1. Collect cells by centrifugation at 500 x g for 5 minutes at 4°C.
2. Re-suspend in 1 ml **pre-warmed PBS** (perform cell count) and spin 500 x g for 5 minutes at RT.
3. Re-suspend pellet in 1 ml PBS then add 27 µl 37% formaldehyde (to give final concentration of 1%) to cross-link DNA/protein complexes.
4. Incubate for 10 minutes at RT on an end to end rotator.
5. Add 1.3 M Glycine (114 µls / ml of sample) and incubate 5 minutes at RT on an end to end rotator.
6. Spin to collect cells at 500 x g for 5 minutes at 4°C.
7. Re-suspend in 1 ml **ice cold PBS**.
8. Spin to collect cells at 500 x g for 5 minutes at 4°C and discard the supernatant. Proceed to step 2.

*At this point the protocol can be continued or the pellet can be frozen and stored at -80°C, if freezing the pellet add 1 µl Protease Inhibitor Cocktail (PIC).*

| Buffer | Cell Count (Millions) | Buffer Volume (ml) |
|---|---|---|
| 0.65 M Glycine* | 1-5<br>5-10<br>10-15 | 3<br>4<br>5 |
| Hypotonic Buffer | 1-5<br>5-10<br>10-15 | 0.4<br>0.8<br>1.0 |
| Lysis Buffer** | 1-5<br>5-10<br>10-15 | 0.3<br>0.3-0.5<br>0.5-1.0 |
| Digestion Buffer for enzymatic digestion | 1-5<br>5-10<br>10-15 | 0.3<br>0.4<br>0.5 |
| Enzymatic Stop Solution<br>for enzymatic digestion | 1-5<br>5-10<br>10-15 | 7.5 µl<br>10 µl<br>12.5 µl |

**Table 3** *Optimal buffer volumes for cell lines (for buffer volumes for less than 1 million cells please see page 23).*

**Step 1c: For fresh or frozen tissue**

The following section describes fixation and chromatin preparation of 5-300 mg fresh/frozen tissue. Keep samples on ice at all times to minimise sample degradation, unless stated otherwise.

1. Thaw frozen tissue on ice.
2. Prepare 5-10 ml fixation solution per tissue sample in fume hood (1% formaldehyde in PBS). It is important all of the tissue is covered in order to fix the cells and cross-link DNA/protein complexes
3. Cut the tissue into small pieces (approximately 1 $mm^3$) in a petri dish using a scalpel blade.
4. Add the fixation solution to the tissue sample in the petri dish and incubate for 10-15 minutes at RT with gentle agitation on a rocking platform (if the tissue sticks to the petri dish, dislodge the tissue using a pipette tip ensuring the tissue is in solution).
5. Remove as much of the fixation solution as possible avoiding the tissue sample.
6. Add 0.65 M glycine solution to quench the fixation reaction
   **N.B. Glycine is supplied as a 1.3 M solution and should be diluted 50:50 with PBS for use – refer to Table 4 for optimum volumes) and incubate samples at RT for 5 minutes with gentle agitation on a rocking platform.**
7. Collect tissue sample in glycine and transfer to a 15 ml centrifuge tube. If some tissue is left behind, add 5 ml PBS to the petri dish and collect the remaining sample and transfer to the 15 ml centrifuge tube.
8. Collect cells by centrifugation at 3500 x g for 5 minutes at 4°C and discard supernatant.
9. Add 5 ml **ice cold PBS** and homogenise sample by pipetting up and down. If large pieces of sample remain, samples can be homogenised in alternative ways such as a hand held tissue homogeniser.
10. Collect cells by centrifugation at 3500 x g for 5 minutes at 4°C and discard supernatant. Proceed to step 2.

| Buffer | Tissue (mg) | Volume of buffer |
|---|---|---|
| 0.65 M Glycine* | <80<br>80-120<br>120-200 | 3 ml<br>5 ml<br>7 ml |
| Hypotonic buffer | <50 mg<br>50-80<br>80-120<br>120-200 | 0.5 ml<br>1 ml<br>2 ml<br>2.5 ml |
| Lysis Buffer** | <50 mg<br>50-80<br>80-120<br>120-200 | 0.3 ml<br>500 µl<br>700 µl<br>1 ml |

**Table 4:** *Optimal buffer volumes for tissue*

** Glycine is supplied as 1.3 M, please dilute 50:50 with PBS buffer to reach a working concentration of 0.65 M.*
*** Lysis Buffer must be pre-warmed to 37°C in a water bath for 30 minutes with occasional shaking before use, to remove any precipitates. The contents of the bottle should be mixed by inverting it a couple of times before putting it into the water bath and (at least) once half-way through the incubation. Bring the buffer back to room temperature when ready to use.*

# Step 2 – Cell Lysis and Chromatin Shearing

Chromatin can be sheared either by a sonication (mechanical using ultrasonic sound waves) or an enzymatic (micrococcal nuclease digestion) approach. It is important to choose the appropriate method of shearing. Section 2a describes chromatin shearing by sonication for $1x10^4$-$15x10^6$ cell preparations and the buffer volumes required are outlined in Table 3, section 2b describes chromatin shearing by sonication for 5 mg - 300 mg fresh/frozen tissue. The protocol assumes shearing conditions have been optimised by the user, if this is not the case please refer to Troubleshooting for optimal shearing conditions. For enzymatic shearing please refer to section 2c.

**Step 2a: Cell lysis and chromatin shearing by sonication for adherent/suspension cells**

1. Re-suspend the cell pellet in Hypotonic Buffer and incubate the samples at 4°C for 10 minutes (refer to Table 3 for optimum volume from starting cell number).
2. Centrifuge the hypotonic slurry at 5000 x g for 5 minutes at 4°C to collect the nuclei.
3. Discard the supernatant and re-suspend the pellet in Lysis Buffer (**ensure the Lysis Buffer has been pre-warmed prior to use to ensure all precipitates are fully dissolved**, refer to Table 3) and incubate samples at 4°C for 10 minutes.
4. Sonicate samples until the desired lengths of DNA fragments are achieved (100-500 bp).
5. Centrifuge the samples for 10 minutes at maximum speed at 4°C and transfer the supernatant to a clean dry microcentrifuge tube.
6. Add 1 µl of PIC to the samples and mix.
7. Chromatin samples are now ready for IP. Chromatin stocks can be stored at -80°C for a maximum of 2 months. It is recommended that the shearing efficiency of each chromatin stock is analysed at this stage.

**Step 2b Cell lysis and chromatin shearing by sonication for fresh/frozen tissue**

1. Re-suspend cell pellet in Hypotonic Buffer (refer to Table 4 for optimal volumes) by pipetting and incubate sample for 10 minutes at 4°C. For efficient cell lysis, flick the tube every few minutes to prevent the cells settling at the bottom of the tube.
2. Centrifuge the hypotonic slurry at 5000 x g for 5 minutes at 4°C to collect the nuclei.
3. Discard the supernatant and re-suspend the pellet in Lysis Buffer (**ensure the Lysis Buffer has been prewarmed prior to use to ensure all precipitates are fully dissolved**, refer to Table 4) and incubate samples at 4°C for 10 minutes. Flick the tube every few minutes to prevent the cells settling at the bottom of the tube for efficient nuclear lysis.
4. Sonicate the samples until the desired lengths of DNA fragments are achieved (100-500 bp).
5. Centrifuge the samples for 10 minutes at maximum speed at 4°C and transfer the supernatant to a clean dry 1.5 ml microcentrifuge tube.
6. Add 1 µl of PIC to the samples and mix.
7. Chromatin samples are now ready for IP. Chromatin stocks can be stored at -80°C for a maximum of 2 months.

**N.B.** *It is recommended that the shearing efficiency of each chromatin stock is analysed at this stage. Shearing efficiency varies greatly and will need to be optimised and confirmed separately, checking the size of the fragments on an agarose gel such as described in the following quantification section (Step 2d).*

**Step 2c: Cell lysis and chromatin shearing by enzymatic digestion**

1. Re-suspend the cell pellet in Hypotonic Buffer and incubate the samples at 4°C for 10 minutes (refer to Table 3 for optimum volume from starting cell number).
2. Centrifuge the hypotonic slurry at 5000 x g for 5 minutes at 4°C to collect the nuclei and discard the supernatant.
3. Re-suspend the pellet (nuclei) in Digestion Buffer by pipetting (refer to Table 3 for optimum volume for starting cell number), immediately add 2 µl PIC to each stock nuclei suspension. Keep stock nuclei suspensions on ice while determining DNA concentration.

**Determining DNA concentration**

- Remove a 10 µl sample of each stock nuclei suspension and add to 490 µl 0.1% SDS, mix well and incubate on ice for 10 minutes.
- Estimate the concentration of DNA on a spectrophotometer and use this to calculate the total amount of chromatin in each stock nuclei suspension in order to determine volume of Shearing Cocktail to be used (eg. Nanodrop reading x 50 x total volume of stock nuclei suspension).

**Example calculation**
Sample measures 9 ng/µl
9 (concentration) x 50 (dilution factor) x 400 (volume of Digestion Buffer)
**= 180,000 ng or 180 µg total chromatin**
1 U Shearing Cocktail per 5 µg chromatin therefore 180/5 **= 36 U Shearing Cocktail**
Shearing Cocktail is supplied as 15 U per µl therefore 36/15 **= 2.4 µl Shearing Cocktail to be added**.

4. Add Shearing Cocktail to each stock nuclei suspension (from step 2c, point 3) at a ratio of 1 U Shearing Cocktail:5 µg chromatin (Shearing Cocktail is supplied as 15 U/µl) and mix thoroughly.
5. Incubate for 5 minutes in a 37°C waterbath then immediately add Enzymatic Stop Solution (refer to Table 3 for optimum volume) and place tubes on ice.
6. Centrifuge for 5 minutes at 12,000 x g at 4°C and discard the supernatant.
7. Re-suspend the pellets (nuclei) in Lysis Buffer (ensure the Lysis Buffer has been pre-warmed prior to use to ensure all precipitates are fully dissolved, refer to Table 3 for optimum volume) and incubate the tubes on ice for 10 minutes to lyse the nuclei.
8. Centrifuge the samples for 10 minutes at maximum speed at 4°C and transfer the supernatant to a clean dry microcentrifuge tube.
9. Add 1 µl of PIC to the samples and mix.
10. Chromatin samples are now ready for IP. If samples are not to be used immediately, store at -80°C for a maximum of 2 months. It is recommended that the shearing efficiency of each chromatin stock is analysed at this stage.

**N.B.** *Shearing efficiency varies greatly and will need to be optimised and confirmed separately, checking the size of the fragments on an agarose gel such as described in the following quantification section.*

**Step 2d: Shearing efficiency**

Chromatin shearing should be checked on a 1% agarose gel to ensure that the appropriate fragment sizes have been generated during shearing. Prior to immunoprecipitation, aliquots of stock chromatin are also used for DNA quantification in order to determine the volume of DNA required for slurry preparation in Step 3.

1. Take a 25 µl aliquot of sheared chromatin from each sample and place in a microcentrifuge capped tube.
2. Add 5 µl of 1 M $NaHCO_3$ and 5 µl of 5 M NaCl. Make up to a final volume of 50 µl with nuclease free water and mix thoroughly.
3. Incubate the samples at 65°C for 2 hours to reverse the cross-linking. If required samples can be left overnight.
4. Briefly centrifuge the samples to remove any liquid from the caps.
5. Add 1 µl of the Proteinase K solution and mix thoroughly. Incubate for 1 hour at 37°C.
6. Return the samples to room temperature and add 2 µl Proteinase K stop solution.
7. Quantify the DNA in the samples using a spectrophotometer at 260 nm. Multiply the reading by 2 to account for the dilution during the reverse cross-linking. This will be used to determine the volume of chromatin to load in Step 3; Slurry Preparation and Immunoprecipitation.
8. To ensure that 100-500 bp fragments have been obtained during shearing the DNA should be run on an agarose gel and visualised against a marker of known size DNA fragments (e.g. 100 bp ladder). A smear of DNA fragments 100-500 bp in length is ideal, fragments of smaller or greater length may affect the efficiency of the ChIP reaction.

**N.B.** *If chromatin is over- or under-sheared refer to the relevant section of the troubleshooting guide and FAQs.*

# Step 3 – Slurry Preparation and Immunoprecipitation

**Step 3a: Slurry preparation and column activation**

An important consideration when performing ChIP-seq is the amount of chromatin that will need to be loaded to the column in order to elute sufficient IPd DNA for library synthesis. The DNA yield obtained from ChIP will depend on the quality of the chromatin, the affinity and avidity of the antibody and the abundance of the target. A minimum of 500 pg of DNA is required for preparation of high quality NGS sequencing libraries using the Chromatrap® Uniq-seq Kit (Cat. no. 500264/500265). As little as 1000 cells can yield this quantity of DNA from ChIP of an abundant target using a good quality antibody. As a starting point we would recommend using 10 µg chromatin per ChIP with 2-5 µg antibody. For the positive control for qPCR analysis, prepare the slurry in a 1 ml volume with a 2:1 antibody : chromatin ratio. Remember to prepare negative controls for both antibody validation and the standard assay described in Table 4.

1. Thaw chromatin stocks at 4°C.
2. Centrifuge sheared chromatin at max speed for 10 minutes at 4°C, even if previously centrifuged.
   **NOTE: Use only the clear supernatant for subsequent steps.**
3. Prepare IP slurries in a fresh microcentrifuge tube according to Table 5. For every antibody IP set aside the equivalent amount of chromatin in a microcentrifuge tube and make up to 100 µl with Column Conditioning buffer (if necessary), label as an input. These will be processed alongside the samples for reverse cross-linking and Proteinase K digestion at Step 4a and will be used as controls in the downstream analysis.
4. Mix well and incubate the IP slurries on an end to end rotor for 1 hour at 4°C.

| Reagent | Immunoprecipitation Slurry (1000 µl total volume) | Positive control (1000 µl total volume) |
|---|---|---|
| Chromatin stock | Up to 100 µl | 1 µg |
| Antibody/IgG | Optimum addition rate | 10 µl (2 µg) |
| PIC | 1 µl | 1 µl |
| Column Conditioning Buffer | Make up to final volume of 1000 µl | Make up to final volume of 1000 µl |

**Table 5:** *Slurry preparation for spin column IP*

**Step 3b: Chromatrap® spin column preparation**

Chromatrap® spin columns are shipped in a storage solution, prior to use, columns must be washed and activated to remove any traces of shipping solution and to prepare them for slurry incubation.

1. Remove the spin column from the collection tube (save for later) and place in an empty 1 ml tip box rack (or alternative holder).
2. Add 600 µl Column Conditioning Buffer to each column and allow to flow through under gravity (~ 30 minutes). **N.B. do not close caps when flow is under gravity.**
3. Discard the flow through and repeat this conditioning step a second time.
4. Discard the flow through. The columns are now ready for the addition of the IP slurries, proceed to step 3c.

**Step 3c: Immunoprecipitation**

The immunoprecipitation step involves the binding of the antibody of interest to the protein A/G attached to the spin column filter disc. This allows the selective enrichment of the target protein/DNA complex and allows any non-specific complexes to be washed away. Target chromatin is then eluted using a specially formulated ChIP-seq Elution Buffer for maximal target recovery.

**N.B.** *If precipitates have formed in the* **ChIP-seq Elution Buffer** *then it should be warmed to 37ºC in a water bath for 30 minutes with regular shaking until precipitates have dissolved* **before use**.

1. Remove slurries from the end-to end rotator following 1 hr pre-incubation and briefly spin down to remove residual liquid from the caps.
2. Load the entire 1 ml slurry and allow to flow completely through the column at RT (approx 30-40 minutes).
3. Position Chromatrap® spin columns back into the collection tubes provided and add 600 µl of Wash Buffer 1 to each column. Close the cap and centrifuge at 4000 x g for 30 seconds at RT. Discard the flow through and repeat.
4. Add 600 µl of Wash Buffer 2 to each column and centrifuge at 4000 x g for 30 seconds at RT. Discard the flow through and repeat.
5. Add 600 µl of Wash Buffer 3 to each column and centrifuge at 4000 x g for 30 seconds at RT. Discard the flow through and repeat.
6. Spin dry at top speed for 30 seconds at RT to remove any remaining liquid from the spin column. The original collection tubes should be discarded at this point and columns transferred into clean dry 1.5 ml collection tubes (provided).
7. Add 50 µl **ChIP-seq Elution Buffer** to each column, cap and incubate at RT for 15 minutes.
8. Centrifuge the columns at top speed for 30 seconds to collect the eluted chromatin.

At this stage samples can also be analysed by Mass Spectrometry, for this Chromatrap® recommends pooling IP'd samples to ensure sufficient protein for sample complexity. For sequencing and qPCR analysis please proceed directly to reverse cross linking.

# Step 4 – Reverse cross-linking

**Step 4a: Reverse cross linking**

Chromatin samples must be reverse cross-linked to release the DNA from protein bound complexes. Protein is then degraded by Proteinase K digestion before being purified in Step 4b of the protocol. Input controls which have not been through the IP process (Step 3a.3) must be reintroduced at this stage and treated as per the sample.

1. To each eluted sample add 5 µl of 1 M $NaHCO_3$, 5 µl of 5 M NaCl and make up to a final volume of 110 µl with water. To each input add 5 µl of 1 M $NaHCO_3$ and 5 µl of 5 M NaCl for a final volume of 110 µl. Mix thoroughly and incubate for 2 hours at 65°C. If required, the incubation at 65°C can be performed overnight.
2. Add 1 µl Proteinase K to each IP and input sample. Vortex briefly and perform a short spin. Incubate for 1 hour at 37°C.
3. Add 2 µl Proteinase K Stop Solution to each IP and input sample. Vortex briefly and perform a short spin.

**N.B.** *If validating by qPCR, take a 10 µl aliquot of the input and dilute 1 in 10 or 1 in 100 with water and deduct 3.3/6.6 Cts respectively, with 100% primer efficiency. With the remaining 100 µl input please proceed to step 4b.*

**Step 4b: DNA purification**

Chromatin must now be purified before proceeding with qPCR or library synthesis. DNA purification columns and reagents are included in all Chromatrap® ChIP-seq kits to recover ultra pure DNA from ChIP samples. Alternatively, DNA can be purified by phenol/chloroform extraction using an inert carrier such as linear polyacrylamide (LPA). The use of glycogen as a carrier is not recommended due to potential contamination with nucleic acids from a biological source.

**N.B. DNA Wash Buffer** must be prepared **before first use**. Add 60 ml ethanol (95-100%) to the DNA Wash Buffer concentrate and note on label that ethanol has been added.

Some of the components of this product are irritants, refer to MSDS sheet for more information and follow safety guidelines of your research facility.

1. Add 5 volumes of DNA Binding Buffer to 1 volume of sample and mix.

DNA Binding Buffer contains an integrated pH indicator. DNA adsorption requires a pH ≤7.5, and the pH indicator in the buffers will appear yellow in this range. If the pH is >7.5 the binding mixture will turn orange or violet and means that the pH of the sample exceeds the buffering capacity of the DNA Binding Buffer and DNA adsorption will be inefficient. In these cases add 10 µl 3 M Sodium acetate, pH 5, to adjust the pH of the binding mixture, the colour of the mixture should turn yellow.

2. Place a Chromatrap® DNA purification column in collection tube provided and transfer sample onto column.
3. Centrifuge at 16,000 x g for 60 seconds. Discard flow through.
4. Add 700 µl DNA Wash Buffer to each Chromatrap® DNA purification column and centrifuge at 16,000 x g for 60 seconds. Discard flowthrough. Centrifuge the Chromatrap® DNA purification column once more at 16,000 x g for 60 seconds to remove residual Wash Buffer.
5. Place the Chromatrap® DNA purification column in a clean 1.5 ml microcentrifuge tube.
6. To elute DNA, add 50 µl DNA Elution Buffer to the centre of the membrane and incubate for 1 minute, centrifuge at 16,000 x g for 60 seconds.

Samples are ready for validation by qPCR and library preparation.

# Step 5 – Quantitative PCR analysis

Prior to sample sequencing, we recommend analysing the IPd DNA by qPCR using at least one positive and one negative control to validate the IP. The Chromatrap® ChIP-seq kit contains positive control primers for human GAPDH.

1. Prepare the qPCR reaction mix as follows for a 10 µl reaction volume:
- 5 µl of a 2x SYBR® Green qPCR mix
- 2.5 µl primer mix (combine primers 1:1)
- 2.5 µl IPd or input DNA

Primer concentrations may need to be adjusted but we recommend a final concentration of 1 µM in the reaction mix for each primer.

Program the thermal cycler as follows for the positive control primers supplied.

Two minutes at 95°C
10 seconds at 95°C
30 seconds at 60°C
15 seconds at 72°C
} 40 cycles

These conditions may require optimisation depending on the primer, qPCR mix and qPCR system used.

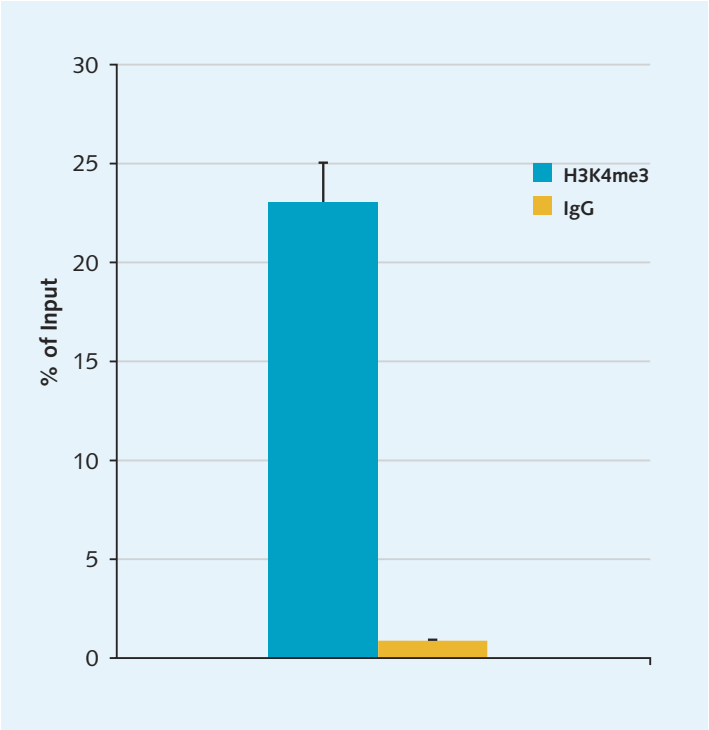


**Figure 3:** *ChIP was performed on human cancer cells using the Chromatrap® UniqSeq Kit (Cat. no. 500264/500265). IP was performed with the positive ChIP control antibody H3K4me3 using 2 µg antibody and 1 µg chromatin, and qPCR performed with positive control GAPDH primer set. The data is presented as mean % input (the relative amount of IPd DNA compared with input DNA after qPCR analysis).*

# Troubleshooting and FAQs

| Process | FAQ | Solution |
|---|---|---|
| **Cross-linking and fixation** | How long should the cells be cross-linked? | Optimal cross-linking of DNA ensures that the chromatin structure is preserved during the isolation and ChIP procedure. Too little cross-linking will result in DNA loss. Over cross-linking can result in elevated background and reduced antigen availability. The optimal time for cross-linking will vary with cell and tissue type. Short incubations may improve shearing efficiency whilst over-incubation can cause inhibition, hampering the ChIP assay. |
| **Cell Lysis** | How do I ensure cells are completely lysed? | Ensure that adequate Lysis Buffer volume is used for the number of cells being processed. Check lysis under a light microscope.<br><br>Ensure that adequate Lysis Buffer volume is used for the weight of the tissue being processed. During the two lysis steps (Hypotonic and Lysis Buffer) make sure the cells do not settle at the bottom of the tube by flicking the tube every few minutes to ensure efficient cell lysis. |
| | How do I prevent protein degradation? | Add protease inhibitors to the chromatin at the appropriate step. Proteases can degrade proteins crosslinked to the DNA, resulting in less efficient IP. If protein degradation is a problem, 1 µl PIC can be added to the ice-cold PBS before collecting the cells for chromatin extraction. Ensure that chromatin extraction steps are performed at 4°C and always keep the samples on ice when processing. |
| | Why is the Lysis and/or ChIP-seq Elution Buffer cloudy? | The Lysis and ChIP-seq Elution Buffers contain detergents which precipitate at 4°C. Warm the buffer to 37°C in a water bath for 30 minutes or until fully redissolved. Return to room temperature before use. |
| **Cell type** | What cell types have been validated for use with this protocol? | This protocol has been optimised for both adherent/suspension cells and fresh/frozen tissue, careful planning for chromatin collection from different sources needs to be optimised by the user.<br><br>If using FFPE tissue, refer to Chromatrap® FFPE ChIP protocol.<br><br>The key requirement of working with tissue samples is to obtain a unicellular starting suspension before proceeding with any sonication steps. More stringent grinding and sonication steps to disaggregate the cells may be required if working with yeasts or plant tissues. |

| Buffer | Cell Count | Buffer volume (µl) |
|---|---|---|
| 0.65 M Glycine | 1000<br>50,000<br>100,000 | 200<br>400<br>800 |
| Hypotonic Buffer | 1000<br>50,000<br>100,000 | 100<br>200<br>400 |
| Lysis Buffer | 1000<br>50,000<br>100,000 | 100<br>100<br>100 |
| Digestion Buffer | 1000<br>50,000<br>100,000 | 100<br>100<br>100 |
| Enzymatic Stop Solution | 1000<br>50,000<br>100,000 | 2.5<br>2.5<br>2.5 |

**Table 6** *Buffer volumes for low cell numbers*

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| **Number of cells** | How do I determine the optimal number of cells for ChIP-seq? | An important consideration when performing ChIP-seq is the amount of chromatin that will need to be loaded to the column in order to elute sufficient IPd DNA for library synthesis. The DNA yield obtained from ChIP will depend on the quality of the chromatin, the affinity and avidity of the antibody and the abundance of the target. A minimum of 500 pg of DNA is required for preparation of high quality NGS sequencing libraries using the Chromatrap® ChIP-seq library preparation kit. As little as 1000 cells can yield this quantity of DNA from ChIP of an abundant target using a good quality antibody. As a starting point we would recommend using 10 µg chromatin per ChIP with 2-5 µg antibody. |
| | What buffer volumes should I use for low cell numbers? | The amount of cross-linking solution and 0.65 M glycine required will depend on the size of the vessel the cells are contained in. Ensure sufficient solution is added to completely cover the cells. For example, 1 ml of solution is sufficient for a 6 or 12 well plate and 500 µl for a 48-well plate. It is recommended when using low cells numbers the cells and nuclei are lysed using 100 µl Hypotonic Buffer and 100 µl Lysis Buffer respectively. Chromatin should be sheared using the protocol optimised by the user and is not cell number dependent. For a general guide see Table 6 above. |
| | How much tissue do I need for chromatin extraction? | This protocol has been optimized using 5 mg – 300 mg fresh/frozen tissue. Chromatin collection from less or more tissue needs to be optimised by the user. |
| | How small do I need to cut the tissue sample? | Chop the tissue into small pieces (approximately 1 $mm^3$) using a scalpel blade. The smaller the pieces of tissue, the more efficient the extraction. |

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| | Some of my tissue sample remains in the petri dish, what should I do? | When transferring the sample from the petri dish to the 15 ml centrifuge tube, tilt the petri dish and wash the cells form the top of the petri dish to the bottom a few times to collect most of the tissue at the bottom of the petri dish. While still tilting the petri dish, transfer as much solution and tissue as possible to a 15 ml centrifuge tube. Cutting the end of a 1 ml pipette tip will help to transfer the tissue in solution. If tissue remains in the petri dish, add 5 ml cold PBS and transfer as much sample as you can to the 15 ml centrifuge tube. |
| **Chromatin shearing by sonication** | Why do I have a poor yield of sheared chromatin? | Cells may be over cross-linked, making them resistant to lysis and shearing. Ensure cells are fixed for the appropriate time or reduce incubation with formaldehyde. Make sure that the appropriate buffer volumes have been used. |
| | What sonication conditions should I use? | We have found that 30 second ON/OFF pulses for 15 minutes at a high power setting produces chromatin fragments of 100-500 bp. Ensure that the sample is kept at 4°C during the OFF phase. Shearing conditions may need optimisation by the user. |
| | Can I use enzymatic shearing? | Yes, enzymatic digestion of chromatin is an ideal method of shearing DNA if a sonicator is not available. Shearing conditions should be optimised to ensure 100-500 bp fragments are generated. |
| **Chromatin shearing by enzymatic digestion** | Why is my chromatin under sheared? | If only larger bands (e.g. 400 bp and above) are seen in the gel the amount of Shearing Cocktail in the digestion may need to be increased. Try increasing the U:chromatin ratio in the reaction (e.g. 1 U Shearing Cocktail per 2 µg chromatin). Cell membranes may not have been lysed efficiently in Hypotonic Buffer to allow the Shearing Cocktail access to the chromatin. Check cell lysis during Hypotonic Buffer incubation (Step 10, Section B) using a phase contrast microscope to ensure all the nuclei are released before resuspension in digestion buffer. If membranes are not efficiently lysed during the 10 minute incubation time in Hypotonic Buffer try incubating the samples for longer, monitoring the cell lysis using a phase contrast microscope to determine the optimum time for your cells. If membranes do not lyse following extended incubation in Hypotonic Buffer then cells may not be suitable for enzymatic shearing try sonication refer to Chromatrap® spin columns protocol. |
| | Why is my chromatin over sheared? | If chromatin is over sheared i.e. completely digested to mononucleosome fragments then the amount of Shearing Cocktail in the digestion may need to be reduced. Try reducing the U:chromatin ratio in the reaction (e.g. 1 U Shearing Cocktail per 10 µg chromatin). |

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| **Shearing efficiency** | How much chromatin should I load into the gel? | Adequate chromatin should be loaded into the gel for visualisation against the ladder. Do not over- or under-load as this may hinder visualisation. Typically 15-30 µl of the reverse crosslinked stock is adequate for analysis. |
| | What percentage of agarose should I use? | Use a 1-2% agarose gel. |
| | What buffer should I use? | Prepare a 1x TAE or TBE buffer for electrophoresis. |
| | What electrophoretic conditions should I use? | Run the gel slowly at 100-120 V until the dye-front has migrated at least 2/3 the length of the gel. |
| **Chromatin IP** | Do the Chromatrap® spin columns require blocking? | There is no requirement to carry out a blocking step as the spin columns and buffers have been formulated to minimise non-specific binding. |
| | How much antibody should be used per ChIP? | This should be determined empirically and is dependent on the amount of chromatin used per IP and the quality of the antibody. We recommend using 1-10 µg antibody per IP taking into account the amount of chromatin used and the quantity of DNA required downstream. Insufficient antibody may result in poor IP whereas excess can cause non-specific binding and lower specificity. |
| | What is causing high background? | The quality of the ChIP antibody has a major impact on the success of the assay. Use only ChIP-seq validated antibodies. Inefficient wash steps can also leave traces of non-specific chromatin alongside enriched DNA. If background remains high include an additional wash step during the IP protocol. |
| | Why do I not have any enrichment? | The antibody used must be ChIP validated. It is essential to include ChIP validated positive and negative antibody controls. Antibodies from other applications may not work in ChIP. The ChIP-seq kit contains a positive control antibody, H3K4me3 (Chromatrap® cat no. 700010), to validate the efficiency of the IP. |
| **Reverse cross-linking** | How long should samples be reverse cross-linked? | A minimum of 2 hours at 65°C. Although, samples can be left overnight if necessary. We recommend the use of DNA LoBind tubes to minimise sample loss during heating. |
| **qPCR** | What SYBR® reagents can I use? | The following SYBR® reagents have all been shown to produce optimal results with the positive and negative antibodies and GAPDH primers provided in the kit; iTaq™ Universal SYBR® Green Supermix, PerfeCTa SYBR® green supermix, SsoAdvanced™ SYBR® Green Supermix, IQ™ SYBR® Green Supermix. |

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| | What primers are included in the kit? | The kit contains GAPDH primers for human cells. The forward primer sequence is TCGACAGTCAGCCGCATCT, the reverse primer is CTAGCCTCCCGGGTTTCTCT and the amplicon is 69 bp. These primers are not compatible with chromatin from non-human species. |
| | What positive and negative controls should I use? | Use the kit supplied GAPDH primers as a positive control for ChIP using the supplied H3K4me3 antibody and a negative ChIP control using the supplied non-specific rabbit IgG (Chromatrap® cat. no. 700014). Alternatively, as a negative control, a gene locus not occupied by the target protein can be amplified in qPCR. The supplied GAPDH primers are only suitable for chromatin from human sources. For non-human chromatin it is recommended that the user design control primers around a region know to be occupied by H3K4me3, or another suitable target is used. In addition, it is important to amplify the Input with all primer sets for data interpretation. |
| **DNA profiling** | Why are the NanoDrop and Qubit readings so dissimilar? | The NanoDrop cannot accurately quantify the typically low concentration of IPd DNA. Use a fluorometer such as the Qubit to accurately quantify DNA before library preparation. |
| | What concentration of DNA should I use for the Bioanalyzer? | The quantitative range of the Bioanalyzer high sensitivity kit is 5-500 pg/µl. IPd DNA may not require diluting; see library synthesis guide for instructions. Load a maximum of 5 ng/µl of sample for analysis using the Bioanalyzer pre-library synthesis. |
| **Library synthesis** | How much DNA is required for library synthesis? | The amount of DNA required to prepare an NGS library will depend on the library preparation kit used, check the manufacturers' guidelines. A minimum of 500 pg of DNA is required to prepare a library of sufficient quality and complexity using the Chromatrap® UniqSeq kit. It is recommended that for optimal library complexity the user prepares libraries using as much ChIP DNA as possible. |
| | How many PCR cycles should I use for library enrichment? | The number of cycles required to amplify libraries to sufficient concentration depends on the quality and amount of input DNA. It is recommended that the minimum number of PCR cycles that yields sufficient library quantity for sequencing is used to maintain library complexity. |
| **Sequencing and data quality** | Why do I have adapter dimers in my Bioanalyser analysis and/or sequencing data? | It is essential that libraries are cleaned and size selected both before and after PCR enrichment to ensure unwanted DNA fragments such as unligated primers or primer dimers are not carried over into the sequencing reaction. Ensure the DNA clean up method used removes DNA fragments ≤200 bp in length. |

| | | |
|---|---|---|
| | Why do I have high levels of duplication? | ChIP-seq enriches specific fragments of DNA associated with a protein of interest. Therefore high duplication levels in the IP are not unusual. However, if the control sample also has high levels of duplication then we recommend loading more starting material during library preparation to reduce PCR sequence bias introduced during library enrichment. Use the minimum number of PCR cycles necessary to achieve the desired library concentration to minimise PCR bias during enrichment. Loading more DNA generally allows the number of PCR cycles to be reduced and leads to improved duplication rates. |
| **IP sequencing controls** | What control should I use? | We recommend using an input as the background control. |
| **Sample storage** | How should I store my IPd DNA? | Ideally at -80ºC for a maximum of three months. We recommend the use of DNA LoBind tubes to minimise sample loss during storage. |

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# Other products available from Chromatrap®

## ChIP products

| Product | Quantity | Catalogue no. |
|---|---|---|
| Chromatrap® ChIP-seq Pro A | 24 | 500189 |
| Chromatrap® ChIP-seq Pro G | 24 | 500190 |
| Chromatrap® HT ChIP-seq Pro A | 1 x 96 | 500214 |
| Chromatrap® HT ChIP-seq Pro G | 1 x 96 | 500215 |
| Chromatrap® Enzymatic ChIP-seq Pro A | 24 | 500191 |
| Chromatrap® Enzymatic ChIP-seq Pro G | 24 | 500192 |
| Chromatrap® HT Enzymatic ChIP-seq Pro A | 1 x 96 | 500216 |
| Chromatrap® HT Enzymatic ChIP-seq Pro G | 1 x 96 | 500217 |
| Chromatrap® FFPE ChIP-seq Pro A | 24 | 500235 |
| Chromatrap® FFPE ChIP-seq Pro G | 24 | 500236 |
| Chromatrap® Native ChIP-seq Pro A | 24 | 500237 |
| Chromatrap® Native ChIP-seq Pro G | 24 | 500238 |
| Chromatrap® UniqSeq kit Pro A | 24 | 500264 |
| Chromatrap® UniqSeq kit Pro G | 24 | 500265 |
| Chromatrap® UniqSeq Enzymatic Pro A | 24 | 500266 |
| Chromatrap® UniqSeq Enzymatic Pro G | 24 | 500267 |
| Chromatrap® *Drosophila* ChIP-seq kit Pro A | 24 | 500279 |
| Chromatrap® *Drosophila* ChIP-seq kit Pro G | 24 | 500275 |
| Chromatrap® *Drosophila* UniqSeq kit Pro A | 24 | 500276 |
| Chromatrap® *Drosophila* UniqSeq kit Pro G | 24 | 500277 |
| Chromatrap® Sonication Shearing | – | 500239 |
| Chromatrap® Enzymatic Shearing | – | 500165 |

## DNA products

| Product | Quantity | Catalogue no. |
|---|---|---|
| Chromatrap® DNA Purification | 50 | 500218 |
| Chromatrap® Gel Purification | 50 | 500219 |
| Chromatrap® HT DNA Purification | 2 x 96 | 500220 |
| Chromatrap® HT DNA Purify and Concentrate | 2 x 96 | 500240 |
| Chromatrap® DNA Extraction | 50 | 500260 |
| Chromatrap® HT DNA Extraction | 2 x 96 | 500261 |
| Chromatrap® Size Selection | 50 | 500262 |
| Chromatrap® HT Size Selection | 2 x 96 | 500263 |

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