ChIP-seq library preparation using fish embryos (Atlantic salmon and Rainbow trout)

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This document describes different steps of ChIP-seq library preparation using fish embryos (Atlantic salmon and rainbow trout). The main steps of library preparation are listed below:

Step 1- Embryo extraction (de-chorionation, pooling of embryos for replicates)

Step 2- Crosslinking DNA-protein interactions using 1% Formaldehyde

Step 3- Cell lysis and chromatin shearing (using Chromatin EasyShear Kit - High SDS from Diagenode # C01020012)

Step 4- Chromatin Immunoprecipitation and Tagmentation (using µChIPmentation kit from Diagenode # C01011011)

Step 5- Library amplification and purification (using 24 UDI for tagmented libraries set I and set II from Diagenode # C0101134 and # C0101135)

Step 6- QC of libraries (Qubit measurements, qPCR validation)

Wherever appropriate, I have included representative figures for some of the steps. Step 2 and 3 were optimised for the fish embryos, therefore, I have provided detailed instructions for these two steps and inserted pages at the relevant sections in the Diagenode's instruction manual. For Step 4 and 5, we essentially followed instructions as per the µChIPmentation Kit manual with slight tweaks, these minor changes in the form of embedded text are mentioned at the respective sections in µChIPmentation manual.

Step 1- Embryo extraction (de-chorionation, pooling of embryos for replicates)

Overview: This step describes the method used to extract salmonid embryos from eggs, and clean them from other egg constituents, such as proteins and oils which can interfere with Trypsin digestions and downstream library preparations for ChIP-seq.

Consumables:

- Petri dishes
- Cold water from egg trays
- Watchmaker forceps
- 1.5 mL Eppendorf tubes
- PBS
- DMEM
- FBS
- Vortex
- Fixed angle centrifuge
- Crushed ice
- Ice tray

1. **Blastulation and early gastrulation.**

During blastulation the cell mass is not very tightly bound to the yolk and can be easily dissociated mechanically. However, it is also very easy to lose or break cells. Because of this, the best approach is to dechorionate eggs under a dissection microscope, and use a bore tip pipette to transfer each blastula to an Eppendorf tube:

1.1 Place required number of eggs in a petri dish with cold water from egg trays on ice.

Required number of eggs is stage-dependant and will need optimization for non-salmonid species. See table 1 for numbers of eggs used for salmonids.

1.2 Place another petri dish with cold PBS in the stage of a dissection microscope.

1.3 Transfer one egg to the petri dish under the microscope.

1.4 Dechorionate using watchmaker forceps. Take care not to damage blastula.

1.5 Use light mechanical pressure from the forceps to separate blastula from yolk.

For this step, a very careful and light scraping motion on the sides of the blastula seems to work best.

1.6 Using a P1000 set to 200 µL, with a bore tip pipette, transfer blastula to a 1.5 mL Eppendorf tube on ice.

1.7 Repeat for required number of eggs for each replicate.

See table 1. For egg numbers used in salmonids for different stages.

1.8 Let blastulas decant to the bottom of the tube, then remove as much supernatant as possible and add fresh 1000 µL of ice-cold fresh PBS.

1.9 Proceed to Crosslinking using formaldehyde (step 2, see below).

2. Mid gastrulation onwards

At some point during gastrulation, normally on the onset of epiboly, cells will be more tightly bound to each other and to the yolk surface. This makes it more challenging to isolate embryos under the dissection microscope, but it also makes it easier to separate the gastrulas from the yolk without accidentally dissociating them. Because of this, eggs at this stage are dechorionated on a well of a six well plate, and vortexed to separate embryonic mass from yolk and oils:

2.1 Pre-chill swinging bucket centrifuge to 4 ^{o}C.

2.2 Place required number of eggs for one biological replicate (see table 1) in a well of a six well plate, fill well with de-chlorinated, aerated, cold water (from egg trays). Place six well plate on ice.

2.3 Using watchmaker forceps, transfer one egg into another well, along with 1 mL of PBS.

2.4 Dechorionate egg and remove chorion, take care not to leave any embryonic contents inside chorion.

2.5 Repeat for all eggs in one biological replicate.

2.6 Transfer contents of well into a 1.5 mL Eppendorf tube, on ice.

2.7 Vortex tube for ~10 seconds.

2.8 Centrifuge on fixed angle centrifuge, 300 RCF for 6min at 4 ^{o}C.

2.9 Remove supernatant and add 1000 µL of fresh cold PBS.

2.10 Repeat stage 2.7 once.

2.11 Proceed to Crosslinking using formaldehyde (step 2, see below).

Table 1: Number of eggs required for one replicate for each downstream technique and stage of development. We decided these numbers based on estimations of cell counts at each stage and required number of cells per assay. Cell numbers per stage will differ for non-salmonids, so this numbers will need to be adjusted accordingly.

Technique	Blastulation	Early gastrula	Mid – late gastrula	Early somito-genesis	Mid somito-genesis	Late somito-genesis	Pharyngula
Cell. isolation	40 embryos	30	30	20	10	10	5
Crosslinking	40	30	30	20	10	10	5
Whole cryopreservation	50	50	50	50	25	25	25

Step 2- Crosslinking DNA-protein interactions using 1% Formaldehyde

Overview: This step describes the method used to crosslink proteins to DNA of whole salmonid embryos.

Consumables:

- 1% PFA solution
- Rotator system
- 1M glycine solution
- PBS
- Fixed-angle centrifuge

Perform each step for all biological replicates.

1. Pre-chill fixed angle centrifuge

2. For each replicate of pooled embryos (from Step 1: embryo extraction), remove the supernatant and resuspend embryos in 875 µL of 1% PFA.

3. Put tubes on a rotator at 50 rpm for X min, at room temperature.

Note: X min = 8 mins for blastula and gastrula, 10 mins during segmentation stages, 15 mins post-segmentation stages

4. Add 125 µL of 1M glycine. Continue rotating for an additional 10 mins at 50 rpm, at room temperature.

5. Centrifuge at 300 RCF, for 6 min at 4 ^{o}C.

6. Discard supernatant and resuspend in 1 mL PBS.

7. Store at -80 ^{o}C.

Step 3- Cell lysis and chromatin shearing

Overview: This step describes shearing of chromatin fragments using a bath sonicator (Diagenode Bioruptor). Chromatin shearing ensures release and solubility of chromatin fragments into the supernatant, the smaller fragments also improve the overall resolution of ChIP-seq peaks. The optimum size range of chromatin fragments for ChIP is 200-800 bp with a peak at 250-400 bp. Care should be taken to not over-sonicate the samples as this might result in loss of epitopes/proteins from chromatin fragments.

Consumables:

- Bath sonicator (Diagenode Bioruptor); hand-held probes could also be used for sonication.
- Rotator system
- Fixed-angle centrifuge
- 2 mL douncer
- Hank's balanced salt solution (HBSS) buffer
- PBS
- Chromatin EasyShear Kit - High SDS from Diagenode # C01020012
- 0.65 mL Bioruptor Pico Microtubes # C30010011
- Eppendorf DNA LoBind tubes 1.5 mL
- RNase (100 mg/mL)
- Proteinase K (20 mg/mL)
- MinElute PCR Purification Kit Qiagen
- Qubit Fluorometer and DNA High Sensitivity reagents
- Tapestation and D1000 High Sensitivity reagents

Take out the crosslinked samples from -80 freezer and leave at the bench until half-thawed, then keep on ice for 15-20 min for complete thawing.

1. Centrifuge the samples at 850 RCF for 5 min at 4 °C, discard the supernatant and wash embryos once with PBS (gently mix by 1 mL pipette).

2. Centrifuge at 850 RCF for 5 min at 4 °C, discard supernatant and dissociate embryos to single cells in PBS + PIC solution (1 mL PBS + 5 µL of 200x PIC supplied with the high SDS kit) using a P200 pipette.

   Dissociation of embryos to single cells (or minimum to a clump of 4-8 cells) is crucial as it later ensures complete lysis of cells prior to sonication step. Big

chunks of tissues are difficult to lyse and will subsequently lead to inefficient sonication.

I. Early stage embryos (165 hpf) will generally dissociate to single cells by pipetting with P200 and should not require dounce homogenisation.

II. For later stages, dounce embryos on ice for 20-60 cycles using a 2 mL douncer (tight pestle). Observe under microscope after every 20 cycles and proceed accordingly until majority of tissue has been homogenised to single cells or to clumps of 4-8 cells. See representative example below.

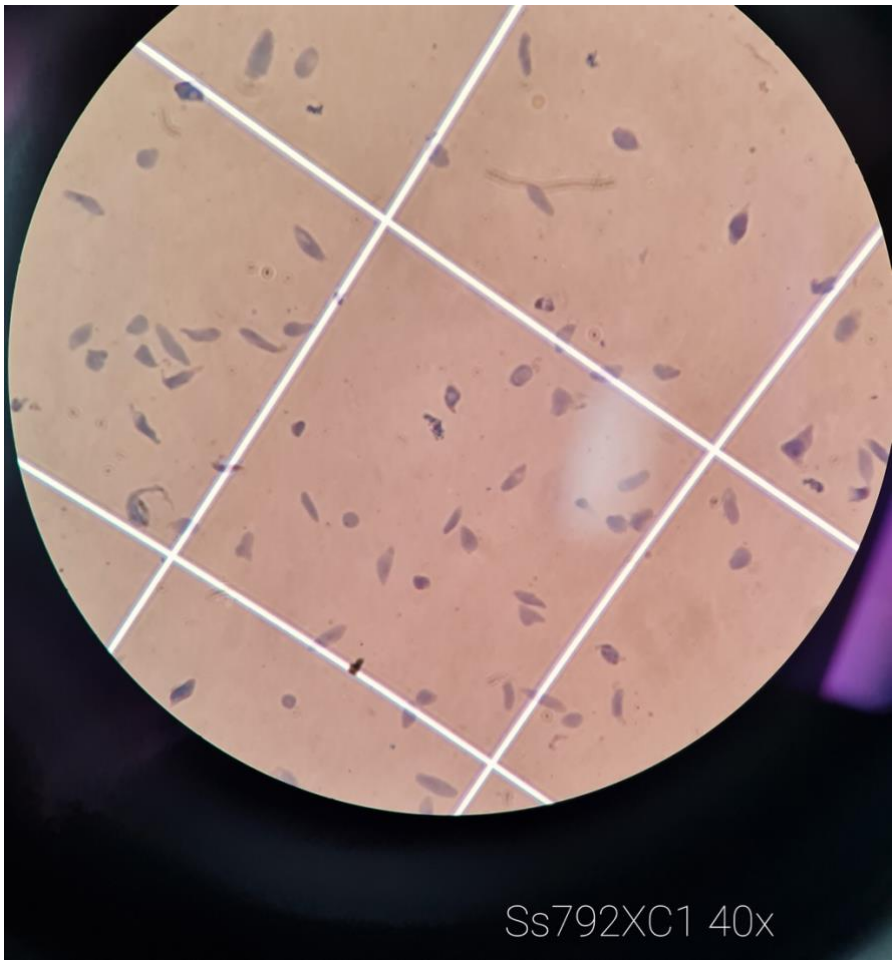

Figure 1: Homogenisation and dissociation of Atlantic salmon embryos (792 hpf) into single cells. Embryos were homogenised in 1 mL PBS + PIC using a 2-mL douncer for 50 strokes with tight pestle on ice.

3. Count cells using haemocytometer (mix 10 µL of the cell suspension with 10 µL of Trypan blue, load 10 µL on haemocytometer chamber).

The main idea here is to collect 500 thousand cells for each sample and then lyse them in final 500 µL of complete lysis buffer, this ensure same density of cells for all samples (i.e. 1,000 cells/ µL) which makes sonication optimisation much easier.

4. Pellet down 5 x 10^5 cells (850 RCF for 5 min at 4 °C). If a clear dense pellet is not visible, re-spin samples at 2,000 RCF for 10 min at 4 °C.

    I. Carefully discard the supernatant and lyse the pellet in 315 µL of tL1 lysis buffer + 2 µL of 200X PIC, mix by pipetting with P200. Vortex for 5-10 seconds and keep on ice for 20-30 min to ensure complete lysis.

    Observe lysis after 20-30 min, if samples look cloudy because of SDS precipitation, leave samples at bench for 2 min and then vortex, and observe. Pale yellow and homogenous solution indicates complete lysis. If big chunks of tissue/cells are visible, mix by P200 and incubate further for 5 min on ice. Complete lysis is crucial for efficient sonication.

    II. Add 185 µL of HBSS buffer and mix by pipetting with a P1000.

    III. Take out 100 µL of lysate into a 0.65 mL tube for sonication on Bioruptor. *Remaining samples can be stores at -80 freezer until further use (for 2-6 months).*

5. Sonicate 100 µL aliquots in 0.65 mL microtubes on Bioruptor bath sonicator (with our protocol, generally 3 cycles on Bioruptor are sufficient to shear chromatin to 200-800 bp; 1 cycle = 30 sec ON & 30 sec OFF).

    This sonication step needs to be optimised for each sample. Sonicate 100 µL aliquots in 0.65 mL microtubes for 3, 5, and 7 cycles and check chromatin shearing profile using Tapestation (High-sensitivity D1000 tapes). Choose number of cycles that gives you a good range of chromatin fragments (200-800 bp) with a peak at 250-400 bp.

6. Analyse chromatin shearing: It is important to analyse size range of chromatin fragments before proceeding to ChIP pull-down.

    Some tissues/embryo stage could be difficult to sonicate and will require more cycles of sonication to obtain chromatin fragments in the desired size range. After sonication, a small amount of the lysate is processed and

analysed on Tapestation and the remaining is stores in -80 freezer until ChIP pull-down. If chromatin shearing analysis suggests suboptimal sonication, the previously sonicated samples can be sonicated again until shearing profile looks good.

I. After 3 cycles of sonication on Bioruptor, centrifuge lysate at 17,000 RCF for 5 min at 15 °C.

II. Carefully transfer the supernatant to 1.5 mL DNA LoBind tube.

III. Of this, transfer 15 µL to a 0.2 mL PCR tube and use for shearing analysis, store the remaining lysate in -80 freezer until further use for ChIP pull-down.

Chromatin shearing analysis: 15 µL lysate in a 0.2 mL PCR tube

- Add 0.5 µL of RNase (100 mg/mL stock) to the lysate.
- Incubate at 50 °C for 1 hr on PCR block.
- Add 25 µL of tE1 and 2 µL of tE2, mix by pipetting.
- Reverse crosslinks at 65 °C for 4 hr on PCR block.
- Next, add 2 µL of proteinase K (stock 20 mg/mL).
- Incubate at 50 °C for 1 hr on PCR block.
- Purify DNA using Qiagen MinElute PCR Purification Kit
  - Note: Before use, add 1 µL of glacial acetic acid per 250 µL of PB buffer in a fume hood (to bring pH to acidic range for efficient binding to spin columns).
  - Finally elute DNA in 15 µL of EB (Elution Buffer).
- Measure DNA concentration using Qubit DNA high-sensitivity assay. Because the volume of original lysate and final elution is the same (i.e. 15 µL), Qubit measurements reflect amount of chromatin ng/µL in the original lysate.

  The amount of chromatin we use per ChIPmentation reaction is approximately 100 ng (equivalent to 34,000 cells for Atlantic salmon, genome size = 2.97 Gb). We use Qubit measurements of the purified DNA to calculate volume of lysate required per ChIPmentation reaction. For example, if shearing analysis and Qubit assays show that lysate concentration is 15 ng/ µL, we use 6.7 µL of lysate per ChIPmentation reaction.

- Check size distribution of chromatin fragments by analysing purified DNA on a Tapestation (D1000 High Sensitivity tapes, representative examples below).

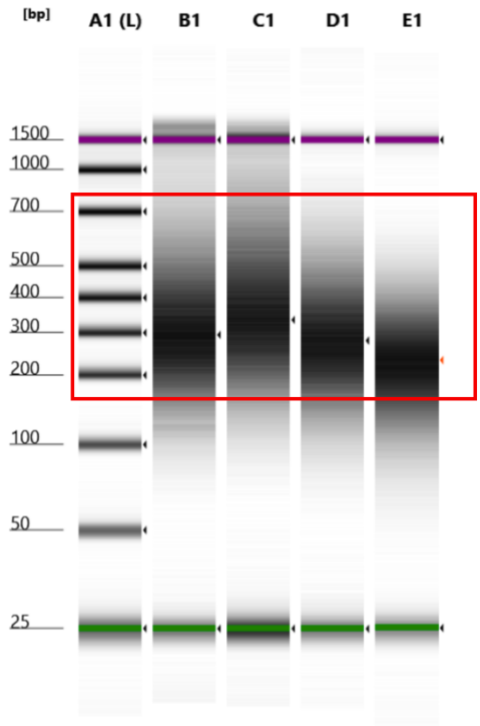

Figure 2: Tapestation profile of sonicated chromatin as seen on a D1000 High-sensitivity Tape. Red rectangle indicates desired size range of chromatin fragments (200-800 bp).

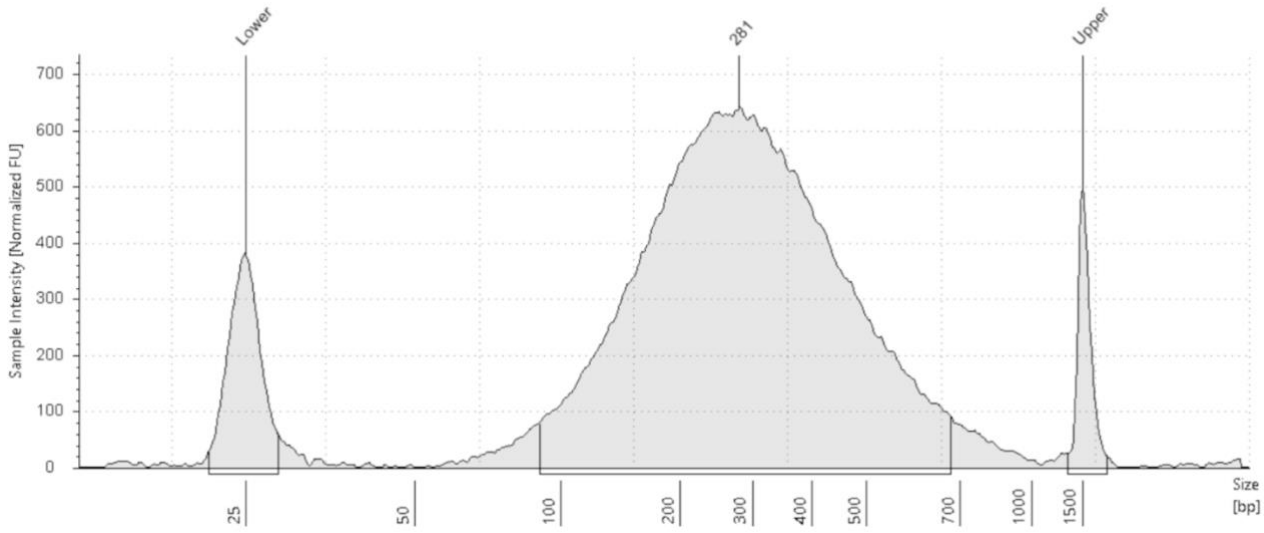

Figure 3: Tapestation profile of sonicated chromatin, peak size (281 bp) is well within the desired 250-400 bp range.

Step 4- Chromatin Immunoprecipitation and Tagmentation (using µChIPmentation kit from Diagenode # C01011011)

For this step, we essentially followed instructions as per µChIPmentation kit manual. The kit suggests using chromatin equivalent to a minimum of 10,000 cells per ChIPmentation reaction, we use approximately 100 ng chromatin per ChIP that is equivalent to 34,000 cells. Depending on the concentration of DNA/chromatin, the amount of lysate that goes into per ChIPmentation reaction varies from 5-20 µL. For detailed protocol, please refer below to µChIPmentation kit manual section- 'Magnetic immunoprecipitation and tagmentation', pages 19-22. Pages inserted below, please move to the next page.

Consumables:

- µChIPmentation kit from Diagenode # C01011011
- 0.2 mL tubes (DNase-free)

Magnetic immunoprecipitation and tagmentation

Day 1-2  1h overnight incubation, 1.5 hours

3.1 Determine the number of IP reactions to be run including the negative and positive control IPs. Take the required amount of DiaMag Protein A-coated magnetic beads and transfer it to a clean 1.5 ml tube. **10 µl of beads are required per IP**.

3.2 Wash the beads 4 times with **50 µl of ice-cold Beads Wash Buffer tBW1 per IP**. To wash the beads, add tBW1, resuspend the beads by pipetting up and down several times and place the tubes in the DiaMag1.5 magnetic rack. Wait for 1 minute to allow the beads to be captured by the magnet and remove the supernatant. Repeat this 3 times.

3.3 After the last wash, resuspend the beads in Beads Wash Buffer tBW1 adding the original volume of beads (this means 10 µl per IP).

3.4 Prepare the Immunoprecipitation mix as described in the table below. Add **150 µl** of **Immunoprecipitation mix** to each chromatin sample.

Component	Volume per reaction
HBSS	50 µl
ChIP Buffer tC1	100 µl
200x Protease Inhibitor Cocktail (black cap)	0.75 µl

Set aside **4 µl** of each sample to be used as an **input sample** and keep at 4°C.

3.5 Add the specific antibody to each tube.

NOTE: *The required amount of antibody per IP varies. Check the supplier's recommendation or perform a titration curve using different amounts of antibody. If a positive control IP is included, use 0.5 µg of the H3K4me3 positive control antibody (white cap). If a negative control IP is included, use 0.5 µg of Rabbit IgG (white cap).*

3.6 Add **10 µl of the washed magnetic beads** to each tube.

3.7 Incubate overnight at 4°C on a DiaMag Rotator.

NOTE: *A tube holder for 0.2 ml tube with a cap can be attached to the DiaMag Rotator with an elastic band.*

3.8 Perform the washes as follows: briefly spin the tubes and place them in the DiaMag0.2 magnetic rack. Wait for 1 minute and remove the supernatant. Add **150 µl of Wash Buffer tW1**: gently shake the tubes to resuspend the beads and incubate for 5 minutes on the DiaMag rotator at 4°C. Repeat the washing step as described above once with Wash Buffer tW2, tW3 and tagW1, respectively.

3.9 Prepare the ChIPmentation mix as described in the table below for the desired number of reactions, including the inputs. Mix thoroughly with a pipette. Keep on ice until used.

Component	Volume per reaction
Tagmentation Buffer (yellow cap)	19 µl
Tagmentase (loaded) (yellow cap)	1 µl

3.10 Put the tubes from step 3.8 on the DiaMag02. Wait until supernatant is clear and discard the supernatant.

3.11 Add **20 µl** of **ChIPmentation mix** to each IP tube and gently resuspend the beads by pipetting.

3.12 Add **20 µl** of **ChIPmentation mix** and **1 µl** of **MgCl2** (blue cap) to each input sample and gently mix by pipetting.

3.13 Incubate IP and input samples for 10 minutes at 37°C in the preheated thermocycler. After 5 minutes of incubation, briefly mix the tubes to resuspend the beads.

NOTE: *The recommended tagmentation time is 10 minutes, but the optimal time can vary depending on the cell number and the antibody used. See "Remarks before starting" section for more details.*

3.14 Put the samples on ice and immediately add **150 µl of cold Wash Buffer tagW2** to each IP sample, while setting aside the input samples. Gently shake the IP samples to resuspend the beads and incubate for 5 minutes on the DiaMag Rotator at 4°C.

3.15 Briefly spin the IP samples and place them in the DiaMag02, wait until supernatant is clear and discard the supernatant.

3.16 Add **150 µl of cold Wash Buffer tagW1** to each IP samples, gently shake the tubes to resuspend the beads and incubate for 5 minutes on the DiaMag Rotator at 4°C.

3.17 Briefly spin the tubes and place them in the DiaMag02, wait until supernatant is clear and discard the supernatant.

End repair, reverse cross-linking

 Day 2  30 minutes

4.1 Remove the strip from magnetic rack and add **25 µl of nuclease-free water** to each IP samples.

4.2 Add **25 µl of 2x High-Fidelity Mastermix** (violet cap) and to each IP and input sample, mix by pipeting and incubate as follows:

Step	Temperature	Time
End repair	72°C	5 minutes
Reverse cross-linking	95°C	10 minutes
	Cooling at 4°C (or ice)	

4.3 Magnetize beads from the immunoprecipitated samples and transfer the supernatant to a new 0.2 ml tube. Keep the samples at 4°C (or on ice).

NOTE: *The total volume of each IP or input sample is 50 µl.*

Step 5- Library amplification and purification (using 24 UDI for tagmented libraries set I and set II from Diagenode # C0101134 and # C0101135)

The kit suggests a pre-amplification qPCR step to determine total number of cycles for each sample, but this results in unnecessary loss of sample and is a bit of hassle, with 33,000 thousand cells most of the samples would anyway need a minimum of 12-15 PCR cycles for amplification. Therefore, we skipped this qPCR step and amplified all the samples for 15 PCR cycles to ensure reasonable yield enough for sequencing. For detailed instructions on PCR conditions for library amplification and purification of amplified libraries, please refer below to µChIPmentation kit manual sections- 'Library amplification' and 'Clean-up, pages 23-25. Pages inserted below, please move to the next page.

Consumables:

- 24 UDI for tagmented libraries set I and set II from Diagenode # C0101134 and # C0101135
- 0.2 mL tubes (DNase-free)
- AMPure XP beads
- Magnetic rack for 0.2 mL tubes

Library amplification

2 Day 2 2.5 hours

Determination of the optimal cycle number for the enrichment PCR

NOTE: *For this step only 2 µl of each library will be used.*

5.1 Dilute 5x the primers with the Primers Dilution Buffer before using them.

Skip qPCR steps and move to 5.7

5.2 Prepare the **Quantification Mix** as described in the table below for the number of desired reactions. Mix by pipetting and keep on ice until use.

Component	Volume per reaction
Primer Pair S1 (diluted)	0.4 µl
2x High-Fidelity Mastermix (violet cap)	5 µl
100x SYBR	0.1 µl
Nuclease-free water	2.5 µl

5.3 Dispense 8 µl of the Quantification Mix into 0.2 ml tubes or strips according to the number of libraries.

5.4 Add 2 µl of IP'ed or input DNA to each tube and mix by pipetting.

5.5 Briefly spin the tubes and run the PCR program described below.

Cycles	Temperature	Time
1	98°C	30 seconds
25	98°C	10 seconds
	63°C	30 seconds
	72°C	30 seconds
1	72°C	1 minute
1	10°C	∞

Keep the IP'ed and input DNA on ice during the qPCR.

5.6 Analyse the Ct values. The optimal cycle number for the amplification of the rest of the ChIPmentation DNA is typically Ct +1.

NOTE: *The Ct value is highly dependent on the thermocycler you use, as well as the way you analyze the qPCR results. Thus when using the kit for the first time you may need to verify that the Ct+1 rule applies well in your conditions.*

5.7 Add **2 µl** of the **diluted Primer Pair** with the appropriate index in each tube from step 4.3 and mix by pipetting.

NOTE: *The tubes already contain the mastermix as it was added at step 4.2.*

5.8 Briefly spin the tubes and run the PCR program described below.

Amplify for 15 cycles

Cycles	Temperature	Time
1	98°C	30 seconds
15 PCR cycles	98°C	10 seconds
	63°C	30 seconds
	72°C	30 seconds
1	72°C	1 minute
1	10°C	∞

Clean-up

2 Day 2 45 minutes

6.1 Carefully resuspend the room temperature AMPure XP beads by shaking and light vortexing until no pellet is visible at the bottom of the container.

6.2 Estimate the library volume and add **1.8x volume of AMPure XP beads** (e.g. for a sample volume of 50 µl, add 90 µl of beads). Mix by pipette 8 – 10 times until the mixture is homogeneous.

6.3 Incubate at room temperature for 10 minutes.

6.4 Place the tube on the DiaMag02 and wait until the beads are completely bound to the magnet (~2 minutes).

6.5 Carefully aspirate by pipette and discard the supernatant without disturbing the pellet.

6.6 Wash the beads pellet 2 times as follows:

- With the tubes on the magnet, add **100 µl** of freshly prepared **80% ethanol** without disturbing the bead pellet and wait for 5 seconds.
- Carefully aspirate by pipette and discard the supernatant without disturbing the pellet.

6.7 Leaving the tube open, let the beads dry on the DiaMag02 for 5 minutes.

6.8 Remove tubes from DiaMag02 and elute DNA by resuspending the beads in **20 µl** of **Resuspension Buffer**.

6.9 Incubate for 10 minutes at room temperature.

6.10 Place the tube on the DiaMag02 and wait until the beads are completely bound to the magnet (~2 minutes).

6.11 Without disturbing the pellet, carefully aspirate and transfer the supernatant containing purified libraries to a new tube.

Step 6- QC of libraries (Qubit measurements, qPCR validation)

Measure DNA concentration of purified libraries using Qubit DNA high sensitivity assays. We typically obtain 0.5 – 3 ng/µL concentration when libraries are eluted in 20 µL volume. Of this, 15 µL aliquot is sent to NGS service provider for sequencing and the remaining is diluted 1:20 in 0.1X TE and stored in -80 °C freezer until further use in qPCR validations. Tapestation profiling of µChIPmentation libraries is not very useful as it does not look very different than the input sample (no shift in size of libraries before/after adapter insertion because of tagmentation chemistry), therefore, we do not analyse libraries on Tapestation. Good yield (Qubit assay) and qPCR validations at positive and negative ChIP regions serve as two-step validation of library preparation success prior to NGS.

Consumables:

- Qubit Fluorometer and DNA High Sensitivity reagents
- PowerUp SYBR Green Master Mix (or any other qPCR master mix)
- PCR primers against positive and negative regions for the ChIP-ed protein
- 0.1X TE buffer

Protocol for qPCR validation:

1. Set up 10 µL volume qPCR reaction on a 96-well plate.

2x SYBR GREEN mix	5 µL
Primer mix (0.5 µM each of forward and reverse)	2 µL
DNA (1:20 dilute library)	3 µL

2. Perform qPCR assay and calculate enrichment using ‘percent input’ method.

An example for the calculation is shown below for three ChIPmentation libraries at TBP active promoter region. Because starting material for Input sample was 6% of the lysate that was used for ChIP samples, we need to adjust Ct values for Input samples by 4.06 ($\log_2$ 100/6 = 4.06).

Sample	Ct (dRn) Rep1	Ct (dRn) Rep2	Ct avg	Ct Adjust Input (Ct Input – 4.06)	Ct Adjusted Input – Ct IP	Enrichment 2^(Ct Adjusted Input - Ct (IP)	% Input (100 * Enrichment)
1 (Input)	32.1	32.7	32.4	28.3			
2 (H3K4me3)	27.0	27.2	27.1		1.2	2.3	228.9
3 (H3K27ac)	28.3	28.5	28.4		-0.1	0.9	94.0
4 (H3K27me3)	38.2	38.9	38.6		-10.3	0.0	0.1

3. Sequences of primer pairs used for qPCR validation are given below. (mix forward (a) and reverse (b) primers, each 0.5 µM final in 0.1X TE)

Atlantic Salmon	Active gene (H3K4me, H3K27ac)	5a_TBP_Act	ACAGCTCGGAGATTTAACGATGGTC
		5b_TBP_Act	CCTGGTTTCTGGCCTTTAACAGAA
		25a_Active	AGAATGTATGAGGAAACGTGTGA
		25b_Active	GCTGCTGCAGTCAGAGAAATA
	Repressed gene (H3K27me3)	29a_K27me3_Hoxc13	CCACCACCACCATACATACAC
		29b_K27me3_Hoxc13	GCAAACACAGTCAACACATCTC
		30a_K27me3_INF_For	ACCCACAACCTACTGAGAGA
		30b_K27me3_INF_Rev	CACATGTTTCACGTTGAGCTATT

Rainbow Trout	Active gene (H3K4me, H3K27ac)	31a_Om_TBP_-1kb	AGCGTTAGATTACGAGCGTTT
		31b_Om_TBP_-1kb	CAAAGCCACTACACAACACTAAAT
		32a_Om_TBP_-1	CGCCACTATTGCTGTCTGTA
		32b_Om_TBP_-1	AACCCTGCTGTCAAGAAACT
		33a_Om_FAS_Active	CATTAGTCACCAGGCTCAGATT
		33b_Om_FAS_Active	CCCACCCAGACTTGTGATAAA
		37a_Om_Kif4_Act	TTTAACTAACGTTCGTGGTGACT
		37b_Om_Kif4_Act	GCTGGCAAGACGTTGGT
	Repressed gene (H3K27me3)	36a_Om_Hox13_Rep	GGCTATTTGTTAGGTATGTCAATGT
		36b_Om_Hox13_Rep	CAAGCGTTATAAACAGTATAGATTGGG
		34a_Om_Hox_Rep	CGGAACCGAGACATGATACTTT
		34b_Om_Hox_Rep	GTGTTGACTGTGTTTGCTCTTT
		35a_Om_INF_Rep	GGACAGCTGGTGCGAATAA
		35b_Om_INF_Rep	CACGTTGTGCTATTCAGTTAAACA