DNA/DNA interactome profiling by crosslinked chromatin and DNA sequencing (Hi-C) in liver cells from chicken (Gallus gallus) and swine (Sus scrofa)

- Fr-Agencode project - Hi-C protocol –

adapted from Rao et al. 2014

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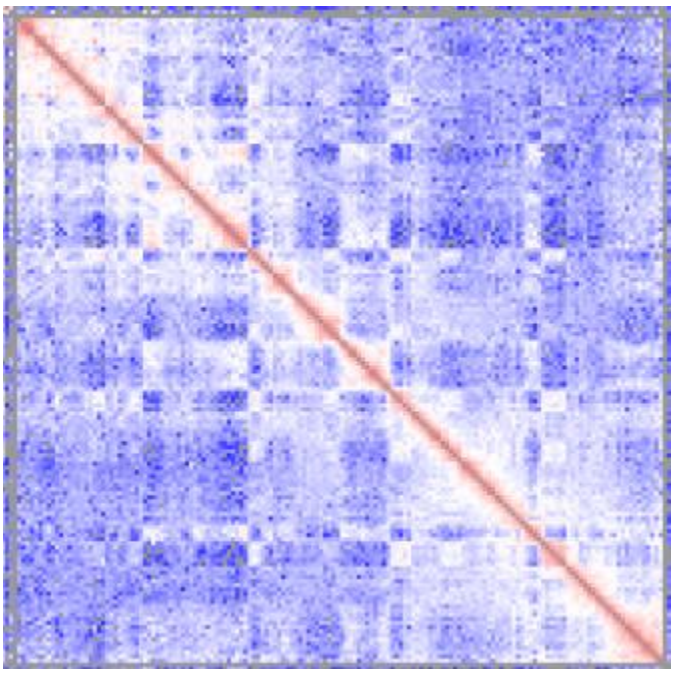

This protocol is divided into three major sections: cell preparation, in situ Hi-C, libraries production. Times are indicatives (it depends on the number of sample to be done). Working in duo is strongly advised to avoid errors.

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I PREPARATION OF SINGLE-CELL SUSPENSIONS FROM LIVER TISSUE (1/2 WORKING DAY)

Equipment

Centrifugation machine supporting 50 ml tubes
Microcentrifuge for Eppendorf tubes
Water bath
A routine microscope
Optional: an epifluorescence microscope

Reagents:

Glycine RNAse Free : Sigma: 50046-50G
Formaldehyde 37% : Sigma F8775-4X25ML
Protease inhibitors: Complete Mini EDTA-Free Roche: 04693159001 or SIGMA P8340-1ML
Accutase : StemPro Accutase (Thermo Fisher A1110501) or from any other provider
DMEM medium: DMEM with Glutamax (Thermo Fisher 10566016) or similar reference from any other provider
PBS w/o Calcium and Magnesium
Nuclear Extract kit (Active Motif, 40010)
Cell grinder: Potter+ glass tube (Sigma: P7734)
Trypan blue
DAPI
Phalloidin-TRITC (Sigma P1951)

Plastics:

Petri dishes (90mm)
Cell strainers 70µm
Falcon tubes 50ml or equivalents
Scalpel blades and forceps
Cell counting system (Malassez cell, Kova glasstic 10 slides…)

Before starting prepare: glycine 1.25M in water, ice cold PBS, ice-cold PBS with protease inhibitors. Pre-warm Accutase and DMEM at 37°C in the waterbath.
For section 1.3: Before starting, prepare ice cold hypotonic solution 1X from 10X plus Complete (protease inhibitor)

Optional: prepare freshly made 18.5% formaldehyde: in 4.8ml of water add 0.925g of Paraformaldehyde and 35µl of 1N KOH (or open a new bottle of formaldehyde 37%). Dissolve by heating in a water-bath at 70°C.

1.1 Liver cells dissociation and single-cell suspension production (60 minutes)

Objective: for each Hi-C experiment, prepare a suspension of 2 to 5 millions of hepatocytes.

- Dissect the liver.
- Keep a few cm^3 piece of tissue (generally around 5-10cm^3 is sufficient to produce around 20-40 millions of cells). Keep unprocessed tissue in PBS on ice (if an insufficient quantity of cells is recovered, additional assays can be performed to increase cell recovery).
- Dilacerate the tissue with scalpel blades as thin as you can. The quality of the chopped liver is really important for getting high yield of cells

- Wash the pieces of liver with PBS to remove blood as much as possible. For that, add room temperature PBS to the pieces, agitate well, decant and remove the supernatant, repeat until the supernatant become transparent. Remove the supernantant
- Add 20ml of 1X Accutase previously warmed at 37C and incubate for 15minutes on a rotating wheel at 37°C. Pipet up and down 20 times to homogenize the suspension (you can use first a 25ml pipet and then a 10ml pipet. if it is well done, you will not have problem to do it with a 10ml pipet, the suspension should look like a tomato soup or juice)
- Filter through a cell strainer of 70µm into a new 50ml tubes, add 20ml of DMEM medium.
- Centrifugate at 250g for 5 minutes
- Remove the supernatant and add 40 ml of medium (DMEM) w/o serum. Using a microscope, evaluate cell quality and count the cells. Checked that cells are well dissociated
- Centrifugate at 250g for 5 minutes
- Resuspend at a concentration of 5 million/cells per 10ml of medium without serum (do not exceed 40ml/tube)

1.2 Fixation of the single-cell suspension using formaldehyde (45 minutes)

Objective: fixe the nuclei architecture using crosslinking reagent

- Add 1% final of freshly made/opened formaldehyde to the growth media *(540µl of 18.5% formaldehyde for 10ml of medium, 270µl of 37% formaldhehyde for 10ml of medium)*.
- Incubate for 10 minutes at 37°C.
- Add 0.125M final Glycine to quench the fixative (*1.1ml of glycine 1.25M for 10 ml of medium*)
- Incubate 5 minutes at RT and cool 5 minutes on ice.
- Centrifugate at 1500rpm 5 minutes to pellet the cells
- Aspirate the supernatant and wash the cells with 10 ml ice cold PBS (with protease inhibitors)
- Remove 10µl of cells and stain the cell preparation with DAPI and phalloidin. Check nuclei quality and integrity under a fluorescent microscope (this could be done later).
- Centrifugate at 1500rpm 5 minutes to pellet the cells
- Keep cell pellets on ice and discard the supernatant
- Flash froze in liquid nitrogen and store at -80°C or proceed to nuclei purification.

1.3 Mechanic disruption of the cytoplasmic membrane (30 minutes)

Objective: break the cytoplasmic membrane to favour nuclei permeabilization

Before starting, prepare ice cold hypotonic solution 1X from 10X plus Complete (protease inhibitor)

If cells have been frozen in the previous step, cells are thawed on ice for 15 minutes

- Resuspend from 1 to 5 million-cells in 1ml of 1X hypotonic buffer.
- Incubate on ice for 15 minutes.
- Add 5µl of detergent mix (Active motif) and vortex 30s at maximum speed.
- Transfer the 1ml suspension into the 3ml glass tube of the cell grinder
- Homogenize the cells on ice with a potter-Elvehjem PTFE pestle. Slowly move pestle up and down 10 times, incubate on ice 1 min to let the cells cool down and then do 10 more strokes.

- Transfer the lysate to a 1.5ml eppendorf tube.
- Remove 10µl of cells and stain the cell preparation with DAPI and phalloidin. Check nuclei integrity and cytoplasmic membrane disruption under a fluorescent microscope (this could be done later).
- Centrifugate for 5 minutes at 2000g and discard the supernatant.
- Flash freeze in liquid nitrogen and store at -80°C or proceed to the next step.

II *IN SITU* DNA DIGESTION, LABELLING AND LIGATION (3.5 WORKING DAYS)

Equipment

Microcentrifuge for Eppendorf tubes
2 dry-baths (thermoblocks) for Eppendorf tubes
A rotating wheel (we use Diagenode DiaMag rotator B05000001)
A 37°C incubator
A vortex
Nanodrop or equivalent spectrophotometer
Optional(but strongly : an epifluorescence microscope

Reagents:

SDS : Sigma SDS 10% solution 71736-100ML
TRITON X-100 : Sigma T8787-50ML
HindIII restriction enzyme (high concentration): New England Biolabs: R0104M (100U/µl):
Digestion buffer: New England Biolabs : NE Buffer 2
Water Dnase/Rnase free (Sigma W4502-1L)
dNTP solution:NE Biolabs: Set 100mM N0446S
Klenow, high concentration: NE Biolabs M0210M (50U/µl)
Biotin-14-dCTP: Thermo Fisher 19518-018
T4 DNA ligase (high concentration) : New England Biolabs M0202 (2000U/µl)
T4 DNA Ligase Reaction Buffer: New England Biolabs B0202S
Proteinase K: Thermo Fisher (20mg/ml)
BSA (20mg/ml): New England Biolabs B9000S
Magnetic beads based DNA clean-up: CleanPCR (CPCR-0050-D)
T4 DNA polymerase: New England Biosciences M0203S
DAPI
Phalloidin-TRITC (Sigma P1951)
5M NaCl
0.5 M EDTA
96% absolute Ethanol

Plastics:

Eppendorf Tubes (safelock, 1.5ml and 2ml)
Eppendorf Tubes DNA LoBind Tube 1.5ml 022431021:

Before starting

If you do not use commercially available buffers, prepare:

10x NEBuffer 2: 100 mM Tris-HCl, 500 mM NaCl, 100 mM $MgCl_2$, 10 mM Dithiothreitol, pH 7.9 @ 25°C

10X Ligation buffer: 500 mM Tris-HCl pH7.5, 100 mM $MgCl_2$, 100 mM Dithiothreitol, 10mM ATP, pH7.5@25°C

Prepare 70%, 80% Ethanol solution (in water)

Before starting put a thermoblock at 62°C, prepare a 0.5% SDS solution in water

2.1 Nuclei lysis and Endonuclease-based DNA fragmentation (16h)

Objective: *in situ* genomic DNA fragmentation using HindIII endonuclease

- For each Hi-C experiment, prepare 3 pellets: one for HiC, one for 3C, one for control DNA. If cells are limiting, favor HiC, then 3C, then controls. Few cells are needed for control DNA (half million is largely enough). Use 2ml safelock Eppendorf tubes.
- Gently resuspend each pellet in 50µl of SDS 0.5% and incubate at 62°C for 10 minutes exactly.
- Remove tubes from the thermoblock and add 145µl of water and 25µl of Triton 10% to quench the SDS.
- Mix well avoiding foaming. Incubate at 37°C for 15 minutes with gentle mixing from time to time.
- Add 25µl of 10X NEbuffer 2.0 and 100U of HindIII and digest overnight at 37°C on the wheel.

2.2 Fill overhangs with marked dNTPs to obtain blunt ends (90 minutes)

Objective: labeling DNA at the restriction sites with biotin

- Prepare the biotin fill-in mix as follow:

MIX HI-C	
Fill-in mix (add in this order)	Per reaction 50µl final
H2O	21 µl
10 mM dATP	1 µl
10 mM dGTP	1 µl
10 mM dTTP	1 µl
0.4 mM biotin-14-dCTP	25 µl
50U Klenow (50U/µl)	1 µl

- Add 50µl of the fill-in mastermix to each Hi-C tube and mix by pipetting. Add 50 µl of water to the 3C and control tubes.
- Incubate the tubes at 37°C with rotation on the wheel for 1hour.
- Incubate at 62°C 20 minutes to inactivate the enzymes with agitation to avoid foaming and cell aggregates.

2.3 Ligation of digested DNA (24 hours)

Objective: *in situ* proximity ligation of cleaved DNA fragments

- While incubating tubes at 62°C, prepare the ligation mix as follows:

Ligation mix	Per reaction
Water	673 µl
10x ligation buffer	120 µl
10% Triton X-100	100 µl
20 mg/ml BSA	6 µl
T4 DNA ligase	1 µl (2000U)

- Add 900 µl of ligation mix to each tube (Hi-C, 3C and control (without ligase) assays)
- Mix by inverting and incubate 4 hours at room temperature with slow rotation on the wheel (that can be followed overnight at 4°C to improve ligation efficiency).
- For the three tubes (Hi-C, 3C and control), degrade proteins by adding 50µl of PK (20mg/ml) and 120µl of 10% SDS and incubate at 55°C for 30 minutes.
- Add 130µl of 5M NaCl and incubate at 68°C for 8 hours (or overnight).

2.4 Precipitating DNA (from 2 to 14 hours)

Objective: recover and concentrate DNA

- Cool tubes at room temperature.
- Split each sample into two 750µl aliquots in 2ml tubes and add 0.1volume of 3M sodium acetate pH5.2. (75µl) and 1.6 volumes 100% EthOH (1200µl). Mix by vortexing and incubate at -80°C for 15minutes or at -20°C for 2 hours (or overnight for maximale recovery).
- Centrifuge at maximum speed at 4°C in a microcentrifuge for 10 minutes.
- Remove the supernatant
- Resuspend in 500µl of 70% EthOH and centrifuge at maximum speed for 5 minutes.
- Repeat this step
- Dry the pellet (but not too much for easier resuspension)
- Dissolve the two pellets corresponding to one Hi-C or 3C reaction in 130µl of nuclease free water and control pellets into TE (10:0.1, Tris 10mM pH8.0, EDTA 0.1mM) (resuspend one pellet first and the second with the same buffer). Quantify DNA using a spectrophotometer (Nanodrop).

2.5 DNA purification using CleanPCR magnetic beads

Objective: desalt and purify DNA

Prewarm the beads at room temperature (at least 30')

- Resuspend 20 µg of DNA from the previous precipitation step (more is not necessary due to the binding capacity of the beads) in nuclease free water to a final volume of 200µl and in a low DNA binding tube.
- Vortex the beads stock 20'' to homogenize the solution
- Add 360µl of beads to samples and incubate 5' at RT.
- Place the tubes in a magnetic rack and let the magnetic beads pellet for some minutes.
- Remove the supernatant and wash beads two times 30'' with 300µl of EtOH 80%.
- After the second wash remove the remaining EtOH carefully using a P10 tip.
- Dry the beads for 3' and resuspend in:130µl of water for HiC samples and 130 µl of TE (10:0.1) for 3C samples.
- Store the 3C samples at -20°C and quantify by spectro (Nanodrop)

2.6 Removing non ligated biotinylated DNA

Objective: remove biotin-14-dCTPat non-ligated DNA ends using the 3'-5' exonuclease activity of T4 DNA polymerase

Always keep tubes on ice. Do not exceed the incubation time.

- To each 130µl of HiC reaction, add the following reagents:: 1 µl 20 mg/ml BSA + 15 µl 10x NEBuffer 2 + 1 µl 10 mM dATP + 1 µl 10 mM dGTP + 30 Units T4 DNA polymerase (10µl).
- Incubate at 12°C for 90 minutes.
- Place the tube on ice and stop the reactions by adding 2 µl 0.5 M EDTA pH8.0 and heat inactivate 20 minutes at 75°C.

2.7 Second DNA purification using CleanPCR magnetic beads

Objective: purify DNA before library preparation

Prewarm the beads at room temperature (at least 30')

- To each 160µl of reaction, add 288µl of beads suspension, mix and incubate 5' at RT.
- Place the tubes in a magnetic rack and let the magnetic beads pellet for some minutes.
- Remove the supernatant and wash beads two times 30'' with 300µl of EtOH 80%.
- After the second wash remove the remaining EtOH carefully using a P10 tip.
- Dry the beads for 3' and resuspend in 130µl of TE (10:0.1).
- Quantify DNA on a spectrophotometer and store eluate at -20°C.
  DO should be: 260/230>1.8 ; 260/280>1.8

2.8 Quality control of Hi-C and 3C experiments

Objective: check the efficiency of Hi-C assays before library preparation and sequencing

A two-step validation protocol: PCR are performed around one HindIII restriction site with to forward primers. Genomic DNA cannot be amplified with this primers. In Hi-C and 3C assays, some religation events allow to switch the sense of one DNA fragment and PCR amplification with these primers is possible. Then the PCR amplification products is digested either with HindIII or NheI. For 3C experiments, HindIII should cleave the PCR products while NheI should not. For Hi-C experiments, NheI should cleave most of the PCR products while HindIII should cleave only a small fraction.

We used the following primers for each species:

Pig (Sus Scrofa)(chr5: 66392739-66393203, sus scrofa 10.2)):
Pig Hi-C FW1 : TCTGGGCAGGTCACTCATT
Pig Hi-C FW2 : TCTCGGGATGCTGAGTGTTT
Pig Hi-C RV1: AAACACTCAGCATCCCGAGA
Size: FW1+RV1: 465 bp
3C/HiC size: FW1+FW2 : 425 bp (digest: HDIII/NHEI 201+215)

Chicken (Gallus gallus) (chr1:76435918+76436558, Galgal4) (gene GAPDH)
Chicken Hi-C FW1: ACCTCAGTGCTCTCTTTCCC
Chicken Hi-C FW2: AAGAGGGTAGTGAAGGCTGC
Chicken Hi-C RV1: GCAGCCTTCACTACCCTCTT
Size: FW1+RV1: 641 bp
3C/HiC size: FW1+FW2 : 610 bp (digest: HDIII/NHEI 299+ 311)

Goat (Capra Hircus) (>gi|541128986:69874000-69875500 Capra hircus breed Yunnan black goat chromosome 1, CHIR_1.0, whole genome shotgun sequence NCBP2)

Capra Hircus HiC FW3 : ACAAACACCACAAAACACTCCA

Capra Hircus HiC FW4 : TAGTAATTCCAGGTCCCAAGGT

Capra Hircus HiC RV3 : ACCTTGGGACCTGGAATTACTA

Size: FW1+RV1: 394 bp

3C/HiC size: FW3+FW4 : 497 bp (digest: HDIII/NHEI 187+ 310)

Cattle (Bos Taurus) (bosTau8_dna range=chr6:90236297-90237639, gene ALB)

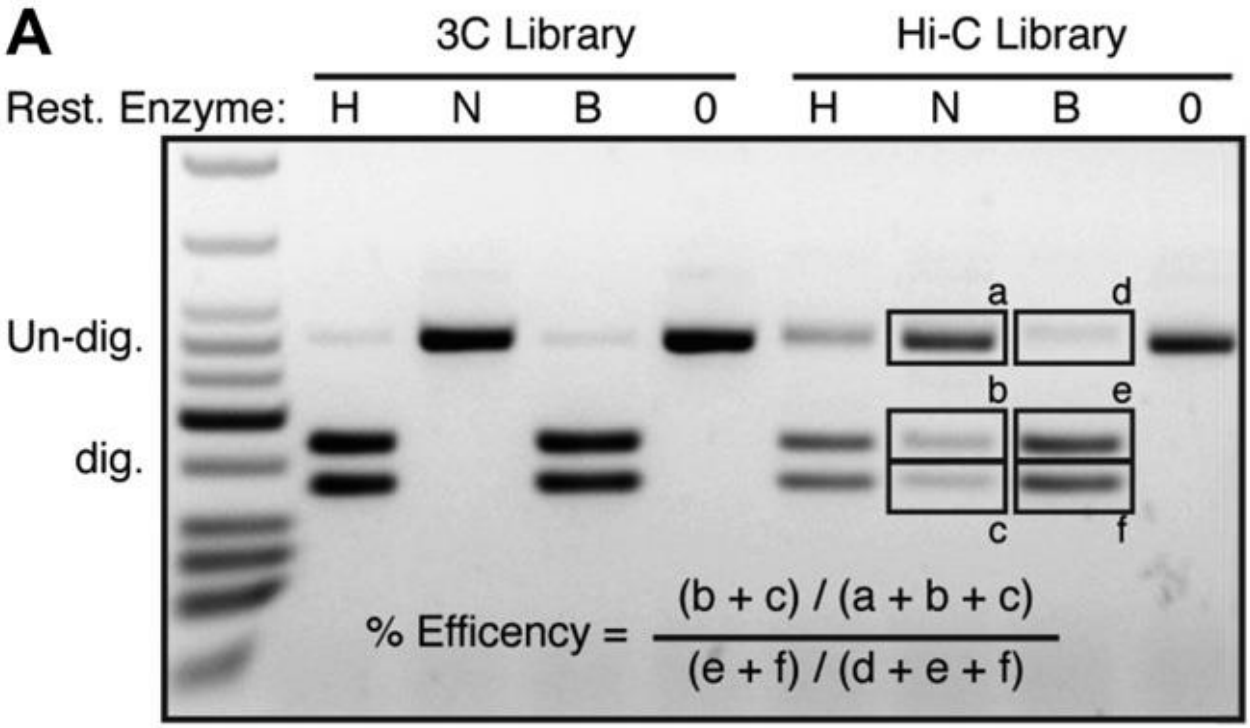
Bos taurus HiC FW5 : GTGTAAAGTTGCATCCCTTCGT

Bos taurus HiC FW6 : TGTGTGTGTGAGTTTGACCTTT

Bos taurus HiC RV5 : AAAGGTCAAACTCACACACACA

Size: FW5+RV5: 584bp

3C/HiC size: FW3+FW4 : 615bp (digest: HDIII/NHEI 210+ 405)



From Belton et al. 2012

III LIBRARIES PRODUCTION

Equipment

Microcentrifuge for Eppendorf tubes
2 dry-baths (thermoblocks) for Eppendorf tubes or a thermocycler
A vortex
Nanodrop or equivalent spectrophotometer
BioAnalyser or Fragment Analyser
Covaris

Reagents:

Nextera_Mate_Pair_Sample Preparation Kit – Box2 Wash Solutions
TruSeq Nano DNA kit (SetA + SetB)
Water Dnase/Rnase free (Sigma W4502-1L)
dNTP solution:NE Biolabs: Set 100mM N0446S
Magnetic beads based DNA clean-up: CleanPCR (CPCR-0050-D)
Magnetic streptavidin M280 Dynabeads
96% absolute Ethanol
AMPure Beads XP Agencourt (Beckman-Coulter, A63880)

Plastics:

Eppendorf Tubes (safelock, 0.5ml, 1.5ml and 2ml)
Eppendorf Tubes DNA LoBind Tube 1.5ml 022431021:

3.1 DNA fragmentation

Objective: cleave religated DNA

- Dilute 1.4µg of DNA in water to a final volume of 130µl and transfert to a Covaris 0.5ml MicroTUBE.
- Switch on the Covaris machine
- Load the microtube in the machine
- With the software Sonolab, choose the protocol **MicroTUBE Snap-Cap 550pb and run it**
- Transfer the sonicated DNA in a new 1.5ml tube.

3.2 Sizing of purified DNA

Objective: keep only DNA fragments between 200-600bp

Prewarm the beads at room temperature (at least 30')

- Add 70µl of water to the 130ul to reach a final volume of 200µl.
- Add 110µl of CleanPCR beads to the 200µl of DNA, mix and incubate 5' at RT.
- Place the tubes in a magnetic rack and let the magnetic beads pellet for some minutes.
- **Keep the supernatant** that contains fragments<600bp and transfer it into a new microtube.
- Add 30µl of CleanPCR beads to the supernatant, mix and incubate 5' at RT.
- Place the tubes in a magnetic rack and let the magnetic beads pellet for some minutes.
- Remove the supernatant and wash beads two times 30'' with 300µl of EtOH 80%
- After the second wash remove the remaining EtOH carefully using a P10 tip.
- Dry the beads for 3' and resuspend in 300µl of **Resuspension buffer (from either Nextera Mate Pair or TruSeq Nano kit)**
- Vortex, centrifuge briefly and incubate 2' at room temperature
- Place the tubes in a magnetic rack and let the magnetic beads pellet for some minutes.
- Transfer the 300µl of supernatant in a new tube of 1.5ml and proceed to next step

3.3 Purification of biotinylated DNA

Objective: keep only DNA with the filled-in religation sites

Wash the Dynabeads

- Vortex M-280 streptavidin magnetic Dynabeads
- Transfer 20µl of Dynabeads to a new 1.5ml tube, for each sample.
- Place the tubes in a magnetic rack and let the magnetic beads pellet for some minutes.
- Remove the supernatant and wash beads two times by:
- Adding 50µl of **Bead Bind Buffer (Nextera Mate Pair),** vortex 10s, place back in a magnetic rack, remove the supernatant
- Resuspend beads in 300µl of **Bead Bind Buffer (Nextera Mate Pair)**

Purify biotinylated DNA

- Mix the 300µl of beads with the 300µl of biotinylated DNA
- Incubate 15min at room temperature and vortex every 2 minutes to resuspend the beads
- Place the tubes in a magnetic rack and let the magnetic beads pellet for some minutes.
- Remove the supernatant and wash beads four times by:
    - Adding 200µl of **Beads Wash Buffer (Nextera Mate Pair),** vortex and centrifuge briefly
    - Place the tubes in a magnetic rack and let the magnetic beads pellet for one minute

    - Remove the supernatant
- After the fourth wash, add 200µl of **Resuspension buffer,** vortex and centrifuge briefly
- Place the tubes in a magnetic rack and let the magnetic beads pellet for one minute and remove the supernatant
- Add 200µl of Resuspension buffer, vortex and centrifuge briefly
- Place the tubes in a magnetic rack and let the magnetic beads pellet for one minute and remove the supernatant
- Immediately proceed to step 3.4

3.4 End-repair DNA ends and 3' A tailing

Objective: repair DNA breaks using Klenow fill-in and add a 3' A for further TA ligation of adapters

Thaw the End Repair Mix 2 on ice (TruSeq Nano kit)
Thaw the A tailing Mix on ice (TruSeq Nano kit)

From step 3.4 to 3.6 (included) all the reactions are made on DNA bound to the M280 streptavidin beads

End-repair

- For each sample, mix 60µl of water and 40µl of End Repair Mix 2
- Add 100µl of the mix to the tube with purified biotinylated DNA on magnetic beads
- Transfer the mix to PCR tubes
- Vortex and incubate 30minutes at 30°C in a thermocycler and hold at 4°C

DNA purification

- Transfer the mix to 1.5ml tube
- Place the tubes in a magnetic rack and let the magnetic beads pellet for one minute
- Remove the supernatant and wash beads four times by:
    - Adding 200µl of Beads Wash Buffer and vortex
    - Place the tubes in a magnetic rack and let the magnetic beads pellet for one minute
    - Remove the supernatant
- After the fourth wash, repeat 2 washes as follow:
    - add 200µl of **Resuspension buffer** and vortex
    - place the tubes in a magnetic rack and let the magnetic beads pellet for one minute and remove the supernatant
- Resuspend the beads in 17.5µl of water
- In case of using a thermocycler, transfer the 17.5µl of DNA to a PCR tube
- Add 12.5µl de A-Tailing Mix
- Mix by pipetting up and down
- Incubate 30 minutes at 37°C
- Inactivate the enzyme at 70°C for 5 minutes
- Store on ice or at 4°C and proceed immediately to step 3.5

3.5 Ligate Adapters

Objective: TA cloning of Adapters to the DNA extremities

Choose your index adequately (for low multiplexing) and write it down on your tracking sheet
Thaw on ice the selected adapters (kit TruSeq Nano or others)
Thaw on ice Resuspension Buffer (Nextera Mate Pair)
Thaw on ice Stop ligation buffer

- Add to the 30µl of beads with A-tailed DNA (step 3.4) :
    - 2.5µl of Resuspension Buffer
    - 2.5µl of DNA Ligase mix
    - 2.5µl of DNA Adapter Index
- Mix by pipetting up and down
- Incubate 10 minutes at 30°C
- Remove the tube from the thermoblock or the thermocycler
- Add 5µl of Stop ligation Buffer
- DNA purification on beads
- Transfer the mix to 1.5ml tube
- Place the tubes in a magnetic rack and let the magnetic beads pellet for one minute
- Remove the supernatant and wash beads four times by:
    - Adding 200µl of Beads Wash Buffer and vortex
    - Place the tubes in a magnetic rack and let the magnetic beads pellet for one minute
    - Remove the supernatant
- After the fourth wash,
    - Add 200µl of **Resuspension buffer** and vortex
    - place the tubes in a magnetic rack and let the magnetic beads pellet for one minute and remove the supernatant
    - Add 200µl of **Resuspension buffer,** vortex and keep on ice
- Immediately proceed to step 3.6

3.6 PCR amplification

Objective: amplify the library. 10 cycles of PCR amplification are largely sufficient when starting the library process with 1.4µg of DNA

Prepare the following mix:

- *25µl of Enhanced PCR mix (kit TruSeq Nano)*
- *5µl of PCR Primer Cocktail Truseq PCR*
- *20µl of H2O*

- Place the tubes in a magnetic rack and let the magnetic beads pellet for one minute and remove the supernatant
- Resuspend the beads in the PCR mix (50µl)
- Perform the following PCR cycles:

98°C - 45 seconds	
10 cycles :	98°C - 15 seconds 60°C - 30 seconds 72°C - 30 seconds
72°C - 60 seconds	
4°C	

3.7 DNA purification using CleanPCR magnetic beads

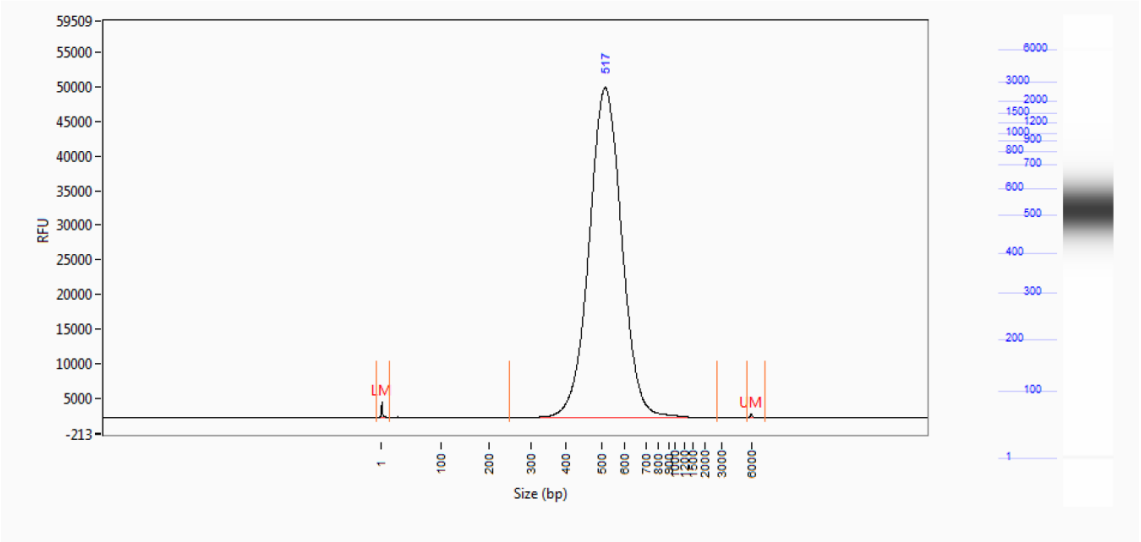
Objective: Purify DNA from PCR mix and enzyme and eliminate fragments < 300pb)

Prewarm the CleanPCR beads at room temperature (at least 30')
Prepare EtOH 80% in water

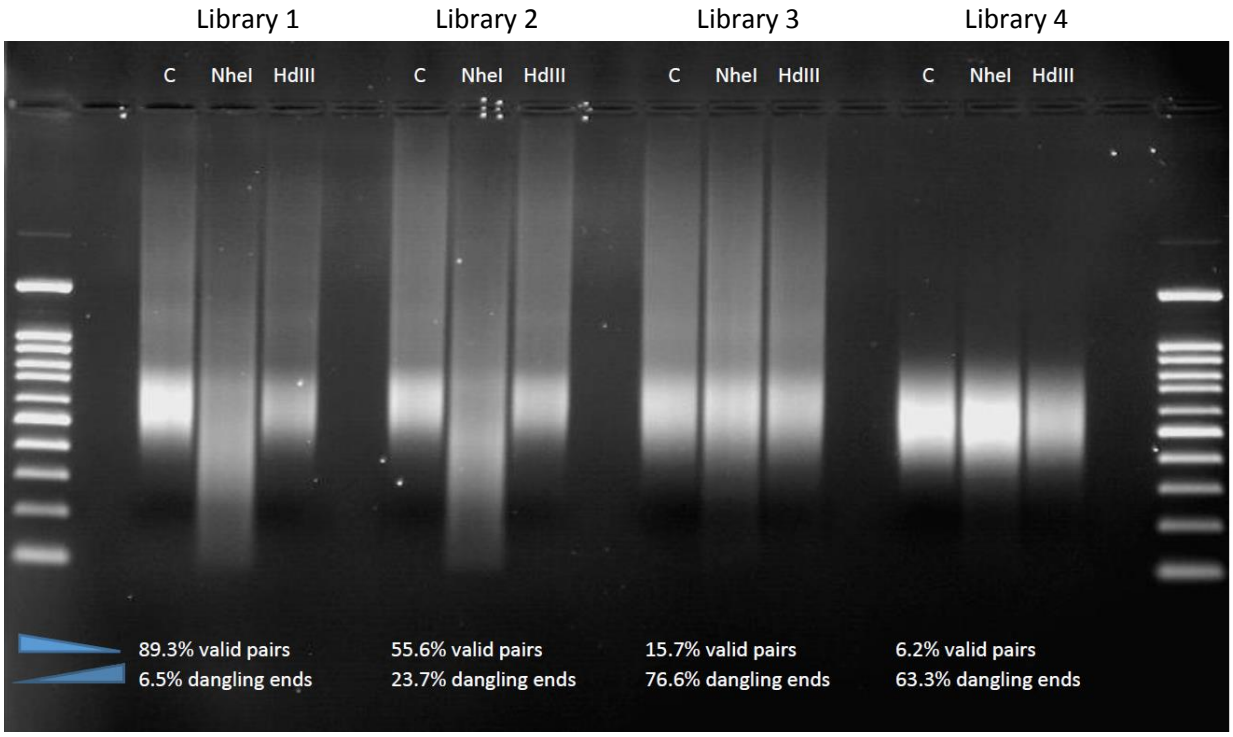
- Transfer the amplified DNA (48µl) in a 1.5ml Eppendorf tube
- Place the tubes in a magnetic rack and let the magnetic beads pellet for one minute
- Keep the supernatant and transfer it to a new 1.5ml tube
- Vortex the CleanPCR beads
- To each 48µl of reaction, add 30µl of CleanPCR beads suspension, mix and incubate 5' at RT.
- Place the tubes in a magnetic rack and let the magnetic beads pellet for some minutes.
- Remove the supernatant and wash beads two times:
    - Add 300µl of EtOH 80%
    - Incubate 30 seconds at RT
    - Remove the EtOH 80%
- After the second wash remove the remaining EtOH carefully using a P10 tip.
- Dry the beads for 3 minutes
- Resuspend the beads in 30µl of Resuspension Buffer and mix by vortexing
- Incubate 2 minutes at RT
- Place the tubes in a magnetic rack and let the magnetic beads pellet
- Transfer 30µl of the supernatant, taking care of not loading beads and transfer to a new 1.5ml tube.
- Libraries are kept at -20°C before sequencing

3.8 QC of libraries

Libraries are controlled with the BioAnalyzer (Agilent) or the Fragment Analyzer
A single clear peak is expected between 450bp and 600bp.
Libraries are quantified using Kappa libraries quantification kit on Quantstudio 6 Flex system (Thermofischer).



In addition, for HiC libraries prepared with HindIII, we digest the libraries using NheI and HindIII restriction enzymes to determine if we have well purified and amplified the biotinilated fragments containing the filled-in religation sites (Belton et al. 2012).



After QC, HiC libraries are sequenced in pool in one HiSeq 3000 lane to validate their quality before depth sequencing.

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