Protocols

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Anneal MEDS

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#TODO
```

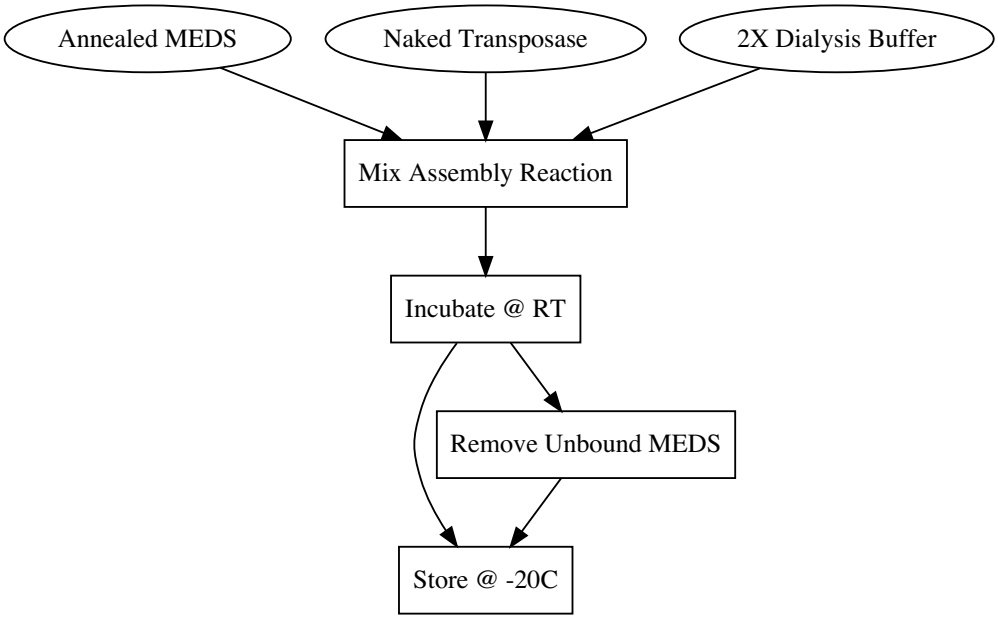
Charging Naked Tn5 with MEDS (Picelli)

Notes

This procotol is adapted from Picelli, et al. 2014 (Picelli et al., 2014).

Yield can be stored at -20C.

Workflow



Reagents

- HEPES
- KOH

- NaCl
- EDTA
- DTT
- Triton X-100
- Glycerol
- Pre-annealed MEDS
- Naked Transposase
- DNA-binding Columns or AMPure XP beads (Agencourt)

Protocol

1. Mix Pre-annealed MEDS Oligos with Tn5.

Table 1: Tn5 Charging Reaction

Reagent	Quantity	Unit
100uM equimolar oligos A/B in TE	0.125	Volume
100% Glycerol	0.400	Volume
2x Tn5 dialysis buffer	0.120	Volume
Tn5, A280=3.0 (1.85 mg/ml)	0.360	Volume

2. Incubate 60 minutes at room temperature.

3. Charged Tn5 solution can be stored at -20. Unbound MEDS oligos should be removed from the solution before use. This can be done with a DNA binding column (Zymo recommended in original protocol) or Agencourt AMPure XP beads (Beckman Coulter).

Buffers

2x Tn5 Dialysis Buffer

Table 2: 2X Tn5 Dialysis Buffer

Reagent	Quantity	Unit	Final.Conc.	Stock
HEPES-KOH (pH 7.2)	1.000	mL	100mM	1M
NaCl	400.000	uL	200mM	5M
EDTA	4.000	uL	0.2mM	0.5M
DTT	20.000	uL	2mM	1M
Triton X-100	20.000	uL	0.2%	100%
Glycerol	2.000	mL	20%	100%
H20	6.556	mL	-	-

ATAC-seq

Notes

Workflow

Reagents

- Illumina Nextera DNA Preparation kit (Illumina Cat #FC-121-1030) (stored at -20 deg C)
- 1X PBS
- Lysis buffer (10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% IGEPAL CA-360)*
- Nuclease-free water
- Customized Nextera PCR primers (see table)
- SYBR Green I dye (Invitrogen #S-7563)
- NEBNext High-Fidelity 2X PCR master mix (NEB Cat #M0541)
- Qiagen MinElute DNA purification kit
- Qiagen DNA purification kit
- Materials for TapeStation/BioAnalyzer/Qubit/KAPA kit for libary QC and quantification

Protocol

1. Harvest cells.
2. Spin 50,000 cells at 500G for 5 minutes at 4C.
3. Gently resuspend in cold 1X PBS to wash.
4. Spin 50,000 cells at 500G for 5 minutes at 4C.
5. Remove PBS and resuspend in 50 uL cold ATAC Lysis Buffer.

Buffers

Lysis Buffer

```
Reagent <- c("TRIS-HCL ph 7.4", "NaCl", "MgCl2", "IGEPAL", "H2O")

Quantity <- c(8.33,5,0.75,0.25,235.67)

# https://stats.idre.ucla.edu/r/codefragments/greek_letters/
```

```
Unit <- c("\\(mL\\)","\\(mL\\)","\\(mL\\)","\\(mL\\)","\\(mL\\)")

Final <- c(paste0(10,"\\(m\\)","M"),
           paste0(10,"\\(m\\)","M"),
           paste0(3,"\\(m\\)","M"),
           paste0(0.1,"%"),
           "-")


Stock <- c("300mM", "500mM", "1M","100%","-")

LysisBuffer <- data.frame(Reagent=Reagent,
                          Quantity=Quantity,
                          Unit=Unit,
                          "Final Conc."=Final,
                          Stock=Stock)
knitr::kable(LysisBuffer, caption = 'ATAC Lysis Buffer',
             format = 'pandoc')
```

Table 3: ATAC Lysis Buffer

Reagent	Quantity	Unit	Final.Conc.	Stock
TRIS-HCL ph 7.4	8.33	mL	10mM	300mM
NaCl	5.00	mL	10mM	500mM
MgCl2	0.75	mL	3mM	1M
IGEPAL	0.25	mL	0.1%	100%
H20	235.67	mL	-	-

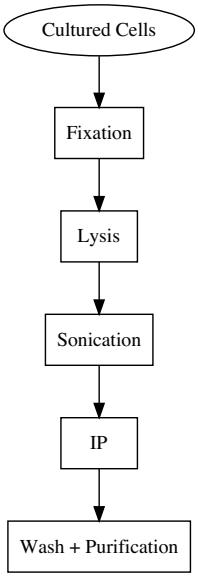
Cell Line ChIP (Bradner Lab protocol)

Notes

This protocol is adapted from the ChIP protocol used in the Bradner Lab. Works well with 10-25 million cells. Unless otherwise noted keep all steps on ice. Work quickly and reduce handling of material as much as possible. For example, never pipette mix when resuspension in a rotator at 4c would suffice.

When using a bioruptor, 0.5% SDS is usually optimal. Before starting, make buffered stocks of HEPES and Tris.

Workflow



Protocol

1. Bead setup

- Wash 100ul Protein G DynaBeads 3x5min in 1ml blocking buffer using magnetic rack and aspirating all buffer with each wash.
- Add 500ul blocking buffer to tub with trapped beads
- Add antibody (usually 10ug) to this buffer. Do not bother resuspending manually. Instead rotate end over end at 4c for minimum 5 hrs.

2. Fixation

   - Make 11% formaldehyde fixation solution fresh (see below).
   - Add volume of fixation solution equal to 1/10 of the culture volume.
   - Incubate at RT on rocker for 10-15 minutes (optimize for cell type and IP target).
   - Quench with volume of 2.5M glycine equal to 1/20 culture volume.
   - If adherent:
     - Rinse 2x with chilled PBS+PIC at 1X concentration.
     - Add small volume of PBS+PIC (dependent on dish size) and scrape with cell scrapers or alternatively bent 200ul pipette tip.
     - Transfer scraped cells to conical (50ml or 15ml).
   - Wash:
     - Spin at 4c @ 1350g for 5min.
     - Aspirate supernatant.
     - Resuspend in cold 1X PBS+PIC.
     - Repeat 2x.
   - Collect cells by spinning at 4c @ 1350g for 5min, aspirate supernatant.
   - Cells are now ready for lysis. Alternatively snap freeze by dunking conical into LN_2 and immediately transfer to -80c.

3. Lysis

   - Resuspend pellet in 5ml chilled LB1+PIC and incubate 10 minutes @ 4c while rotating.
   - Pellet cells at 1350g for 5min @ 4c, aspirate supernatant.
   - Repeat the previous two steps using LB2+PIC.
   - Resuspend in appropriate volume SB+PIC with SDS at optimized percentage.

4. Sonication

   - Sonicate using parameters optimized for instrument and cell type.
   - Spin sonicated material at 20,000g for 10min at 4c to clarify.
   - Collect supernatant and transfer to a new tube. Discard pellet (debris).
   - Set aside ~50ul for use as input (non-IP control) and store at -20c.
   - Dilute remaining sonicated chromatin with SB+PIC such that %SDS $< 0.1\%$, ideally targeting $<$ 15ml total volume.

5. IP

   - Wash antibody-bead complex 3x5min in cold blocking buffer.
   - Resuspend in 500ul cold blocking buffer.
   - Gently add bead suspension to sonicated chromatin.
   - Rotate end over end at 4c overnight.

6. Bead Wash + Elution

   - Collect bead-antibody-chromatin on magnetic rack, aspirate SB + debris.
   - Wash end over end 5min at 4c in each of the following buffers. After each wash, collect beads with magnet, aspirate.
     - Twice in SB
     - High salt SB
     - LiCl wash buffer
     - TE+NaCl (add 10ul 5M NaCl for every 1ml TE)

- Remove as much of final wash as possible while beads are trapped by the magnetic rack. If necessary, spin at 4c at 960g for 3 minutes then return to magnetic rack and aspirate again.
- Gently resuspend in 210ul EB. Pipette mix vigorously or vortex.
- Elute chromatin by incubating at 65c for 15min. Vortex or pipette mix once every 5min.
- Spin at 20,000g to pellet beads and/or trap with magnetic rack.
- Transfer supernatant to fresh tube
- If input chromatin was saved, add EB to input chromatin such that IP and input are in the same volume.
- Reverse crosslinks overnight, ~16hrs at 65c.

7. Purification

- Add 200ul TE to each tube.
- Add RNAseA to a concentration of 0.2mg/ml to each tube (8ul if stock is 10mg/ml).
- Mix gently then incubate at 37c for 2hrs.
- Add 7ul 300mM CaCl in 10mM ph8 Tris-HCL.
- Add proteinase K to a concentration of 0.2mg/ml (4ul if stock concentration is 20mg/ml).
- Mix and incubate 30min at 55c.
- Options:
  1. Proceed with phenol:chloroform:isoamyl extraction
  2. Use column based extraction (e.g. minelute).

Buffers

Buffers are shown as recipes for a reasonable volume and use stock solutions and/or reagents listed below.

Table 4: 50mL 11% Formaldehyde Fixation Buffer

Ingredient	Target.Conc	To.Add
1M HEPES-KOH, pH7.5	50mM	2.5ml
5M NaCl	100mM	1ml
0.5M EDTA, pH 8	1mM	100ul
0.5M EGTA, pH 8	0.5mM	50ul
37% Formaldehyde	11%	14.9ml
ddH_2O	NA	31.5ml

Table 5: 50mL Blocking Buffer

Ingredient	Target.Conc	To.Add
BSA	0.5%	50mg
1X PBS	NA	50mL

Table 6: 250 mL Lysis Buffer 1 (LB1)

Ingredient	Target.Conc	To.Add
1M HEPES-KOH, pH7.5	50mM	12.5mL
5M NaCl	140mM	7mL
0.5M EDTA, pH 8	1mM	0.5mL
glycerol	10%	25mL

Ingredient	Target.Conc	To.Add
IGEPAL CA-630	0.5%	1.25mL
Triton X-100	0.25%	625uL
PIC	See product	See product
ddH_2O	NA	203mL

Table 7: 250 mL Lysis Buffer 2 (LB2)

Ingredient	Target.Conc	To.Add
1M TRIS-HCL, pH8	10mM	2.5mL
5M NaCl	200mM	10mL
0.5M EDTA, pH 8	1mM	0.5mL
0.5M EGTA, pH 8	0.5mM	0.25mL
PIC	see product	see product
ddH_2O	NA	236.75mL

Table 8: 250 mL Sonication Buffer (LB)

Ingredient	Target.Conc	To.Add
1M HEPES-KOH, pH7.5	50mM	12.5mL
5M NaCl	140mM	7mL
0.5M EDTA, pH 8	1mM	0.5mL
0.5M EGTA, pH 8	1mM	0.5mL
Triton X-100	1%	2.5mL
10% Na-Deoxycholate	0.1%	2.5mL
10% SDS	optimize	optimize
PIC	see product	see product
ddH_2O	NA	vol up to 250mL

Table 9: 250 mL Sonication Buffer High Salt (LBHS)

Ingredient	Target.Conc	To.Add
1M HEPES-KOH, pH7.5	50mM	12.5mL
5M NaCl	500mM	25mL
0.5M EDTA, pH 8	1mM	0.5mL
0.5M EGTA, pH 8	1mM	0.5mL
Triton X-100	1%	2.5mL
10% Na-Deoxycholate	0.1%	2.5mL
10% SDS	0.1%	2.5mL
PIC	see product	see product
ddH_2O	NA	vol up to 250mL

Table 10: 250 mL LiCl Wash Buffer (LWB)

Ingredient	Target.Conc	To.Add
1M Tris-HCL, pH8	20mM	5mL

Ingredient	Target.Conc	To.Add
0.5M EDTA, pH 8	1mM	0.5mL
5M LiCl	250mM	12.5mL
IGEPAL CA-630	0.5%	1.25mL
10% Na-deoxycholate	0.5%	12.5mL
ddH_2O	NA	218.25mL

Table 11: 250 mL Elution Buffer (EB)

Ingredient	Target.Conc	To.Add
1M Tris-HCL, pH8	50mM	12.5mL
0.5M EDTA, pH 8	10mM	5mL
10% SDS	1%	25mL
ddH_2O	NA	207.5mL

Stocks

Table 12: ChIP stock solutions

Stock	Target.Vol	Target.Conc	Reagent.MW	solid	Adjustment
1M HEPES-KOH, pH 7.5	200mL	1M	238.30	47.66g	KOH
5M NaCl	500mL	5M	58.44	146.1g	-
1M Tris-HCL, pH 8	200mL	1M	121.14	24.228g	HCL
5M LiCl	40mL	5M	42.39	8.478g	-
10% Na-Deoxycholate	200mL	10%	NA	20g	-
10% SDS	200mL	10%	NA	20g	-
1M (~10%) $CaCl_2$	100mL	1M	110.98	11.1g	-
2.5M Glycine	400mL	2.5M	75.07	75.07g	-

Other Reagents + equipment

- Bovine Serum Albumin
- Triton X-100
- 1X PBS
- 37% formaldehyde (with methanol)
- glycerol
- IGEPAL CA630
- Sonicator and associated attachments
- TPX tubes (if sonicating in BioRuptor)
- Cell scrapers (alternatively bend a sterile pipette tip to a 90_o angle)
- DynaBeads (usually protein G)
- Desired antibody
- TE Buffer
- Protease inhibitors (Roche tablets or HALT cocktail; add phosphatase inhibitors for phosphorylated marks such as pSer polII)
- Proteinase K
- RNAse A

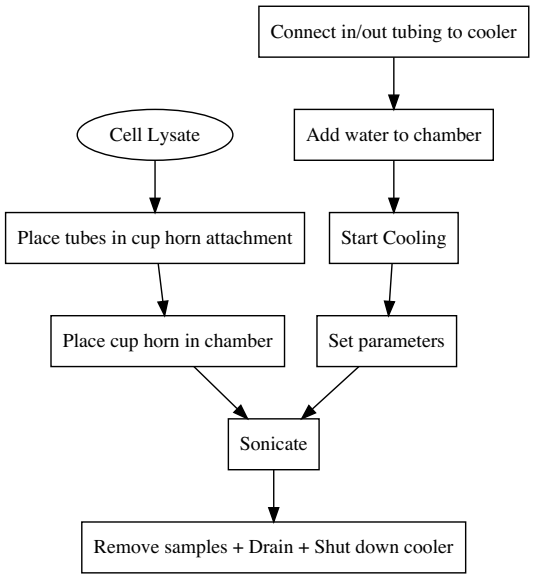
- Magnetic Racks for 1.5ml eppendorf and optionally 15ml conical
- Heat block, incubator, or water bath
- Nuclease-free H_2O

QSONICA Operation (ChIP-seq)

Notes

This protocol explains operation of the Q800R3 with automated cooler for chromatin shearing as part of a ChIP-seq protocol.

Workflow



Protocol

1. Prep cell lysate early in the day.
    - Lysate can sit at 4c in sonication buffer or several hours at least. Note that for QSONICA sonication 1% SDS is recommended.
    - Divide the lysate such that approximately 1e7 cell equivalents of lysate are in 300ul
    - Use 500ul thin wall tubes
2. Place tubes in bottom of sample clamp.

- The clamp is composed of two 3d printed discs with holes for tubes around the disc as well as a threaded central hole.
- Screw these discs together firmly but without flexing the 3d printed plastic.

3. Slot the clamp+tube complex into the cup horn attachment
    - This should just gently fit into the cup horn, no forcing necessary
4. Attach chiller tubes and fill with appropriate amount of H_2O.
5. Place the cup horn attachment in the sonication chamber
    - Make sure that cup horn is seated flat and the the gears on the top of the cup horn and the sonication chamber are completely meshed
6. Set desired sonication strength and on/off timing
    - :15/:45 at 70% for 30 minutes of total sonication is a good starting point
7. Make sure that the chamber is closed and turn the machine on.
    - You can now walk away from the machine until it is finished. This will take several hours depending on the parameters used.
8. Drain machine by attaching drainage hose and briefly running cooler pump.

Bioruptor Operation

Common Stock Solutions

1M MgCl2, 250ml

1. $MgCl_2$ - $6H_2O$ (203.3 g/M): 50.825 g
2. Bring up to 250ml

5M NaClm

Bibliography

Picelli, S., Björklund, Å. K., Reinius, B., Sagasser, S., Winberg, G., and Sandberg, R. (2014). Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. *Genome Research*, 24(12):2033–2040.