ChIPmentation 12- 14.7.2014

K562 fresh collected 14.7.2014

**Fixing cells for ChIP (vortex cells in all steps briefly)**

1. Collect cells by centrifugation, discard media/buffer etc. (KEEP few μl of supernatant to prevent cell loss) (500rcf)
2. Resuspend cells in **400 μl PBS at room temperature**
3. Add **26,46 μl fresh formaldehyde** to a concentration of 1 % final to cell/PBS suspension/(add 0.1%BSA for low cell numbers), mix carefully and rock the cell suspension for 5 minutes at room temperature.
4. Quench the formaldehyde by **21,3 μl 2.625 M glycine** to a 0.125 M final concentration and 0.1 %BSA, mix carefully and rock the cell suspension for 5 minutes at room temperature.
5. Collect cells by centrifugation at 500 x g for 8 minutes, 4°C. Wash GENTLY twice with 400 μl ice-cold PBS/PMSF. (KEEP few μl of supernatant to prevent cell loss).

*The cell pellets can be snap-frozen in a methanol/dry ice bath or liquid N2 and stored at -80°C.*

**Prepare nuclei (1k-3 mio cells)**

Prepare buffers on ice:

|  |  |
| --- | --- |
| 3 mio cells | component |
| Cell lysis buffer | Cell Lysis buffer |
|  | 50x PIC |
|  | 100x PMSF |
| Sonication buffer | Nuclear Lysis buffer |
|  | 50x PIC |
|  | 100x PMSF |

1. Add **400 μl Lysis buffer**, resuspended cells, mix carefully and incubate on ice for 5 minutes
2. Spin 5 min **1700 x g at 4 °C,** discard supernatant without disturbing the pellet
3. Resuspend nuclei in **130 μl 0.1%SDS sonication buffer** containing protease inhibitor and transfer in a AFA tube

**Shearing the chromatin and IP**

|  |  |
| --- | --- |
| Low cell chromatin shearing | S220 series |
| Target base pair range | 200-700 |
| Duty cycle | 2% |
| Peak incident power | 105 Watts |
| Cycles per burst | 200 |
| Processing time | 10 min |
| Bath temperature | 6 °C |
| Power mode | Frequency sweeping if available |
| Degassing mode | Continous |
| Volume | 130 μl (ALWAYS FILL to 130 μl) |
| Max. cell number | 3 Mio cells |
| AFA intensifier | Is integrated in holder (?) |
| Water level (RUN) | Level 12 (1 mm below bottom of microtube cap |

Measure c(DNA) of chromatin with the NANODROP

1. After shearing, transfer samples into a pre-chilled microcentrifuge
2. Centrifuge samples at 14,000 x g, 4 °C for 5 minutes to pellet insoluble material, transfer 130 μl to a new tube

**Histone ChIP with magnetic beads**

1. For a ChIP pipet the following scheme:

|  |  |  |
| --- | --- | --- |
|  | 1 IP | X IPs |
| H2O | 152 -x - y μl |  |
| 50 X protease inhibitor cocktail (PIC) | 4 μl |  |
| Phenylmethylsulfonylfluorid 100x | 2 μl |  |
| 5x Incubation buffer | 40 μl |  |
| BSA (then mix) | 2 μl |  |
| chromatin | X |  |
| Antibody | y |  |

1. Rotate o/n 4°C
2. Per IP, wash 25 µl protein A Dynabeads beads (PAD) (or protein G Dynabeads, e.g. when using goat pAB or mouse IgG1/IgG3 or IgM) 2x with 2x original volume 0.1% BSA/PBS, resuspend in 25 ul 0.1% BSA/PBS per IP and rotate o/n 4°C.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| content | K562 500k  10’ soni | K562 500k  10’ soni | K562 500k  10’ soni | K562 500k  10’ soni | K562 10k  10’ soni | K562 10k  10’ soni | K562 10k  10’ soni | K562 10k  10’ soni |
| Ab | H3K4me3  C’54’0003 | H3K27me3  07-449 | IgG  Rab Erika | PU.1  Sc-352 | H3K4me3  C’54’0003 | H3K27me3  07-449 | IgG  Rab Erika | PU.1  Sc-352 |
| Ab amount | 1 ug | 1 ug | 1 ug | 2 ug | 1 ug | 1 ug | 1 ug | 2 ug |
| Ab ul | 1 | 1 | 1(1:5 diluted) | 1 | 1 | 1 | 1(1:5 diluted) | 1 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | 9 | 10 | 11 | 12 |
| content | K562 500k  10’ soni | K562 500k  10’ soni | K562 500k  10’ soni | K562 500k  10’ soni |
| Ab | H3K4me3  C’54’0003 | H3K27me3  07-449 | IgG  Rab Erika | PU.1  Sc-352 |
| Ab amount | 1 ug | 1 ug | 1 ug | 2 ug |
| Ab ul | 1 | 1 | 1(1:5 diluted) | 1 |

The next day- IP and wash

1. Add 20 ul of blocked beads to each IP and rotate 2h 4°C
2. Precool magnet on ice
3. Wash Samples with:

|  |  |  |
| --- | --- | --- |
| buffer | Times washing | |
| WB I | | 2 |
| WB II | | 1 |
| WB III | | 1 |
| Tris pH 8 | | 2 |

**On-site Transposition**

1. Pipett tagmentation reaction:

|  |  |  |
| --- | --- | --- |
| Component Tagmentation | volume | X reactions |
| 10x Tagmentation buffer MgCl | 3 μL |  |
| H2O | 27-x μL |  |
| Tagment DNA Enzyme | x μL |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *cup* | *Sample* | *antibody* | *Cell Nr ChIP* | *TagDNA Enzyme* |
| *1* | CM\_12-1 | Diagenode H3K4me3 | 500 000 | *1 ul (undiluted)* |
| *2* | CM\_12-2 | Millipore H3K27me3 | 500 000 | *1 ul (undiluted)* |
| *3* | CM\_12-3 | IgG rab | 500 000 | *1 ul (undiluted)* |
| *4* | CM\_12-4 | santacruz PU.1 | 500 000 | *1 ul (undiluted)* |
| *5* | CM\_12-5 | Diagenode H3K4me3 | 10 000 | *1 ul (undiluted)* |
| *6* | CM\_12-6 | Millipore H3K27me3 | 10 000 | *1 ul (undiluted)* |
| *7* | CM\_12-7 | IgG rab | 10 000 | *1 ul (undiluted)* |
| *8* | CM\_12-8 | santacruz PU.1 | 10 000 | *1 ul (undiluted)* |

1. Incubate 20 minutes at 37°C**,** mix carefully every 5 minutes and stop immediately by putting reaction on ice
2. Wash the beads with 150 μl for 30s at 4°C with the following buffers:

|  |  |
| --- | --- |
| buffer | Times washing |
| Wash buffer 1 | 2 |
| WB 4 (transfer before last step) | 2 |

1. After washing, add 68 μl EB buffer and 2 ul Prot K
2. Incubate at 55°C 1h and 65°C for 8 hours. (decrosslinking). Don’t forget the input! Rev-crosslink in cycler, cooling to 8°C.

* **MinElute Purification, elute in 13 ul EB**
* **Dilute 2 ul with 18 ul EBtween for qPCR (3+27 for the input)**
* **Use 1ul to check enrichment cycles of undiluted material 10 ul left for final amplification**

**Try tagmentation-based library prep**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Well in qPCR* | *Sample* | *antibody* | *Cell Nr ChIP* | *Tag Enzyme* | *Custum Adapter* | *Cq* | *Ct qPCR*  *plateau* | *Enrichment cycles* | *Ng/ul* |
|  | CM\_12-1 | Diagenode H3K4me3 | 500 000 | *1 ul (undiluted)* | *5* | *11.14* | *14.26* | *12* |  |
|  | CM\_12-2 | Millipore H3K27me3 | 500 000 | *1 ul (undiluted)* | *1* | *11.44* | *14.55* | *12* |  |
|  | CM\_12-3 | IgG rab | 500 000 | *1 ul (undiluted)* | *2* | *15.41* | *18.57* | *16* |  |
|  | CM\_12-4 | santacruz PU.1 | 500 000 | *1 ul (undiluted)* | *6* | *12.24* | *15.59* | *13* |  |
|  | CM\_12-5 | Diagenode H3K4me3 | 10 000 | *1 ul (undiluted)* | *7* | *15.6* | *18.72* | *16* |  |
|  | CM\_12-6 | Millipore H3K27me3 | 10 000 | *1 ul (undiluted)* | *4* | *10.78* | *14.49* | *12* |  |
|  | CM\_12-7 | IgG rab | 10 000 | *1 ul (undiluted)* | *3* | *17.48* | *22* | *18* |  |
|  | CM\_12-8 | santacruz PU.1 | 10 000 | *1 ul (undiluted)* | *8* | *17.72* | *21.24* | *18* |  |

1. Pipet qPCR

(Preheat MMix 98°C 45s- Kapa is a hotstart polymerase and not suitable for nick translation in the first PCR step or use NEBnext MM)

|  |  |
| --- | --- |
| Component qPCR | volume |
| Nextera custom primer 1 noMX | 0.3 μL |
| Nextera custom primer 2 barcode | 0.3 μL |
| Kapa HIFI polymerase | 5 μL |
| 100x SYBR | 0.1 μL |
| H2O | 3.3 μL |
| Tagment DNA mix from previous step | 1 μL |

1. Perform PCR with the following program (“CM63”):
   1. 72°C 5 minutes
   2. 98°C 30 seconds
   3. 25 cycles of:
      1. 98°C 10 seconds
      2. 63°C 30 seconds
      3. 72°C 30 seconds
   4. 72°C 1 minute
   5. hold at 12 °C
2. Pipet in the following order in a PCR tube for each reaction (Preheat MMix 98°C 30s- Kapa is a hotstart polymerase and not suitable for nick translation in the first step)

|  |  |
| --- | --- |
| Component qPCR | volume |
| Nextera custom primer 1 noMX | 1.5 μL |
| Nextera custom primer 2 barcode | 1.5 μL |
| Kapa HIFI polymerase | 25 μL |
| H2O | 22-x ul |
| Tagment DNA mix from previous step | x μL |

1. Seal tubes and move to Post-PCR area
2. Perform PCR with the following program(“Enrich CM63”):
   1. 72°C 5 minutes
   2. 98°C 30 seconds
   3. x cycles of:
      1. 98°C 10 seconds
      2. 63°C 30 seconds
      3. 72°C 30 seconds
   4. 72°C 1 minute
   5. hold at 12 °C

**MinElute-**