**G4 ChIP-seq**

**Step 1: Cell collection and fixation (after trypsinization)**

* Fix 20 million cells in DMEM (25ml for 20 million cells)containing 1% PFA and 15% FBS for 15min at RT.
* Quench with 0.13 M glycine at RT for 10 min.
* Centrifuge for 4 min, 4°C, 1200rpm.
* Wash cells twice in ice-cold PBS.
* Centrifuge for 4 min, 4°C, 1200rpm.
* Remove supernatant.
* Store cells in -80°C.

**Step 2: Cell lysis and Chromatin shearing**

* Resuspend the cell pellet in 1ml Hypotonic buffer and incubate the samples at 4 °C for 10 min.
* Centrifuge the hypotonic slurry at 5000 ×g for 5 min at 4°C to collect the nuclei.
* Discard the supernatant and resuspend the pellet in 180μl Lysis buffer(ensure the Lysis buffer has been pre-warmed prior to use to ensure all precipitates are fully dissolved) and incubate samples at 4°C for 10 min.
* Sonicate 180μl lysed nuclei suspension for 100 cycles (30s ON/60s OFF) in 1.5ml specific sonication tubes using Bioruptor.
* Centrifuge the samples for 10 minutes at maximum speed at 4°C and transfer the supernatant to a clean dry microcentrifuge tubes.
* Keep 30ul suspension on ice.
* Add 1350μl blocking buffer to bring up the suspension to 1500 μl( so the SDS will be diluted to 0.1%)

**Step 3: Chromatin immunoprecipitation**

* Thoroughly resuspend the protein A beads in the solution and transfer 100 μl(1mg at 10mg/ml) of protein A beads to 1.5ml tubes. Magnetize beads and discard supernatant.
* Wash protein A beads with 1000 μl PBS-T(PBS+0.1% Tween 20) and discard the supernatant, 3×.
* Resuspend beads with 400 μl blocking buffer.
* Dilute 1H6 antibody to 8μg (80μg/ml, Stock: 1mg/ml) in 100μl blocking buffer.
* Add 400 μl beads in blocking buffer to the diluted antibody giving 500 μl total of beads/antibody.
* Rotate at RT for 1hr in a Thermo shaker at 1000rpm.
* Magnetize beads and discard supernatant.
* Wash with 1000 μl PBS-T 3×:
  + 1. Resuspend the beads thoroughly
    2. Magnetize beads and discard supernatant
* Add 1500 μl chromatin lysate to beads/antibody
* Rotate for 1hr at 16°C in a Thermo shaker at 1000rpm.
* Magnetize beads and discard supernatant.
* Wash with 1000 μl wash buffer(10mM Tris pH 7.4, 100mM KCl, 0.1% Tween 20)

1. Resuspend beads in 1000 μl wash buffer and incubate on a rotating platform at 37°C for 10min.
2. Repeat once.
3. Transfer the resuspended beads to a new tube.
4. Resuspend beads in 1000 μl wash buffer and incubate on a rotating platform at 4°C for 10min
5. Remove supernatant.

* Spin down all tubes for several seconds.
* Magnetize beads and aspirate residual buffer from the tubes manually.
* Resuspend beads with 200μl elution buffer.
* Perform elution at 37°C with 2ul RNase A for 1hr and at 65°C O/N in elution buffer with Proteinase K(O/N at 65deg for the reverse cross-link step, 1300rpm shaking.).
* Purify eluted DNA with column.

**Recipe**

**Hypotonic buffer**

Tris-HCl 10mM(stock: 1M)

NaCl 10mM

EDTA 1mM

Triton X-100 0.2%

pH8@4°C

Protease inhibitor complete 1×

**Lysis buffer**

Tris-HCl 10mM

NaCl 150mM

EDTA 1mM

SDS 1%

pH8@RT

Protease inhibitor complete 1×

**Blocking buffer**

HEPES pH7.5 25mM

NaCl 10.5mM

KCl 100mM

CaCl2 130nM

MgCl2 1mM

Protease inhibitor complete 1×

**Wash buffer**

Tris pH7.4 10mM

KCl 100mM

Tween 20 0.1%

Protease inhibitor complete 1×

**Elution buffer (200ul)**

1×TE buffer, 5ul proteinase K