Sugden Lab.

ChIP Protocol for 05/01/05-05/03/05

Samples: Total, No Ab, IgG, E2F4, EBNA1, ORC2, ORC3
Sun 05/01/05

1. Thaw 2 100ul-aliquots of Staph A cells (stored in liquid nitrogen) on ice. Combine together in a 1.7ml microfuge tube. Block cells by addition of 20ul 10mg/ml herring sperm DNA and 20ul 10mg/ml BSA.
2. Incubate @ 4C, with rocking **Start: 1pm**

Mon 05/02/05

1. Wash Staph A cells: **Start: 11am**
   - Spin 10k rpm, 4C, 3 min; pipette off supernatant
   - Wash pellet twice in 1ml 1x dialysis buffer (w/o sarkosyl)
   - Resuspend pellet in 200ul 1x dialysis buffer (+PMSF), store @ 4C
2. Prepare PMSF from dry stock at 100mM in 100% Isopropanol (0.087g/5ml)
3. Thaw BJAB/EBNA1 (FR/8xRep*) cells on ice (3.4x10^7 cells/aliquot, use 2 aliquots, will use 1.1x10^7 cells/IP)
4. Prepare 5ml Cell Lysis solution (*NOTE: Make fresh, use only for 1 day)
   Add 10ul/ml Igepal (NP40), incubate at 37C to dissolve, cool on ice
   Add 10ul/ml PMSF, 1ul/ml aprotinin, 1ul/ml leupeptin ("+PAL")
5. Resuspend each aliquot in 2ml cell lysis buffer (+PAL), transfer 1ml aliquots separately to a chilled B dounce, dounce 10 times
6. Separate into 1ml/pink-capped tube ("p-c tube")
7. Spin 5k rpm, 5min, 4C to pellet nuclei
8. Resuspend 3.4x10^7 cell equivalents in 1ml nuclei lysis buffer (+PAL) in a single p-c tube (e.g. 2 tubes total).
9. Sonicate nuclei at setting 2 twice for 10 seconds, resting on ice 2min between.
10. Spin 14k rpm, 10min, 4C (chromatin in supernatant)
11. Transfer supernatant to a new p-c tube
12. Add 20ul washed/blocked Staph A cells per 3.4x10^7 cell equivalents
13. Incubate 4C 15min on rocker (rotating wheel)
14. Spin 14k rpm, 5min, 4C
15. Transfer supernatant to p-c tube, measure total volume, divide by N+.2 (where N is the number of IP reactions, and .2 is for the total fraction) (e.g. 6.2)
    N =_____ul Total = ____ul
16. Aliquot appropriate N ul into N p-c tubes and 0.2N into a p-c tube for Total. Store the Total sample at 4C without further treatment until X-link reversal.
17. Add N ul into a p-c tube as a mock sample.
18. Add 2 volumes IP dilution buffer (+PAL) to all samples.
19. Add primary antibody to appropriately labeled tube

	Lot #:
No Ab: n/a	n/a
IgG: added at secondary antibody step so n/a	053K4814
E2F4: 5ul Oc PAb anti-HsE2F4 c-term (~10ug antibody)	C0504
EBNA1: 100ul Rn MAb anti-EBNA1 (~10ug antibody)	A. Schepers Fall 2004
ORC2: 10ul Rn PAb anti-ORC2 (concentration?)	A. Schepers Fall 2004
ORC3: 10ul Rn PAb anti-ORC3 (concentration?)	A. Schepers Fall 2004

20. Incubate chromatin+Ab's at 4C o/n with rocking (rotation)

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Tues 05/03/05

1. Add secondary antibody Oc anti-Rn IgG (10ul, 20ug) to all IPs, incubate 4C 1hr rocking
2. Add 15ul washed/blocked Staph A cells per IP sample, incubate on rotating platform, RT, 15 min
3. Spin 14k rpm, 4min, 4C
4. Pipette off supernatant, resuspend pellet/spin pellet as below:
   700ul dialysis buffer (+PMSF) +700ul more of same, 3 min, RT, rock, spin
   700ul dialysis buffer (+PMSF) +700ul more of same, 3 min, RT, rock, spin
   700ul IP wash buffer (+PMSF) +700ul more of same, 3 min, RT, rock, spin
   700ul IP wash buffer (+PMSF) +700ul more of same, 3 min, RT, rock, spin
   700ul IP wash buffer (+PMSF) +700ul more of same, 3 min, RT, rock, spin
   700ul IP wash buffer (+PMSF) +700ul more of same, 3 min, RT, rock, spin
5. Spin again as above, pipette off residual supernatant
6. Add 150ul IP elution buffer to each sample, shake on vortex setting 3, RT, 15 min
7. Spin 14k rpm, 4 min, 4C, transfer supernatant to p-c tube
8. Repeat addition of 150ul IP elution buffer, shaking, and spin; transfer supernatant to same p-c tube as previous elution.
9. Spin 14k rpm 4 min 4C to remove residual Staph A cells, transfer supernatant to p-c tube with conservative removal of supernatant near the bottom.
10. Reinstate treatment of Total sample along with all other samples. Bring the Total sample volume to 300ul with IP elution buffer.
11. Add 5M NaCl to a final concentration of 0.2M (i.e. 300ul total volume, add 12ul).
12. Incubate all samples 65C, 4-5 hours to reverse X-links.
13. Use Qiagen PCR Clean-Up Kit to prepare DNA for PCR analysis
    - Starting volume = 312ul
    - Add 5 volumes Buffer PB (1.5ml each), mix by pipetting
    - Apply to a spin column, spin 10k rpm 30 sec RT, reapply and respin
    - Wash column with 600ul Wash Buffer, decant buffer, respin to remove residual buffer
    - Elute DNA twice with 30ul Gibco dH2O

NOTES OF IMPORTANCE:
CalBioChem Staph A cells (Pansorbin, lyophilized 1 gram #507862) can bind 3.75ug HsIgG per 1ul washed/blocked cells
(1.5ug HsIgG bound per 100ug wet weight Staph A cells)
10ug 1^{o} Ab, 20ug 2^{o} Ab (2:1 molar excess), 15ug Staph A cells can bind 56ug IgG (2.8:1 molar excess)