Dynabeads® Protein A/G and ChIP

· Curran et al 2009 **Nature** 459, 1079-1084 (25 June 2009*) A soma-to-germline transformation in long-lived Caenorhabditis elegans mutants*

- **Dynabeads® Protein A**

- **Keywords:** germline characteristics, *C. elegans* mutants, chaperonin complex, Chromatin immunoprecipitation

- **Application:** Chromatin immunoprecipitation. - In brief, ground frozen worm powder was crosslinked using 1% formaldehyde in PBS and subjected to sonication. Immunoprecipitation was performed using Protein A Dynabeads (Invitrogen) and polyclonal rabbit anti-GFP antibody (Clontech) or polyclonal rabbit-anti-mCherry antibody. Protein–DNA complexes were then eluted from proteinA beads and treated with RNaseA and proteinase K. Precipitated DNA fragments were purified and subjected to qPCR analysis.

· Lim at al 2009 **Nature** 458, 529-533 (26 March 2009), "*Chromatin remodelling factor Mll1 is essential for neurogenesis from postnatal neural stem cells"*

- **Dynabeads® Protein A**

- **Keywords:** Chromatin immunoprecipitation, neurogenesis, Trithorax group (trxG) and Polycomb group (PcG) gene products

- **Application:** quantitative ChIP (qChIP), quantitative ChIP (qChIP), chromatin was prepared from cells fixed with1% formaldehyde and then sheered by sonication. Chromatin was incubated overnight with the indicated antibodies, and then collected by incubation with Protein A Dynabeads (Invitrogen). DNA eluted from the washed immune complexes was extracted, precipitated and then subjected to qPCR analysis with SybrGreen. Recovery of genomic DNA as a percentage input was calculated as the ratio of copy numbers in the precipitated immune complexes to the input control.

· Hammoud et al 2009 ***Nature*** 460, 473-478 (23 July 2009), "*Distinctive chromatin in human sperm packages genes for embryo development"*

- **Dynabeads**

- **Keywords:** epigenetic marking, methylation, developmental promoters, nucleosomes

- **Application:** Chromatin immunoprecipitation. Standard ChIP methods were used35, but we omitted crosslinking and used the following salt concentrations in the numbered buffers35: (1) 150mM NaCl, (2) 250mM NaCl, (3) 200mM LiCl, and (4) 150mM NaCl (the PBS wash). Antibodies used were: anti-H3K27me3 (Upstate 07-449), H3K4me3 (Abcam 8580), H3K4me2 (Abcam 32356), TH2B (Upstate 07-680), H2A.Z (Abcam 4174) and H3K9me3 (Abcam 8898). For each, 4 ml of antibody was coupled to 100 µl of Dynabeads (Invitrogen). After ChIP, samples for sequencing were not amplified, whereas for arrays the DNA was amplified (WGA, Sigma) before hybridization.

· Khadaroo et al 2009 **Nature Cell Biology** 11, 980 - 987 (2009) *The DNA damage response at eroded telomeres and tethering to the nuclear pore complex*

- **Dynabeads® Protein G**

- **Keywords: telomeres, DNA damage response, Cdc13, replication protein A, DNA damage checkpoint proteins, DNA repair protein Rad52**

- **Application: Chromatin immunoprecipitation (ChIP).** Briefly, 50 ml cultures of yeast cells at OD600 = 1 were fixed with

1% formaldehyde for 15 min and lysed with glass beads. The fixed chromatin was fractionated by ultracentrifugation (centrifuge from Beckman Coulter). The chromatin fraction was broken by sonication using a Bioruptor (Diagenode, Liege). After clearing chromatin by centrifugation at 16,000*g* for 15 min, target proteins were immunoprecipitated by specific antibodies overnight and with Dynabeads protein G (Invitrogen Dynal AS) for 3 h at 4 °C, or alternatively with an tibodies crosslinked to agarose beads. After washing, the chromatin was eluted from the beads at 65 °C for 10 min and transferred to a new tube. Crosslinks were reversed by incubation at 65 °C overnight. DNA was purified using a Purelink PCR purification kit (Invitrogen SARL) and eluted in of water (50 µl).

· Nielsen et al 2009 ***Nature Methods*** 6, 753 - 757 (2009), "*A Flp-nick system to study repair of a single protein-bound nick in vivo"*

- **Dynabeads**

- Keywords: Flp-nick system, ***Saccharomyces cerevisiae***

- ***Application:*** Chromatin immunoprecipitation. ChIP was performed using either monoclonal antibodies to HA (Santa Cruz) to precipitate HA-tagged FlpH305L or polyclonal antibodies to phosphorylated histone H2A (Abcam). In all cases, cells were grown in YP with 2% raffinose medium and synchronized in G1 with alpha factor (Lipal) at 30 °C; after 60 min galactose was added to 2% and induction carried out in G1 phase–arrested cells for 120 min before release into S phase at 25 °C in the presence of glucose. The ChIP protocol was as described previously

· Sorefan et al 2009 **Nature** 459, 583-586 (28 May 2009) *A regulated auxin minimum is required for seed dispersal in Arabidopsis*

- **Dynabeads® Protein A**

- **Keywords :** development of multicellular structures and organs, Arabidopsis, INDEHISCENT

- **Application:** ChIP. 35S::IND-GR seeds were grown for 7 days in 50 ml of 0.5% glucose (w/v) 0.53 Mureshige and Skoog medium with constant shaking. Seedlings were then treated with 50 mM IAA610 mM DEX and ChIP was performed as described in ref. 37 with the following modifications. 2 mg of anti-GR antibody (AB3580, Abcam) was used for approximately 200mg of tissue. Dynabeads-protein A (100-02D, Invitrogen Ltd) was used.

· Classen et al 2009 **Nature Genetics** 41, 1150 - 1155 (2009) *A tumor suppressor activity of Drosophila Polycomb genes mediated by JAK-STAT signaling*

- **Dynabeads® Protein A**

- **Keywords:** Polycomb Group (PcG) proteins, *Drosophila melanogaster,* PRC1, JAK-STAT pathway, tumor suppression

- **Application:** Chromatin immunoprecipitation. ChIP was carried out as previously described53 on imaginal tissue from 50 third-instar larvae for H3K27me3 ChIP and 200 third-instar larvae for Pc ChIP. Fixed and sheared chromatin was precipitated using a mouse antibody against H3K27me3 (Lake Placid, no. AM-0174) or a rabbit antibody against Polycomb (kindly provided by V. Pirrotta) and Protein A-coupled Dynabeads (Invitrogen).

· Guan et al 2009 **Nature** 459, 55-60 (7 May 2009) *HDAC2 negatively regulates memory formation and synaptic plasticity*

- **Dynabeads® Protein G**

- **Keywords:** Chromatin modifications, histone-tail acetylation, deacetylases, HDAC family member

- Application: Chromatin immunoprecipitation (ChIP). ChIP was performed with mouse forebrains fixed with 4% PFA

solution and stored at 280 uC before use. Brains were chemically cross-linked by the addition of a one-tenth volume of fresh 11% formaldehyde solution for 15 min at room temperature, ten homogenized, resuspended, lysed in lysis buffers, and sonicated to solubilize and shear crosslinked DNA. Sonication conditions varied depending on the cells, culture conditions, crosslinking and equipment. We used a Misonix Sonicator 3000 and sonicated at power 7 for ten 30-s pulses (90-s pause between pulses) at 4 uC while samples were immersed in an ice bath. The resulting whole-cell extract was incubated overnight at 4 uC with 100ml of Dynal Protein G magnetic beads that had been preincubated with 10 mg of the appropriate antibody. Beads were washed five times with RIPA buffer and once with TE (10mM Tris-HCl pH7.5, 1mM EDTA) containing 50mM NaCl. Bound complexes were eluted from the beads by being heated at 65 uC with occasional vortex-mixing, and crosslinking was reversed by incubation overnight at 65 uC.

· ***Kolasinska-Zwierz et al 2009*** **Nature Genetics** *41*, 376 - 381 (2009) *Differential chromatin marking of introns and expressed exons by H3K36me3*

- **Dynabeads® Protein A**

- **Keywords:** *Caenorhabditis elegans*, histone H3 tail methylations, H3K36me3

- Application: Chromatin immunoprecipitation and expression profiling. Each ChIP was prepared in 500 ml of FA buffer containing 1% sarkosyl. The following antibodies and extract amounts were used: anti-H3 (3 mg abcam 1791 with 300 mg extract); anti-H3K4me3 (5 ml Active Motif AR0169 serum with 300 mg extract); anti-H3K36me3 (3 mg abcam ab9050 with 1 mg extract); anti- H3K9me3 (3 mg Upstate 07-442 with 1 mg extract). Additionally, 10% of extract was saved as a reference. After overnight rotation at 4 1C, 40 ml of blocked and washed magnetic protein A Dynabeads (Invitrogen) were added, and the incubation continued for 2 additional hours. Beads were washed at room temperature two times for 5 min in FA buffer, once in FA with 500 mM NaCl for 10 min, once in FA with 1 M NaCl for 5 min, once in TEL buffer (0.25 M LiCl, 1% NP-40, 1% sodium deoxyholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.0) for 10 min and two times in TE pH 8.0 for 5 min. DNA was eluted twice with 57 ml elution buffer (1% SDS in TE with 250 mM NaCl) at 65 1C, 15 min each time. Eluted DNA was incubated with 20 mg of RNase for 30 min at 37 1C and then with 20 mg of Proteinase K for 1 h at 55 1C.

· Pai et al 2009, **Molecular Biology of the Cell** Vol. 20, Issue 4, 1213-1222, February 15, 2009, *GINS Inactivation Phenotypes Reveal Two Pathways for Chromatin Association of Replicative and DNA Polymerases in Fission Yeast*

Dynabeads® Protein G

- **Keywords:** GINS complex, fission yeast, Psf1 and Psf2

- **Application:** ***Chromatin Immunoprecipitation*** Chromatin immunoprecipitation (ChIP) was carried out basically as previously (Strahl-Bolsinger *et al.,* 1997). Briefly, cells were fixed for 15 min in 1% formaldehyde, and the reaction was stopped by adding glycine to 125 mM. After cell breakage, the extracts were sonicated in an MSE Soniprep 250, using six 15-s pulses. Immunoprecipitations were carried out using protein G Dynabeads (Invitrogen, Carlsbad, CA), preadsorbed with anti-GFP (monoclonal 3E1) or anti-FLAG (M2, Sigma F3165).

· Demetriades et al 2009, **J. Virol.** doi:10.1128/JVI.00097-09, *The LMP1 promoter can be transactivated by NF- B directly*

- **Dynabeads® Protein A**

- **Keywords:** NF-B transactivates the LMP1 promoter

- **Application: Chromatin immunoprecipitation.** The EBV-transformed lymphoblastoid cell line WTLCL was used for Chromatin immunoprecipitation experiments. 50x106 cells were fixed with 1% formaldehyde in medium for 10min at room

temperature. For the isolation of chromatin, cells were collected by centrifugation, washed twice with ice-cold PBS and lysed in Lysis solution (50mM Hepes pH 7.9, 1mM EDTA, 0.5mM EGTA, 140mM NaCl, 10% v/v glycerol, 0.5% v/v NP-40, 0.25% v/v Triton X-100 and 1mM PMSF) for 10min on ice. Nuclei were collected by centrifugation, washed in Wash buffer (10mM Tris-Cl pH 8.0, 1mM EDTA, 0.5mM EGTA, 200mM NaCl and 1mM PMSF), resuspended in 2ml RIPA buffer (10mM Tris-Cl, 1mM EDTA, 0.5mM EGTA, 140mM NaCl, 1% v/v Triton X-100, 0.1% Nadeoxycholate, 0.1% SDS and 1mM PMSF) and subjected to sonication on ice (15-20 rounds of 30sec each) in order to obtain chromatin fragments of an average of 500bp. A small portion of each sample was used for immunoprecipitation in RIPA buffer, following preclearing with protein-A magnetic Dynabeads (Dynal Biotech ASA, Norway). Complexes were subsequently washed three times with each of the following buffers: RIPA buffer containing 140mM NaCl, RIPA buffer containing 500mM NaCl, LiCl buffer (10mM Tris-Cl, 1mM EDTA, 0.5mM EGTA, 250mM LiCl, 1% v/v NP-40, 0.1% Na-deoxycholate and 1mM- PMSF) and TE buffer containing 1% v/v Triton X-100 and 1mM PMSF.

Wan et al 2009, **MOLECULAR AND CELLULAR BIOLOGY**, May 2009, p. 2346–2358, *Role of the Histone Variant H2A.Z/Htz1p in TBP Recruitment, Chromatin Dynamics, and Regulated Expression of Oleate-Responsive Genes*

- **Dynabeads® Protein G**

- **Keywords: transcriptional regulation, *Saccharomyces cerevisiae,* transcriptome profiling, chromatin immunoprecipitation, *POT1*, *POX1*, *FOX2*, and *CTA1***

- **Application: ChIP and real-time PCR.** For each chromatin immunoprecipitation (ChIP) experiment, yeast strains were first grown in glucose medium (YPD) to a density of _1 _ 107 cells/ml and then transferred to oleate medium (SCIM) for the times indicated in each figure. The ChIP experiments were performed as described in reference 33 with the following modifications. For the hemagglutinin (HA)- Htz1p ChIP, cells were crossed-linked with 1% formaldehyde for 45 min at room temperature. Two micrograms of anti-HA antibody (12CA5) was prebound to 50 _l of pan-mouse immunoglobulin G Dynabeads (Dynal Biotech) and then incubated with 1 mg (protein) of supernatant from the sheared chromatin overnight at 4°C. The TBP (Spt15p-Myc) ChIP was performed as described in reference 33. Cells were cross-linked with 1% formaldehyde for 2 h at room temperature. Two microliters of anti-Myc antibody (9E11; Abcam) was prebound to 50 _l of pan-mouse immunoglobulin G Dynabeads and then incubated with 1 mg (protein) of supernatant from sheared chromatin overnight at 4°C .

Klopf et al 2009, **Molecular and Cellular Biology**, September 2009, p. 4994-5007, Vol. 29, No. 18, *Cooperation between the INO80 Complex and Histone Chaperones Determines Adaptation of Stress Gene Transcription in the Yeast Saccharomyces cerevisiae,*

- **Dynabeads® pan Mouse IgG, Dynabeads® Protein G**.

- **Keywords**: **INO80 complex,** *Saccharomyces cerevisiae,* **histone homeostasis**

- Application: Chromatin immunoprecipitation (ChIP) assays were performed essentially as described previously (2). Briefly, 50 ml of yeast culture per sample was treated as indicated in the figure legends and cross-linked with 1% formaldehyde for 10 to 20 min at room temperature. Antibodies against histones H3 and H4 (ab1791 and ab10158; Abcam, Cambridge, United Kingdom), anti-HA monoclonal antibody (12CA5), anti-Arp4 antibody (53), and RNA Pol II antibody (8WG16; Covance Inc.) were used. For immunoprecipitation, Dynabeads panMouse immunoglobulin G (Invitrogen) (for TAP tags) and Dynabeads protein G (for all other tags and antibodies) were used. Precipitated DNA was analyzed by multiplex PCR.

Koinuma et al 2009, **Molecular and Cellular Biology**, January 2009, p. 172-186, Vol. 29, No. 1, *Chromatin Immunoprecipitation on Microarray Analysis of Smad2/3 Binding Sites Reveals Roles of ETS1 and TFAP2A in Transforming Growth Factor β Signaling,*

- **Dynaebads® Protein A, Dynabeads® anti-mouse IgG**
- **keywords: Smad2 and Smad3 (Smad2/3) proteins, transcription factors, gene expression**
- **Application: ChIP.** Cells were cultured in 15-cm plates to approximately 80% confluence, and one plate was used per immunoprecipitation. Cells were fixed with 1% formaldehyde for 10 min at room temperature with swirling. Glycine was added to a final concentration of 0.125 M, and the incubation was continued for an additional 5 min. Cells were washed twice with ice-cold phosphate-buffered saline, harvested by scraping, pelleted, and resuspended in 1 ml of sodium dodecyl sulfate (SDS) lysis buffer (50 mM Tris-HCl [pH 8.1], 1% SDS, 10 mM EDTA, protease inhibitors [P8340]). Samples were sonicated four times for 15 s each time at intervals of 30 s with a UH-50 sonicator (SMT, Japan). Alternatively, 0.2 ml of lysis buffer per 10-cm cell culture plate and a Bioruptor UCW- 201 (output, H; 15 cycles of 30 s of sonication with 30-s intervals; Cosmobio, Japan) were used for samples for some of the conventional ChIP-quantitative PCR (qPCR) analyses. Samples were centrifuged at 14,000 rpm at 8°C for 10 min. After removal of a control aliquot (whole-cell extract), supernatants were diluted 10-fold in ChIP dilution buffer (20 mM Tris-HCl [pH 8.0], 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, Complete EDTA-free protease inhibitors [Roche Diagnostics, Rotkreuz, Switzerland]). Samples were incubated at 4°C overnight in 2-methacryloyloxyethyl phosphorylcholine polymer-treated 15-ml polypropylene tubes (Assist, Japan) with protein A or anti-mouse
IgG-Dynabeads that had been preincubated with 5 to 10g of antibodies in phosphate buffered saline–0.5% bovine serum albumin. The beads were then moved to 1.7-ml siliconized tubes (catalog no. 3207; Corning, Corning, NY) and washed five times with ChIP wash buffer (50 mM HEPES-KOH [pH 7.0], 0.5 M LiCl, 1 mM EDTA, 0.7% deoxycholate, 1% Igepal CA630) and once with TE buffer (pH8.0). Immunoprecipitated samples were eluted and reverse cross-linked by incubation overnight at 65°C in elution buffer (50 mM TrisHCl [pH 8.0], 10 mM EDTA, 1% SDS). Genomic DNA was then extracted with a PCR purification kit (Qiagen).

Liang et al 2009, **MCB** Accepts, published online ahead of print on 24 August 2009, *Defective erythropoiesis in transgenic mice expressing dominant negative upstream stimulatory factor*

- **Dynabeads® Protein A**
- **Keywords:** Regulation of erythroid genes by USF
- **Application: Chromatin Immunoprecipitation (ChIP) and μChIP** Conventional ChIP assays were performed as described previously (13, 35).Spleens from anemic mice were homogenized and cell suspensions were subjected to ChIP analysis. The following antibodies were used in this study: USF1 (H-86), USF2 (N-18), (Santa Cruz Biotechnology); RNA Pol II (CTD45H8), (Upstate Biotechnology, Inc). In addition, for the yolk sac samples, MicroChIP (μChIP) was used according to a protocol with minor modifications (15, 16). Antibody-bead complexes were prepared as described using Dynabeads® Protein A beads (Invitrogen). Embryonic yolk sacs were crosslinked with 1% formaldehyde in 500 μL PBS and quenched with 125 mM glycine. Prior to sonication, yolk sacs were homogenized using a glass tissue grinder (Radnoti) and washed with PBS. Sonication conditions were optimized to yield fragments of ~500 bp, and sonication products were diluted 10-fold. Sonicated chromatin was incubated with various antibodybead complexes, and after a series of washes, DNA was purified using a QIAprep® Spin Miniprep Kit (Qiagen).

Outwin et al 2009**, Molecular and Cellular Biology**, August 2009, p. 4363-4375, Vol. 29, No. 16, *Smc5-Smc6-Dependent Removal of Cohesin from Mitotic Chromosomes,*

- **Dynabeads® Protein G**

- **keywords: Smc5-Smc6 complex, checkpoint maintenance, chromosome segregation Separase independent pathway,**

- Application: ChIP. A GFP-tagged allele of Rad21 was crossed into the relevant backgrounds, and chromatin immunoprecipitation (ChIP) was performed on 50-ml samples as described previously (2), with the exclusion of dimethyl adipimidate from fixation, using rabbit polyclonal anti-GFP antibodies (A11122; Molecular Probes) and protein G Dynabeads (Invitrogen). FACS was used to ensure that samples were in G2 prior to the inactivation of Mis4. ChIP primers are described in Table 1 and were designed with Primer 3 software (59). Data were generated by quantitative PCR (Opticon 3; MJ Research) and represent means standard errors (SE) (*n* 3 to 5).

Huang et al 2009**, Molecular and Cellular Biology**, August 2009, p. 4103-4115,
Vol. 29, No. 15, *Differentiation-Dependent Interactions between RUNX-1 and FLI-1 during Megakaryocyte Development*,

- **Dynabeads protein A**

- **Keywords: transcription factor RUNX-1, megakaryocyte differentiation, myelodysplastic syndrome and leukemia, transcriptional regulation**

- **Application: ChIP assays.** Primary murine fetal liver cells were harvested from E13.5 C57BL/6 mouse embryos and cultured in the presence of 1% thrombopoietin (TPO) conditional medium (56) for 4 days, and mature megakaryocytes were enriched by the discontinuous bovine serum albumin density gradient, as previously described (7). Murine L8057 megakaryoblastic cells were induced with 50nM TPA for 3 days. GATA-1 antibody (N-6), Fli-1 antibody (c-19), normal rabbit serum, and normal rat serum were purchased from Santa Cruz, and Runx-1 antibody (catalog no. 39000) was purchased from Active Motif. Chromatin immunoprecipitation (ChIP) was performed as previously described, with modifications (65). For GATA-1 ChIP, beads were incubated with rabbit anti-rat immunoglobulin G(H_L) [IgG(H_L)] (Jackson Research Laboratories, Inc.) before incubation with the antibody. Antibodies were incubated with beads for 3 h before being incubated with sonicated chromatin overnight, and two washes with 0.25MLiCl were added before the washes with Tris-EDTA. Precipitated DNA was quantified using real-time PCR and a QuantiTect SYBR green PCR kit (Qiagen) on an iCycler system (Bio-Rad).