

Pombe ChIP Dynabeads protocol w/ column DNA clean up

(modified from CHP_10v2 by **MF** and **SB**; updated by **PG** 22/05/2016 and **SB** 30/08/2016)
reformatted w/ additional comments by **SB** 15/05/2026

Day 1: Set up pre-cultures. Growth for 1 day at 30°C.

Day 2: Set up main 100 ml main cultures w/ YES media.

Depending on individual strain growth, use a dilution of 1:100 – 1:500 of post-log phase pre-culture (main culture should have a starting OD of 0.01-0.05).
Grow 14-16 hrs at 30°C.

Alternatively: main cultures can also be i/o directly from plate w/ starting OD of 0.02-0.04 OD.

Day 3: Growth, formaldehyde (FA) crosslink (X-link), and harvest

- Grow cultures to OD = 0.4-0.6
(OD up to 1-1.2 is also possible)
- **Add FA** (37%, Roth p.a.) to f.c. = 1% and **X-link 10 min** at RT with occasional mixing
i.e. for **100 ml** culture -> **2.7 ml FA**,
(or: for 400 ml -> 10.8 ml, for 500 ml -> 13.5 ml)
Optional: If exact cross-linking efficiency is critical, use **fresh** FA (25 mL bottles/Fisher Scientific)
- **Quench** remaining FA by adding 2.5 M glycine to f.c. = 125 mM and incubate for 10 min
i.e. for 100 ml -> 5 ml glycine
(for 400 ml -> 20 ml, for 500 ml -> 25 ml)
- **Collect cells** by centrifugation in 2x 50 ml Falcon tubes for 5 min at 700g at 4°C.
- **Decant and re-suspend** pellets in **25 ml ice-cold PBS** and pool aliquots in one tube. Pellet, decant and repeat wash 1 more time, then re-suspend pellet in **1ml ice-cold PBS**.
- **Transfer** cell suspension to a **2 ml screw-cap tube**, shortspin for 12 s at max. speed, decant, and freeze pellets in liquid nitrogen.
If larger cell cultures have been grown, split into several aliquots (e.g. 40-60 OD for each aliquot).
- **Store** at -80°C or proceed to step 1 of Day 4 (skip freezing).

Day 4: Lysis, sonication, and IP

Estimated time total 4-5 hr

- for lysis + sonication + IP preparation: 2-3 hr;
- antibody incubation 1.5-3 hr
- magnetic beads incubation O/N or > 4 hr

- Thaw cell pellets on ice and re-suspend pellets in **500 µl ice-cold lysis buffer w/ protease inhibitors**
(for a harvest volume and OD of 100 mL and 0.5, respectively, this corresponds approx. to 0.1 OD/µl).
Add protease inhibitors prior to use at the following final concentrations
(indicated volumes for 10 ml working solution)
 - 1 mM **AEBSF** (100 µl of 100X stock, 100 mM),
 - 100 µg/ml **Leupeptin** (10 µl 1000X stock, 100 mg/ml),
 - Roche complete** protease inhibitor cocktail (400 µl from 25X stock = 1 tablet in 2 ml PBS);
- Add 2 scoops (i.e. a 250 µl PCR tube ~ 250 µl vol equivalent) of zirconia beads.
- Lyse cells **6x** for 20 sec (max) in *PrecyLLis* cell disruptor (*PeqLab*) w/ intervals **on ice**.
important: use only screw-cap tubes! (to avoid leakage!)
- Optional: Check cell lysis under the microscope and proceed if more than 80% of the cells are lysed (otherwise do one more lysis cycle).
- Invert tubes and puncture bottoms with a **hot** (use gas burner) **22-gauge (grey) needle** and place tubes in 2.0 ml tubes, centrifuge for 3 min at 700 xg.
- **Transfer** samples to **polystyrene sonication tubes** (*Active Motif*, #53071 or #53072)
(no vortexing! produces foam!)
- **Shear** chromatin of samples by sonicating in a water bath **sonicator** (**Q800R**, *QSonica*) **for 30 min** with cycles of 30"ON/30"OFF (total incubation time: 60 min)
- **Transfer** sonicated material into 1.5 ml tubes and pellet cell debris for 10 min at 16,000g at 4°C; transfer supernatant into a fresh tube
- Repeat pelleting and transfer to reduce variability in results due to remaining debris in later steps ("lysate" or supernatant "SN").
- Optional: Input samples can be stored at -20°C, lysates at -80°C.

Day 4: continue with IP

- For each **IP**, use crude lysate corresponding to **5-30* ODs** and add lysis buffer (w/ protease inhibitors) to a final volume of 540 µl

e.g. for a concentration of crude lysate of 0.1 OD/µl, this corresponds to 125-250 µl lysate.

** for H3 and H3K36me3 use 5-10 ODs*

** for H3K9me2 use 10-15 ODs*

** for FLAG IP use 30 ODs*

- **Add 2-4 µl of antibody** per sample and incubate 4 hrs or o/n on a nutator; alternatively, place eppendorf tubes in a Falcon tube on a rolling mixer at 4°C. Final volume is 600 µl.
 - for H3K9me2 use 2 µl of Abcam ab1220
 - for FLAG use 2 µl of Sigma F3165
 - Recommendation: dilute the antibody prior addition w/ lysis buffer and pipette larger volumes to the samples to minimize pipetting errors; e.g. prepare a working 1:50 dilution and add 100 µl to each sample
- **INPUT:** Remove **40 µl of lysate** and add **160 µl of TE/1% SDS** solution. This is the “Input DNA” sample. The Input DNA will be processed along with the IP’d chromatin on Day 5 at step 6.
- **Prepare ProtG/Dynabeads** (Invitrogen). Per sample calculate **25 µl of magnetic beads:**
 - **Wash** the magnetic beads 2x with PBS/0,1%Tween (incubation is not required, beads for several IP samples can be processed together, e.g. for 100 µl beads use 1ml PBS/T)
 - **Wash** once with 1 mL lysis buffer to reduce residual detergent.
 - **Resuspend the beads** in lysis buffer in a total volume corresponding to 100 µl (or 50 µl) per sample. Add 100 (50) µl to each sample and incubate on a nutator (or in a Falcon tube on a rolling mixer) at 4°C for **>4 hours or O/N**. (Final volume is 700 µl)
 - **Alternatively:** Add ProtG/Dynabeads next day

Day 5: IP wash; reverse X-link

Estimated time: wash 1-2 hr; reverse cross-link 4 hrs

- Put samples onto the magnetic stand, incubate for 1min. Remove SN with a pipette or vacuum bottle and pipette tipped holder (exchange tip after each step).
- **Wash** beads as follows (at RT with cold buffer):
 - 2X with 0.5 ml **Lysis buffer** (buffer 1)
 - 2X with 0.5 ml **High salt lysis buffer** (buffer 2)
 - 2X with 0.5 ml **Wash buffer** (buffer 3)
 - 1X with 150 µl TE and transfer all into a **fresh tube**, keep IPs with TE on ice when not used

optional: spin down samples for 10s at 0.1g to remove buffer from the lids before TE addition and transfer.

 - Put samples onto the magnetic stand and completely remove the SN
(This step is important, as proteins and DNA bind non-specifically to the tube wall).
- Add **200 µl Elution buffer 3** (TE, 0.8% SDS)
- **Reverse X-link**: incubate IP samples together with INPUT samples **10 min at 95°C and max speed** on thermomixer, short spin to remove condensation from the lid and transfer the supernatant to a new tube **3 hr at 65°C**

Reverse X-link of eluate together with beads before transfer into fresh tube is possible and does not affect the efficiency of the rev. X-link; the extended incubation may actually increase the elution efficiency

Day 6: Proteinase K treatment; DNA clean-up; qPCR

(estimated time for Proteinase K: 2 hrs; clean up: 0.5 hr; qPCR setup 1-2 hr; qPCR run 1.5 hr)

- Prepare **fresh** Proteinase K (4mg/ml), add 10 µl to each sample and incubate for 2x 1hr at 55°C, vortex and briefly spin after the first hour to keep the suspension homogeneous.
- Clean-up the DNA with *Zymo Research*, ChIP DNA Clean & Concentrator™, #D5201:
 - Add **5 volumes** of **DNA-binding buffer** to samples
(**1x 1 ml Binding Buffer + 200 µl sample**)
 - Add the mix onto the column in two subsequent steps (each ~600 µl) and spin for 30"
 - Add 200 µl Wwash Buffer and 30" spin (use the wash buffer that contains EtOH!)
 - Add 200 µl Wash Buffer and 30" spin (use the wash buffer that contains EtOH!)
 - Do empty spin for 2',
 - Transfer columns into new tubes and add Elution Buffer: **50 µl** for input, **15-25 µl** for IP.
 - Elute samples by a 1' spin
- **Optional:** Treat INPUT samples with **RNaseA** (f.c. 10 ug/ml) for 30 min at 37°C prior to ProtK

This allows the analysis of mono-nucleosome preparation by agarose gel electrophoresis: load 25-50% of INPUT material on 1.8-2% agarose gel **w/out** EtBr; run at 50-75 V for 45-60 min; stain with EtBr for 2-3', destain with running buffer for 1' with agitating)

- for qPCR use **5 µl** sample (**1:20** dilutions for **ChIP**, **1:100** dilutions for **large tiling arrays** (test on small scale before) and **INPUT**)

2.5 µl Primer For/Rev mix (1.5 uM)

7.5 µl PowerUp! SYBR Green Mastermix

Use one of the input samples for normalization (standard curve). Prepare 6 standard samples (1/10 serial dilution), starting with a 1/20 dilution of the non-diluted input sample (use non-tag or WT if possible)

Use **96-well MicroAmp® Optical 96-Well Reaction Plate** and **qPCR Seal**

- qPCR program stored on Dell™ Notebook of 7500 Fast Real-Time PCR System machines as PowerUp w/o melting curve (use w/ for untested primers)

50°C: 2 min, 95°C: 2 min,

[95°C: 20 s, 50°C: 20 s, 72°C: 40 s, 73°C: 1 s, read] X40 cycles

record melting curve 70°C to 90°C; cool down to 10°C.

Buffers and Solutions:

Formaldehyd (fresh - stock: 37%)
 2.5 M glycine (MW 75.07): 93.75 g in 500 ml (sterile filt.)
 Zirconia beads

Tris/HCl 1 M, pH 8
 TBS (20mM Tris/HCl pH 7.5, 150mM NaCl) (autoclaved)
 TE (10 mM Tris/HCl pH 7.6, 1 mM EDTA) (autoclaved)

0.5 M HEPES/KOH, PH 7.5 (sterile filtered)

5 M NaCl (autoclaved)
 0.5 M EDTA (autoclaved)
 10% SDS (autoclaved)
 10% Triton X-100 (H₂O)
 10% Na-Deoxycholate (H₂O)
 10% Tween 20 (H₂O)

Buffers:	final conc (mM or %)	stock solution	stock conc (mM or %)	100 ml	500 ml
Lysis buffer	50	HEPES/KOH pH 7.5	500	10	50
	140	NaCl	5000	2.8	14
	1	EDTA	500	0.2	1
	1	Triton X-100	10	10	50
	0.1	Na-Deoxycholate	10	1	5
Lysis buffer high salt	50	HEPES/KOH pH 7.5	500	10	50
	500	NaCl	5000	10	50
	1	EDTA	500	0.2	1
	1	Triton X-100	10	10	50
	0.1	Na-Deoxycholate	10	1	5
Wash buffer	10	Tris/HCl, pH 8	1000	1	5
	250	LiCl	4000	6.25	31.25
	1	EDTA	500	0.2	1
	0.5	NP-40	10	5	25
	0.5	Na-Deoxycholate	10	5	25
PBS-Tween	0,1%	PBS-Tween	100x	10	50
Elution buffer 3	50	Tris/HCl, pH 8	1000	5	
	10	EDTA	500	2	
	0.8	SDS	10	8	
TE + 1% SDS	10	Tris/HCl, pH 8	1000	1	
	1	EDTA	500	0.2	
	1	SDS	10	10	

Antibodies used for ChIP:

anti-H3:	polyclonal (rabbit), box B	Abcam ab1791	3-4 µl
anti-dimeK9H3:	monoclonal (mouse), box G	Abcam ab1220	3 µl
anti-trimeK9H3:	polyclonal (rabbit), box C	Upstate 07-523	2 µl
anti-trimeK4H3:	polyclonal (rabbit), box B	Abcam ab8580	3 µl
anti-Flag:	monoclonal (mouse)	Sigma F3165	2 µl

CHP_10v4b: Quick protocol guide

Growth, crosslink, and harvest

- At OD = 0.4-0.6, add FA (f.c. 1%) and **x-link 10'** at RT, (i.e. 2.7 ml for 100 ml culture)
- Quench with 2.5 M glycine (f.c. 125 mM) for 10 min (i.e. 5 ml for 100 ml culture)
- Pellet at 700x g at 4°C for 5', wash 2x w/ 25 ml ice-cold PBS, re-suspend in 1 ml ice-cold PBS,
- Transfer cell suspension to a **2 ml screw-cap tube**, pellet, decant, and freeze pellets in liquid nitrogen.

Lysis, sonication, and IP

- Add **500 µl ice-cold lysis buffer** w/ protease inhibitors + 2 scoops of **zirconia beads**; for 10 ml working solution prepare 100 µl **AEBSF**, 10 µl **Leupeptin**, 400 µl **Roche complete**
- Lyse cells 6X for 20 sec (max) in Precytlis (PeqLab) w/ intervals on ice.
important: use only screw-cap tubes! (otherwise leakage!)
- Puncture tubes with a hot 22-gauge needle, place in 2 ml tubes, centrifuge 3 min at 700 xg, and transfer to polystyrene sonication tubes
- Sonicate w/ **Q800R (QSonica)** for **30 min** with cycles of 30"ON/30"OFF
- Pellet cell debris for 2x 10 min at 16,000Xg at 4°C and transfer supernatant to a fresh tube.
- For each IP, use **5-30 ODs** of lysate and add lysis buffer to 540 µl
- For input, remove **40 µl of lysate** and add **160 µl of TE/1% SDS** solution
- Add **2-4 µl** of antibody and incubate 4 hrs or o/n on a nutator at 4°C. Final volume = 600 µl
- ProtG/Dynabeads — 25 µl/sample; wash 2x PBS/0.1% Tween + 1x lysis buffer; incubate at 4°C for >4 hrs or O/N.

IP wash; reverse crosslink

- Put the samples in the **magnet stand**, wash beads as follows (at RT with cold buffer):
 - 2X with 0.5 ml **Lysis buffer** (buffer 1)
 - 2X with 0.5 ml **High salt lysis buffer** (buffer 2)
 - 2X with 0.5 ml **Wash buffer** (buffer 3)
 - 1X with 150 µl TE and transfer all into a **fresh tube**
- Add 200 µl Elution buffer 3 (TE, 0.8% SDS)
- **Reverse X-link:** incubate IP + INPUT 10 min at 95°C/max. rpm; short-spin, transfer IP SN to fresh tube, then 3 hrs at 65°C.

Proteinase K treatment; DNA clean up; qPCR

- Prepare **fresh Prot K** (4mg/ml), add 10 µl to each sample and incubate for **2x 1hr at 55°C**.
 - **Clean-up** the DNA with *Zymo Research, ChIP DNA Clean & Concentrator™*
 - Add **5 volumes** of DNA-binding buffer (2x 600 µl) ->load -> 30" spin
 - Wash 2x w/ 200 µl Wash Buffer -> 30" spin each
 - Empty spin 2' -> transfer column into new tube -> add 50 µl (input) or 15-25 µl (IP)
 - Elute samples by 1' spin
 - **qPCR mix** **5 µl sample (1:20 ChIP; 1:100 INPUT; for large tiling arrays test 1:100 on small scale)**
 - 2.5 µl Primer For/Rev mix (1.5 µM)**
 - 7.5 µl PowerUp! SYBR Green Mastermix**
- Use one input sample as standard: 6 points, 1/10 serial dilution, starting at 1:20**
Use 96-well MicroAmp® Optical 96-Well Reaction Plate and qPCR Seal
- **qPCR program: PowerUp w/o melting curve (use w/ for untested primers)**
50°C: 2 min, 95°C: 2 min
[95°C: 20 s, 50°C: 20 s, 72°C: 40 s, 73°C: 1 s, read] X40 cycles
record melting curve 70°C to 90°C; cool down to 10°C.