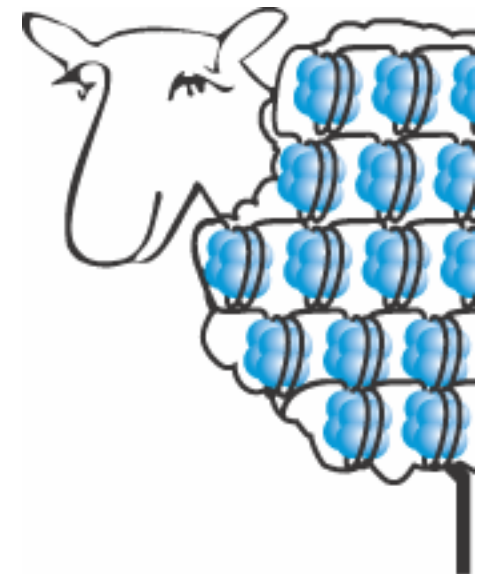


# ChIP grade antibodies: selection and validation

Rachel Imoberdorf, PhD  
Senior Development Scientist





- Introduction
- Antibody selection
- Antibody validation
- CHIP at [www.abcam.com](http://www.abcam.com)

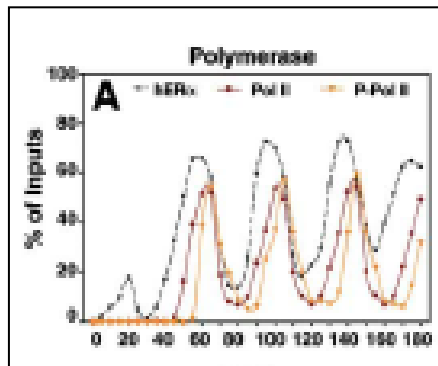
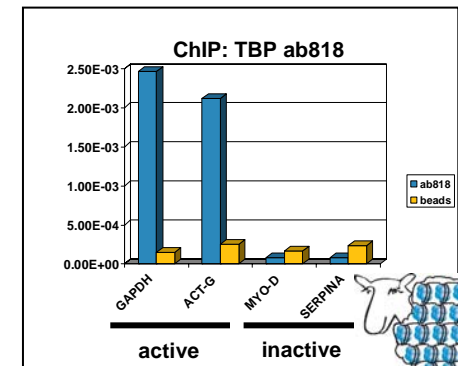


# Introduction

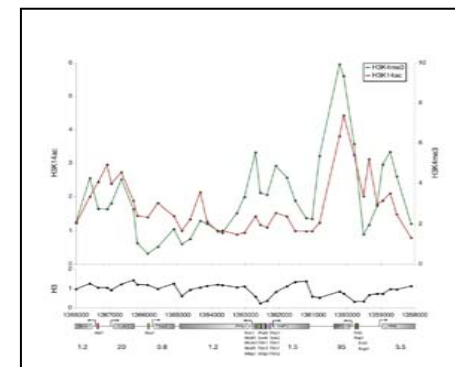
# Chromatin Immunoprecipitation (ChIP)

Allows quantitative analysis of Protein-DNA interactions *in vivo*.

- Localisation of proteins associated with DNA

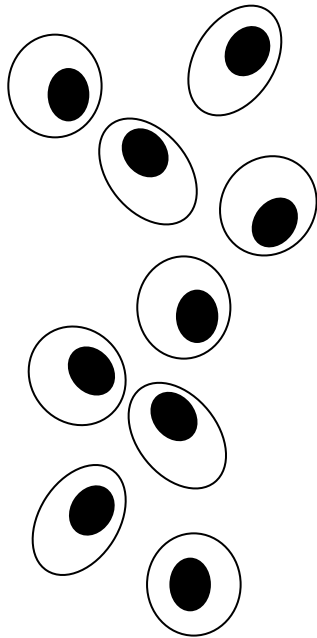


- Follow changes at promoters

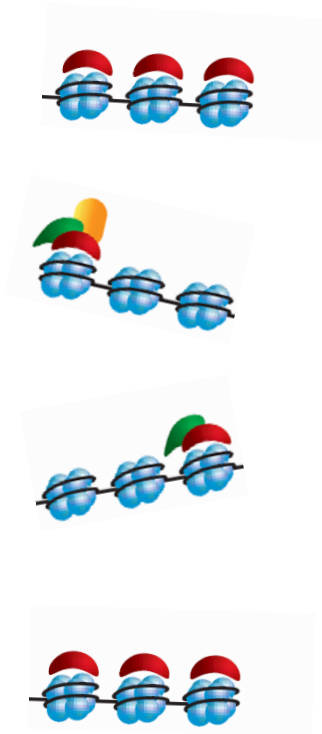


- Map distribution of factor over entire genome

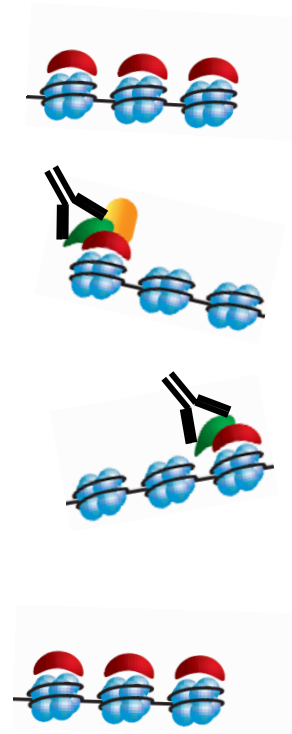
**Cross-linking**



**Sonication**



**Immunoprecipitation**



**DNA analysis**

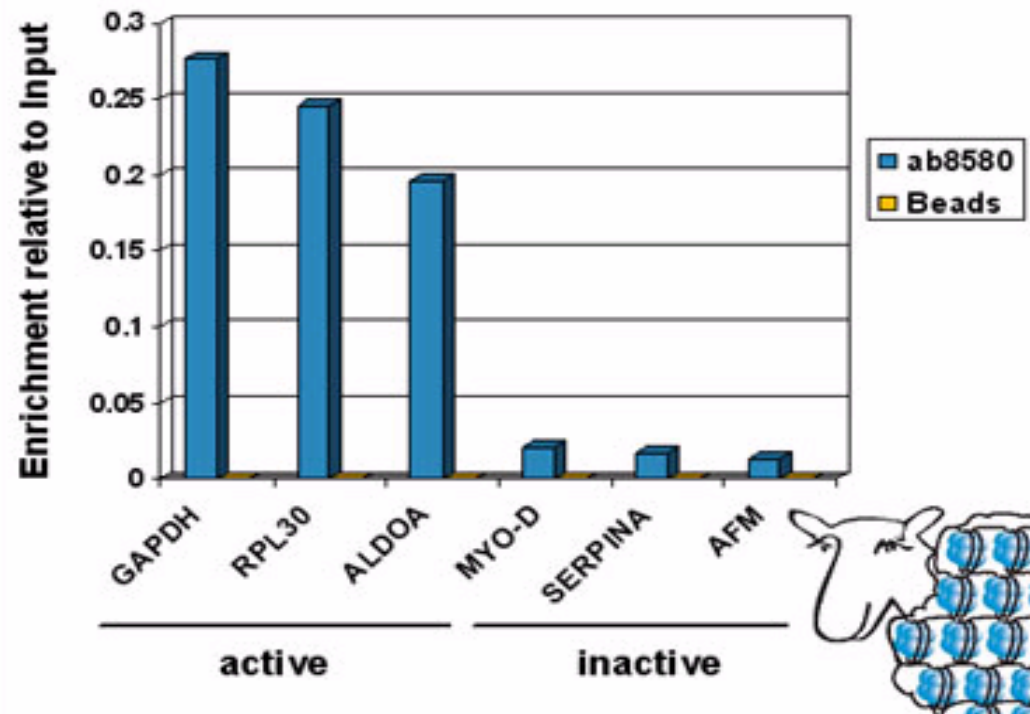
Quantitative  
PCR

Microarray

Cloning

## Data

ChIP: H3 tri-methyl K4 (ab8580)



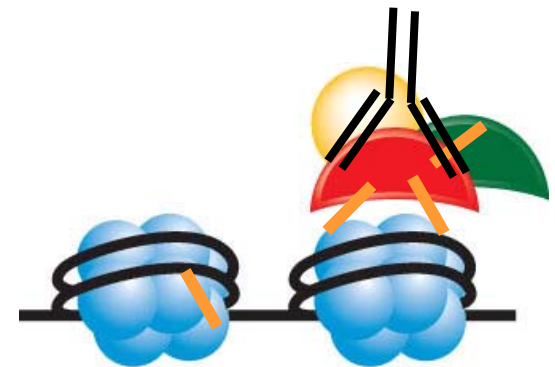
- Active genes marked by H3 K4 tri-methylation



# Antibody selection

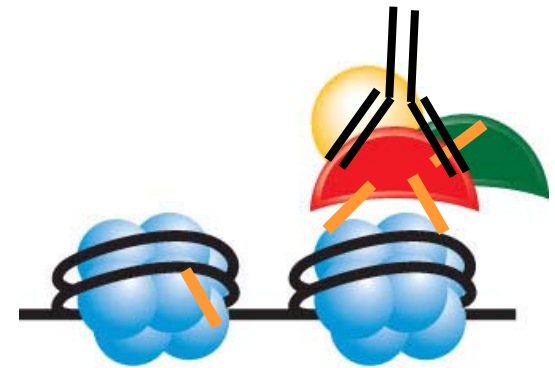
## Antibody is the most important player in ChIP

- Cross-link
- Sonication
- **Immunoprecipitation**
- DNA analysis



## Pass rate for ChIP is low

- Non histone antibodies: 20% (24/120)
- Histone antibodies: 50% (25/50)



## Antibody should be fully characterised and specific

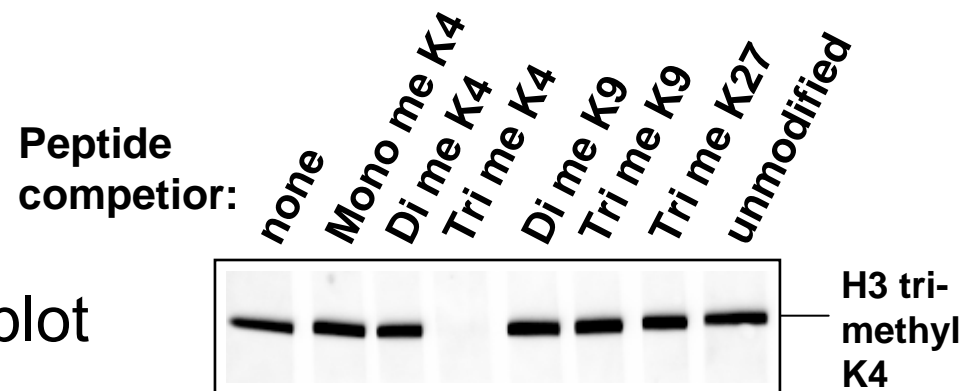
- Affinity purified
- Specificity tested in:

-ELISA

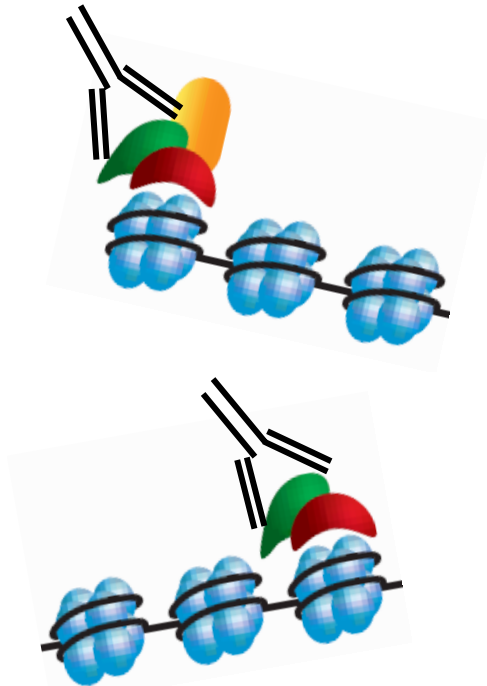
-Peptide inhibition western blot

### Peptide inhibition western blot

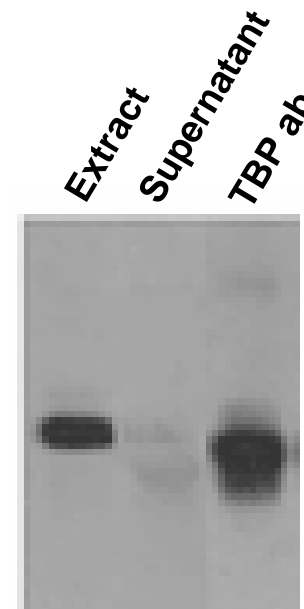
H3 tri-methyl K4 (ab8580)



## IP is good indicator for success in ChIP



Immunoprecipitation –  
TBP antibody (ab28176)

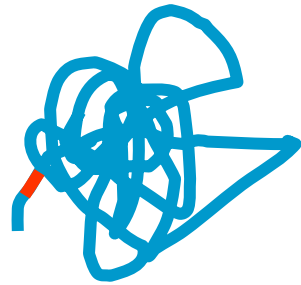


## IHC is also good indicator for success in ChIP

Protein  
conformation:

**Native**

**Denatured**

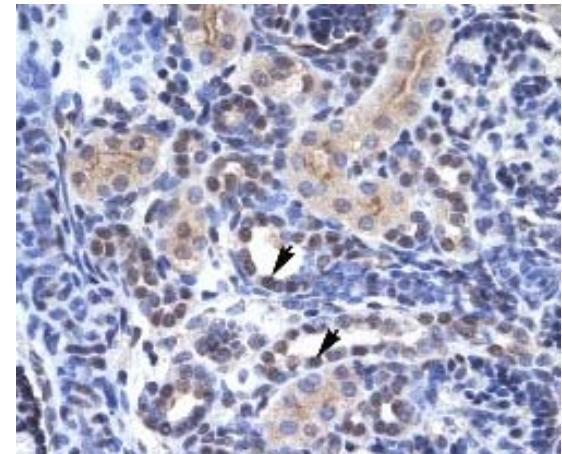


Technique:

**ChIP/IP/IHC**

**Western blot**

**Immunohistochemistry –  
TAF1 antibody (ab28450)**





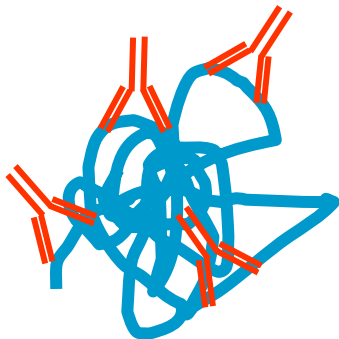
## Antibodies working in IP or IHC

Code	Target	IP	IHC	ChIP
ab15597	BRM/SMARCA2	yes	ND	yes
ab3652	CBP	ND	yes	yes
ab13939	CenPA	yes	yes	yes
ab1791	Histone H3	yes	yes	yes
ab9057	HP1 a	ND	yes	no
ab3752	MeCP2	ND	yes	yes
ab2605	Menin	yes	yes	yes
ab5408	RNA polymerase II CTD repeat [4H8]	yes	yes	yes
ab12405	Suv39H1	yes	yes	yes
ab818	TBP	yes	ND	yes

**Antibodies  
working in IP  
or IHC**

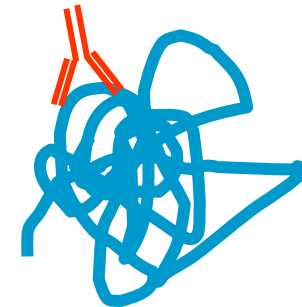
## Polyclonal antibodies have higher success rate in ChIP

### Polyclonal antibodies



- + recognise several epitopes
- batch to batch variation

### Monoclonal antibodies



- recognise single epitope
- + minimal batch to batch variation

## Summary

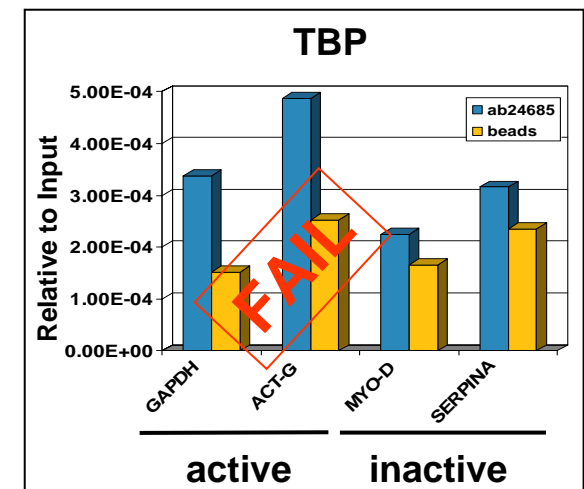
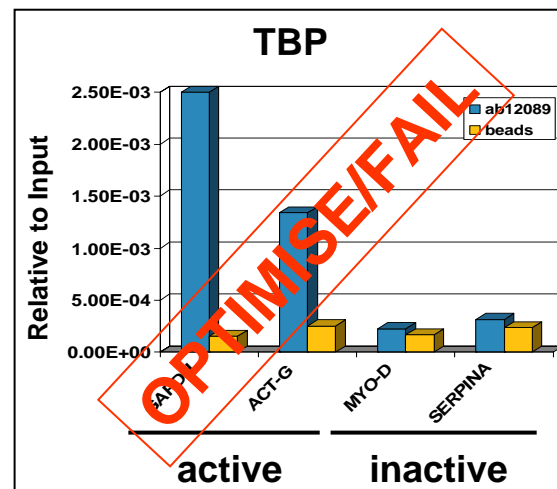
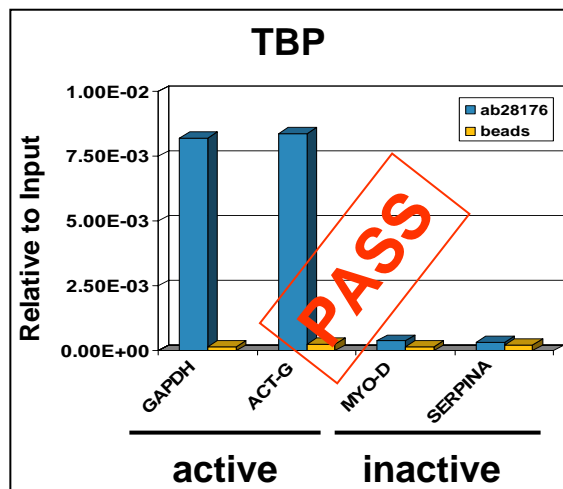
- Well characterised and specific
- IP and IHC are good indicators for success in CHIP
- Polyclonal antibodies have higher success rate in CHIP



# Antibody validation

## ChIP using standard conditions

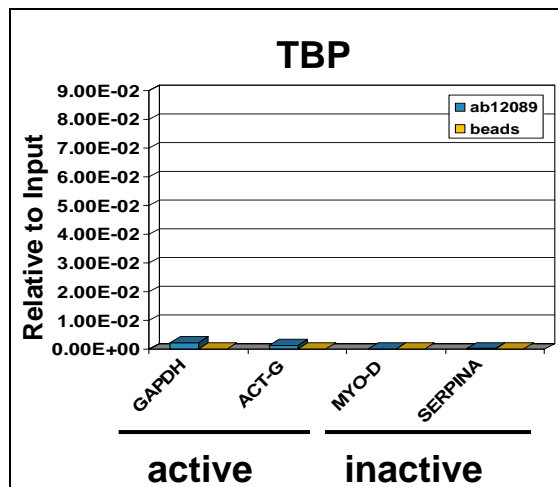
- Requirement: Control loci (+/-)



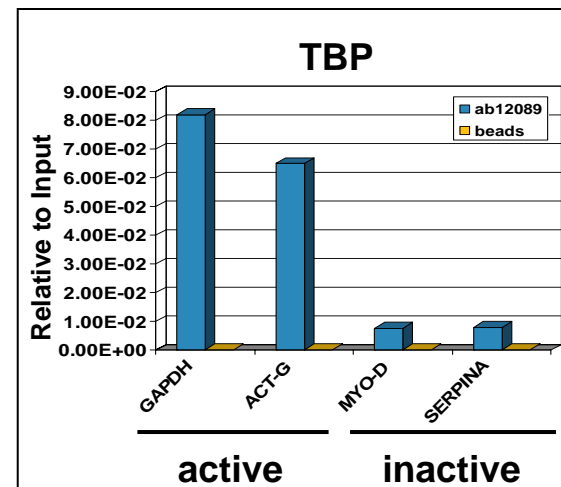
## Antibody concentration should be optimised

- 1–10ug of antibody for every 25ug of chromatin

Antibody: 2ug



10ug

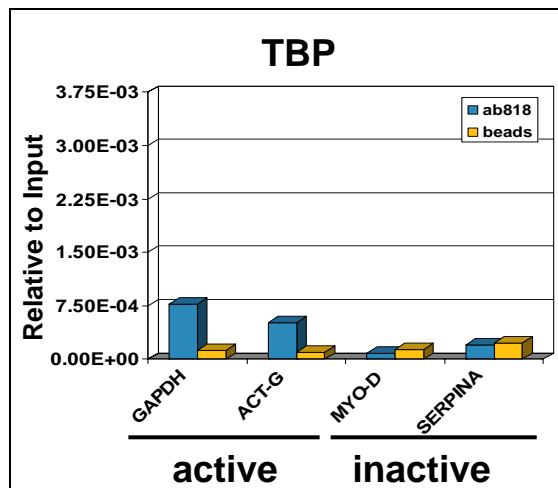


## Test different washing conditions

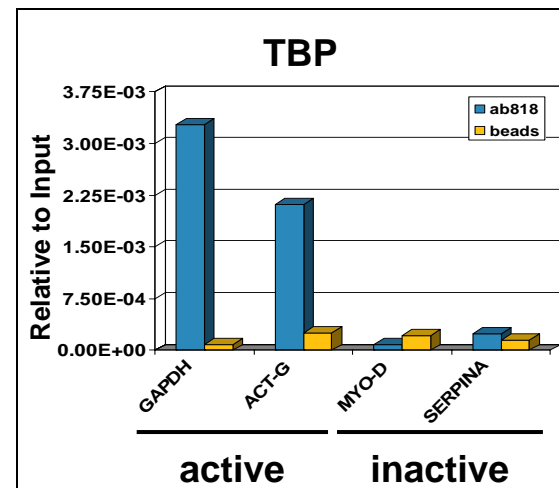
- Salt concentration of last wash: 150-500mM NaCl

[NaCl]:

500mM



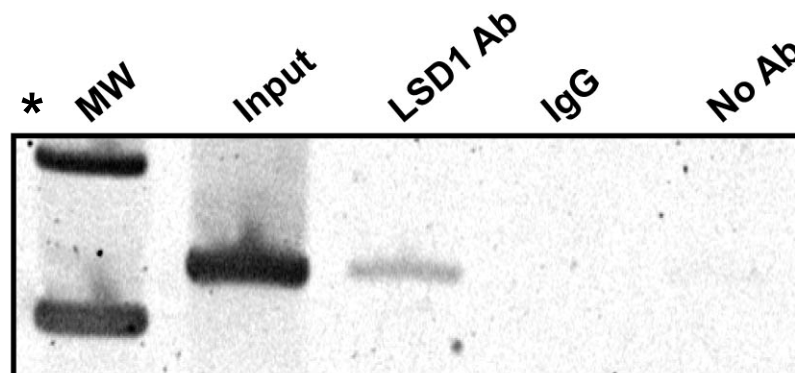
250mM



- Washes should be as stringent as possible

## Western blot can be used to test whether the target has been immunoprecipitated

- Perform ChIP up to the final wash
- Boil samples in SDS loading buffer and resolve on SDS page gel



\*Picture from Abreview

## Summary

- Perform standard ChIP experiment
- Antibody concentration can be optimized
- Washing conditions can be optimized
- Test whether target is immunoprecipitated



# Chromatin immunoprecipitation at Abcam



# ChIP at Abcam

- >160 ChIP grade antibodies



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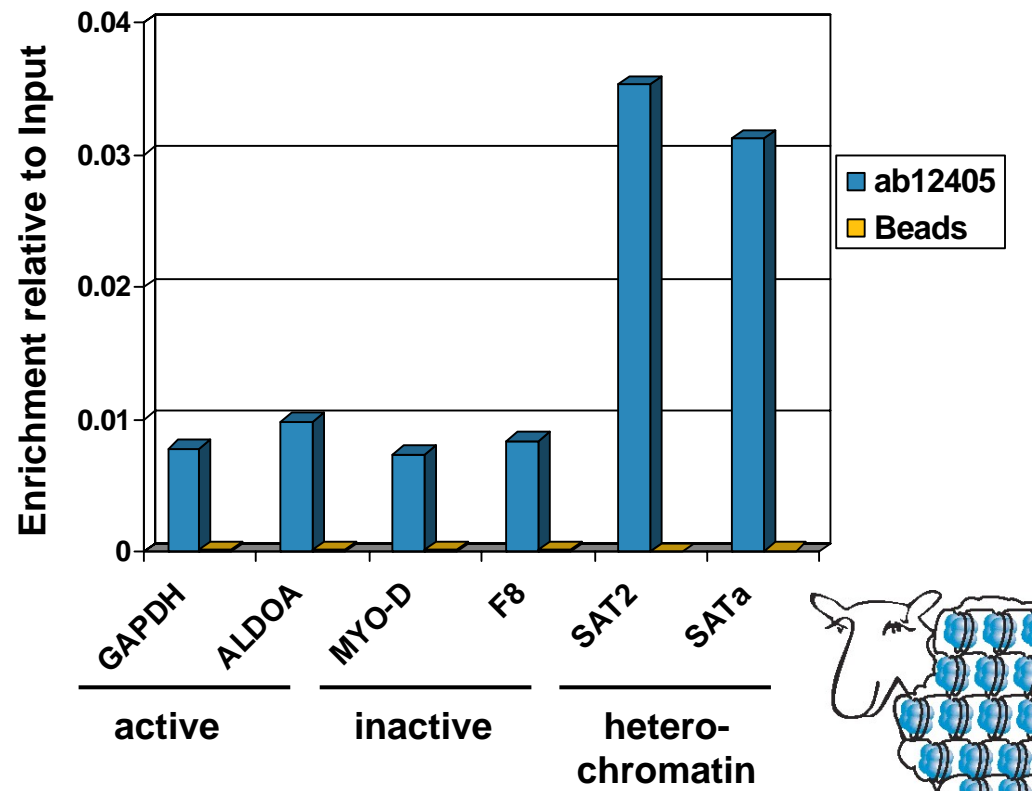
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## Abcam ChIP testing program

- Antibody passes ChIP if:
  - Signal specific
  - Enrichment over background >10x
- Data and experimental conditions published on datasheet

ChIP: SUV39H1 ab12405

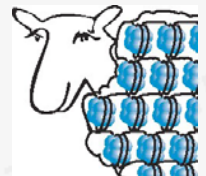
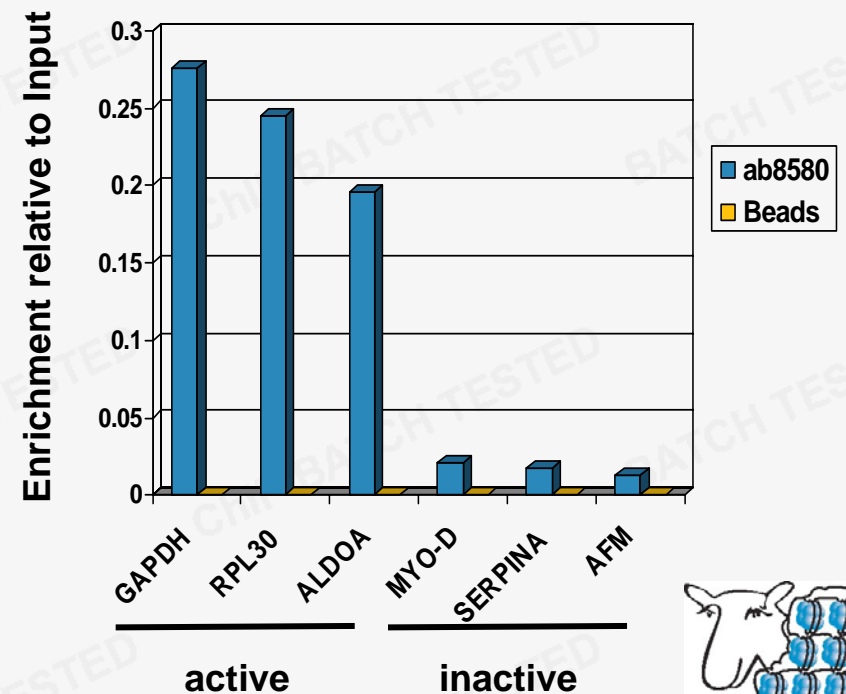


## ChIP batch tested antibodies

- >40 ChIP batch tested antibodies:

- Histones
- Histone modifications
- Other modified proteins

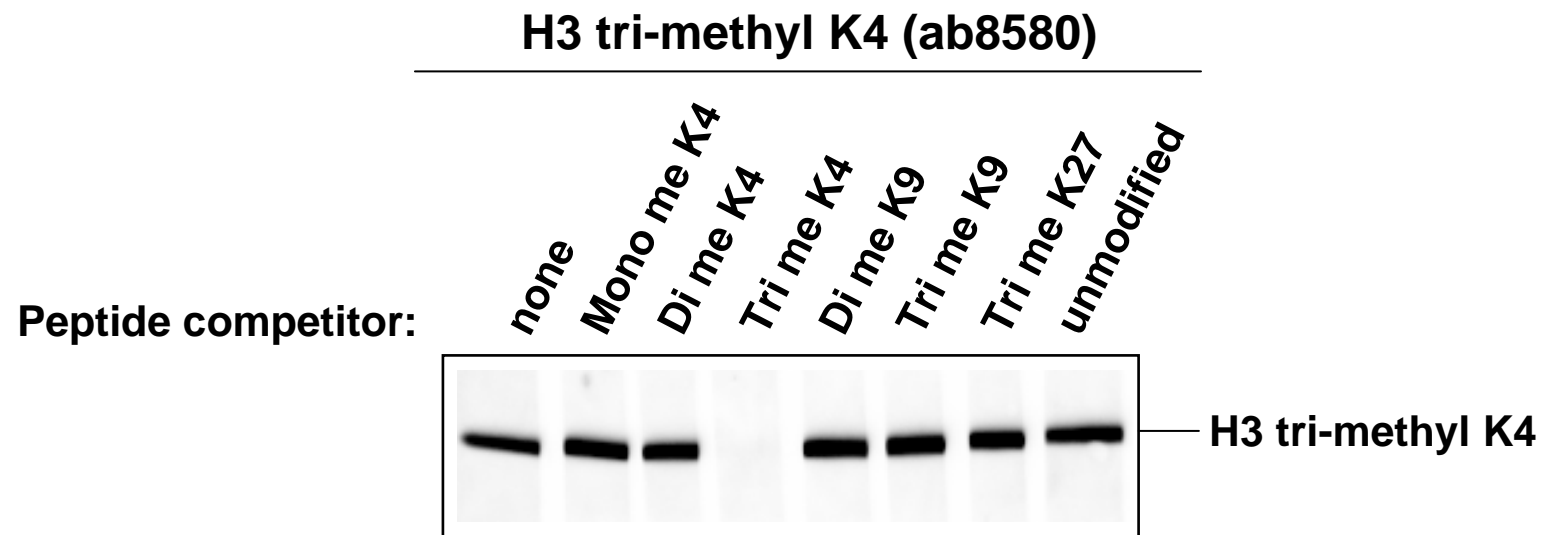
ChIP: Histone H3 (tri methyl K4) ab8580



Every new batch of this antibody is tested in ChIP.

## ChIP batch tested Histone modification antibodies

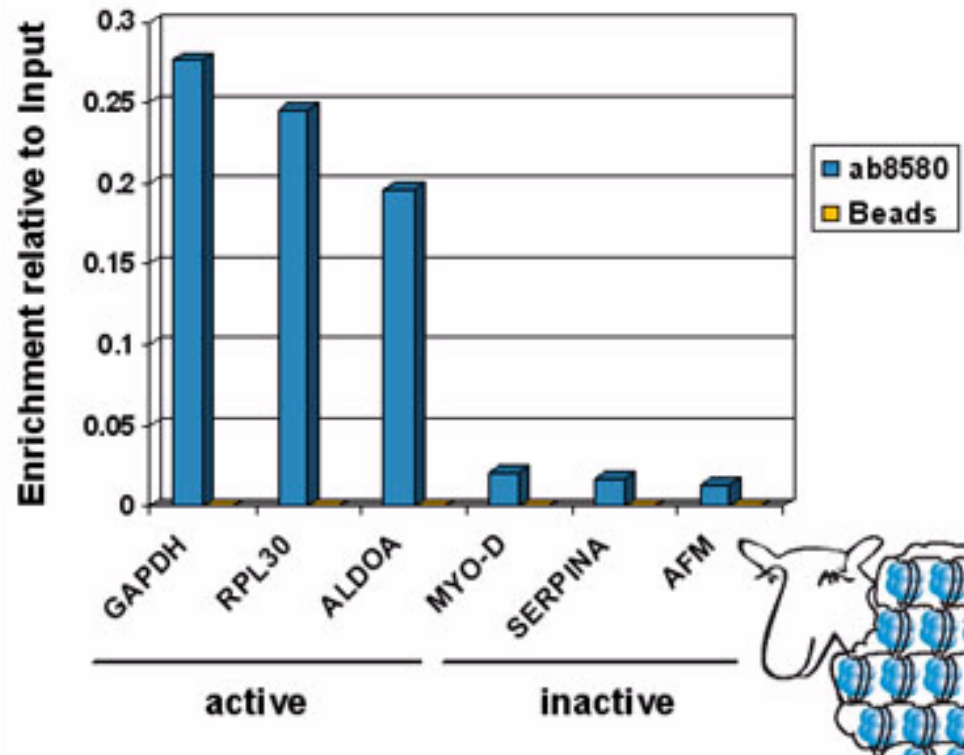
- Specificity tested by peptide inhibition western blot



- Antibody does NOT cross react

## ChIP batch tested Histone modification antibodies

ChIP: H3 tri-methyl K4 (ab8580)



- Antibody passes ChIP if:
  - Signal strength comparable to original batch
  - Pattern of enrichment over a set of 6 loci similar to original batch

- ChIP batch testing pass rate: 75%



# Primer sequences for ChIP on datasheet

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## Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580) protocols

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### PCR primers used to test ab8580 in ChIP at Abcam

The following primers were used to test ab8580 by chromatin immunoprecipitation (ChIP)

*Quantitative PCR is performed using the Taqman approach to amplify the loci shown. Primers are shown in capital letters and the probes in lower case. Primers were all used at 0.9uM final and the probes at 0.25uM final.*

BC025925 (accession number) hsGAPDH exon 1 F1 hsGAPDH intron 1 R1 hsGAPDH exon 1	TCG ACA GTC AGC CGC ATC T CTA GCC TCC CGG GTT TCT CT tcg cca ggt gaa gac ggg cg
BC0327000 (accession number) hsRPL30 first exon F1 hsRPL30 first exon R1 hsRPL30 exon 1	CAA GGC AAA GCG AAA TTG GT GCC CGT TCA GTC TCT TCG ATT tct cgc taa caa ctg ccc agc ttg gag
BC004333 (accession number) hsALDOA second intron F hsALDOA second intron R1 hsALDOA intron 2	TCC TGG CAA GAT AAG GAG TTG AC ACA CAC GAT AGC CCT AGC AGT TC atc cac gag aaa agg cat ttc cgg c
X56677 (accession number) hsMYO-D exon 1 F1 hsMYO-D exon 1 R1 hsMYO-D exon 1	CCG CCT GAG CAA AGT AAA TGA GGC AAC CGC TGG TTT GG tca agc gct gca cgt cga gca at
AF113676 (accession number) - Liver specific hsSERPINA1 (anti trypsin) intron 1 F1 hsSERPINA1 (anti trypsin) intron 1 R1 hsSERPINA1 (anti trypsin) intron 1	GGC TCA AGC TGG CAT TCC T GGC TTA ATC ACG CAC TGA GCT TA ggc tca agc tgg cat tcc t
L32140 (accession number) - Liver specific hsAFM first intron F1 hsAFM first intron R1 hsAFM exon 1	GCA GAA CCT AGT TCC TCC TTC AAC AGT CAT CCC TTC CTA CAG ACT GAG A aca gtt tga aca tcc ctc ctg agc ctg g

*The PCR is performed with the TaqMan Universal PCR Master Mix from ABI.*

Amplification:  
Stage 1 - 2 min at 50°C 1 repetition  
Stage 2 - 10 mins at 95°C 1 repetition  
Stage 3 - 30 sec at 95°C, 1 min at 60°C 35 repetitions



# ChIP protocol at [www.abcam.com/ChIP](http://www.abcam.com/ChIP)

## CROSS-LINKING CHROMATIN IMMUNOPRECIPITATION (X-ChIP) PROTOCOL

### Cross-linking and Cell Harvesting

1. Start with 2 large dishes when confluent ( $1 \times 10^7$ -  $5 \times 10^7$  cells per dish). Cross-link proteins to DNA by adding formaldehyde drop-wise directly to the media for a final concentration of 0.75% and rotate gently at room temperature (RT) for 10mins.
2. Add 1.5ml of glycine (2.5M) to the media and incubate with shaking for 5mins at RT.
3. Rinse cells 2 x with 10ml cold PBS.
4. Scrape cells into 5ml cold PBS and transfer into 50ml tube.
5. Repeat with 3ml PBS.
6. Centrifuge for 5mins at 3,000rpm.
7. Carefully aspirate off supernatant and resuspend pellet in FA Lysis Buffer (750 $\mu$ l per  $1 \times 10^7$  cells).

### Sonication

8. Sonicate lysate to shear DNA to an average fragment size of 500 to 1000bp. Follow the fragment size on a 1.5% agarose gel.
9. Centrifuge for 30secs at 13,000rpm and transfer supernatant to new tube.\*
10. Remove 50 $\mu$ l of each sonicated sample and add to 80 $\mu$ l (when using the DNA purification kit) / 400 $\mu$ l (when using phenol:chloroform) Elution buffer. This sample is the INPUT. This is used for obtaining the DNA concentration for subsequent IP's (see below) and as control in the final PCRs.

### Determination of INPUT DNA concentration

11. Add either 2 $\mu$ l RNase A (0.5mg/ml) when purifying DNA using QIAquick (see 12a) or 5 $\mu$ l of proteinase K (20mg/ml) when purifying DNA using Phenol:Chlorophorm (see 12b) to each INPUT sample in Elution Buffer and heat at 65 °C for 4-5hrs (or overnight) to reverse cross-linking.\*\*
- 12a. The DNA is then purified by using a DNA purification kit (e.g. QIAquick PCR purification kit) following the manufacturer's instructions.
- 12b. Alternatively the DNA can be Phenol:Chlorophorm extracted and ethanol precipitated in presence of 10 $\mu$ l glycogen (5mg/ml) and taken up in 100 $\mu$ l H<sub>2</sub>O.
13. Transfer 5 $\mu$ l of the sample in a tube containing 995 $\mu$ l TE to give a 200-fold dilution and read the OD<sub>260</sub>. The concentration of DNA in  $\mu$ g/ml is OD<sub>260</sub> x 10,000.



**Technical support for ChIP at [www.abcam.com](http://www.abcam.com)**



**Dr Hugh Spotswood**

## Summary

- Large range of ChIP grade antibodies (>160)
- In house ChIP testing program
- Data and experimental conditions published on datasheet
- ChIP resources

## The chromatin team



**Dr Rhian  
Hayward**  
Marketing  
manger



**Candy  
Smelly**  
Marketing  
coordinator



**Dr Jonathan  
Frampton**  
Business  
Development  
Associate

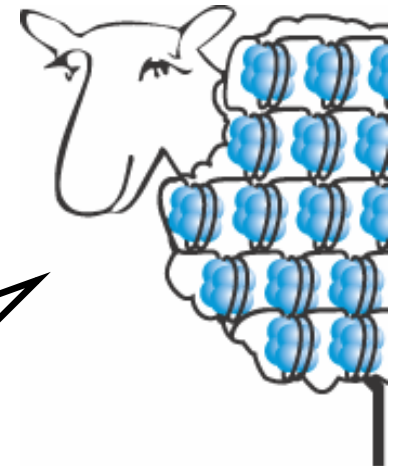


**Dr Rachel  
Imoberdorf**  
Senior Development  
Scientist



**Dr Hugh  
Spotswood**  
Technical Support

**Thank you for your attention!**

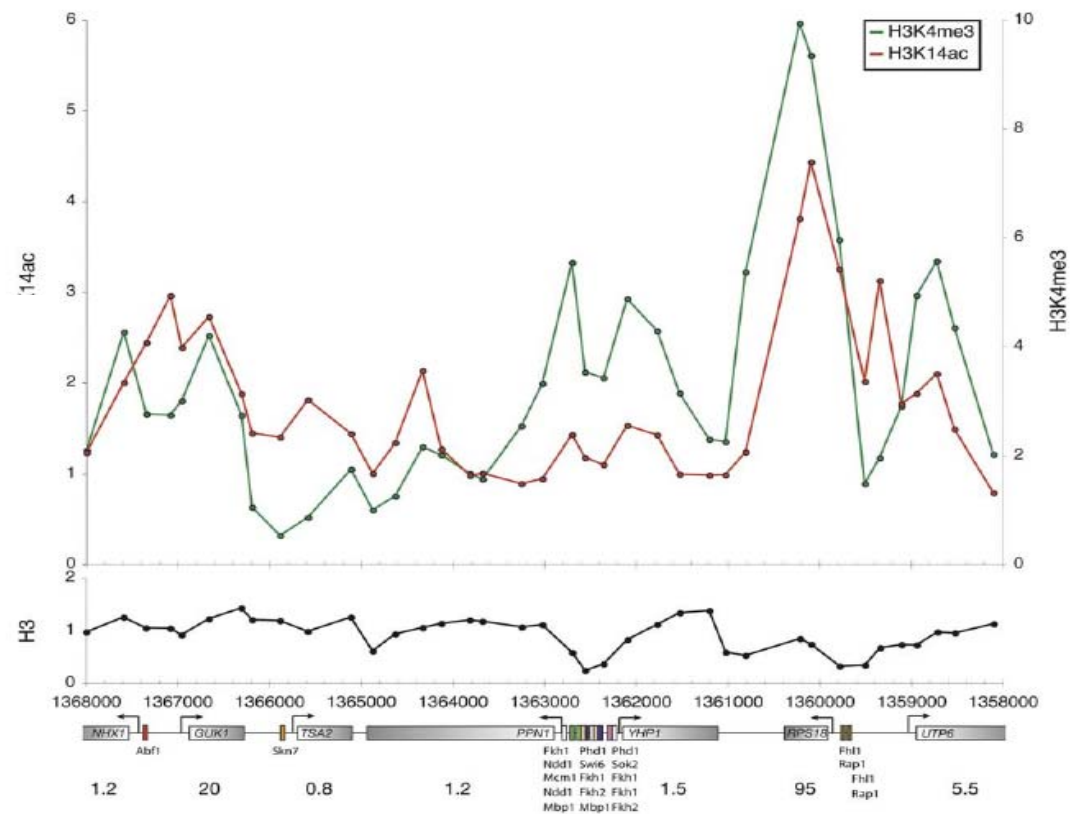


## ChIP on ChIP

- Pokholok et al., (2005) Genome-wide Map of Nucleosome Acetylation and Methylation in Yeast. *Cell*, **122**, 517–527

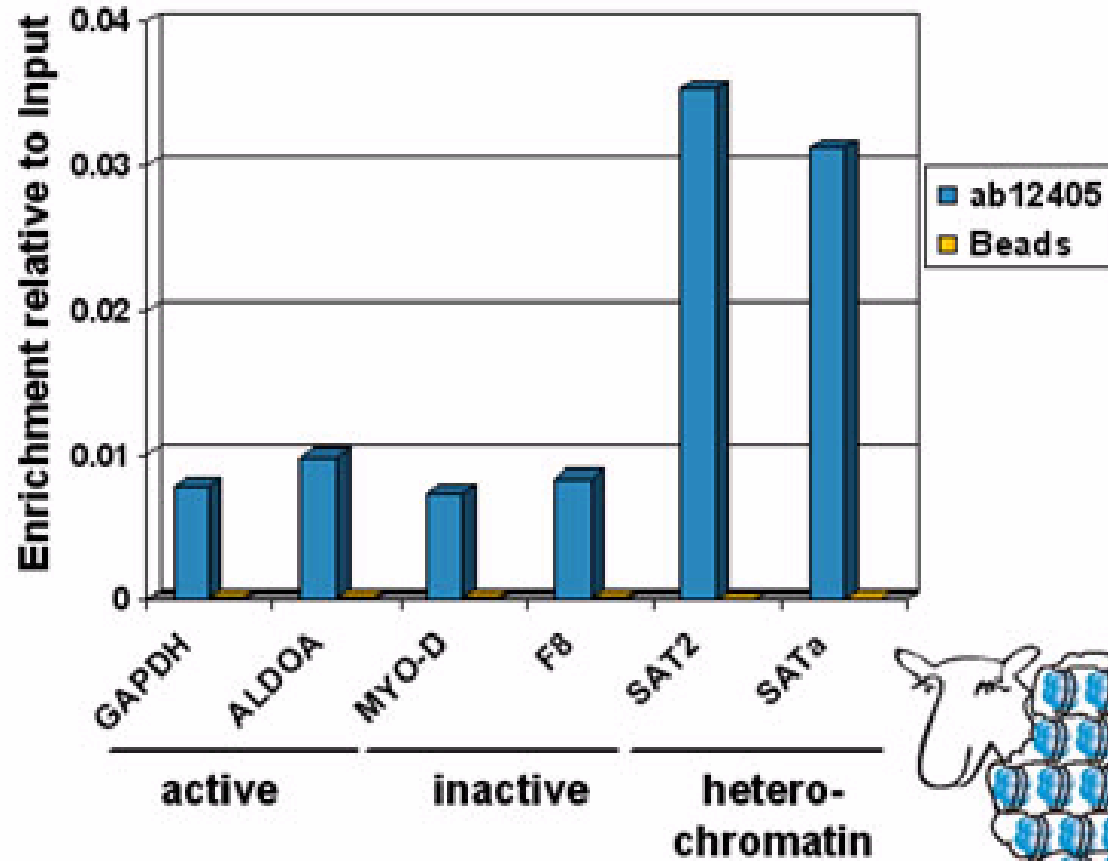
Table 1. Antibodies Used in This Study

Specificity	Catalog #
Anti-Histone H3	ab1791
Anti-Histone H4	ab10156
Anti-H3K4me3	ab8580
Anti-H3K4me2	ab7766
Anti-H3K4me1	ab8895
Anti-H3K36me3	ab9050
Anti-H3K79me3	ab2621



# In house tested antibody

ChIP: Suv39H1(ab12405)



- SUV39H1 enriched at heterochromatin

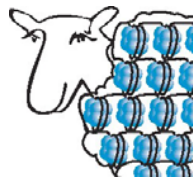
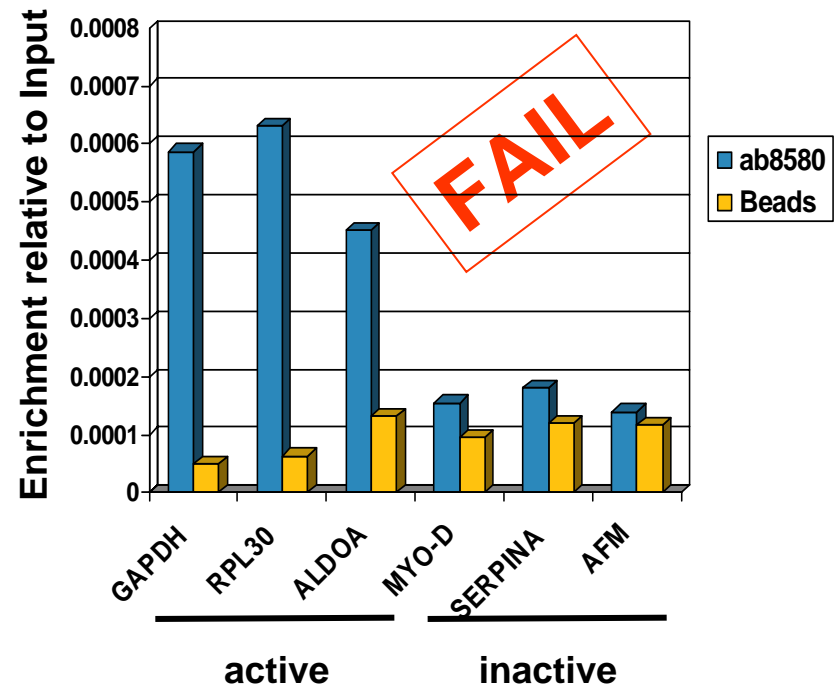
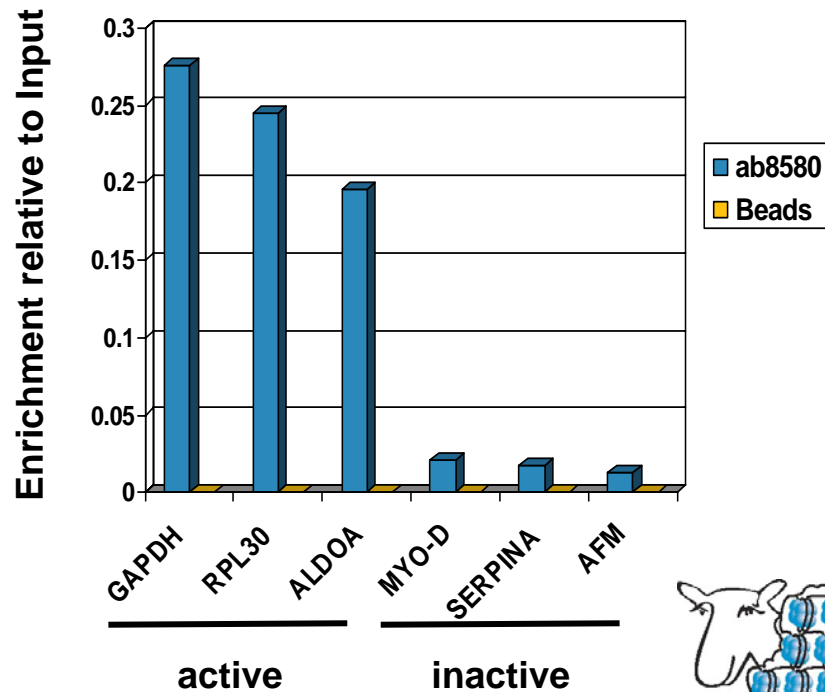
# ChIP batch tested antibodies

Stock batch

New batch

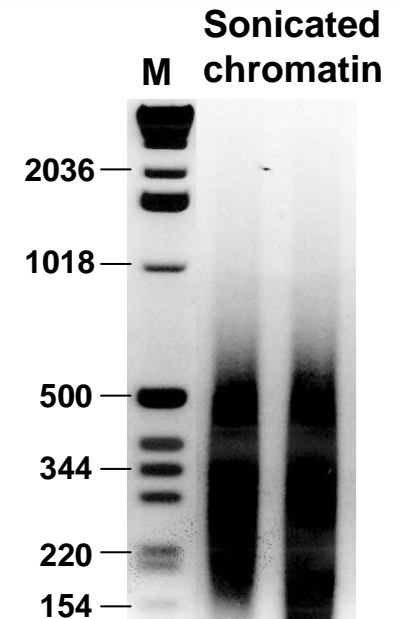
ChIP: Histone H3 (tri methyl K4) ab8580

ChIP: Histone H3 (tri methyl K4) ab8580



Sonication efficiency affected by:

- Foam
- Cell type
- Cell density
- Extent of cross-linking



**TIP: Optimise sonication conditions for every cell type and growth condition.**