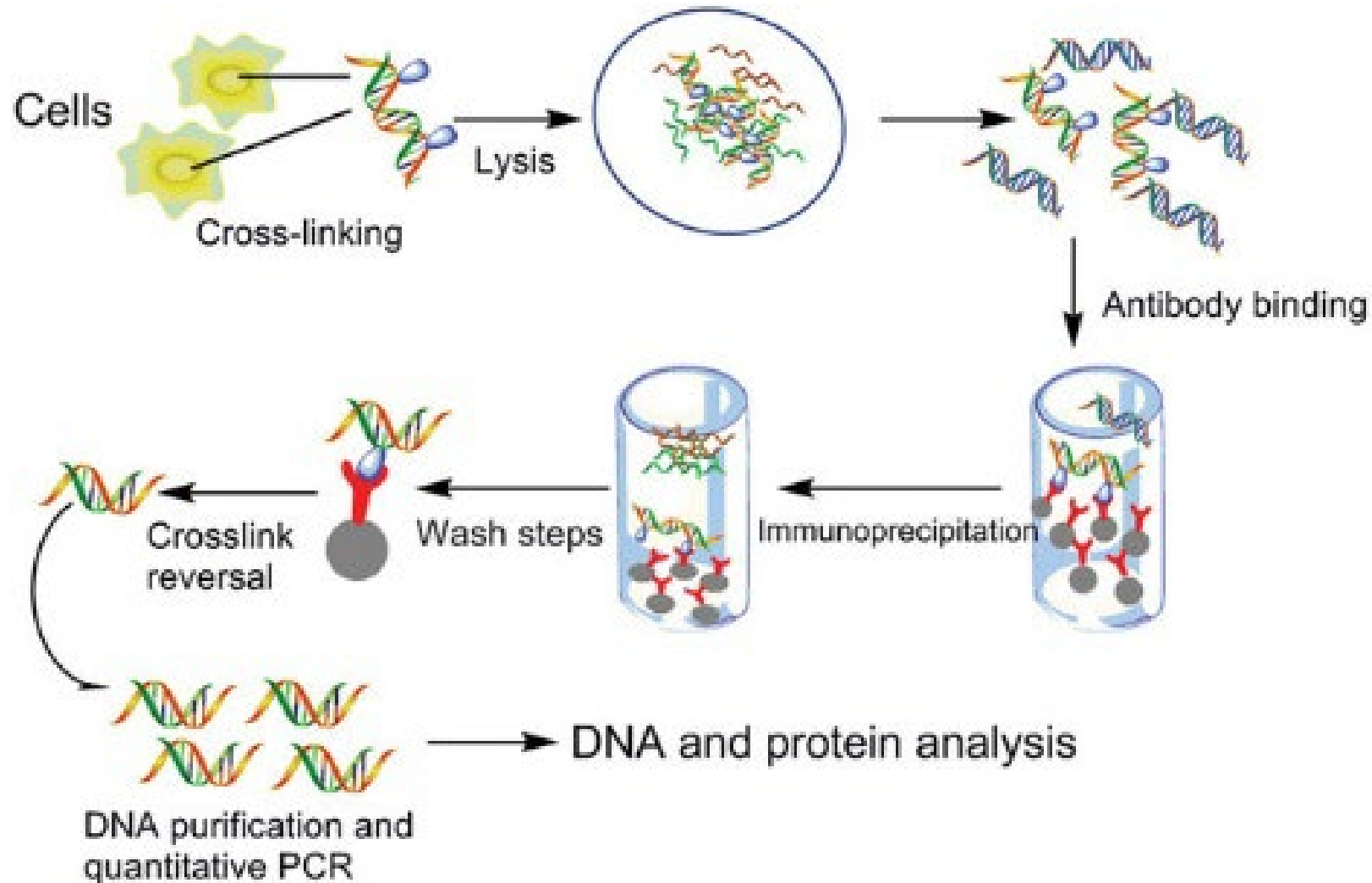


ChIP-ChIP-seq and analysis

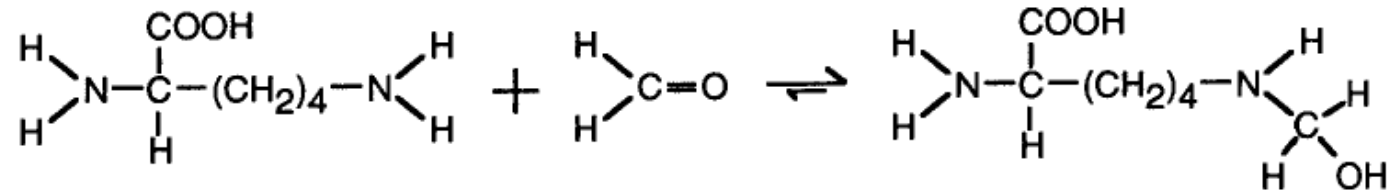
Chromatin Immunoprecipitation

A little bit of information on Chromatin Immunoprecipitation

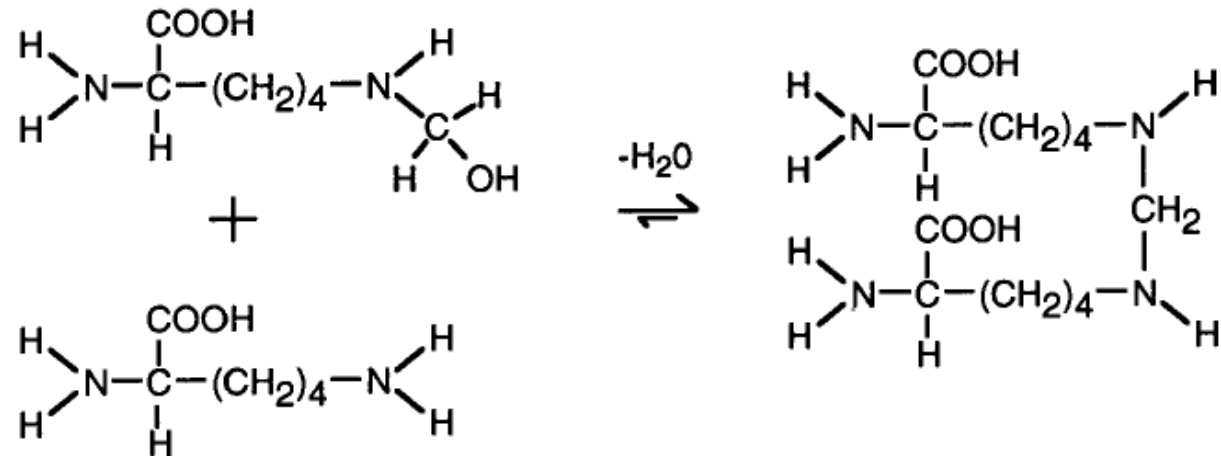


Schiffsche Base Reaction PP

a Reaction I



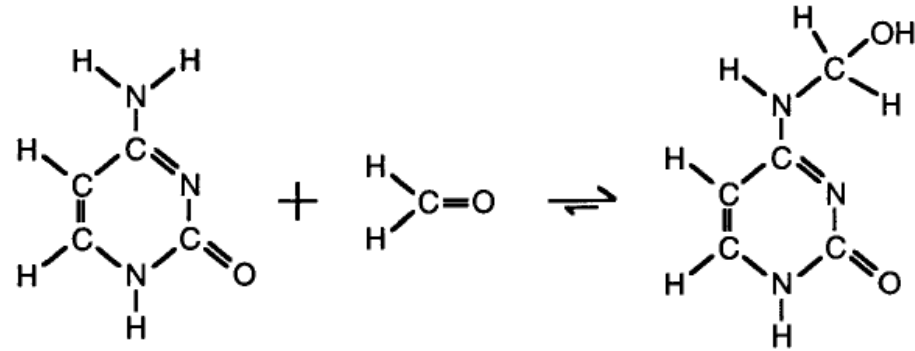
Reaction II



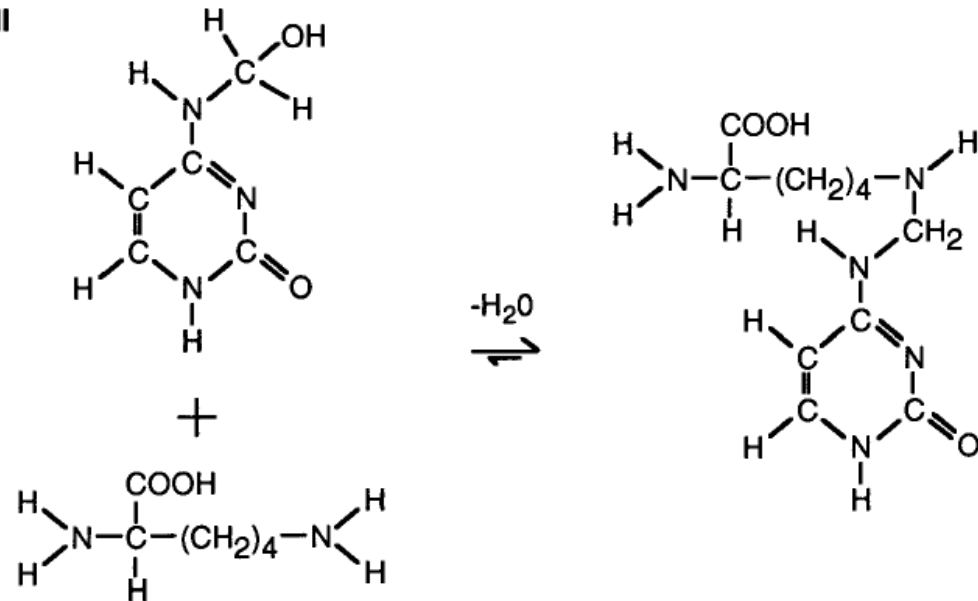
Protein-Protein Cross-link

Schiffsche Base Reaction PD

b Reaction I

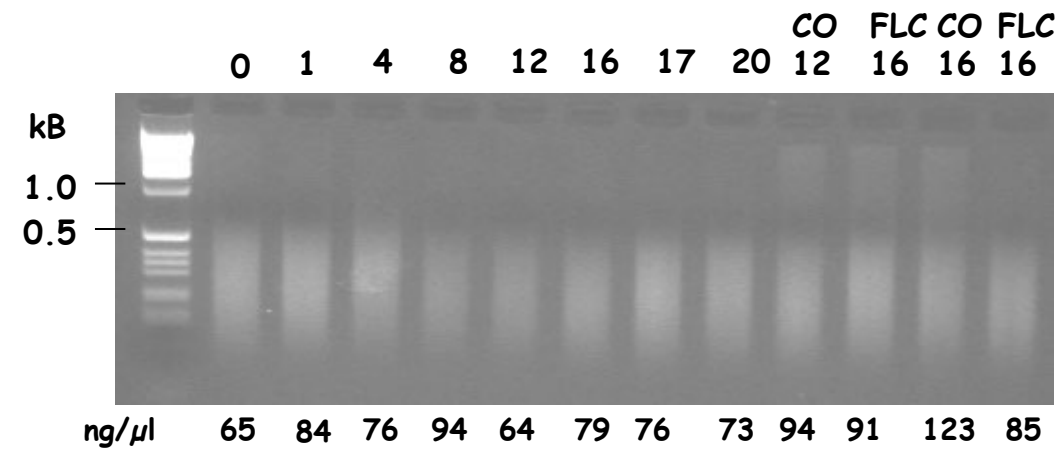


Reaction II



Pilot de-crosslinking

- evaluation of size
- quantitation of DNA

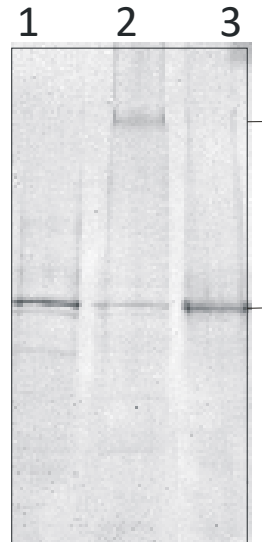


DNA-detection after
agarose gel
electrophoresis

Different crosslinked samples after sonication

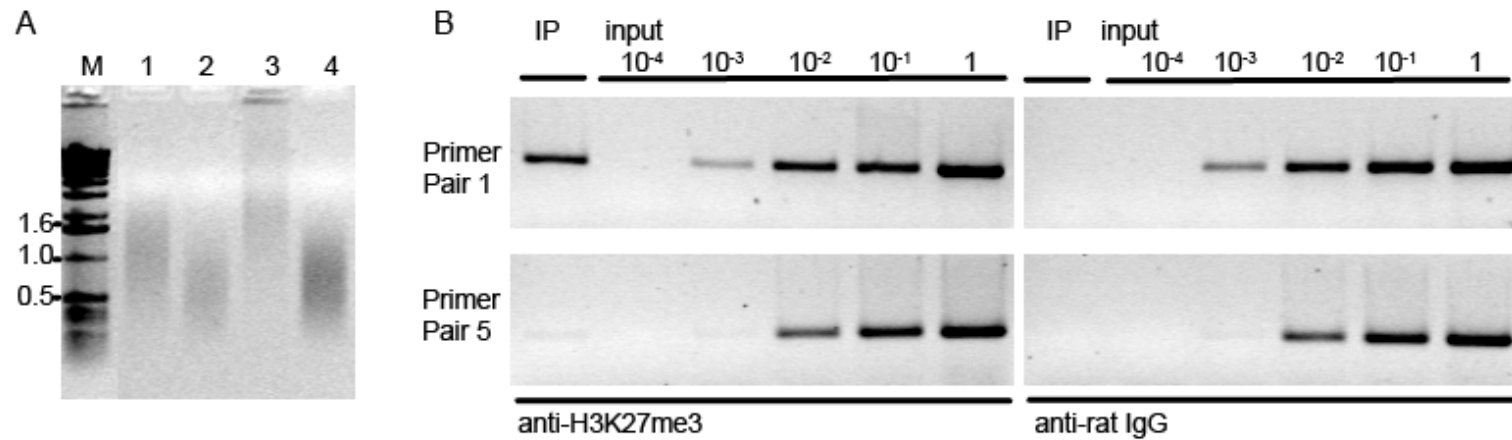
Analysis of Crosslinked Protein/Reversal of Crosslinks

Western blot against
WRKY transcription factor



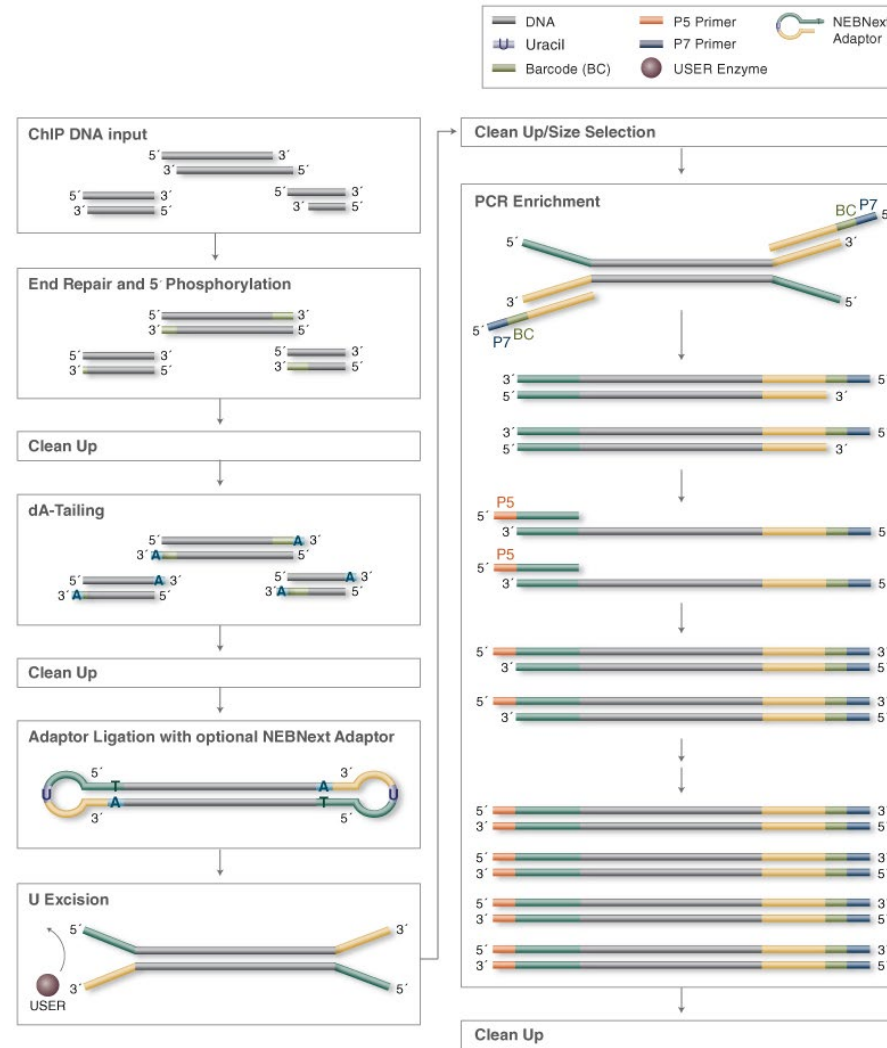
1: non-crosslinked nuclear extract
2: cross-linked nuclear extract
3: reverse of cross-linking by
incubation at 95°C for 1h

ChIP PCR result



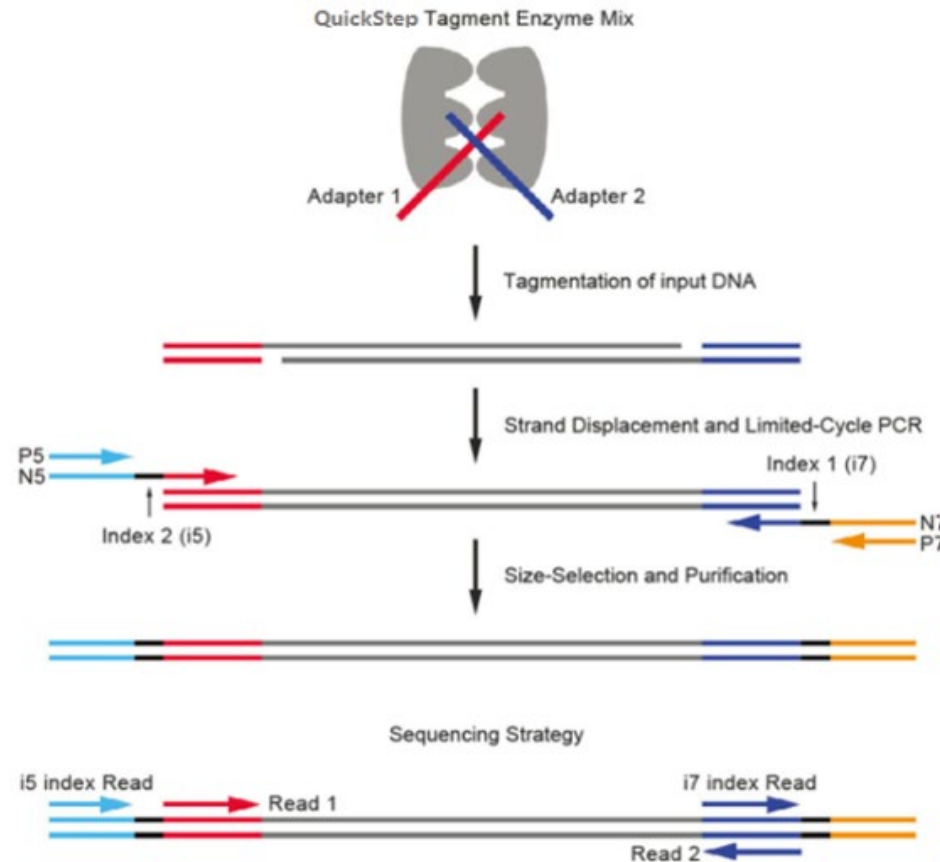
Making a ChIP-seq library

Making a NGS library out of ChIPed DNA



Example from NEB:

ChIPmentation: using transposable elements as helper



Adapter 1 and Adapter 2, two oligos embedded in QuickStep Tagment Enzyme
P5 and P7, two universal PCR Primers
N5 and N7, two index primers containing index 2 (i5) and index 1 (i7) respectively

Bioinformatics

Mapping

Dataset 1 (published data)
TRB1-GFP-IP-R1
TRB1-GFP-IP-R2
TRB1-GFP-IP-lhp1-R2
TRB1-GFP-IP-lhp1-R2

Col-0-IP-R2
Col-0-IP-R2

QC reads
[FastQC]

Filter bad
reads and
adaptors?

Map reads to
reference
genome
[Bowtie2]

Filter for uniquely mapped
reads
[Samtools]

Metageneplots
[DeepTools]

Visualize sequence tracks
[IGV]

Motif Enrichment
[Homer or MEME-ChIP]

Extract fasta sequence of
peaks [Bedtools]

Meta-
analysis

GO-term enrichment

Map to target genes [Homer
or Bedtools]

Peak
Enrichment
and QC

Quality assessment:
1.) enriched peak prediction
in pseudoreplicates across
samples
2.) enriched peak prediction
in pseudoreplicates within
samples

Identify enriched regions per
bio-replicate against control
ChIP of corresponding
dataset
[EPIC2 or MACS]

Calculate coverage for all
input and Col-0 IP samples
for 200bp windows across
genome
[bedtools, BAMscale cov]

Identify regions with outlier
coverage in input or control
IP and merge these to
custom blacklist
[Rstudio, Bedtools]

Overlap and merge
replicates based on IDR
framework
[Encode ChIP-seq pipeline]

Filter enriched peaks
against blacklist
[bedtools]

Merge and intersect all TRB
target regions
[Bedtools]