A high-throughput ChIP-Seq for large-scale chromatin studies

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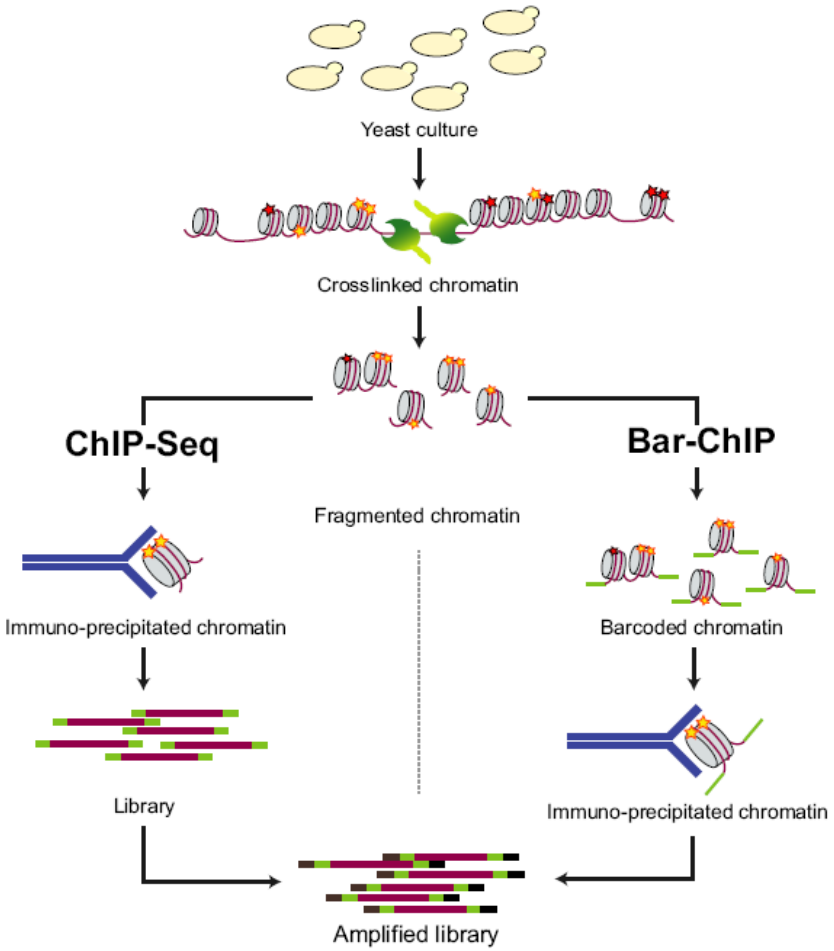
Background

- ChIP-Seq and ChIP-on-chip
  - a single protein modification per experiment
  - cumbersome and costly to most laboratories

- Bar-ChIP
  - A DNA barcoding step prior to chromatin immuno-precipitation
  - Highthroughput ChIP-Seq method

Background


Yeast culture
Crosslinked chromatin
ChIP-Seq
Bar-ChIP
Fragmented chromatin
Immuno-precipitated chromatin
Barcoded chromatin
Library
Immuno-precipitated chromatin
Amplified library

Bar-ChIP captures genome-wide distribution of H3K4me3

- Evaluate the impact of chromatin barcoding on the recovery of genome-wide patterns of H3K4me3 by both Bar-ChIP and classical ChIP-Seq methods.

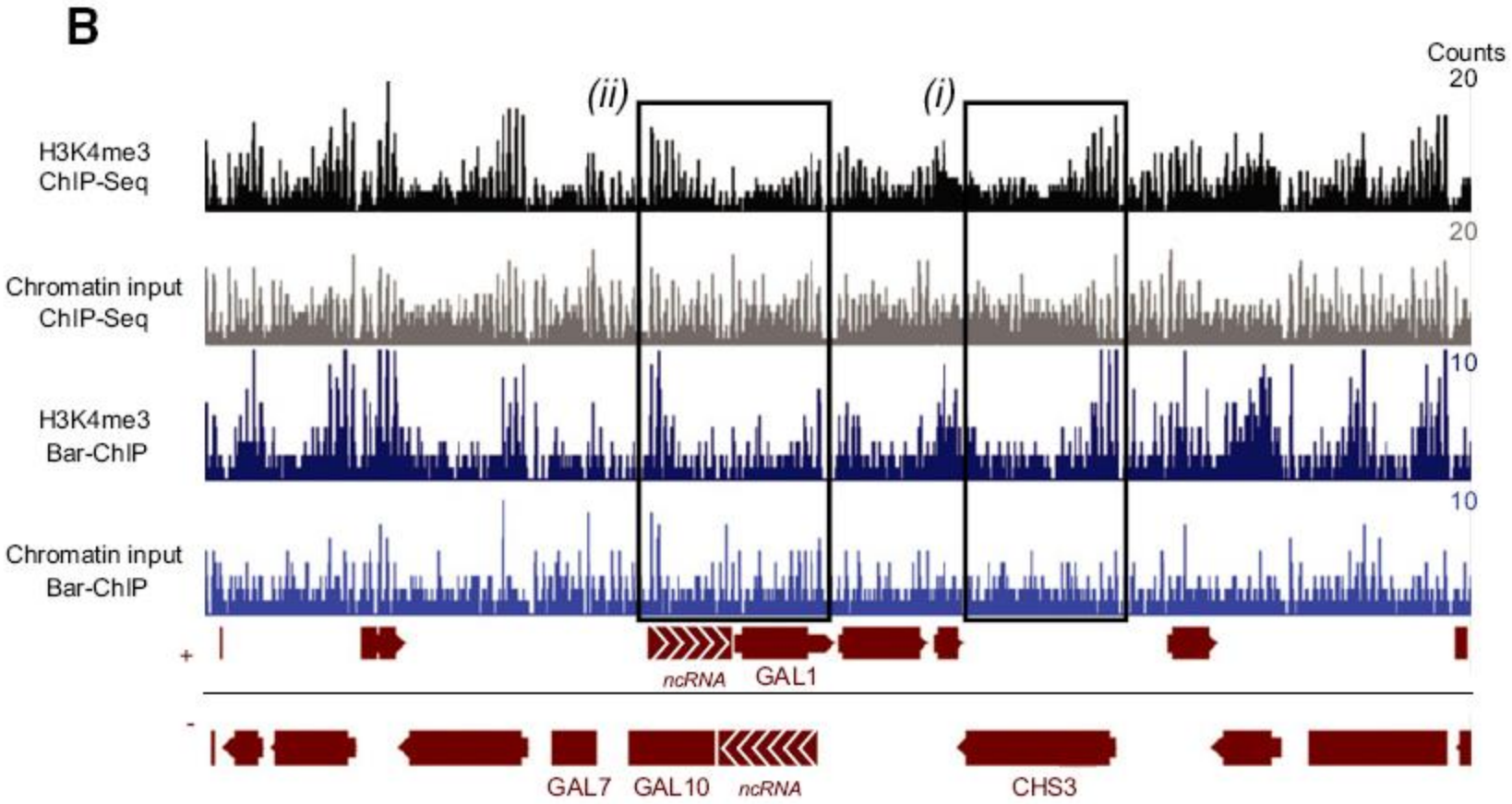
- Result：

  — mono-nucleosomes deriving from the fragments following the Bar-ChIP generated and recovered after the IP were slightly longer

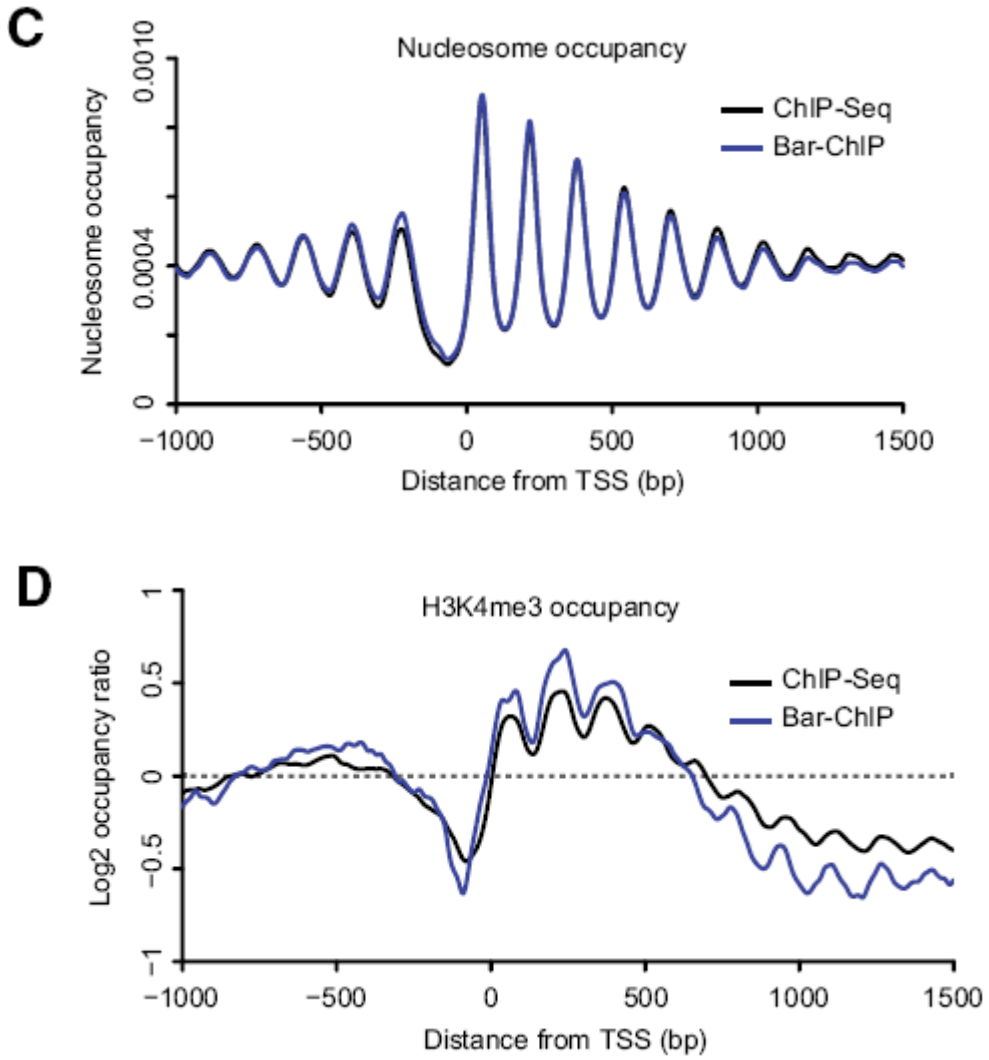
  — A very good reproducibility was observed between biological replicates for both ChIP- and Bar-ChIP-Seq, a high correlation between the two techniques was obtained for each IP DNA and input DNA

  — Signals for the presence of the H3K4me3 mark were equally well recovered

Bar-ChIP captures genome-wide distribution of H3K4me3



Bar-ChIP captures genome-wide distribution of H3K4me3

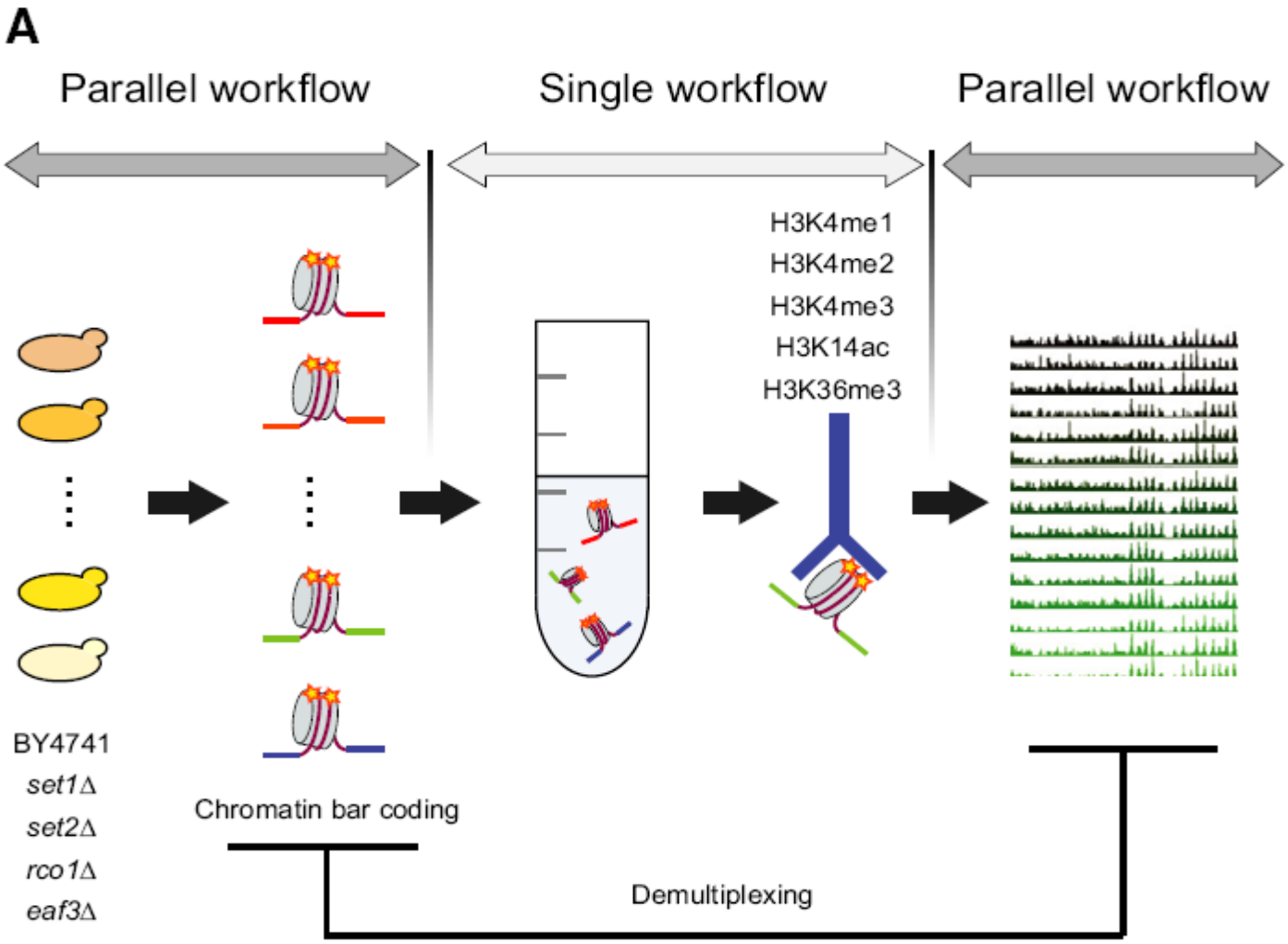


the same genomic regions were identified by both techniques for H3K4me3 enrichment or depletion, indicating that Bar-ChIP faithfully captured the distribution of H3K4me3-marked nucleosomes.

Bar-ChIP enables rapid and simultaneous generation of ChIP-Seq data sets

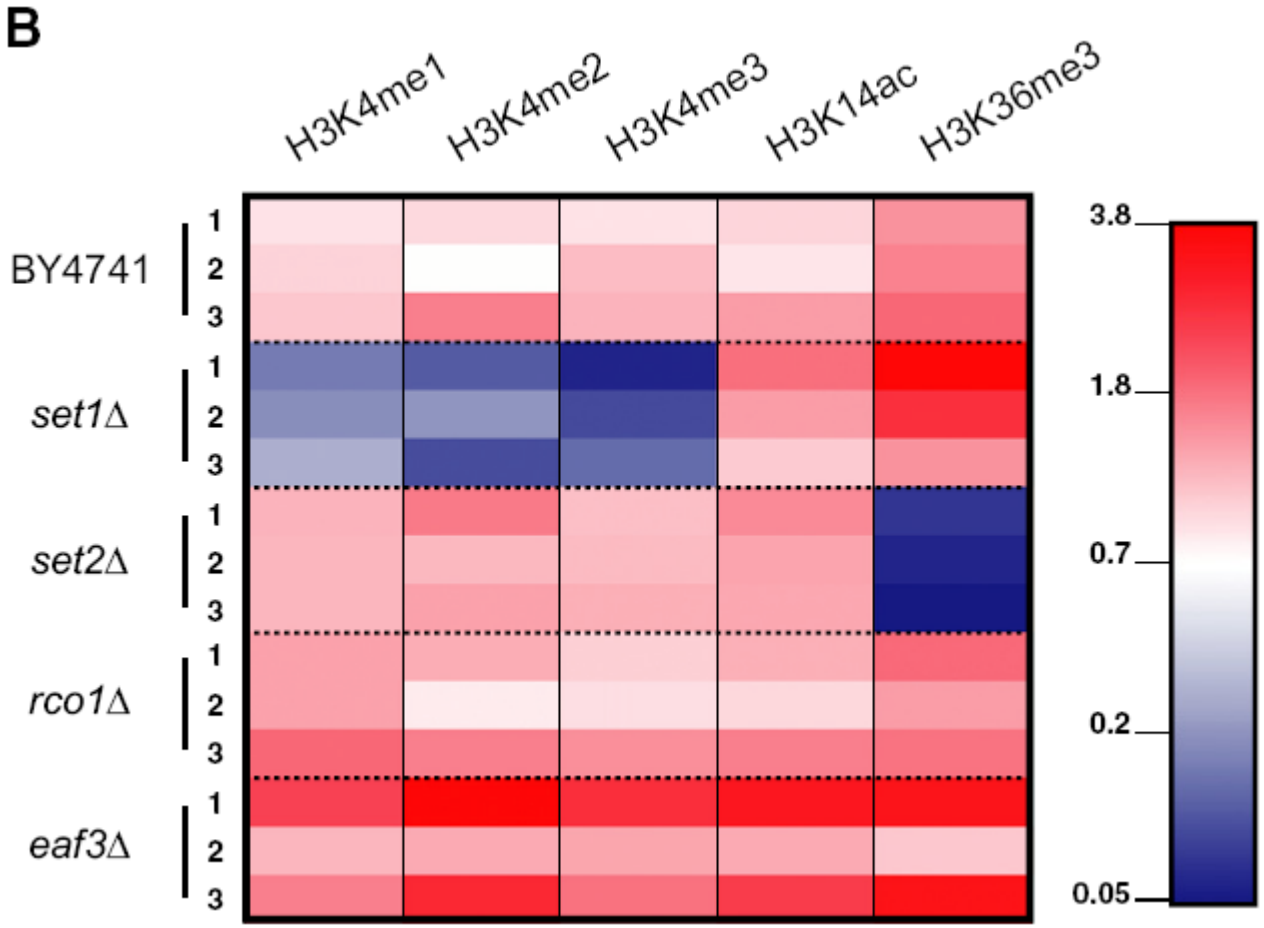
- All derived from the BY4741 strain background
- Mutant：
  - set1△：deleted for Set1p，the only protein capable of catalyzing the deposition of mono-, di- and trimethyl groups on lysine 4 of H3 in S. cerevisiae
  - set2△：deleted for Set2p，the only histone methyltransferase responsible for deposition of methyl groups on lysine 36 of H3 (H3K36me1, 2, 3) in S. cerevisiae
    - Rpd3S：Rco1p, Eaf3p
- Five distinct histone modifications:
  - H3K14ac ,H3K4me3, H3K36me3, H3K4me2 and H3K4me1

Bar-ChIP enables rapid and simultaneous generation of ChIP-Seq data sets



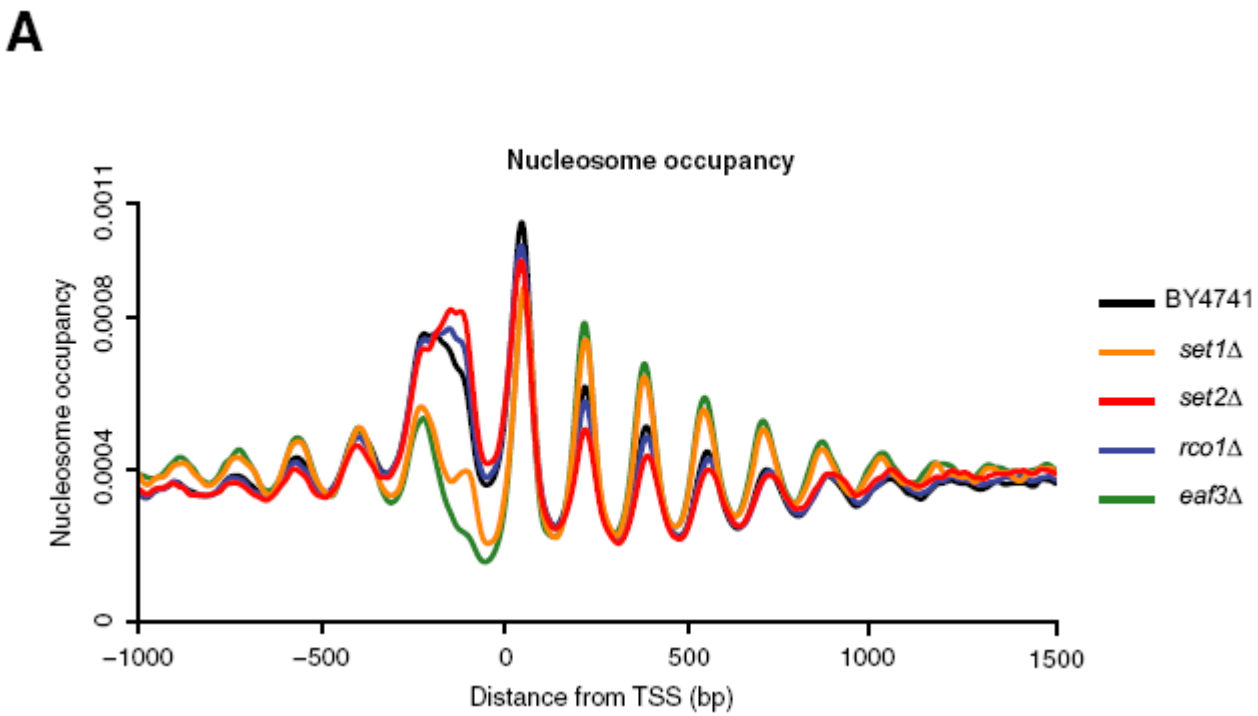
Schematic representation of the experimental design

Bar-ChIP enables rapid and simultaneous generation of ChIP-Seq data sets



- Bar-ChIP can be used to study the genome-wide patterns of histone marks.

Multiplexed experiments provide overview of genome-wide distribution of histone marks



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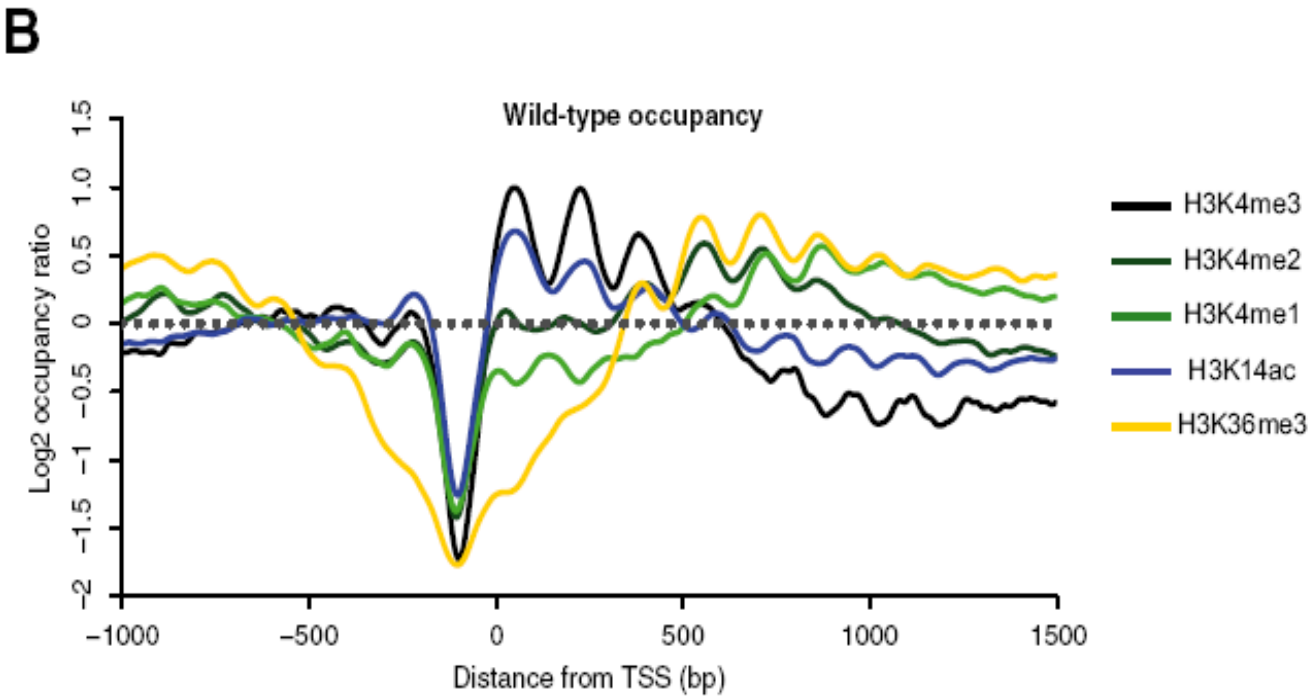
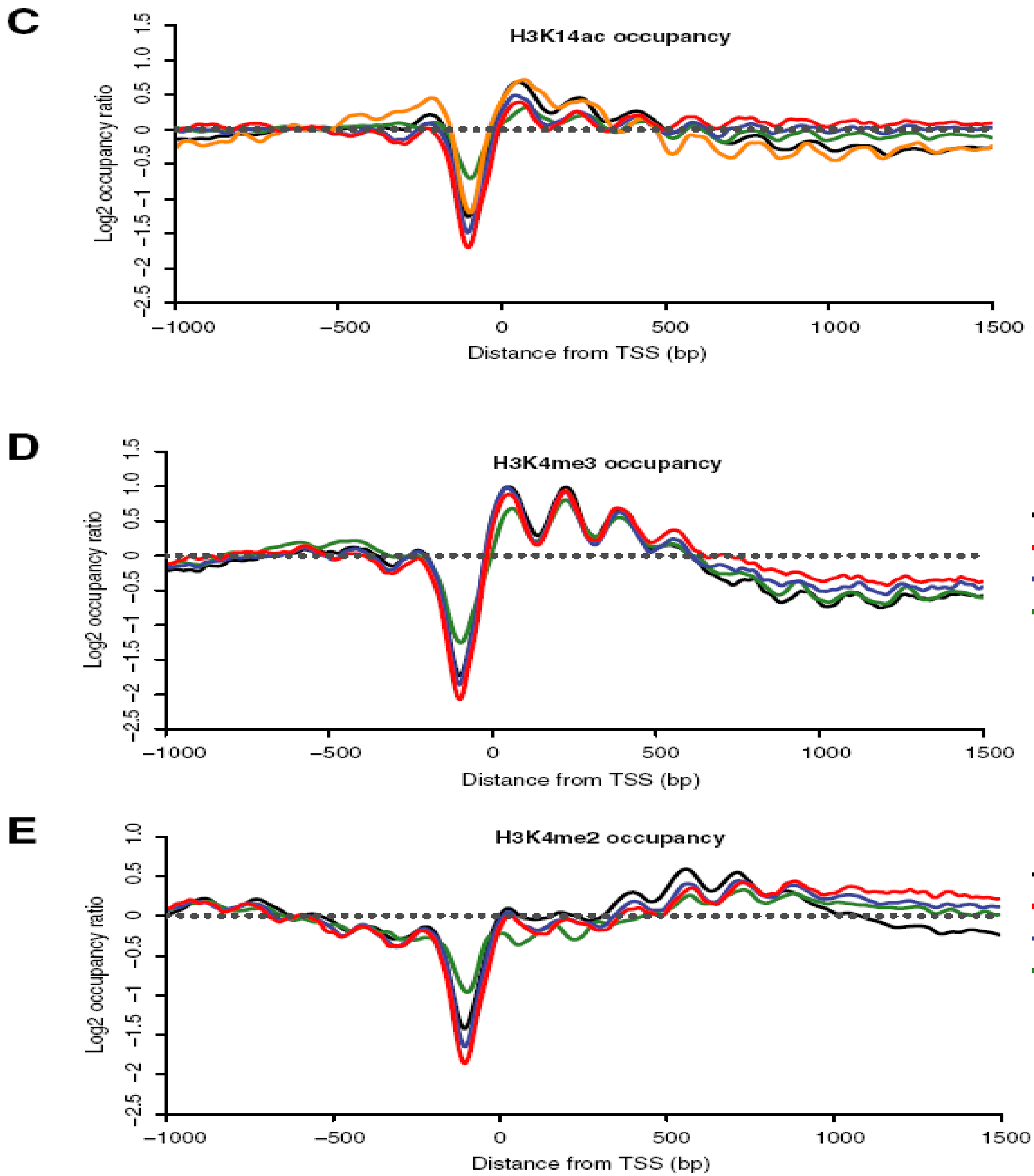
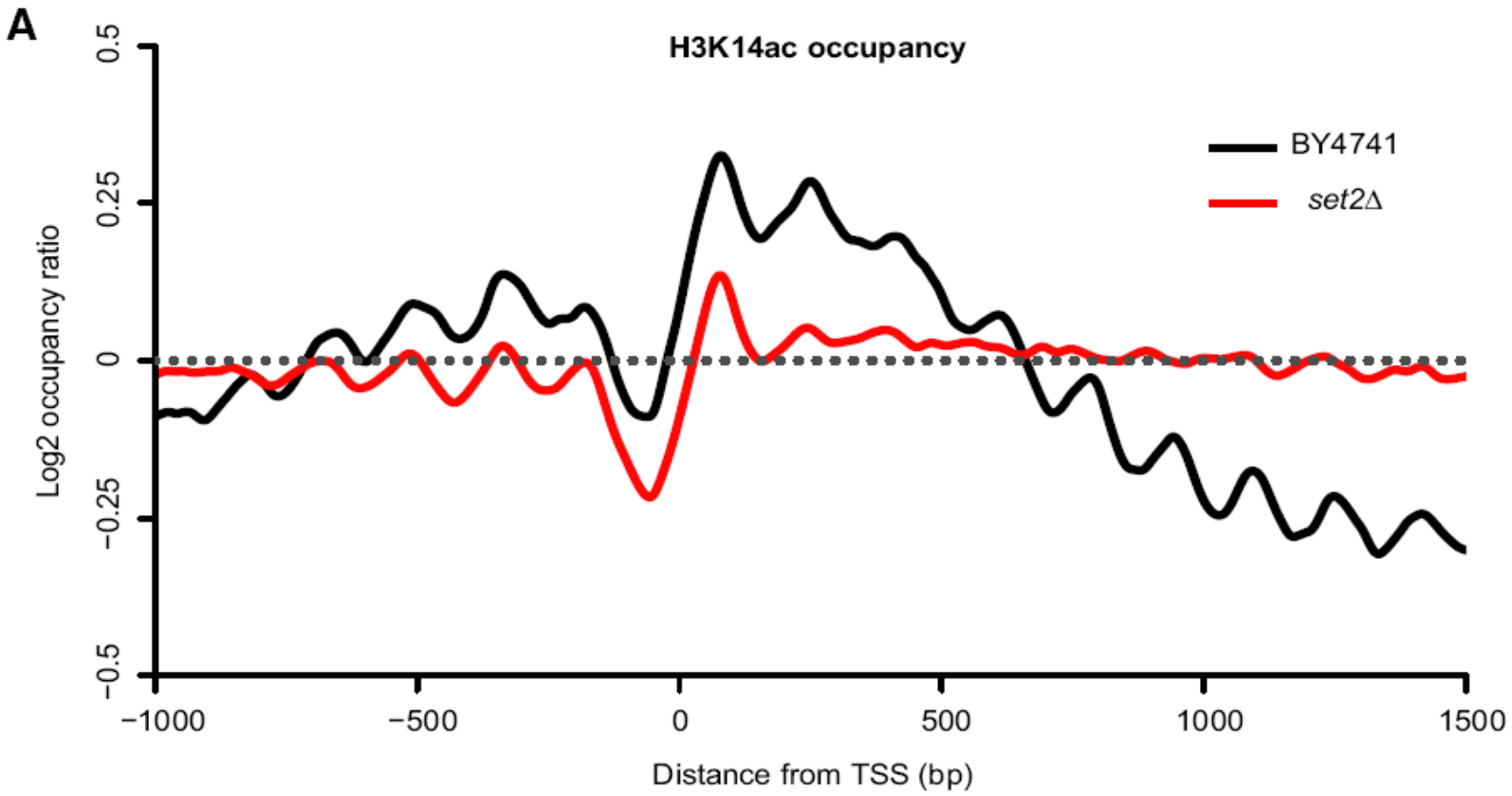




Altered distribution of chromatin marks in the set2△ mutant associates with the emergence of cryptic transcription



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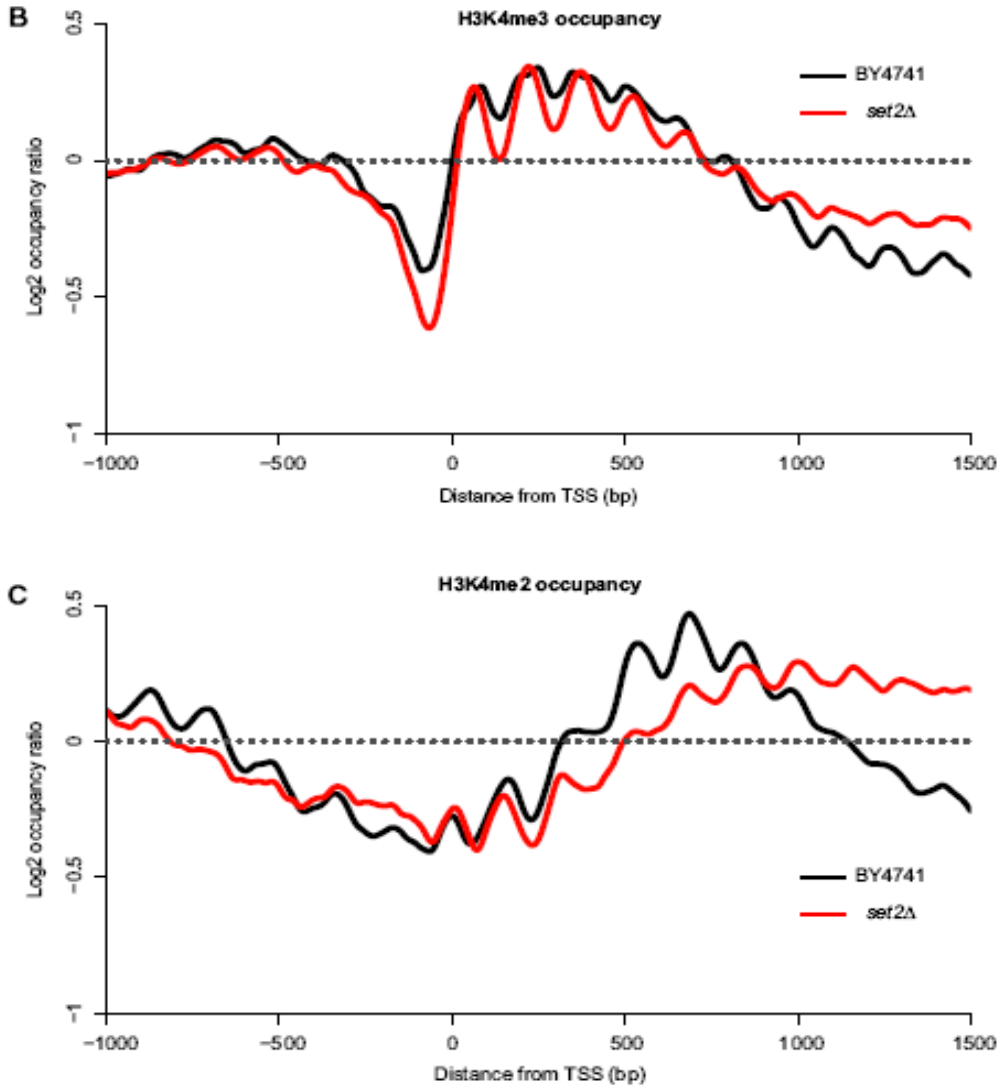

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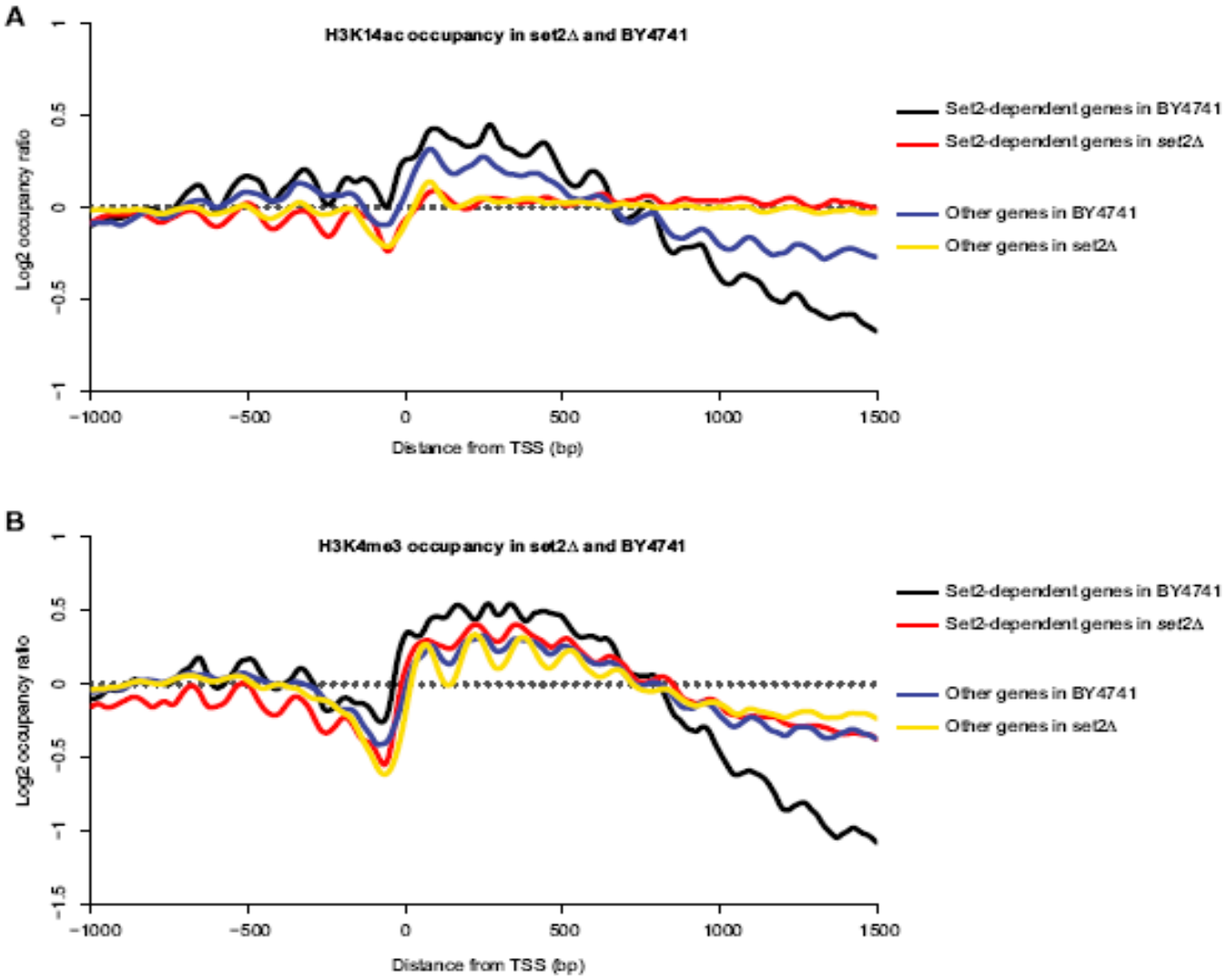

Origin of internal H3K14 acetylation and H3K4 methylation in the set2△ strain

- Test whether the global accumulation in H3K14ac observed in set2 △ and the two Rpd3S-associated mutants (rco1 △ and eaf3 △) may also originate from an increased incorporation of acetylated histones.
  - while H3K14ac appears to be maintained at low levels within gene bodies through the activation of Rpd3S, the mechanism of its incorporation in the set2D mutant does not seem to involve the classical histone exchange pathway.

- address a potential crosstalk between H3K14ac and H3K4me3/2 in the 3’ region of genes
  - In set2△ada2△sas3△， did not display any striking difference in the genome-wide distribution of H3K4me3，substantial decrease in H3K4me2
  - abrogation of H3 acetylation resulted in an increased level of H3K4me2

Conclusion

- 创新点：
  - 在免疫沉淀前加入DNA标记，使得实验更加准确
  - 可进行同步免疫沉淀
- 要善于思考
- 需改进的问题：
  - Mnase消化的不均一性
  - 染色体标记和免疫沉淀的偏好问题
  - 染色体标记的有限效率问题：IP后所得的分子数量有限

Thanks