

Scaling normalisation for ChIP-seq with exogenous chromatin

Workshop on **ChIP-seq data analysis**

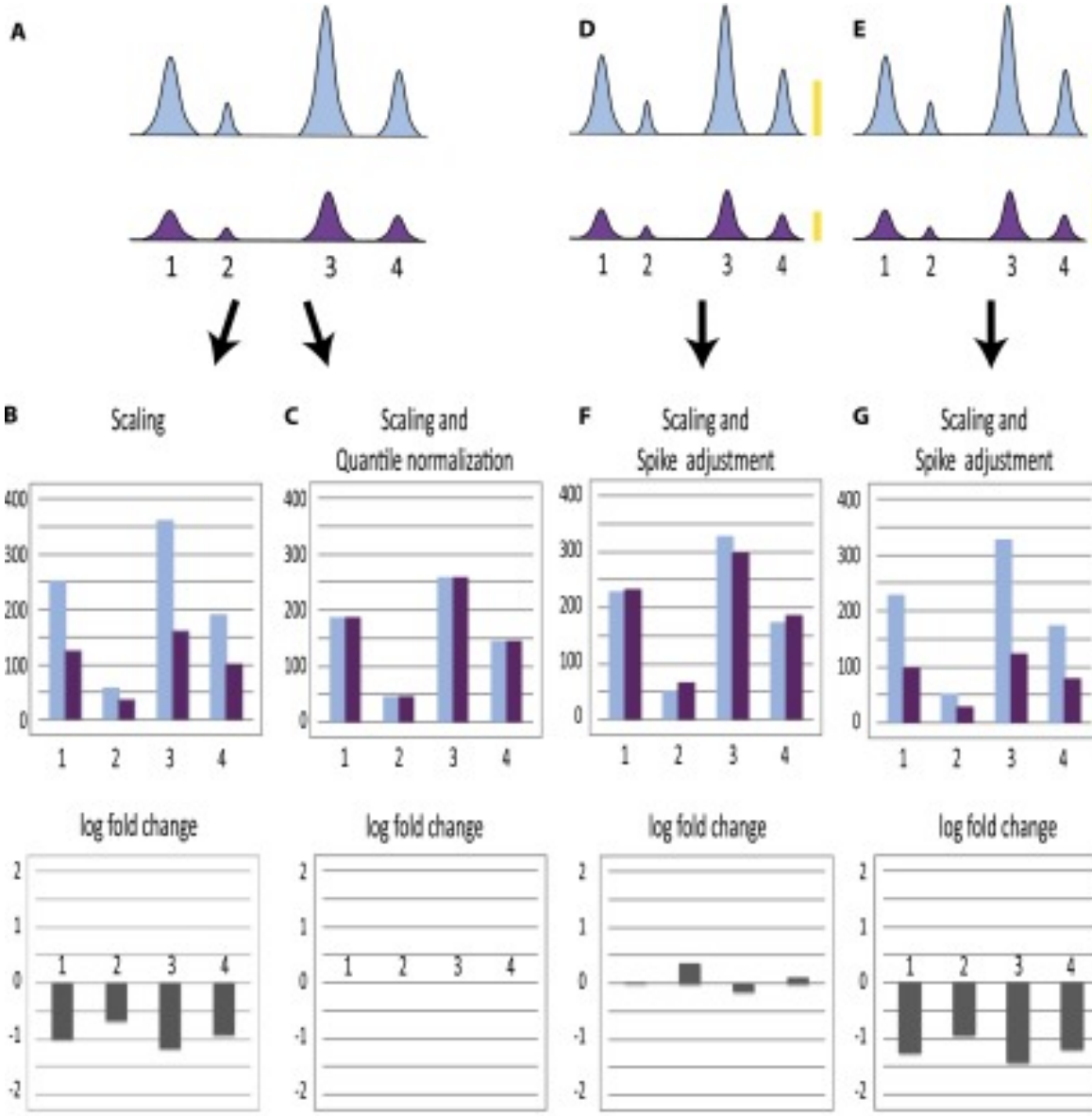
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Local vs. global change in abundance



Global difference due to
 * global change in signal
 * technical issue

B: scaling to library size

C: B & quantile normalisation

D vs. E:

D: technical issue

E: genuine difference

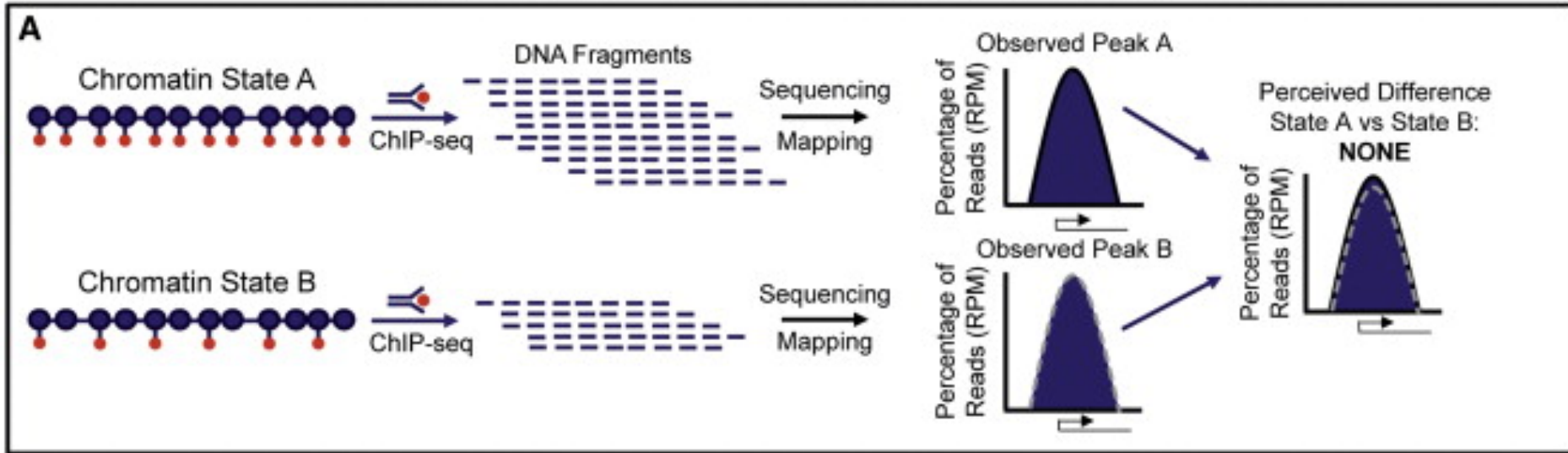
Biological difference or not

No biological difference
(replicates)

Biological difference

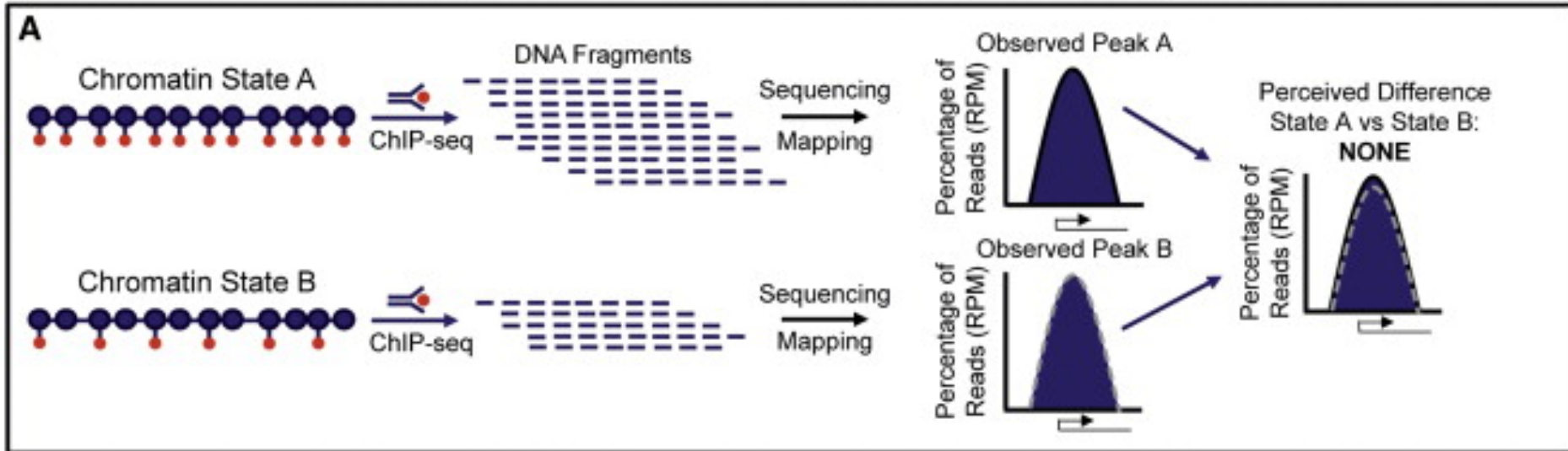
Global change in abundance

Traditional normalization (RPM) obscures epigenomic differences

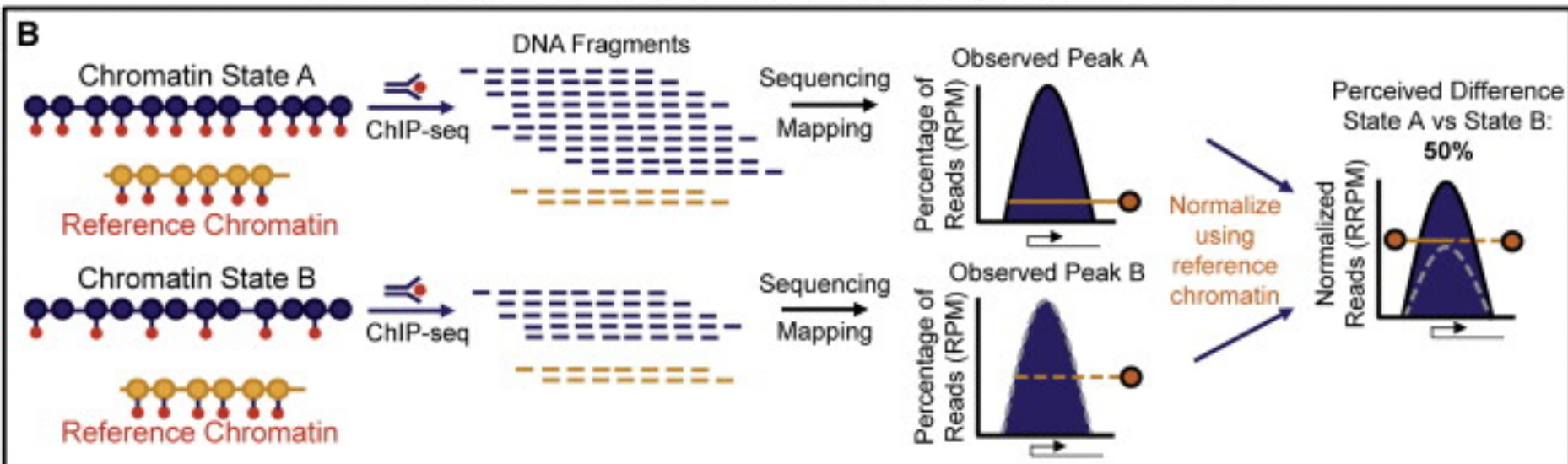


Global change in abundance

Traditional normalization (RPM) obscures epigenomic differences

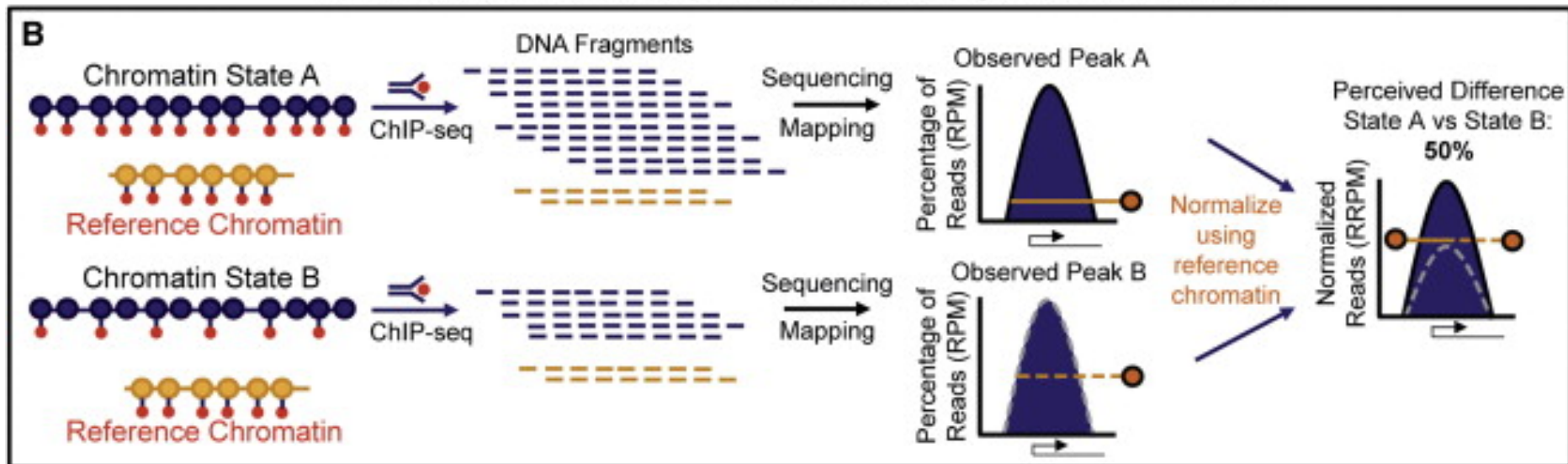


Reference normalization (RRPM) reveals epigenomic differences



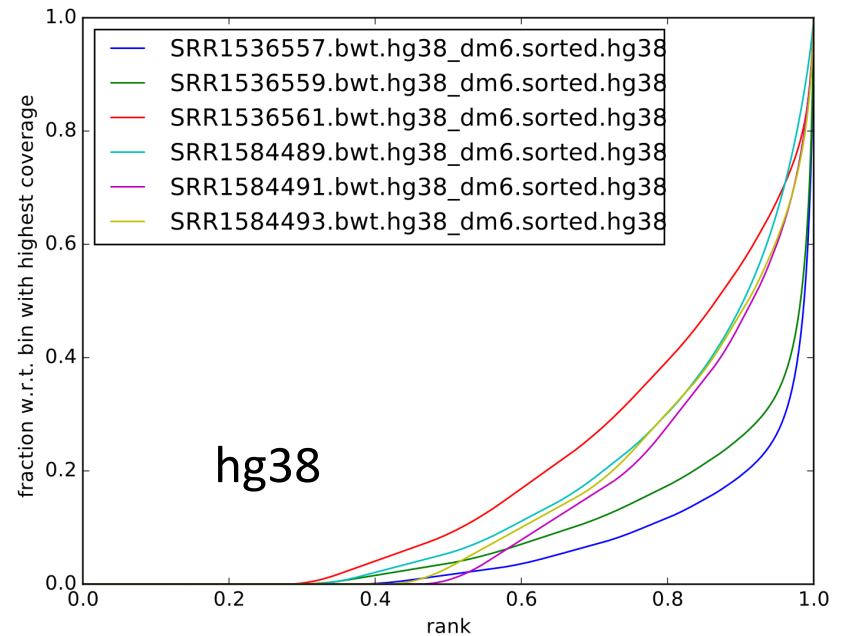
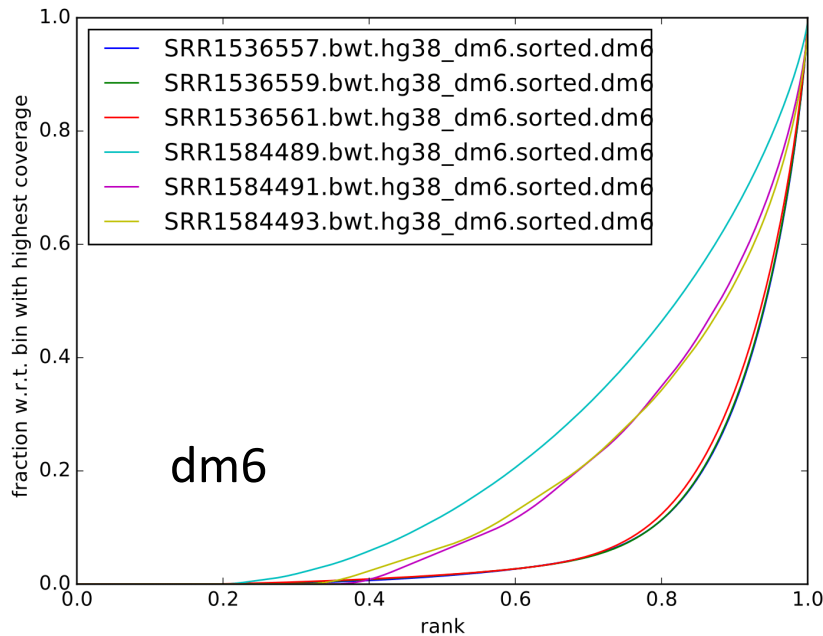
The principle of scaling normalisation

- Scaling to number of mapped reads in exo chromatin
- Scaling to average background in exo chromatin



Things to be aware of...

- Chromatin quantification
- Different IP efficiency for exo- and endo- chromatin



H3K79me2 content:

ChIP: 57 > 59 > 61; Input: 89 > 91 > 93