

ChIP on chip

Sample amplification

Affymetrix Chip on chip forum
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Plate forme génomique

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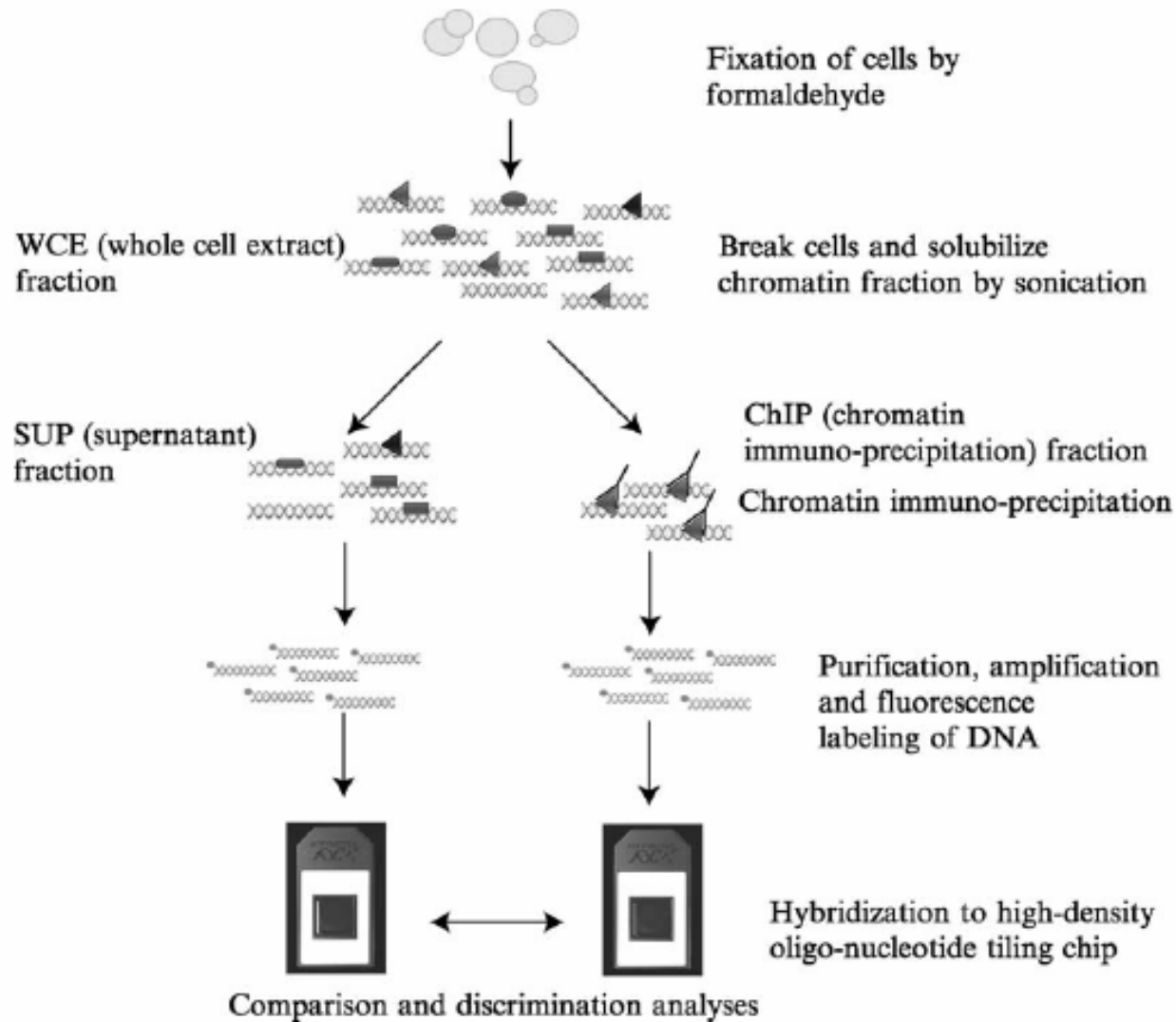
The Genomics Platform

- Core facility providing various technologies for quantification of RNA and DNA
- Proposes complete solution, from advice in experimental design to full data analysis and interpretation
- Technologies currently available:

- **Microarrays**
- **Real-time PCR**
- **Differential display**



Workflow



QC and amplification pipeline

IP (clean!)



Q PCR validation

→ Reject if failed

IP



IP quantification/conditioning/storage

IP



IP amplification

→ Reject if failed

Amplified IP



Q PCR validation (UNG!!)

→ Reject if failed

Prepare for hybridization

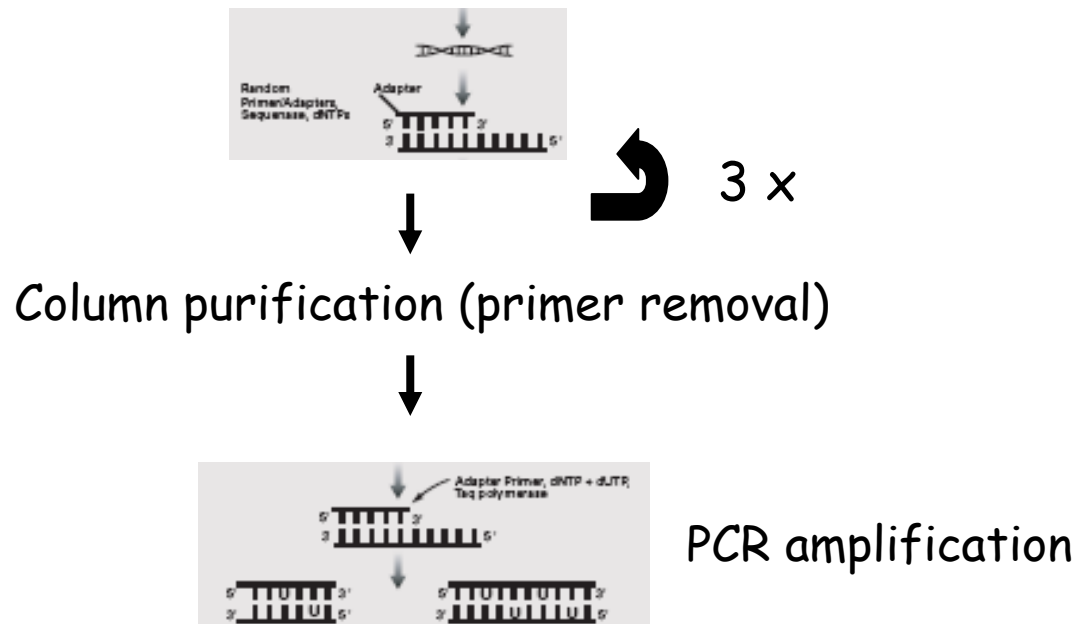
Amplification method

- Fidelity, no bias
- Reproducibility
- Yield (μg range)
- Reliability

- Ease to manipulate
- Incorporation into Affymetrix workflow

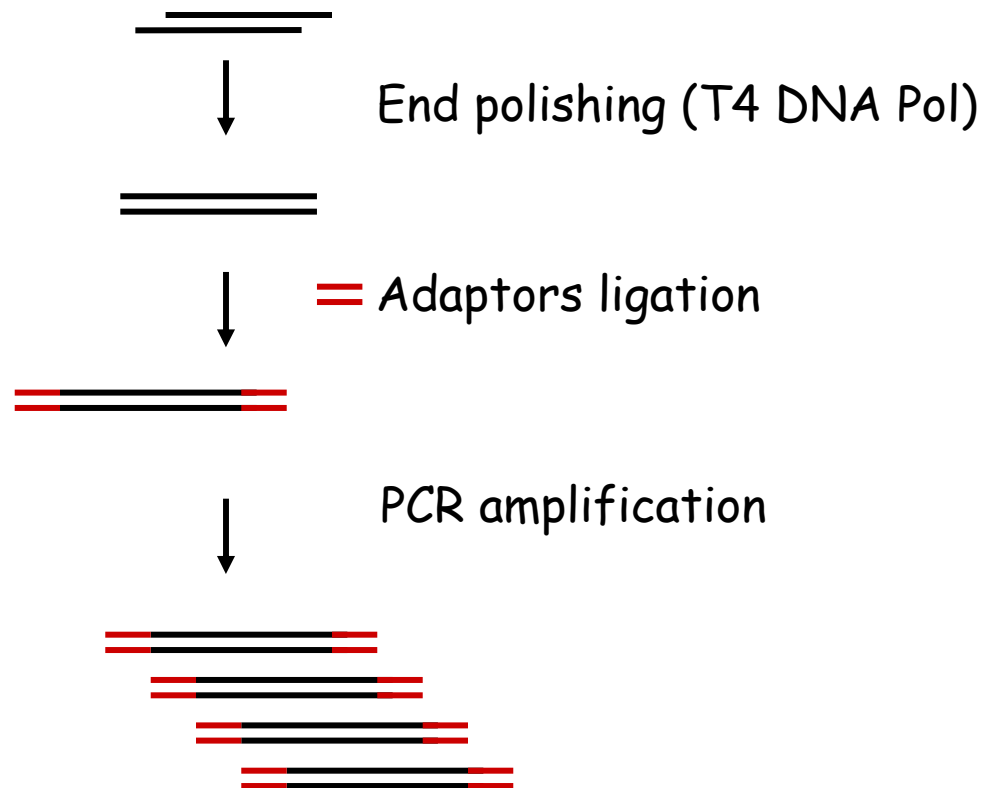
Amplification 1

- Round A B (Affymetrix, developed by DeRisi Lab, UC San Francisco, June 2001; adapted from Bohlander et al. *Genomics* 13 (1992))



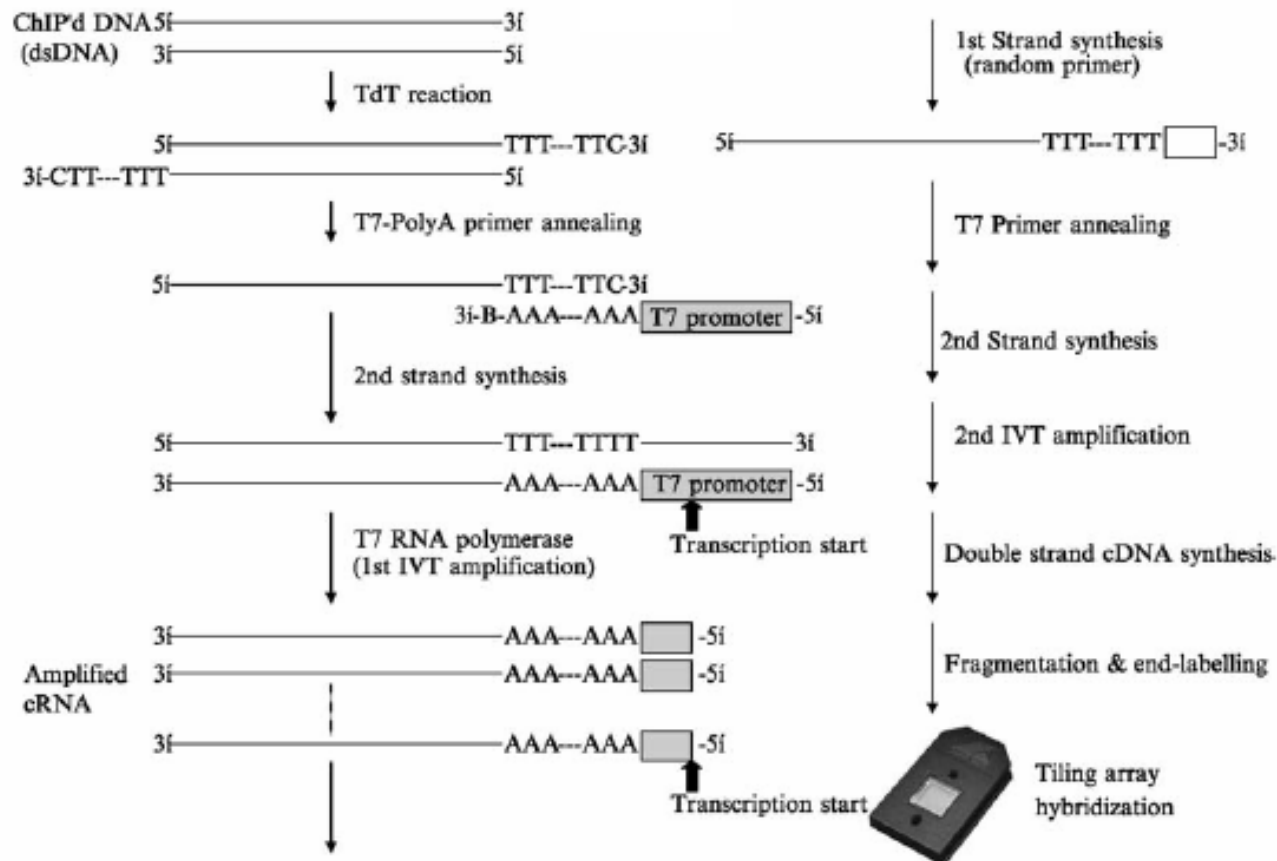
Amplification 2

- LM PCR



Amplification 3

- T7 IVT

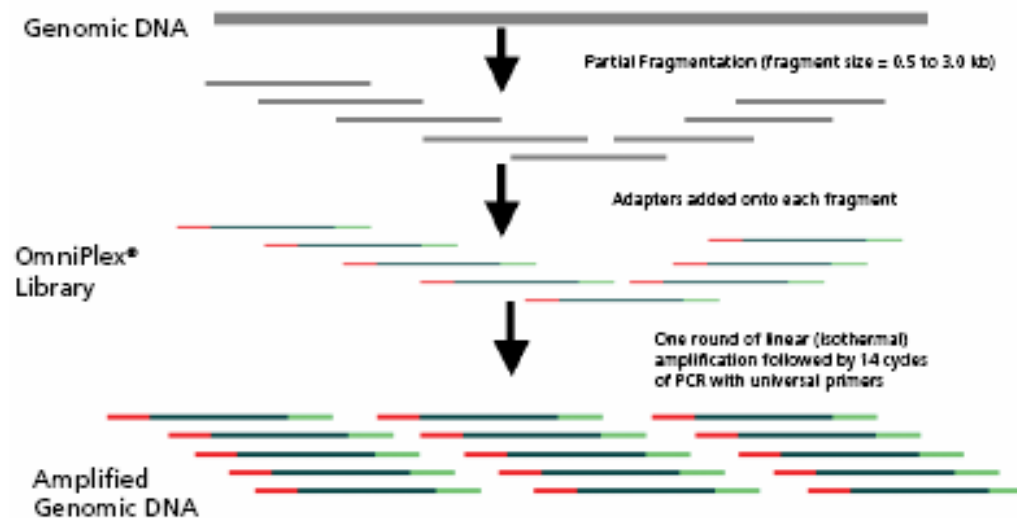


Common characteristics

- Numerous steps in the procedure
- Intermediate purifications often required
- Time consuming
- Some introduce different bias (related to complexity)

Amplification 4

- GenomePlex WGA kit (Sigma Aldrich)

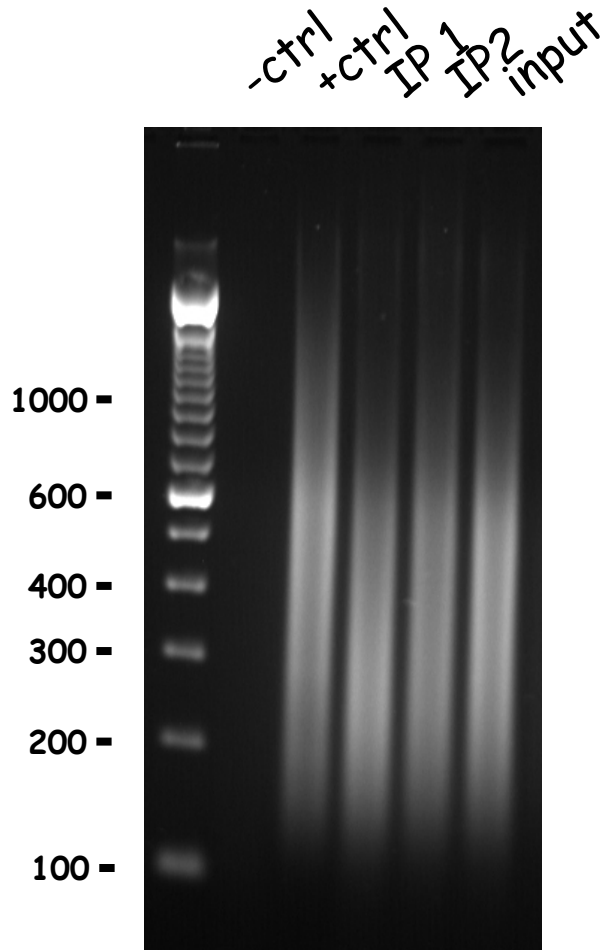


O'Green et al (Farnham lab) *BioTechniques* 41:577-580 (November 2006)

WGA kit

- Designed for whole genome amplification
- Requires low input (less than 10 ng)
- Simple to use
- Whole process in a single tube
- No purification step involved
- Rapid (half a day)

Amplification yields/pattern

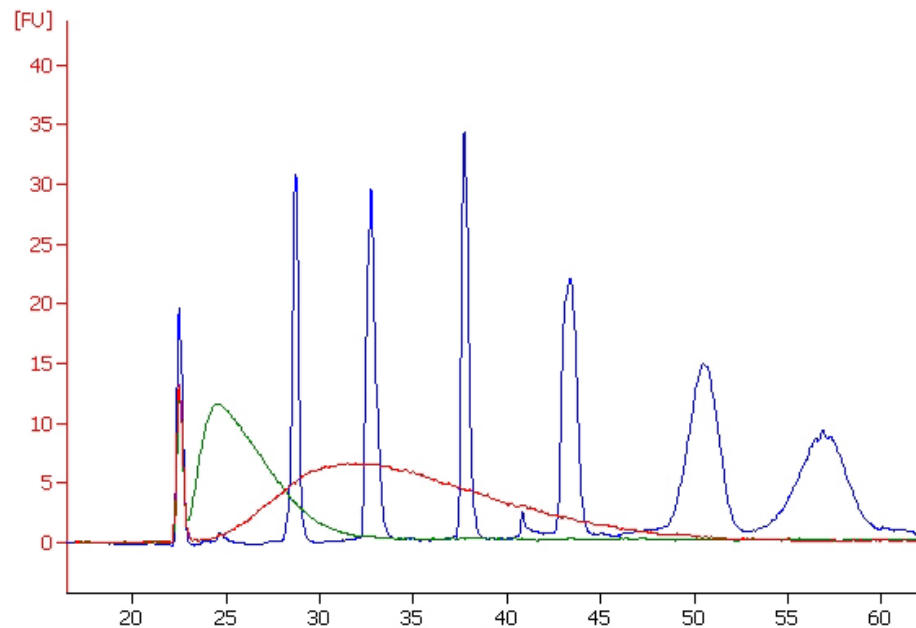


- 2 to 10 ug of DNA from IP
- Size distribution similar to IP

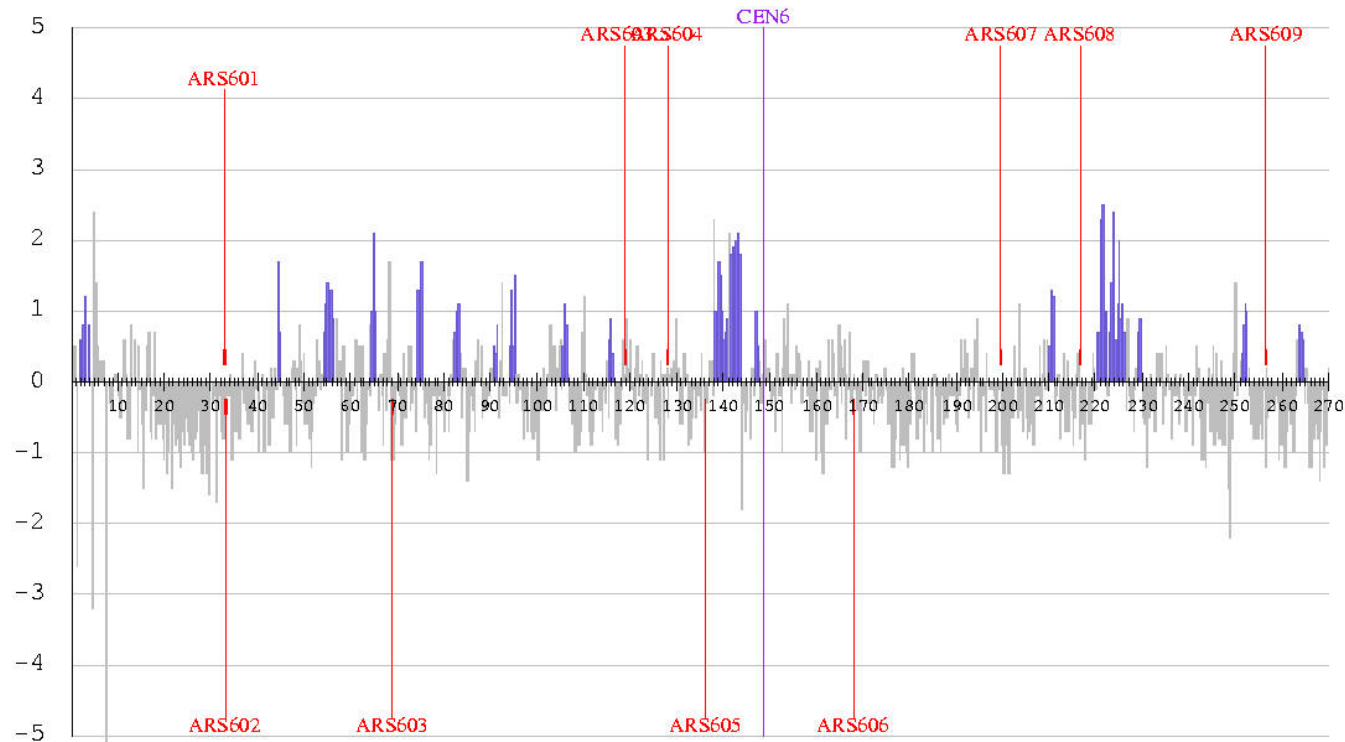
Work with reagents
in a DNA-free zone
Pre-PCR

Fragmentation-labeling

- Direct incorporation of dUTP in PCR in a 1:4 ration with dTTP, followed by use of Affy kits with UNG-APE1 and TdT-biotin



TFIIB-cMyc on *S. Cerevisiae* Chr VI



Ratio IP vs gDNA

Conclusions

- *GenomePlex WGA* kit (Sigma Aldrich)
- Robust
- Reproducible
- Simple
- Rapid
- Easily incorporated into our *Affymetrix ChIP on chip* workflow

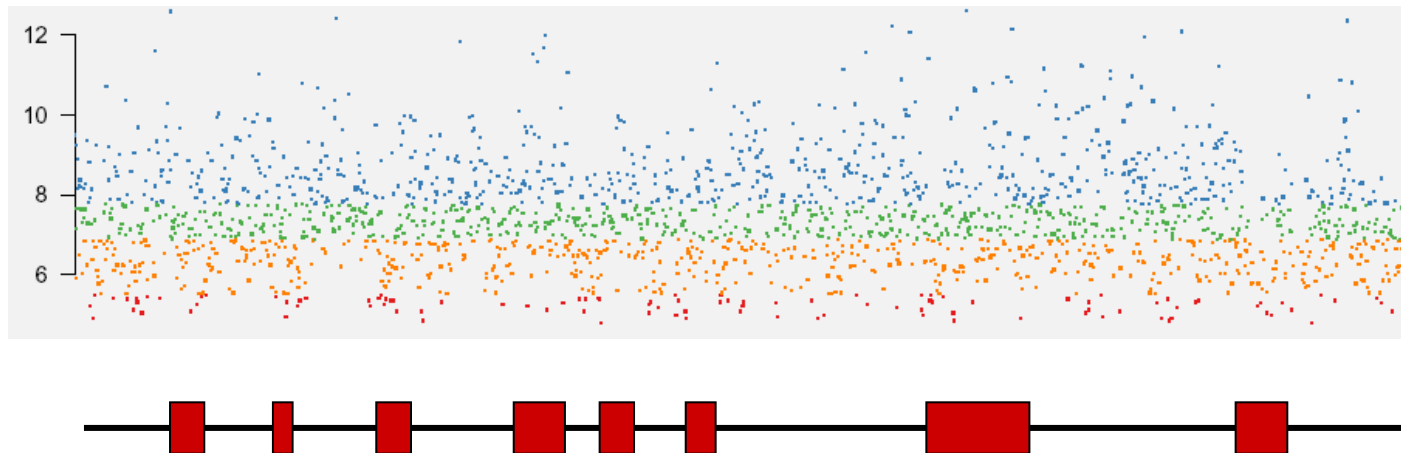
Control vs reference

- Control:
 - Specificity of IP
 - Non specific Ab
 - Mock IP
 - Untagged strain
 - RNAi-treated sample
- Reference
 - Calculate local enrichment
 - Input
 - gDNA (amplified or not ????)

Data treatment-signal correction

- Signal variability
 - Abundance of material
 - Amplification bias
 - Labeling efficiency
 - Hybridization kinetics ($GC\%$, T_m on 25 bases oligos)
 - Probe quality
- How can we assess this and correct to extract mostly abundance differences?
 - MAT model based
 - Use of gDNA as ref
 - TileMap
 - Huber

gDNA signals



Under dev:

use of a set of three replicates of non amplified gDNA for all ChIP and transcriptome profiling studies (same strain-genetic Background!!)

The Genomics Platform

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- Didier CHOLLET
- Céline DELUCINGE
- Cécile DELUEN-SAGNE
- Mylène DOCQUIER
- Olivier SCHAAD
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