

*Development of high-density NimbleGen
microarray chip for genotyping of a
radiation-hybrid panel of wheat
chromosome 7B*

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Aim of the study

- Develop a CGH NimbleGen high-density microarray chip for genotyping RH-population and construction of a high density RH-map of wheat chromosome 7B
- Use a subset of deletion lines to construct 7B chromosome bin map

Radiation hybrid panel

Irradiate seeds at 25,35,45 krad



Langdon 7D(7B)

Chr 1-6: AABB

Chr 7: AADD

Langdon

Chr 1-7: AABB



1200 RH₁ panel for chromosome 7B

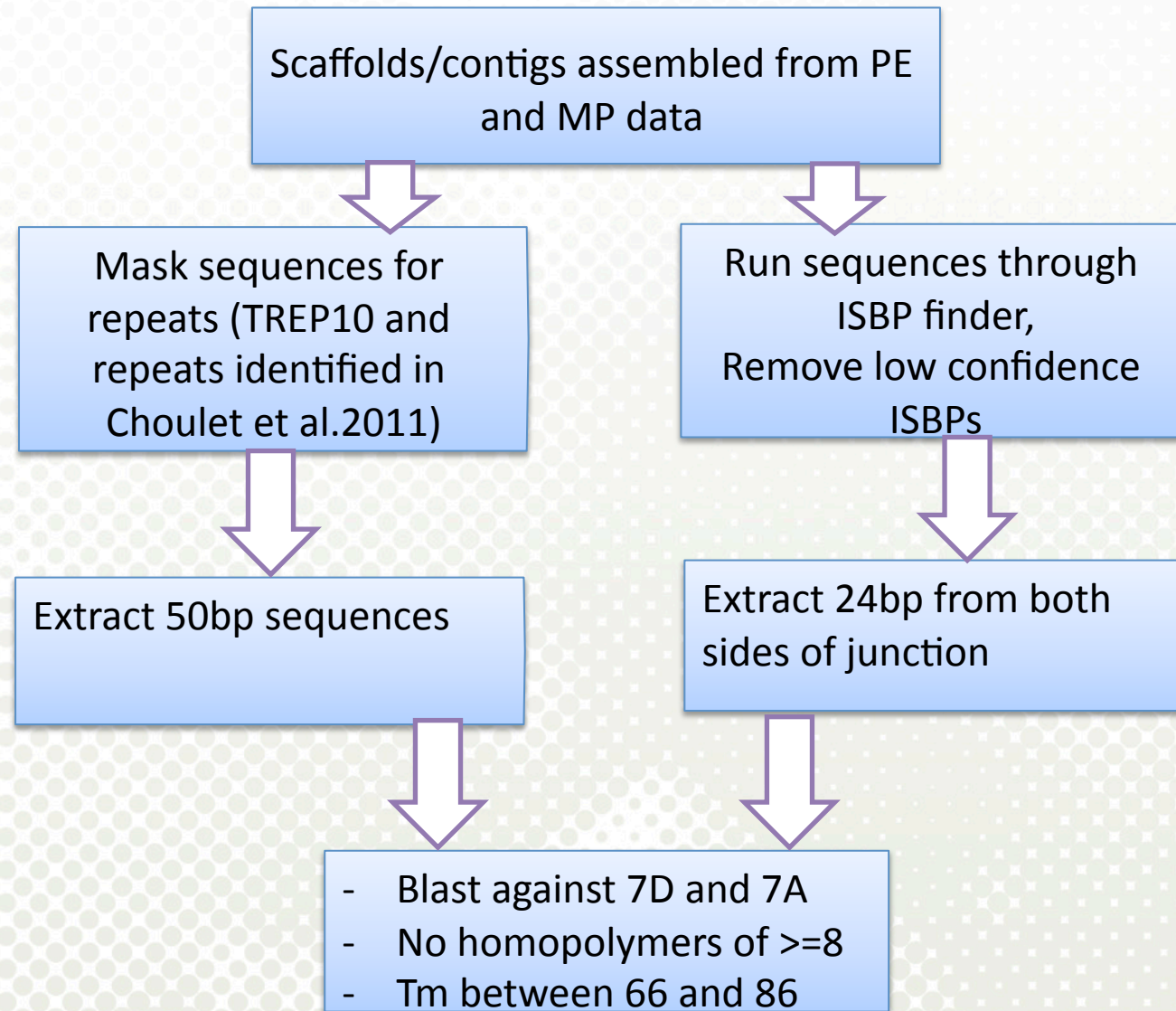
Chr 1-6: A**ABB**

Chr 7 : A**ABD**

300RH₁ lines selected with retention frequency 10-90%

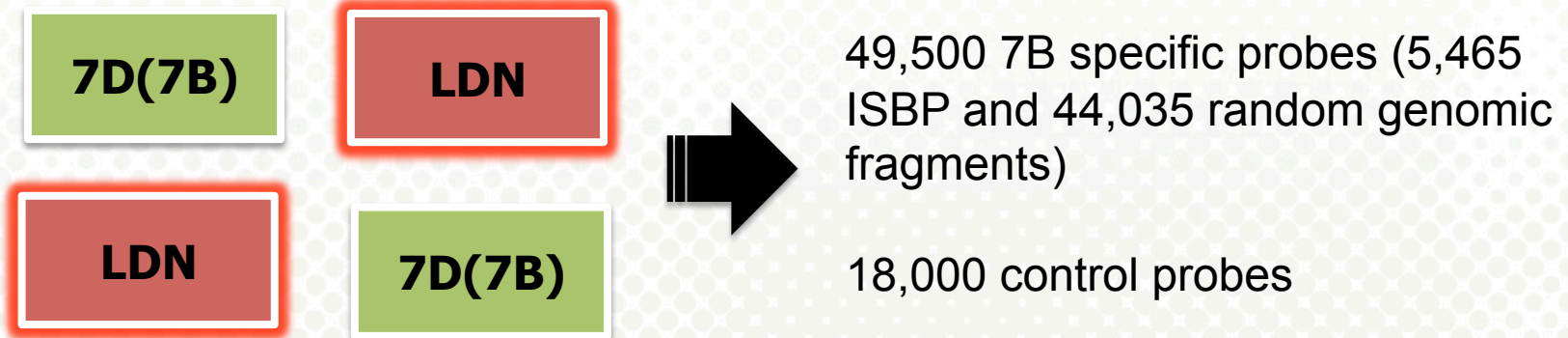


Design of probes for NimbleGen microarray



Identifying 7B specific probes

- 3*720K CGH chip



- 12*270K CGH chip

7B deletion bins and reference (Langdon)



Normalization of Microarray Data

- To correct for systematic differences between samples on the same slide, or between slides, which do not represent true biological variation between samples but arise due to:
 - different labelling efficiencies of the dyes
 - different amounts of Cy3- and Cy5-labeled DNA
 - differences in scanning
 - print-tip, spatial, effects, etc.



Two-step normalization of CGH-arrays

Spatial intensity normalization



Print-tip Loess normalization

- Produce MA plot

$$M = \log(\text{cy3}) - \log(\text{cy5})$$

$$A = (\log(\text{cy3}) + \log(\text{cy5})) / 2$$

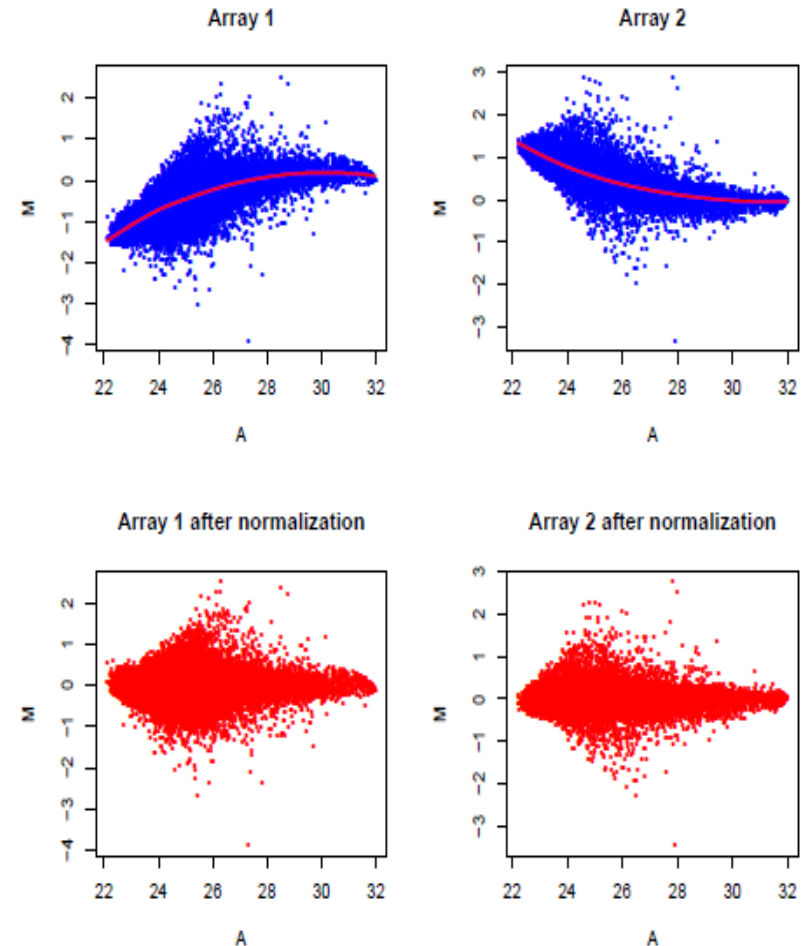
- Apply a Loess regression to the data

- Calculate the normalized M value by

$$M_{\text{normalized}} = M_{\text{original}} - M_{\text{predicted by Loess}}$$

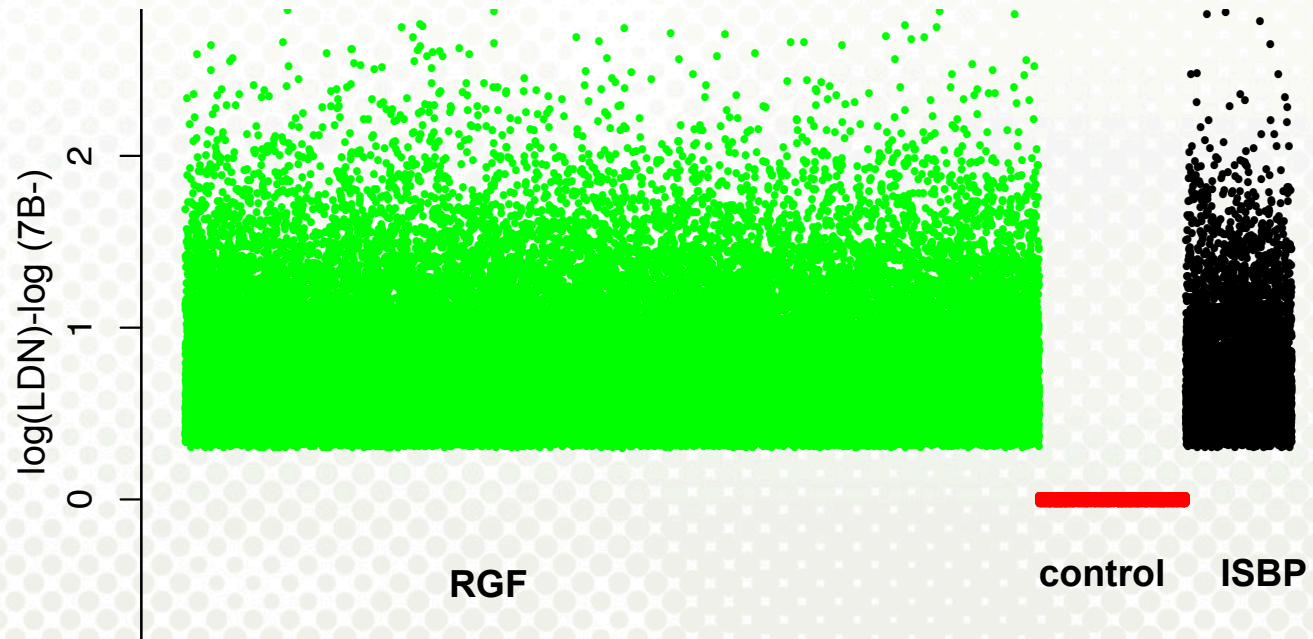
How does Lowess work?

- Lowess performs a large number of local regressions in overlapping windows
- Each regression is then then combined to form a smooth curve

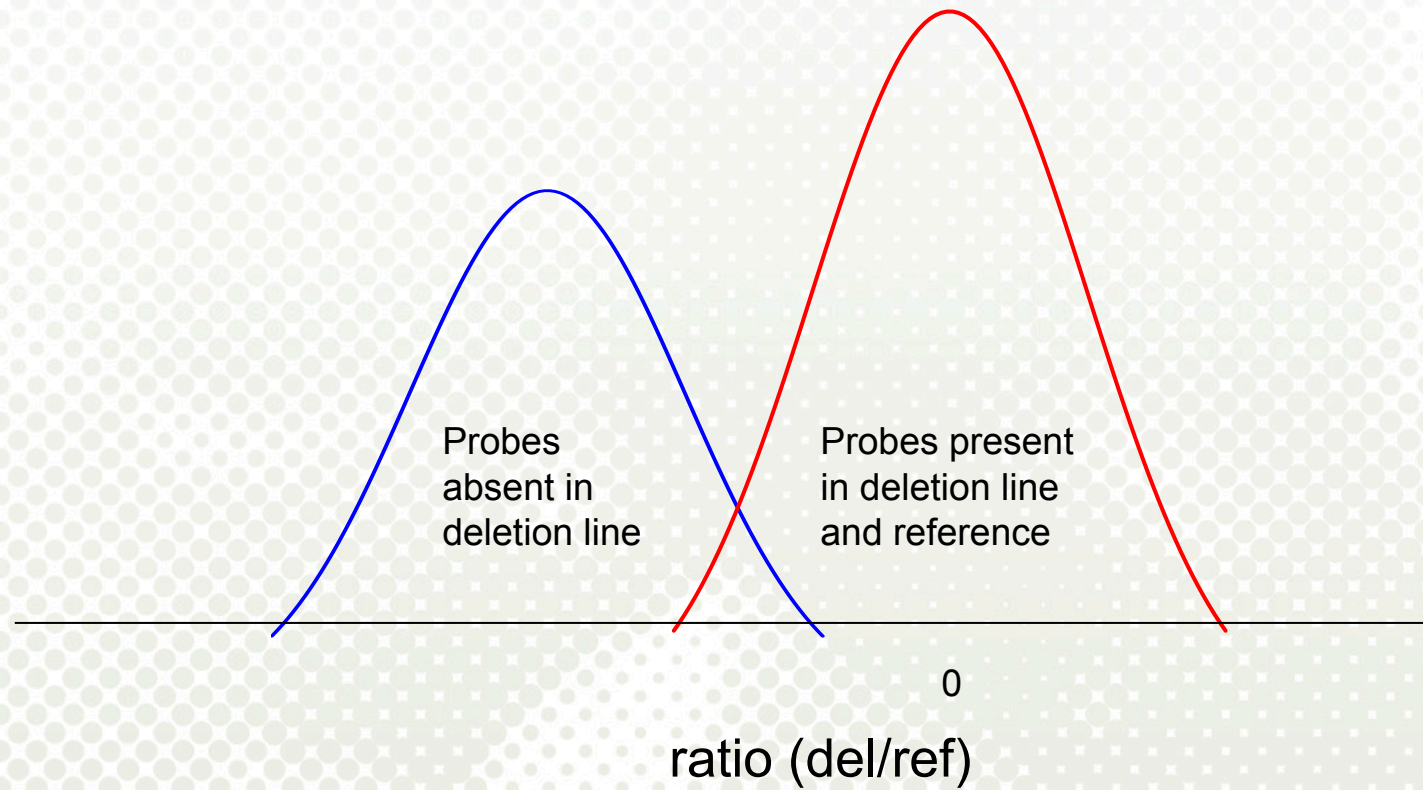




Selecting 7B specific and control probes

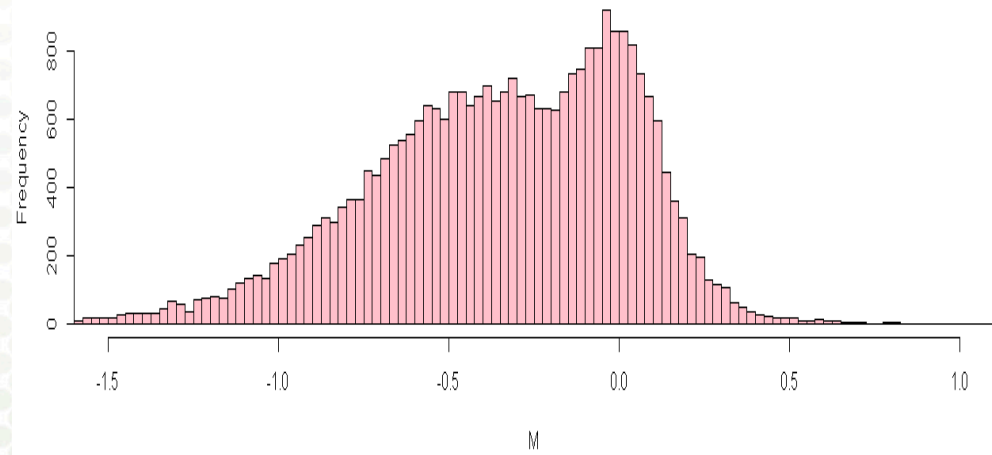


Identification of bin probes

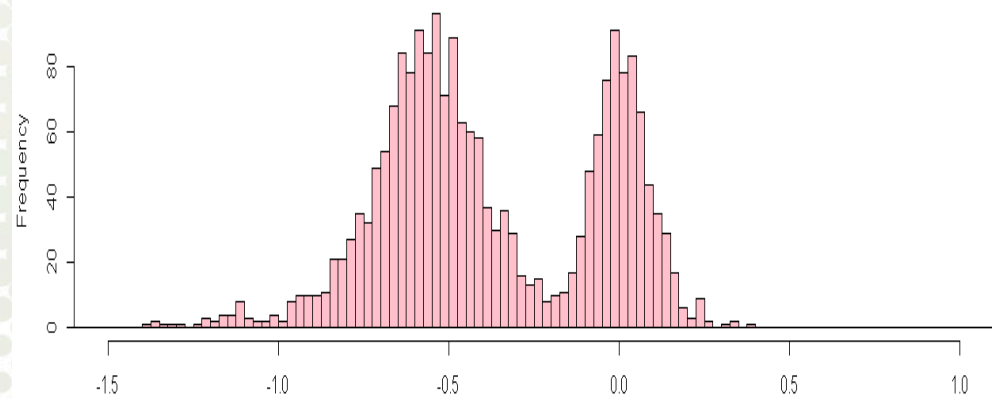


Averaging over probe replicates vs scaffolds

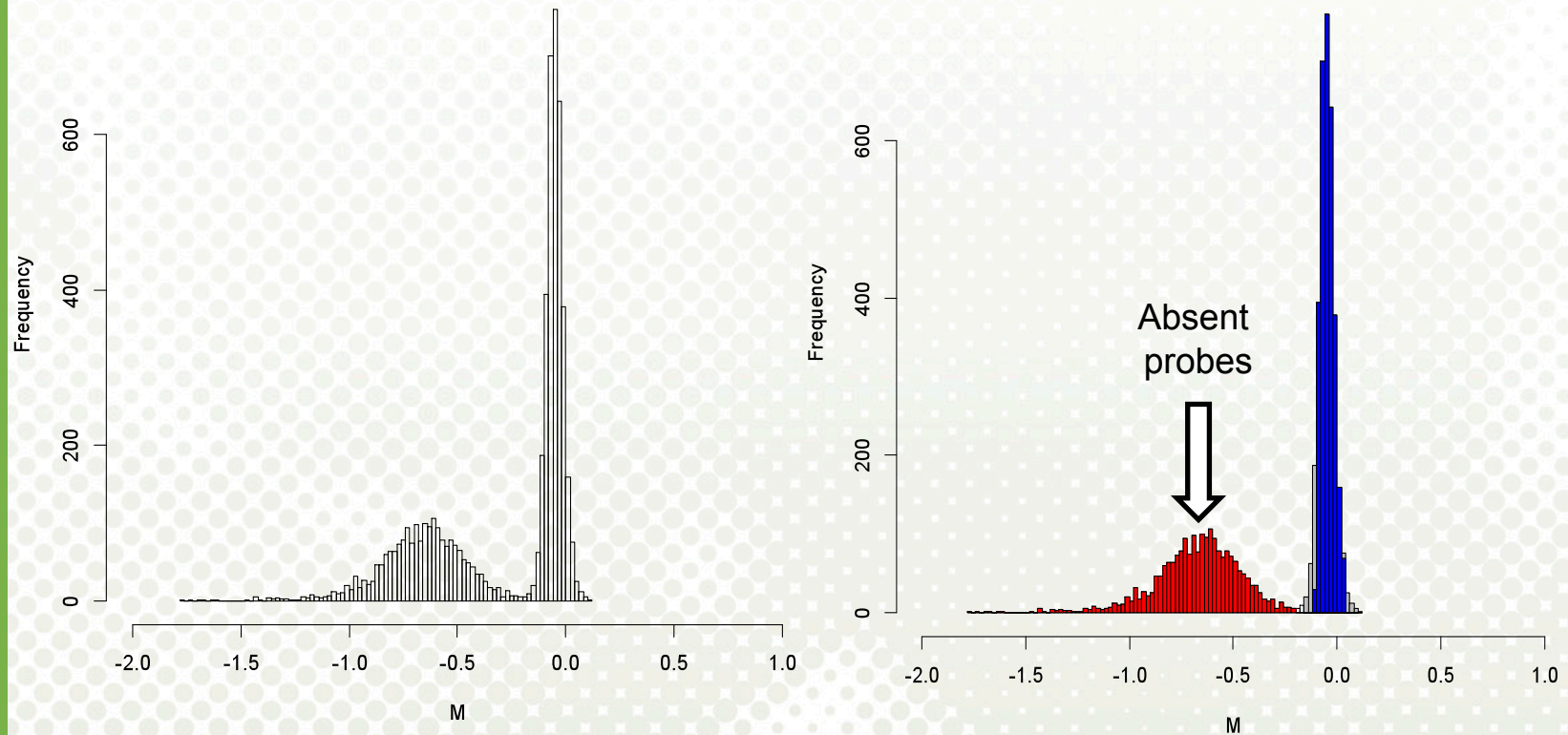
On probes



On scaffolds (>3 probes)



Identification of present/absent probes in deletion lines

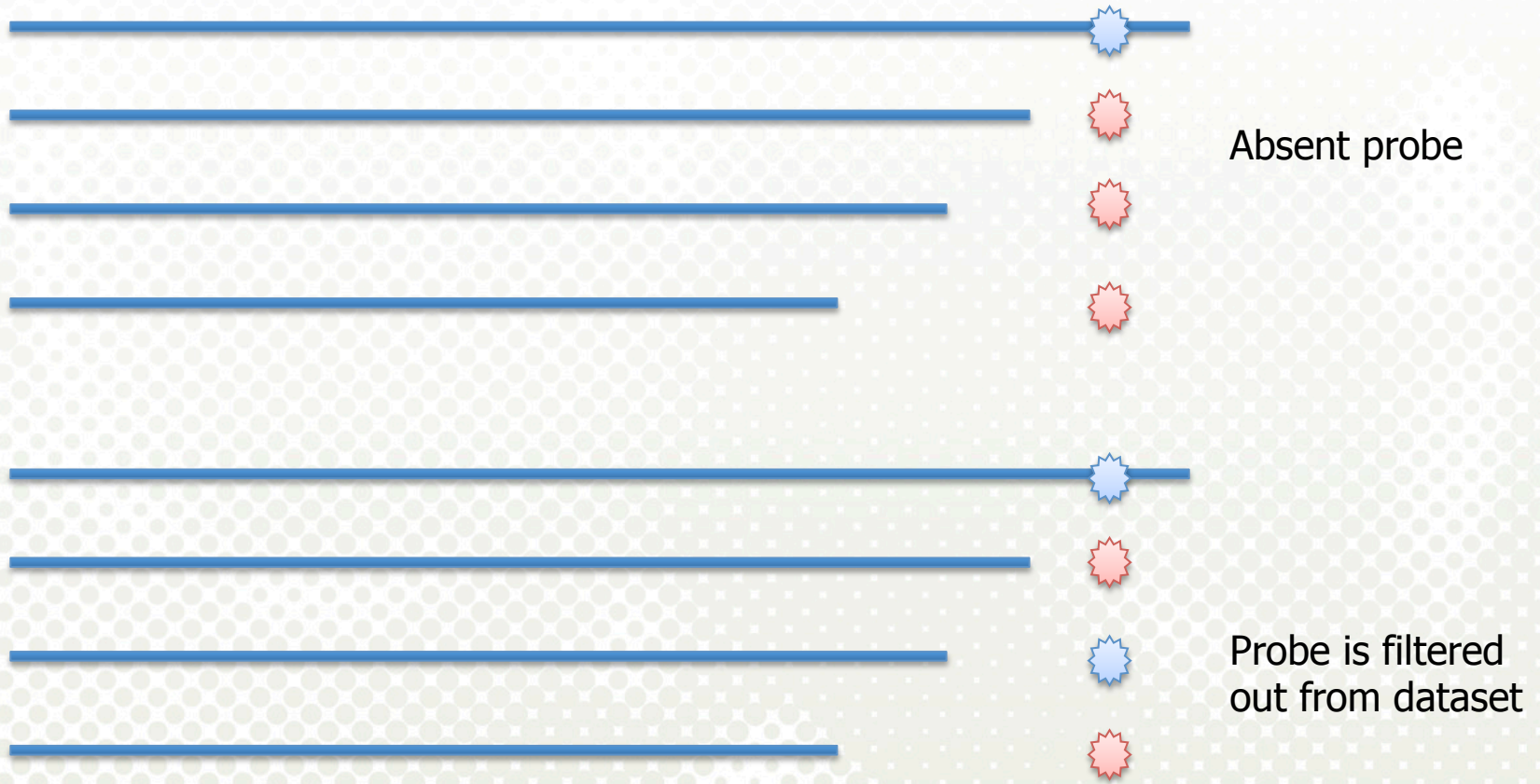


Mclust in R



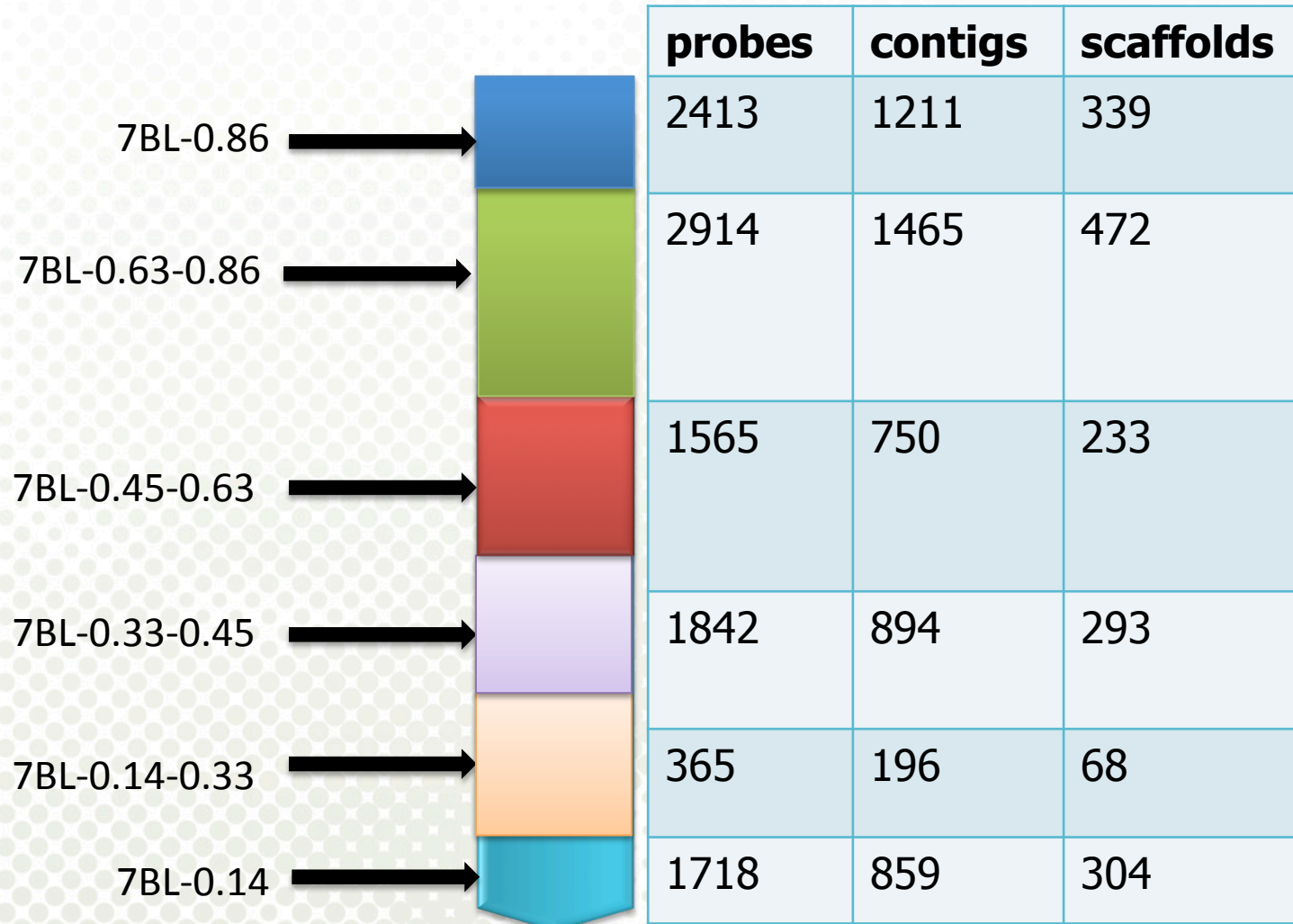


Deletion bin mapping





Deletion bin map of 7B



Summary

- Developed CGH microarray chip will be used for genotyping radiation-hybrid panel
- We have bin mapped 5357 contigs (1709 scaffolds with >3 probes) for 7BL
- 10817 probes were bin mapped for 7BL among which 1396 ISBPs and 9421 RGF

Collaborators

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Thanks for your attention!