The AFLP package: generating objective and repeatable genetic AFLP data

useR! 2011

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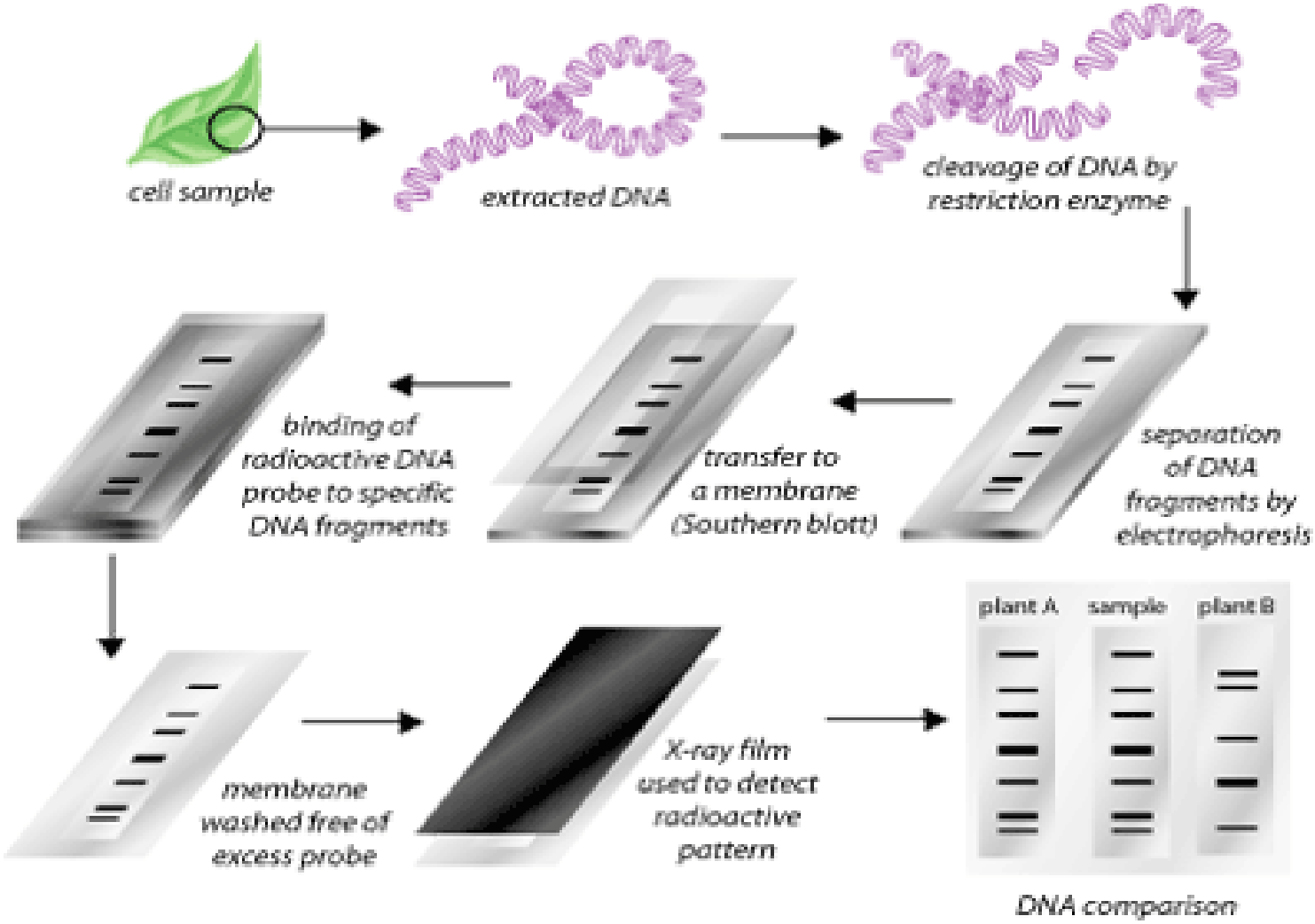
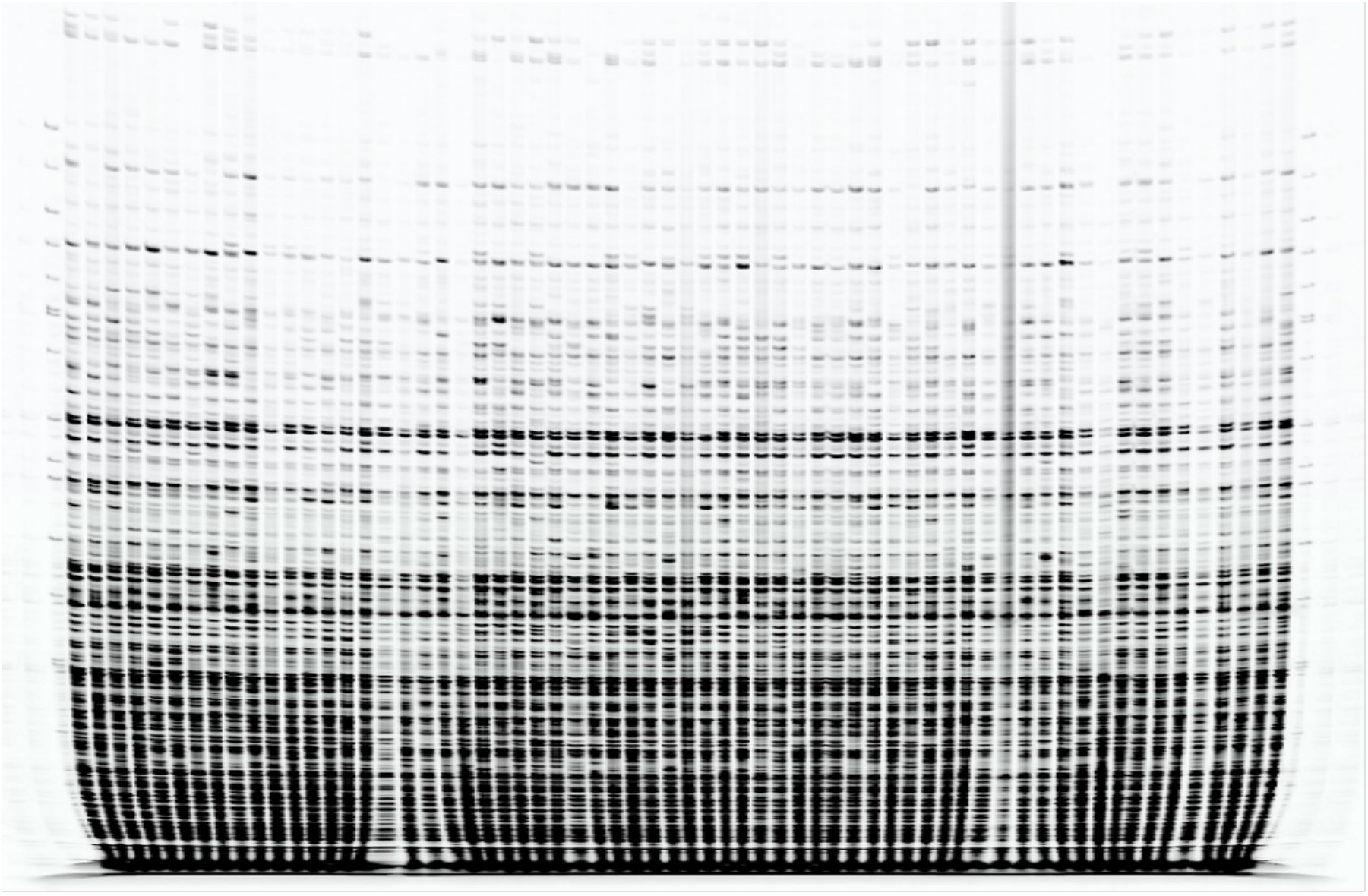
Outline

AFLP?

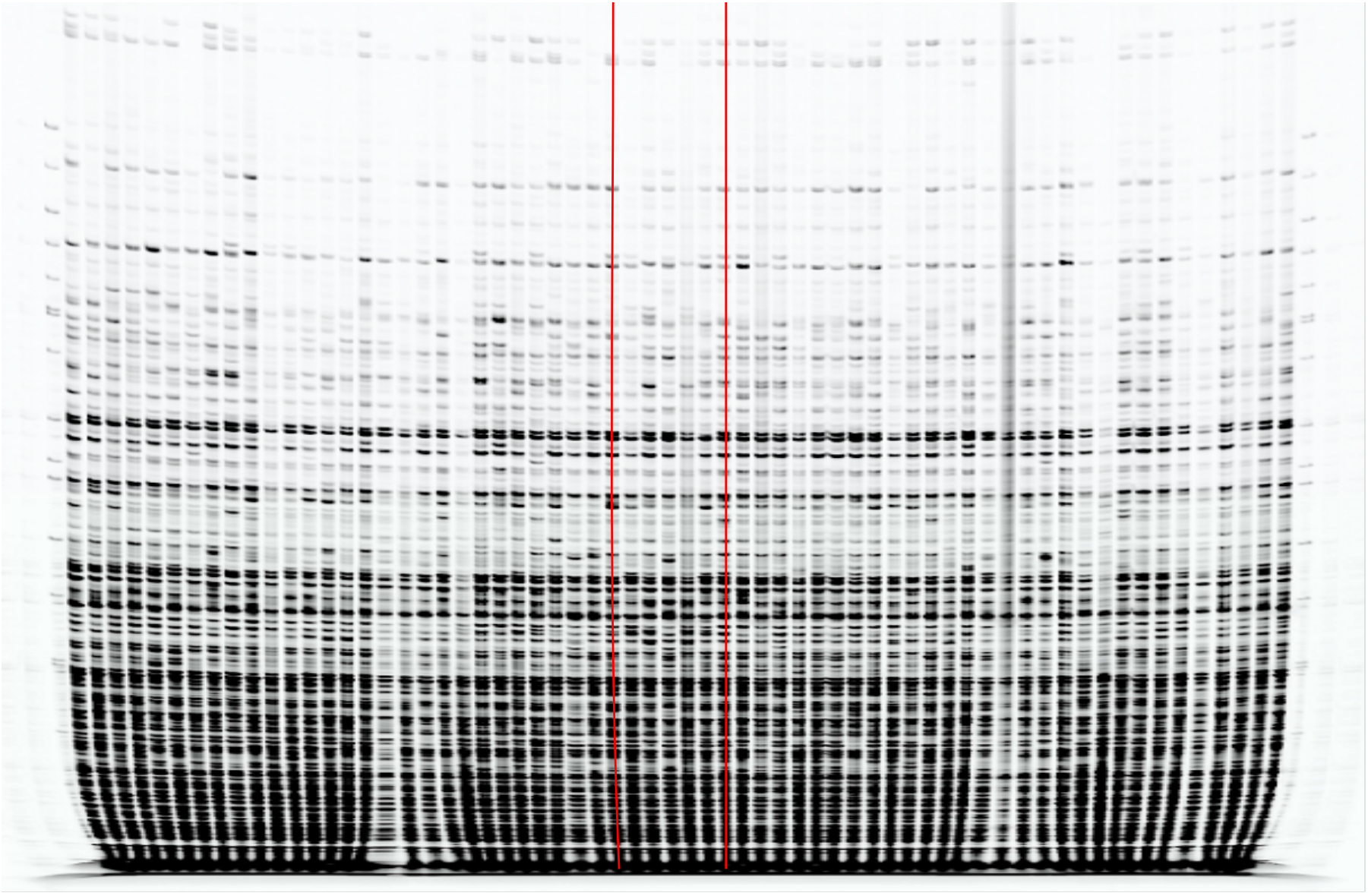
Amplification **F**ragment **L**ength **P**olymorphism



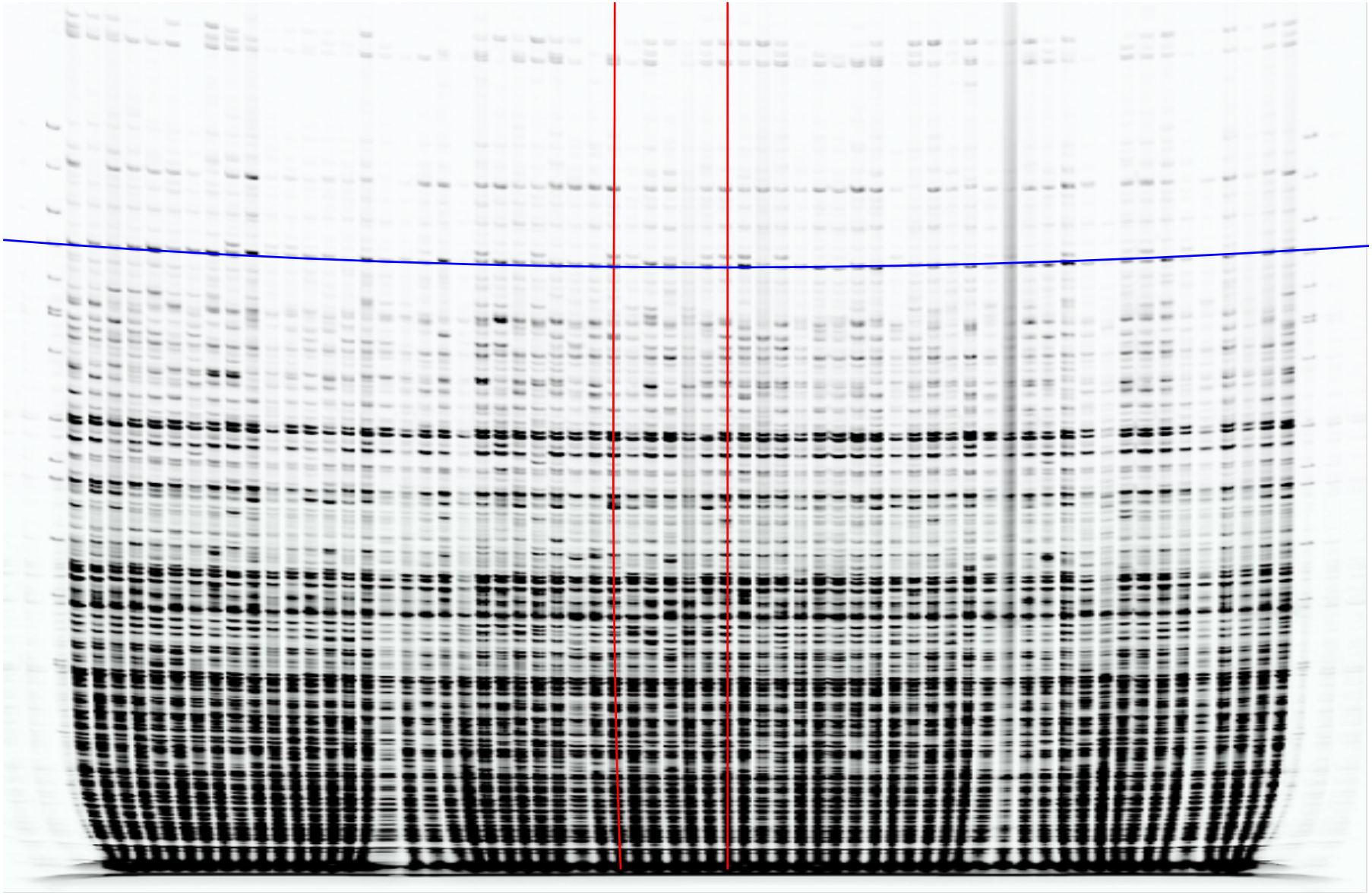
One plate of an AFLP slab gel



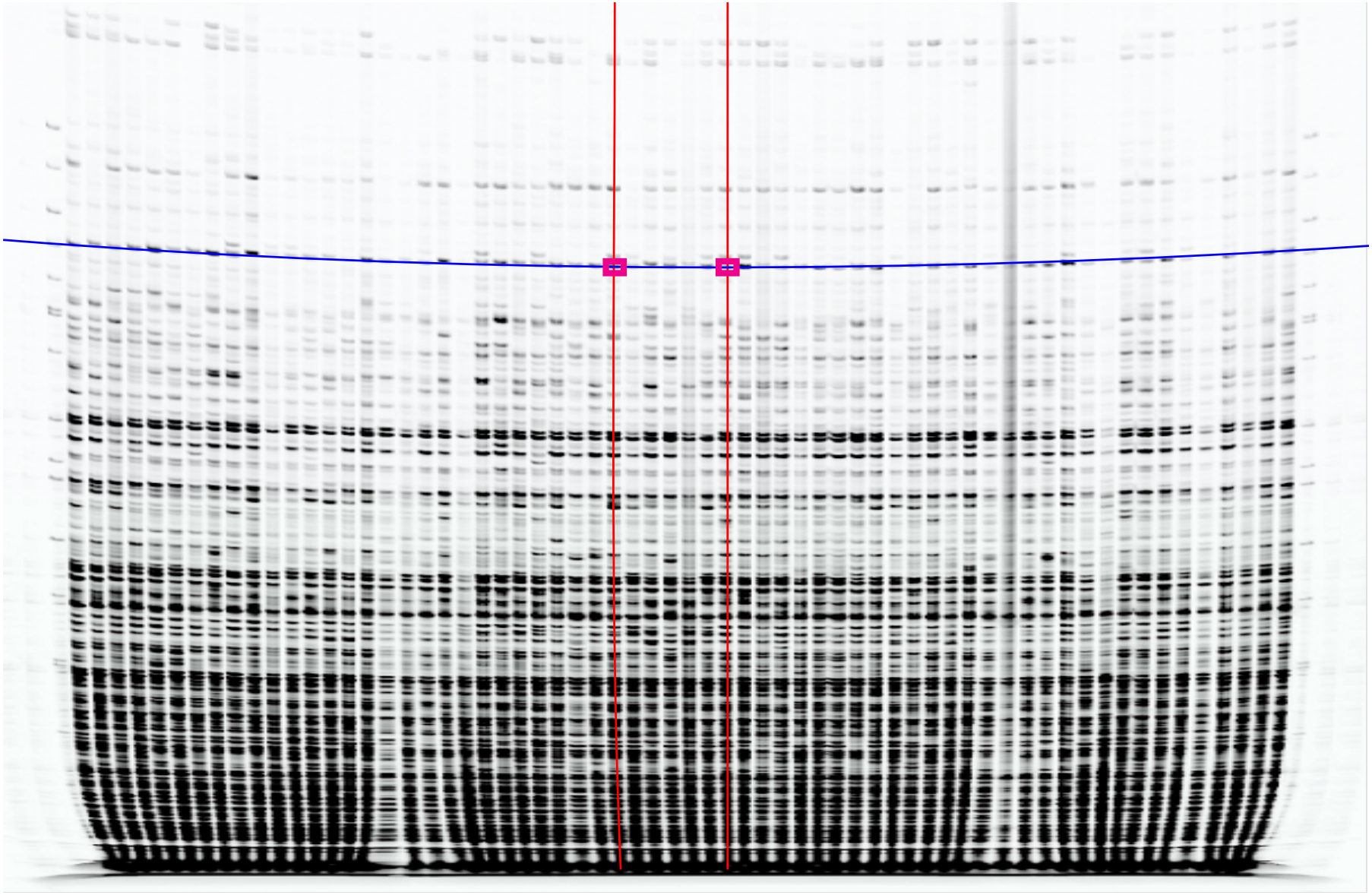
Red line: replicates



Blue line: marker = fragments with same length



Magenta rectangles: bands with fluorescence



Problems

- Binary data required
  - Fluorescence $\Rightarrow$ presence/absence (scoring)
- Manual scoring
  - Tedious
  - Subjective
  - If scientist uses knowledge about population $\Rightarrow$ possible bias
- Lots of steps in the lab
  - Some affect entire subset (e.g. replicate, marker, plate,. . .)
  - Hard for humans to take into account
  - Replication is required to asses lab effects
- Single threshold is not a good option
  - Adjustments required for lab effects
  - Gradient in signal strength along fragment lengths

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4. Do it fast, objective and reproducible

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```
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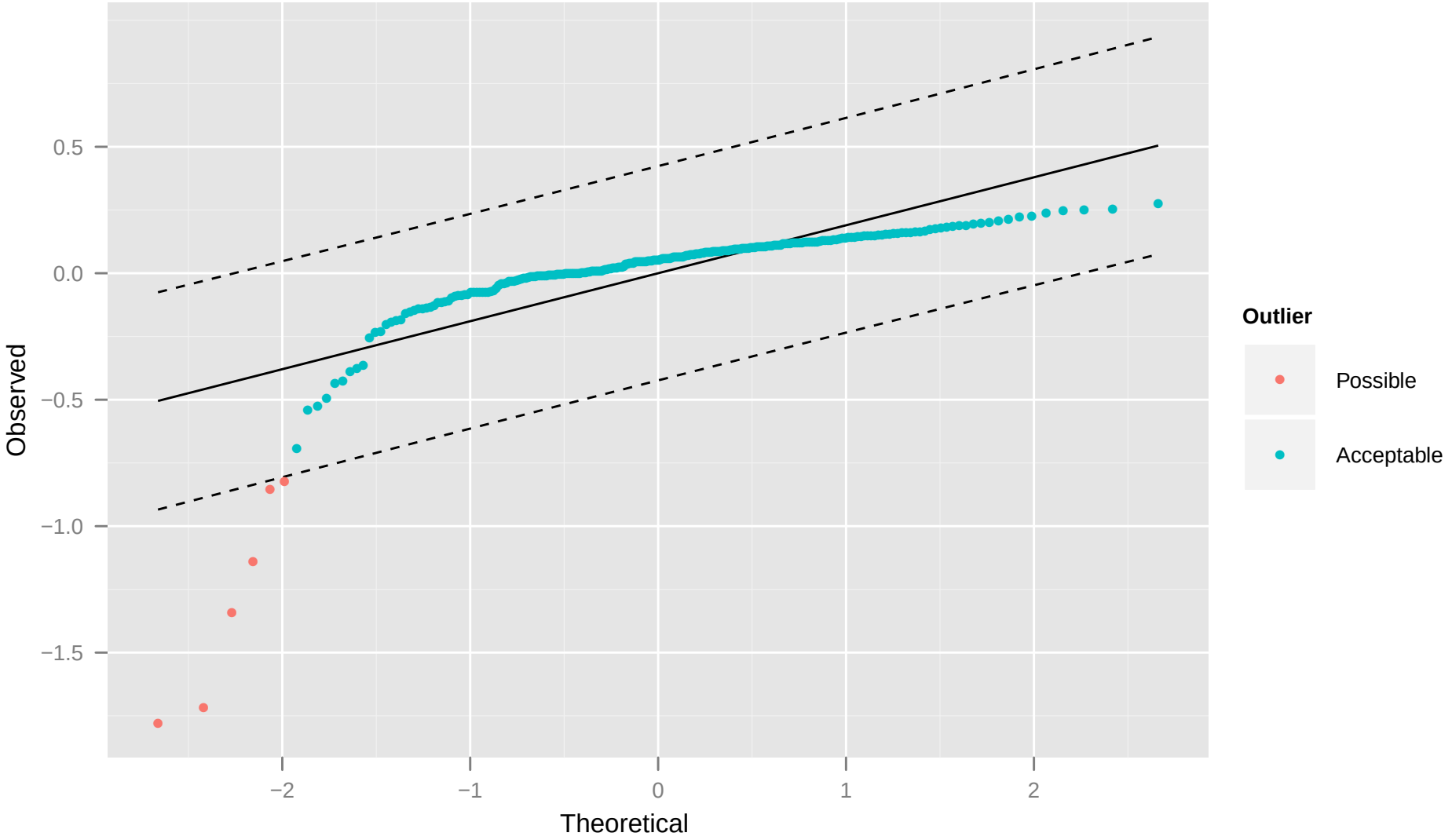
- QQ-plots random effects and residuals $\Rightarrow$ outliers
- Outliers marked, not deleted

QQ-plot random effect replicates



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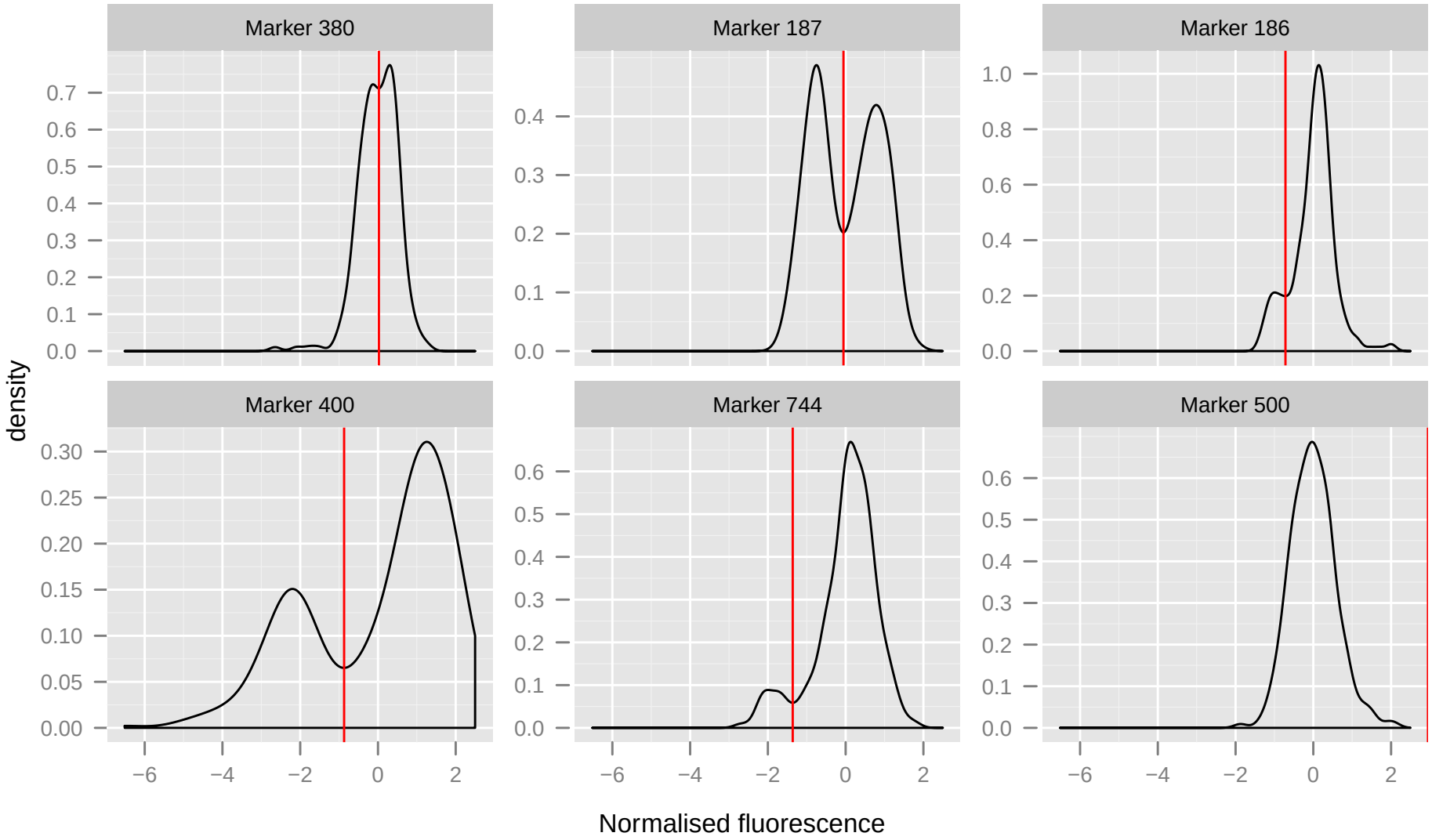
- Threshold $\Rightarrow$ minimum density between two maxima $\Rightarrow$ variable among markers
- (Normalised) fluorescence above threshold $\Rightarrow$ present

Densities of the normalised fluorescence per marker with threshold



Repeatability

- Compare classification of different replicates from same specimen
- $R = 1 - \frac{\sum E_{ij}}{\sum M_{ij}}$
- Varies between 0 ($\forall i,j : E_{ij} = M_{ij}$) and 1 ($\forall i,j : E_{ij} = 0$)
- Our repeatability score = complement of 'technical difference rate' TDR (Bonin et al., 2004)
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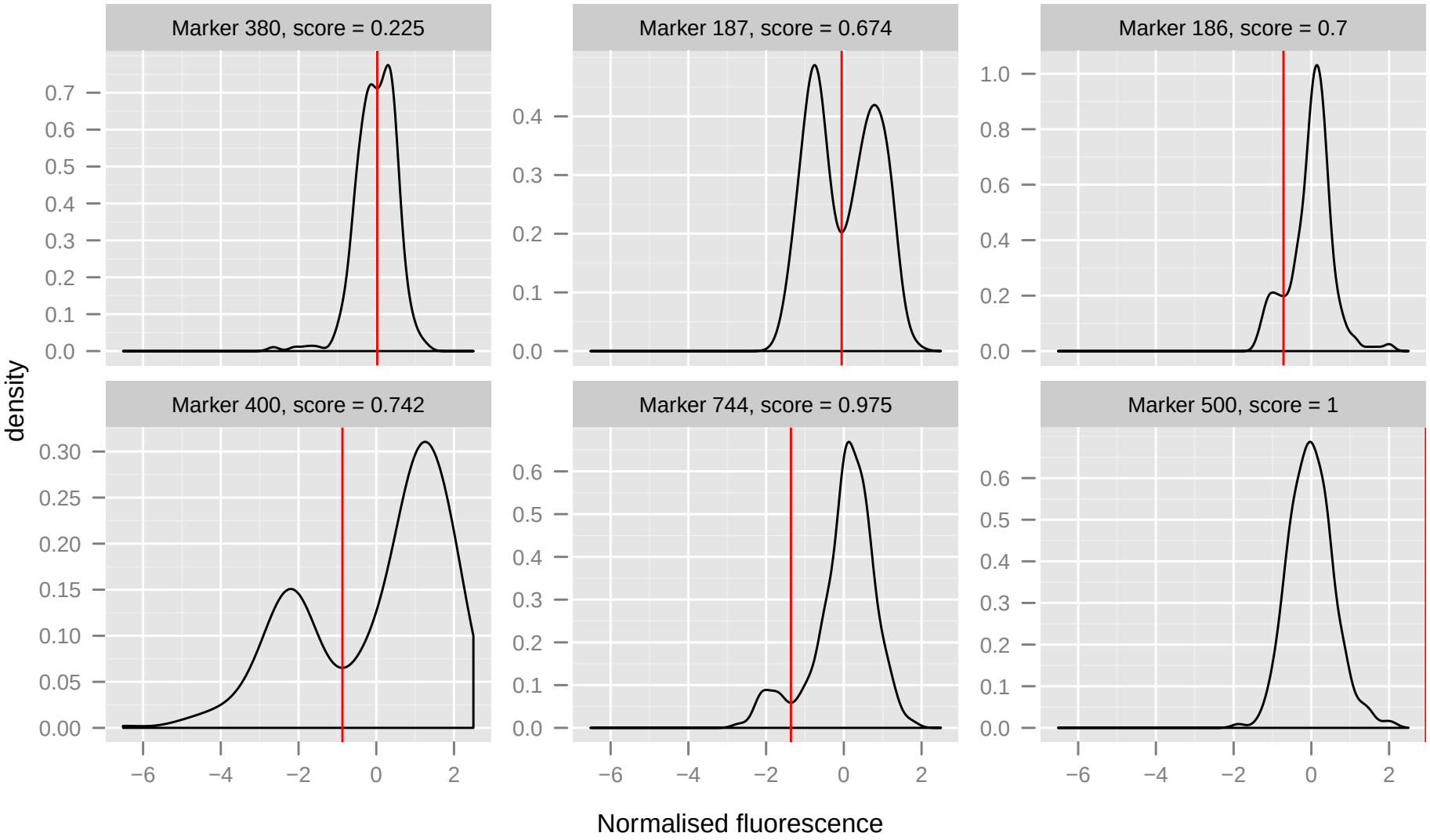
Densities of the normalised fluorescence per marker with threshold and repeatability score



Further analysis

- Classification = **start** genetic analysis
- Results available as data.frame $\Rightarrow$ easy to use with another package
- Direct methods for `hclust()` and `princomp()`
- Suggestions welcome

Genetic analysis of lime trees

- Two species: *Tilia cordata* and *T. platyphyllos*
- Different morphology (e.g. small vs large leaves,. . .)
- Hybrid: *Tilia europea (x)*
- Intermediate morfology
- In situ determination difficult?

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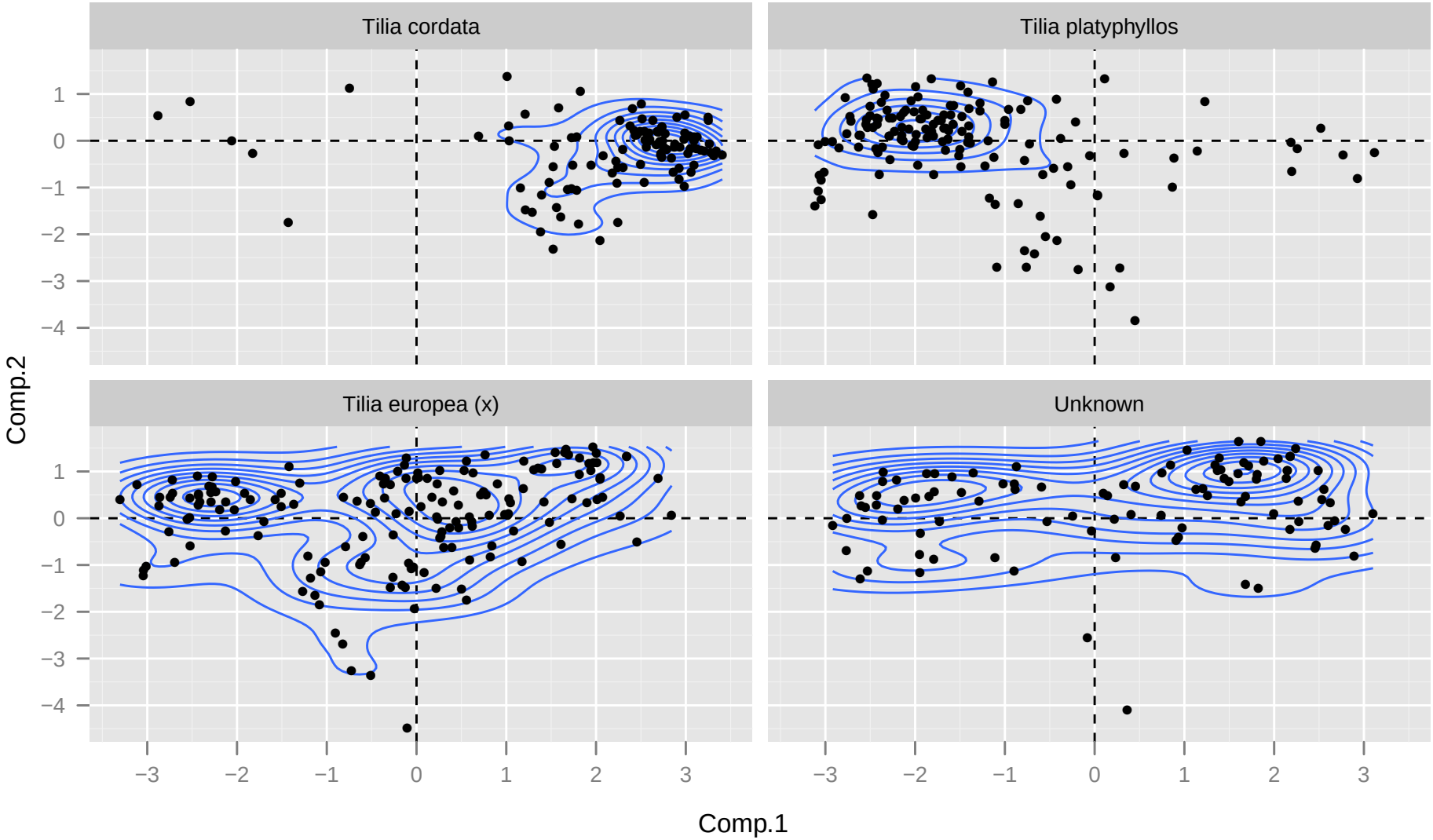
PCO using Jaccard distance split by field determination



The AFLP package

- Available on R-Forge
  ```
  install.packages("AFLP",
  repos="http://R-Forge.R-project.org")
  ```
- Each step $\Rightarrow$ seperate function
- Additional functionality
  - Generation of random lab design with replication
  - Imports two data formats: SAGA (Li-Cor) and ABI
  - Automatic binning of peaks available (algorithm of Arrigo et al. (2009))
- All information in one S4-object
- Output on screen, to LaTeX or no output

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- Extra markers = little extra effort $\Rightarrow$ more information
- Scoring doable in the lab $\Rightarrow$ fast feedback on quality

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Weakness

- Depends on quality of fluorescence measurements

Thank you for listening

Questions?

References

Arrigo, N., J. W. Tuszynski, D. Ehrich, T. Gerdes, and N. Alvarez (2009). Evaluating the impact of scoring parameters on the structure of intra-specific genetic variation using RawGeno, an R package for automating AFLP scoring. *BMC Bioinformatics*, 10:33.

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Onkelinx, T., P. Verschelde, S. Neyrinck, G. Genouw, and P. Quataert (2011). AFLP package. `https://r-forge.r-project.org/projects/aflp/`.