

Tuberculosis Gene Deletion Typing, Not YATM

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Bigarrays

- **New tool for TB epidemiology**

 - Genetic event: deletions**

 - Detection: membrane hybridization**

- **Advantages**

 - Phylogenetic**

 - Functional genomics**

TB Molecular Epidemiology

Typing systems:

IS6110 RFLP

Spoligotyping

Lesson learned

Outbreaks

Laboratory Contamination

Evaluate Control Programs

International Transmission of MDR TB

Dangerous Disseminator

Dangerous Locations

Progression in AIDS Patients

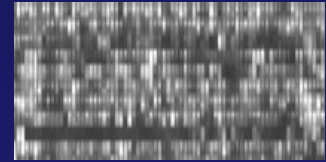
Exogenous Reinfection

Recent Transmission

Virulent Strains

High Tech: Microarray

Deletions detected by microarray using GeneChip[®]



More than 100 deletions

From 21 bases to 15 k bases

High Tech: Microarray

Deletions detected by microarray using GeneChip^(TM)

Affordable Tech: Macroarray

membrane-based hybridization method

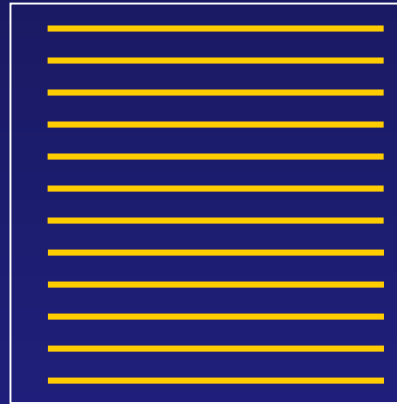
Simultaneous detection of multiple, deleted genomic regions

Bigarray

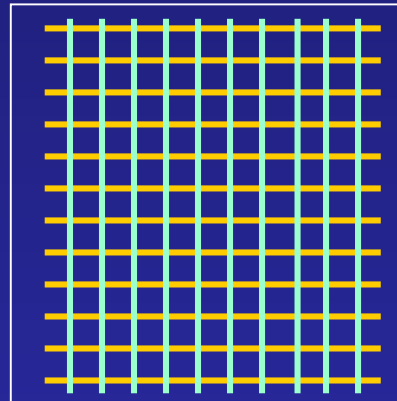
Hybridization Based Technique: **Targets** and **Probes**

Targets:

- Oligonucleotides
- Deletion specific
- Bound to membrane



Membrane before and after utilization

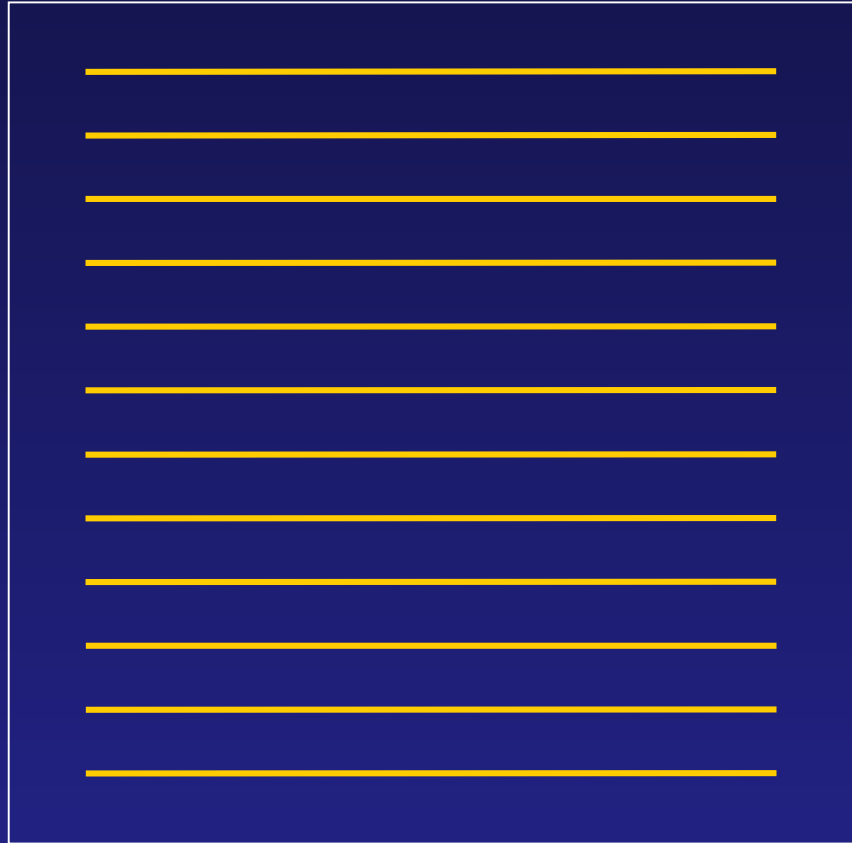


Probes

- PCR products
- Strains specific
- In solution

Target Design





fixing targets on membrane

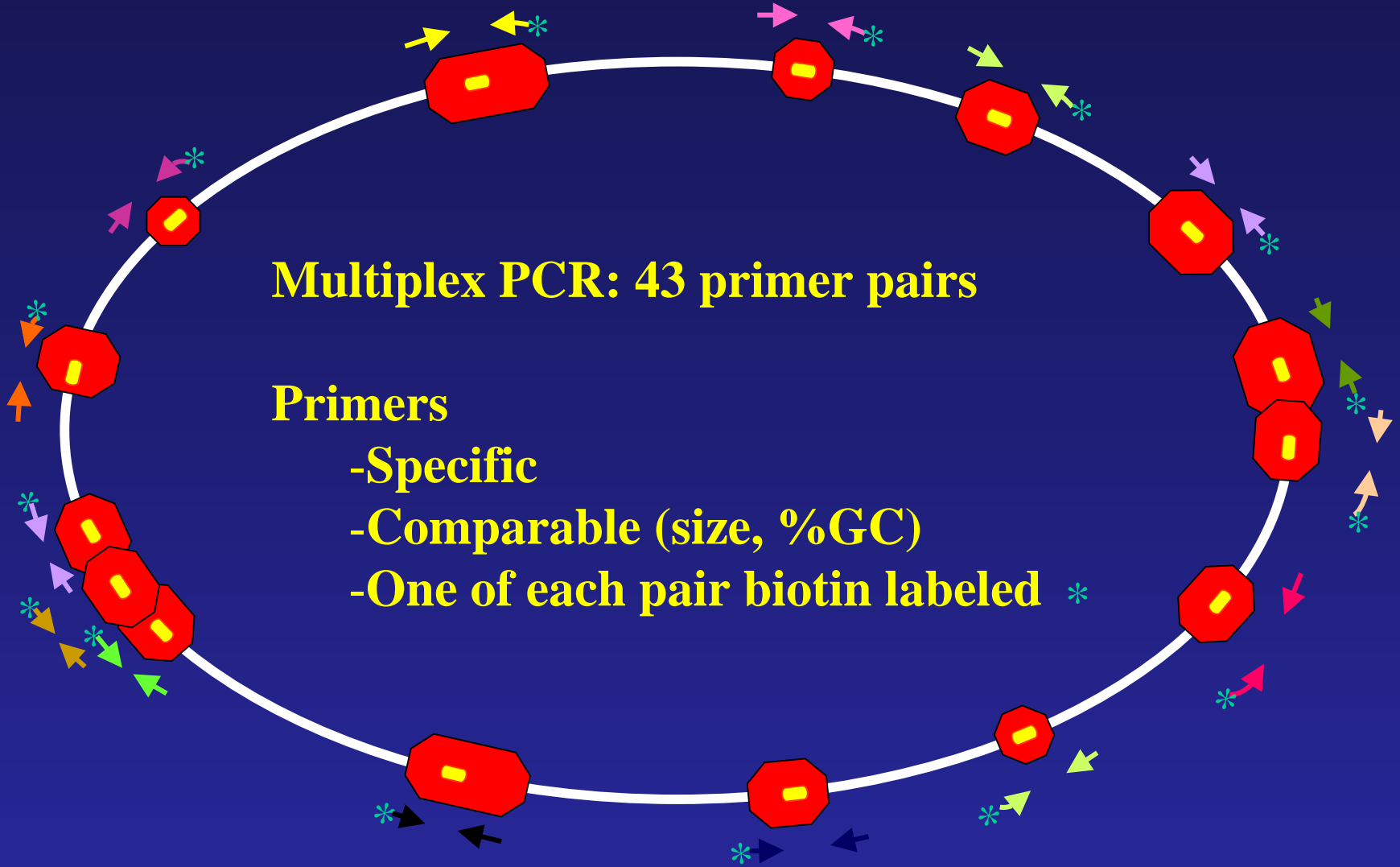
Chemical covalent link allow reusing the membrane many times (>10)

Probe Design

Multiplex PCR: 43 primer pairs

Primers

- Specific
- Comparable (size, %GC)
- One of each pair biotin labeled *

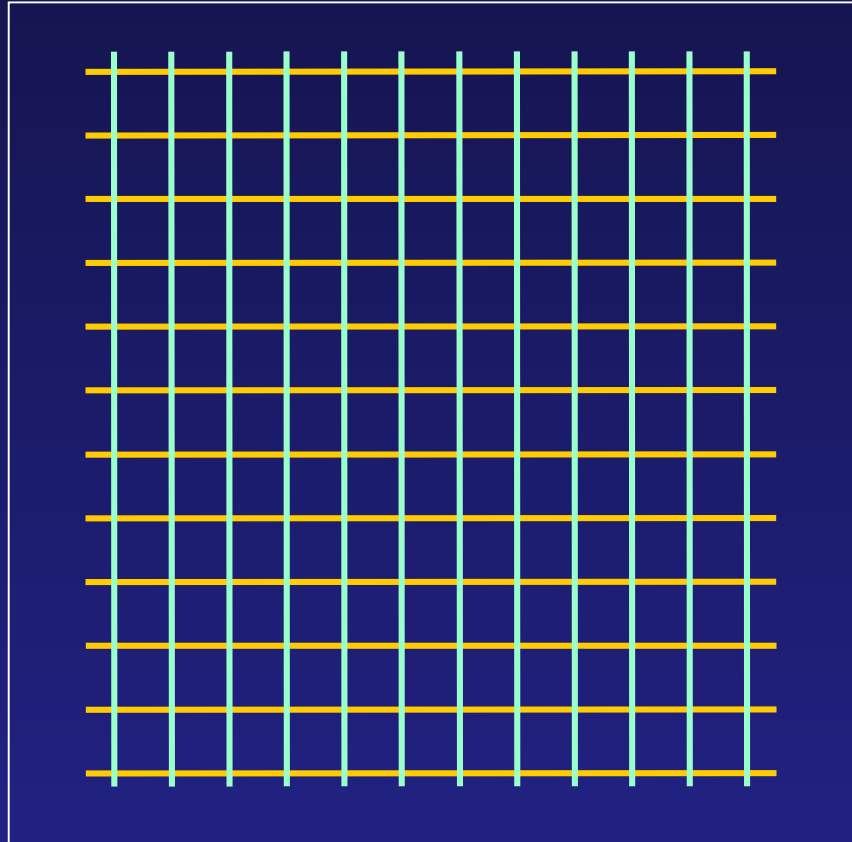




strain DNA



Multiplex PCR:
specific
amplification
and labeling



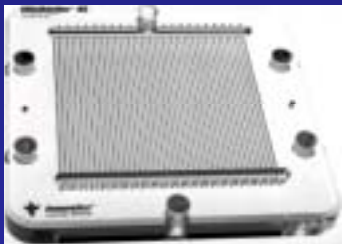
stringent wash



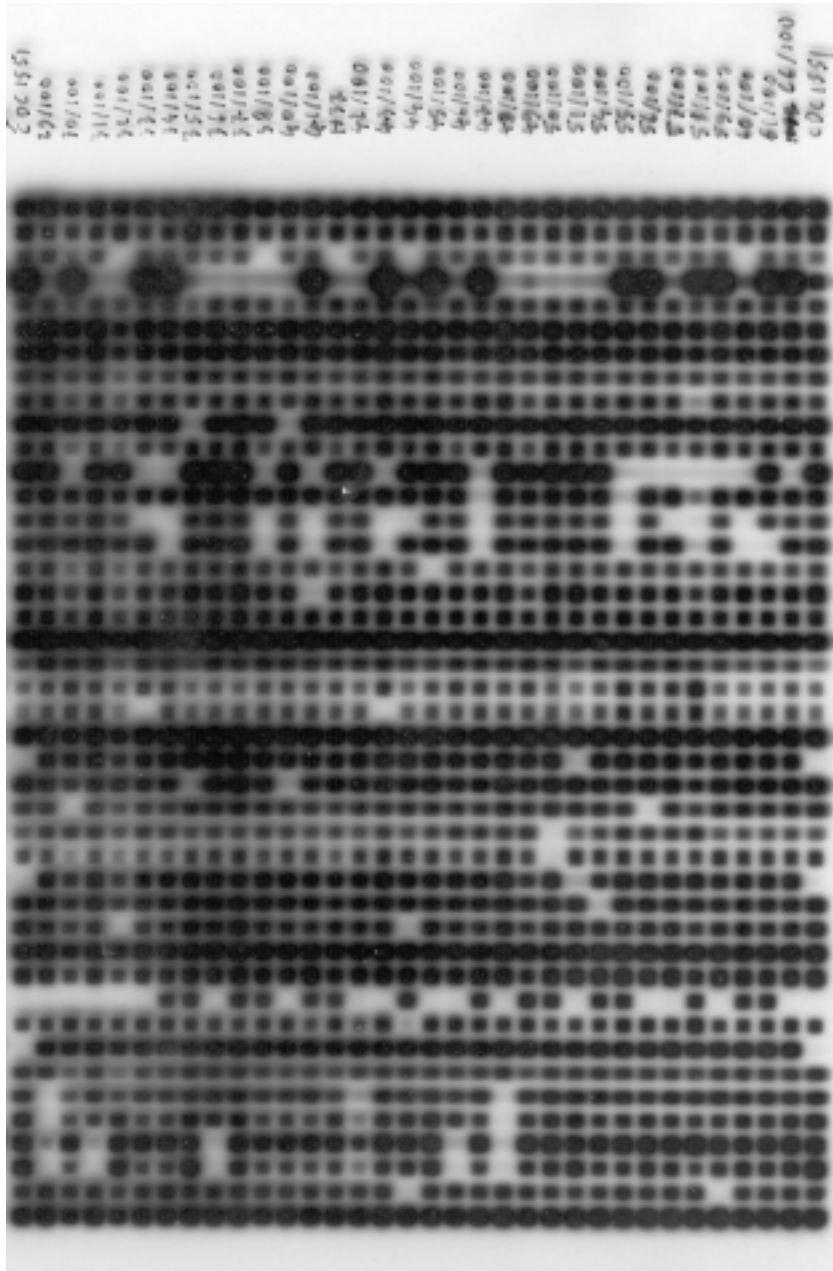
peroxidase
labeling



ECL-
detection
system

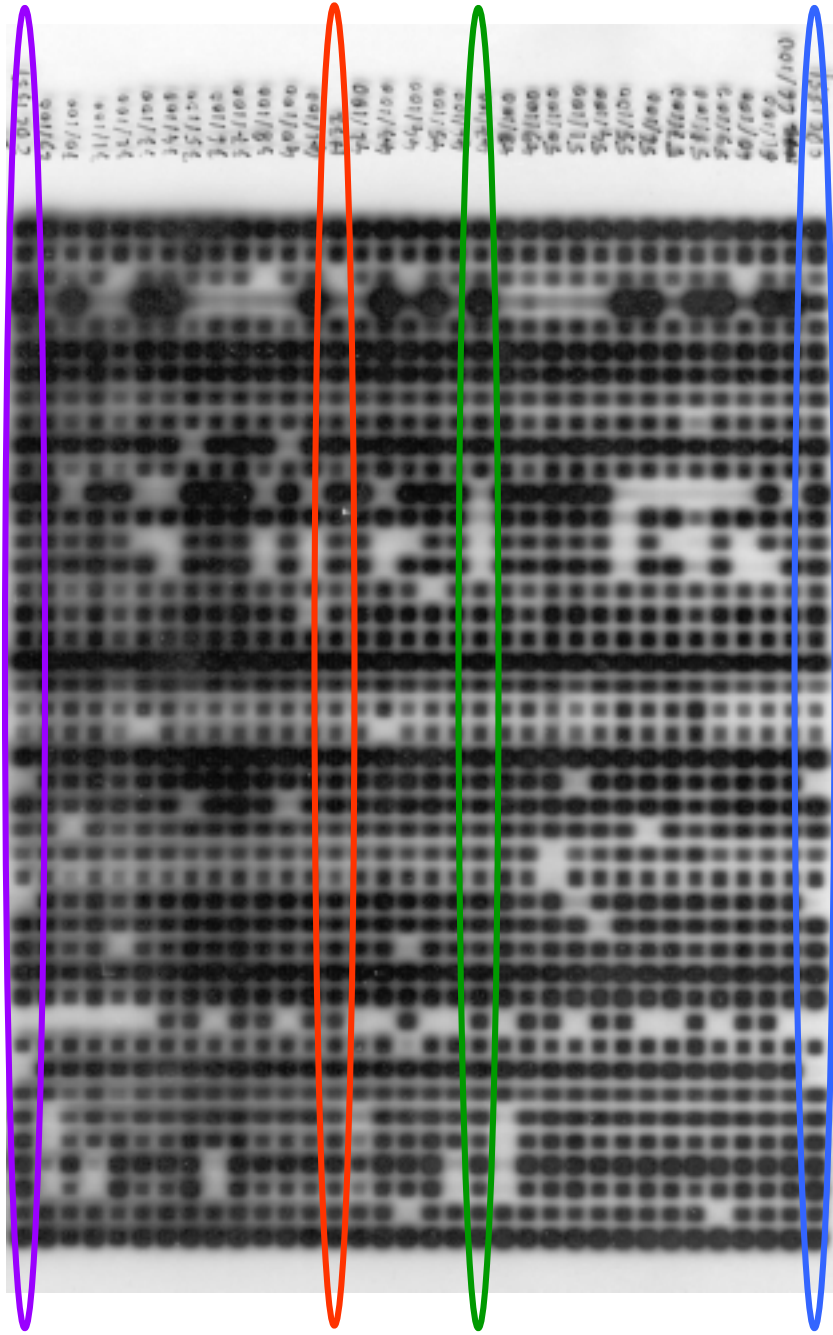


Using of the membrane

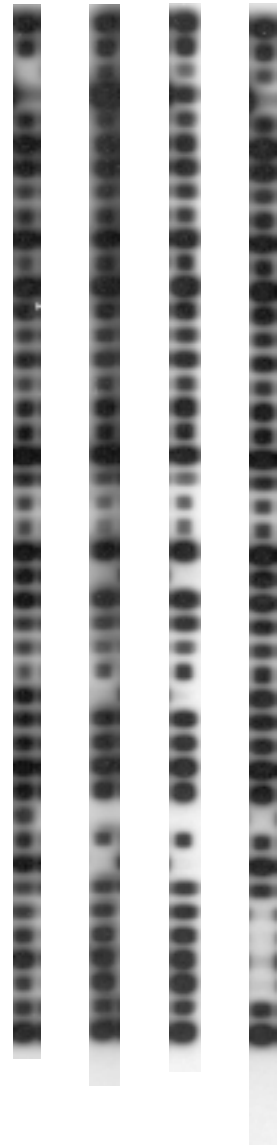


2001551
32/100
39/100
31/100
34/100
35/100
34/100
37/100
38/100
40/100
41/100
43/100
44/100
49/100
44/100
45/100
44/100
42/100
48/100
49/100
50/100
51/100
54/100
55/100
55/100
57/100
58/100
59/100
48/100
61/100
62/100

0001100
1999 24/100
c 00 1551



H37Rv
CDC1551
CDC1551
48/100



What we expect to see ...

For H37Rv:

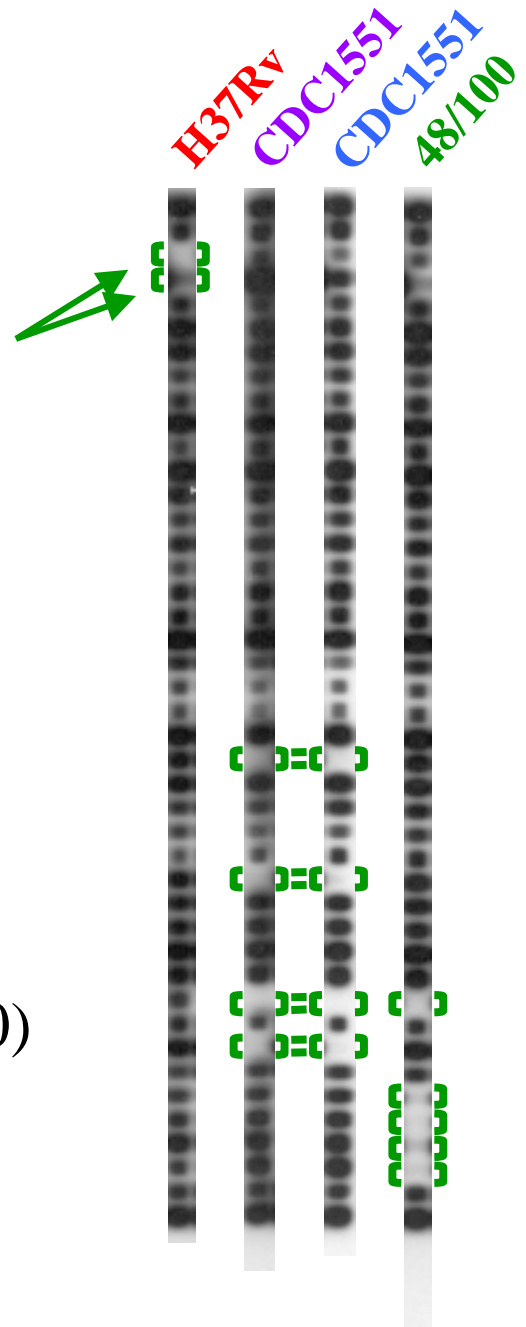
- H37Rv DNA is present
- DNA present in CDC1551 missing from H37Rv

For CDC1551:

- CDC1551 DNA is present
- DNA present in H37Rv missing from CDC1551

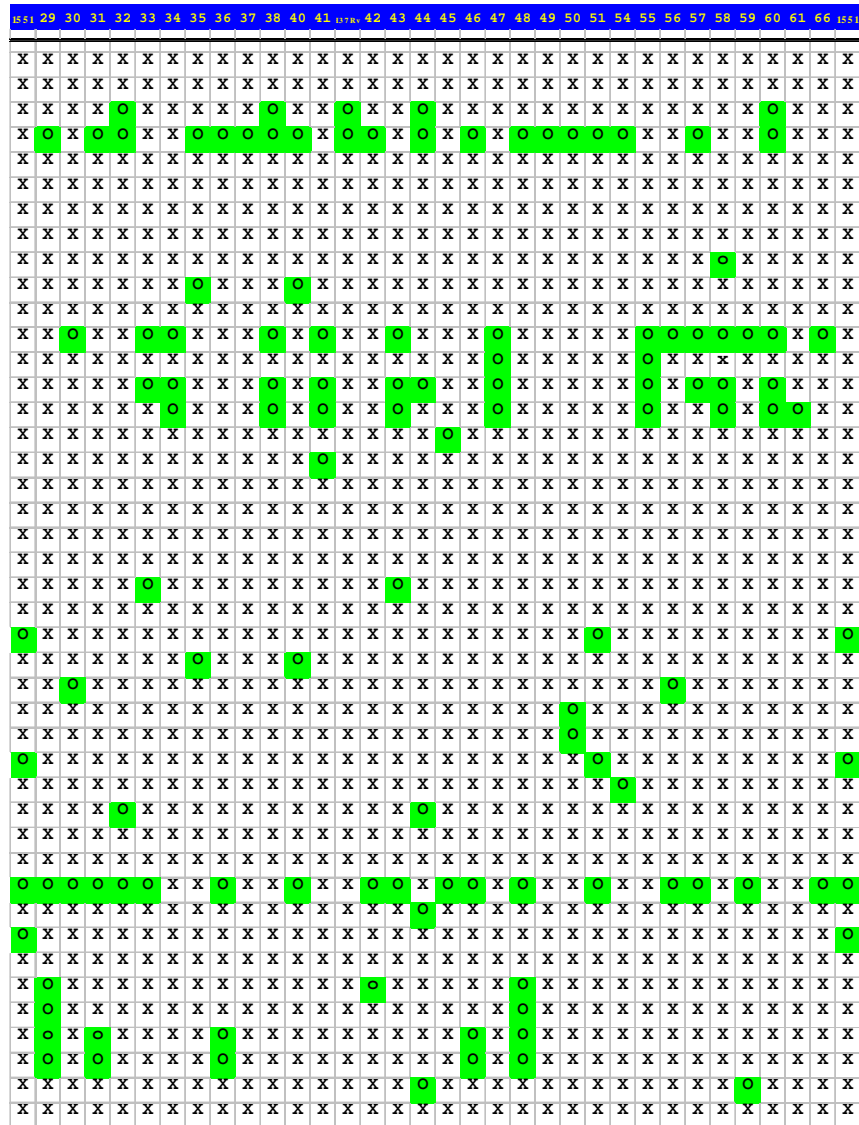
Two strains repeated display the same profil:
Example of the reproducibility for CDC1551

A different pattern for a non related strain (48/100)

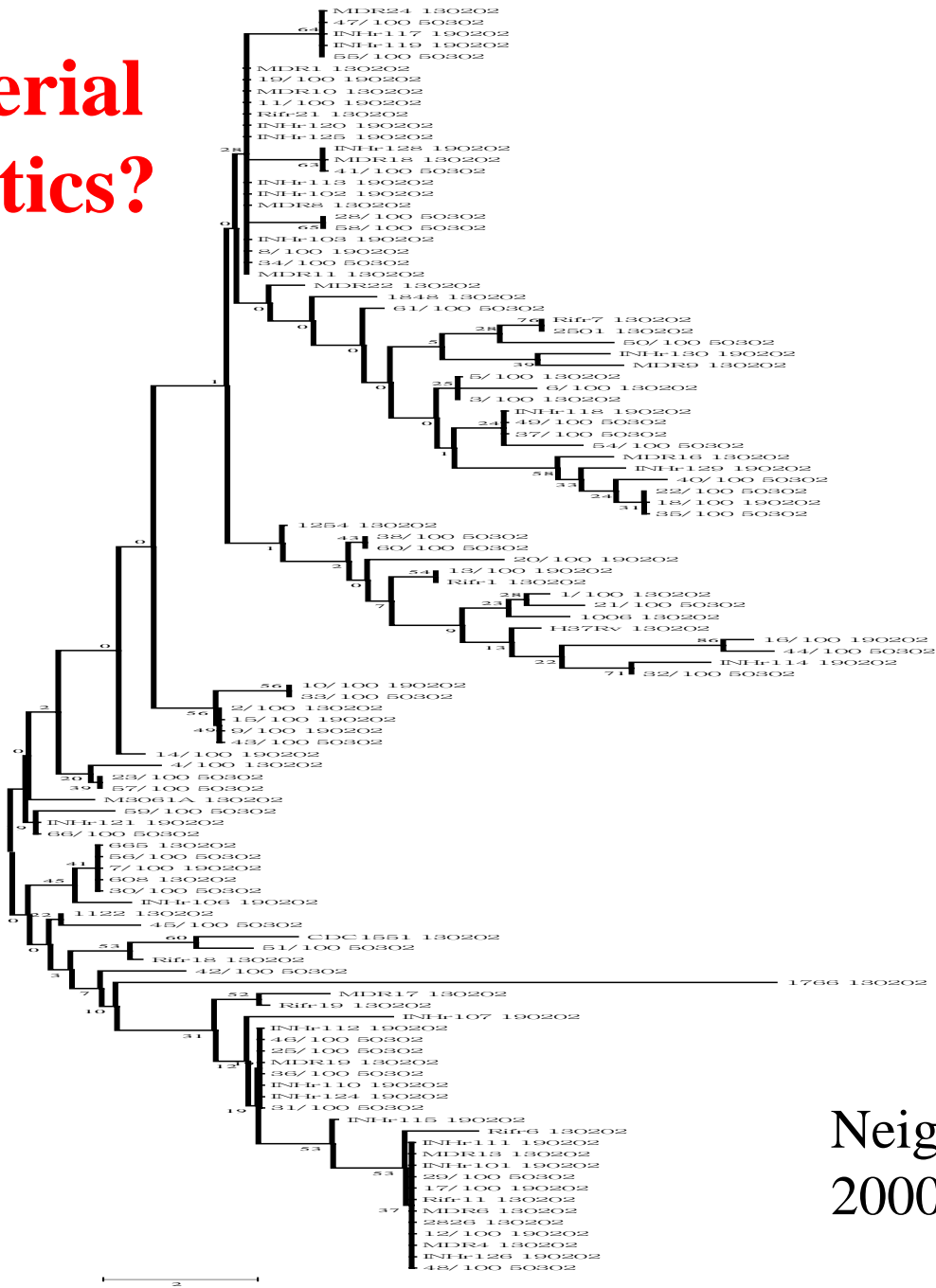


Results:

- Easy to record
- Easy to analyze

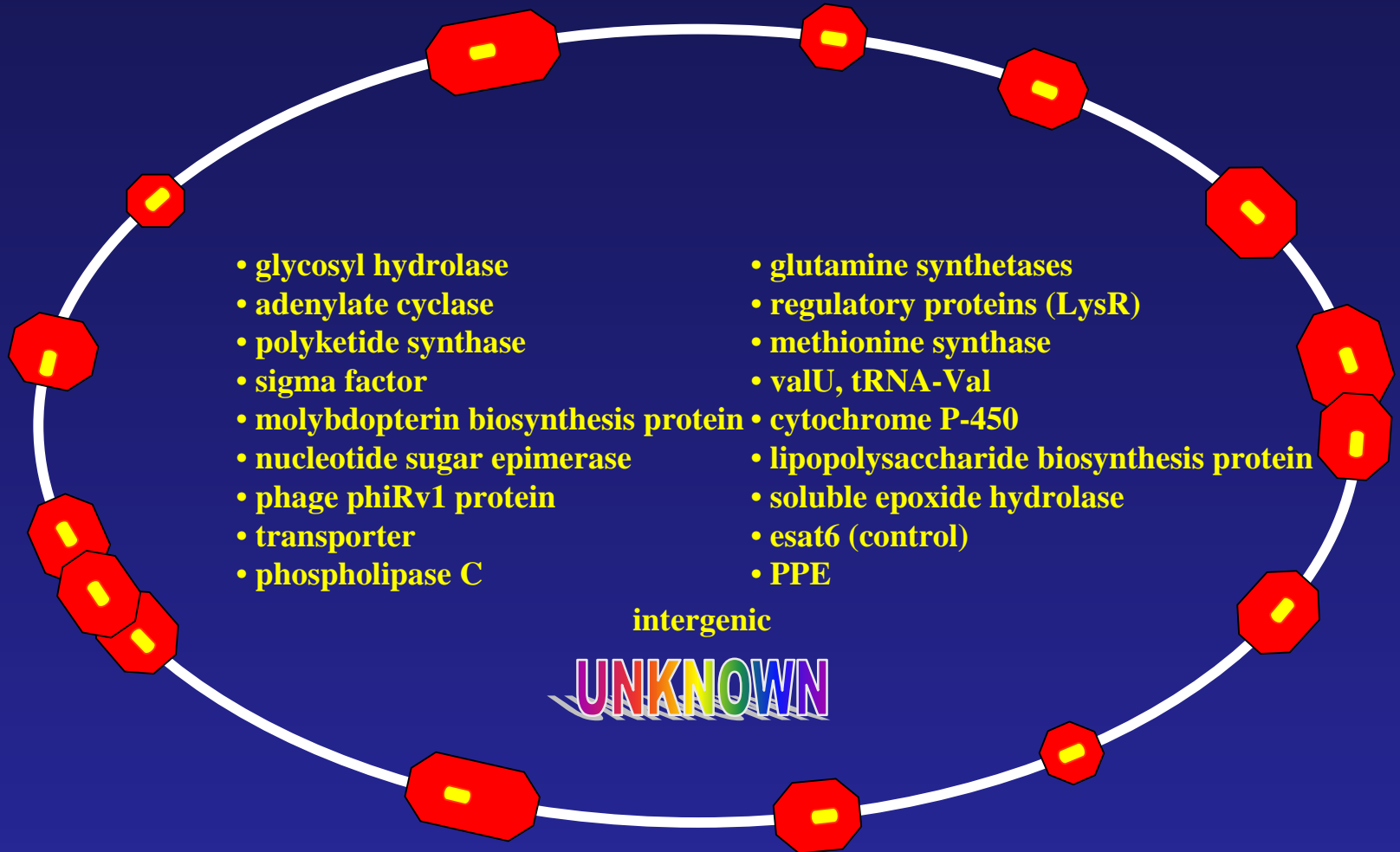


Mycobacterial Phylogenetics?



Neighbor-Joining
2000 bootstrap tests

Functional Genomics?



Functional genomics

Bigarray allow to characterize strains by ORFs absence

Hypothesis:

Try to correlate stains with special behavior to defined deletions

Probable importance of degenerative evolution

- described for different intracellular bacteria (*Rickettsia* ...)
- suspected for mycobacteria considering the comparative analysis of the *M. tb* and *M. leprae* genome

Mycobacteria from the tuberculosis complex

- are 99.9% at the nucleotide level
- have identical 16S rRNA sequences

BUT have different tropism, phenotype and pathogenicity

SUMMARY

Membrane based MTB deletion detection

The technique is simple, inexpensive, rapid to perform and analyze ... and robust.

Different informations:

Differentiates between strains

Correlates an ORF deletion with a phenotype

Yields “reliable phylogenetic” information

Could be modified for other species

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