Molecular surveillance based on mass spectrometry

Lilia M. Ganova-Raeva, PhD

Molecular Epidemiology and Bioinformatics Team

OVERVIEW

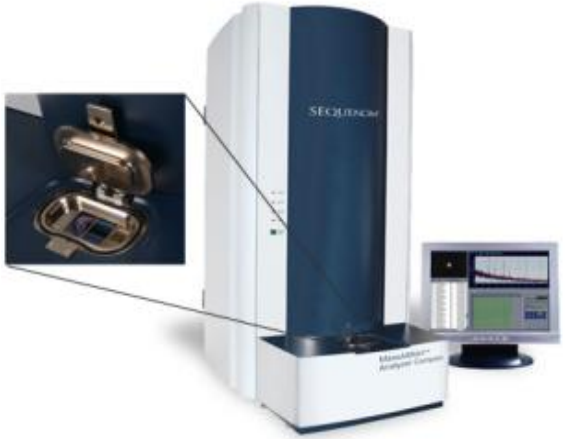
- Mass spectrometry
- Detection of viral transmission
  - Surveillance
  - HCV transmission
  - Disease chronicity
  - Improve prevention practices
  - Assess variability
- Virus genotyping
  - Surveillance
  - Disease severity and HCC development
  - Antiviral therapy
  - Disease chronicity
  - HBV transmission
  - Liver transplantation

MALDI-TOF MS

The MassARRAY® System – a laboratory in one platform

Only two biochemistries cover multiple qualitative and quantitative applications

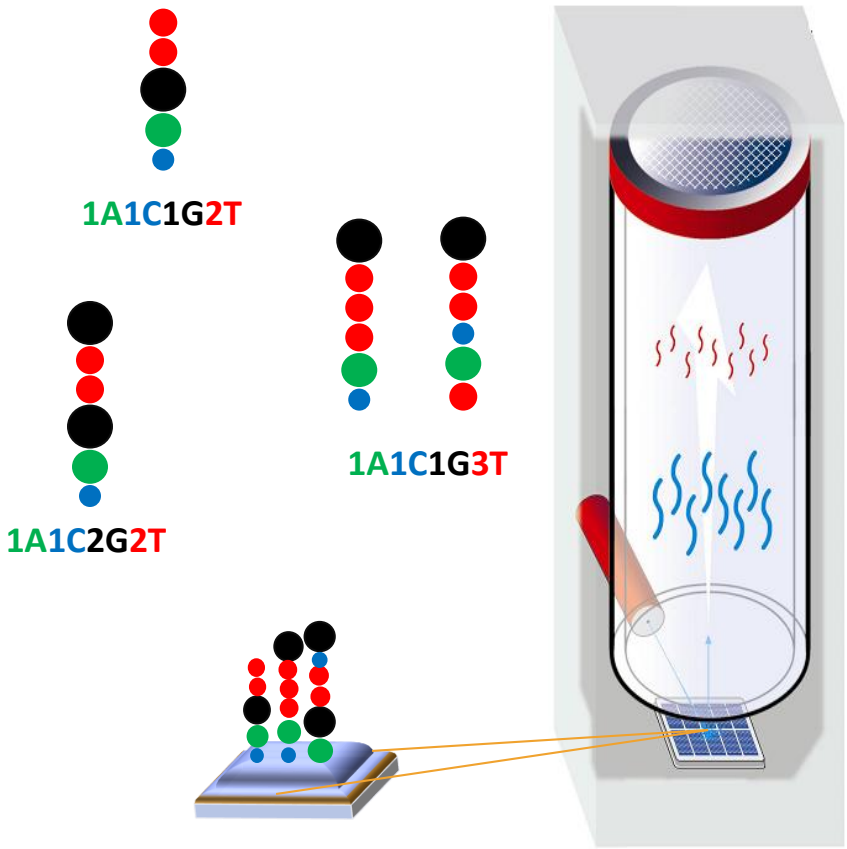
- SNP Genotyping:
- Automated assay design for high level multiplexing
- Detection, Identification
- Fine-mapping
- Quantitative Applications
- Comparative Sequence Analysis
- Molecular Typing
- Methylation Analysis



Great feasibility for public health applications

MALDI-TOF MS

Direct measurement of an intrinsic property of the NA molecule



DNA nucleotides alphabet:

A, C, G, T

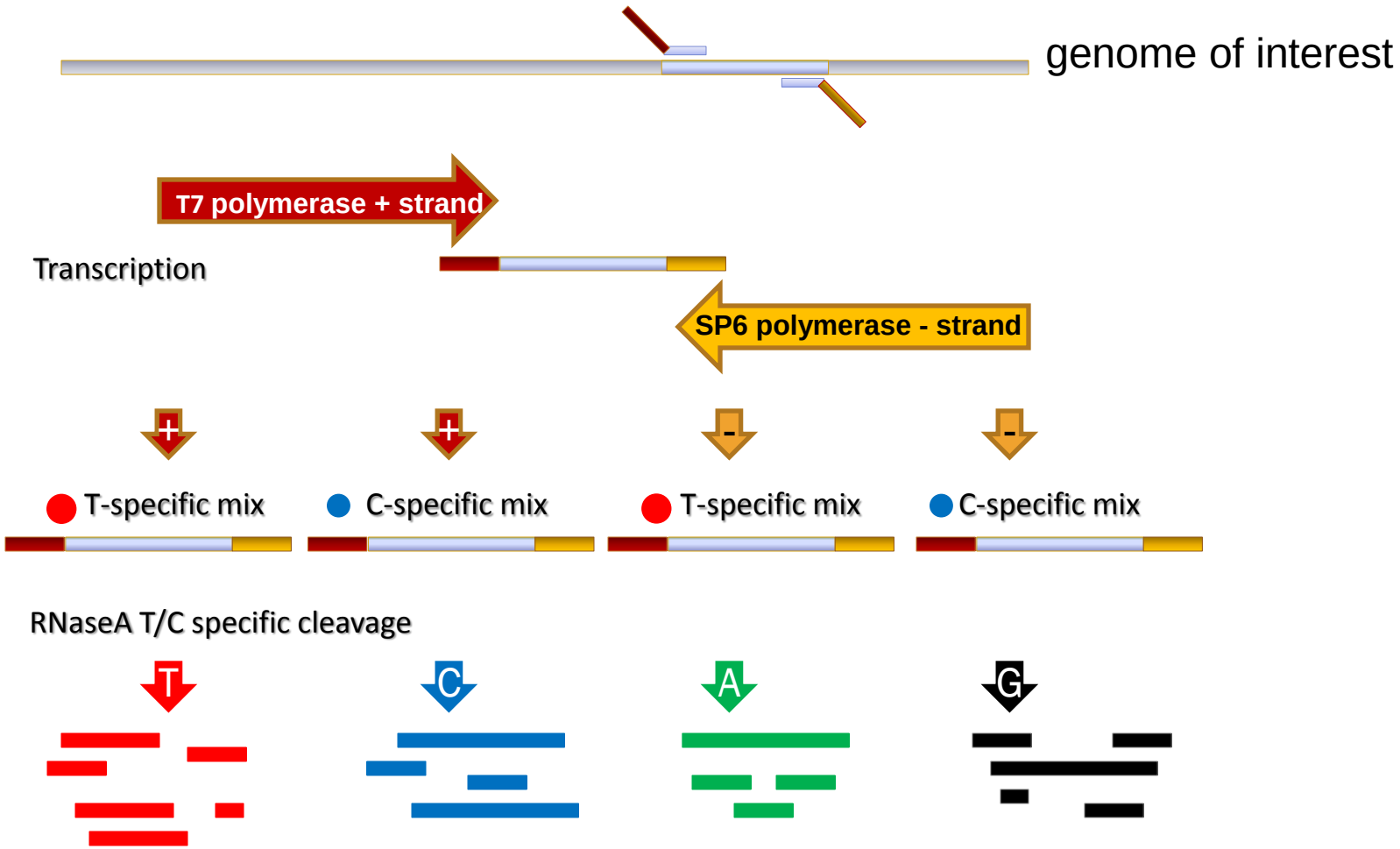
Each base has defined molecular mass:

A = 313.2 Da
C = 289.2 Da
G = 329.2 Da
T = 304.2 Da

Jurinke et al, **2002**, Advances in Biochemical Engineering

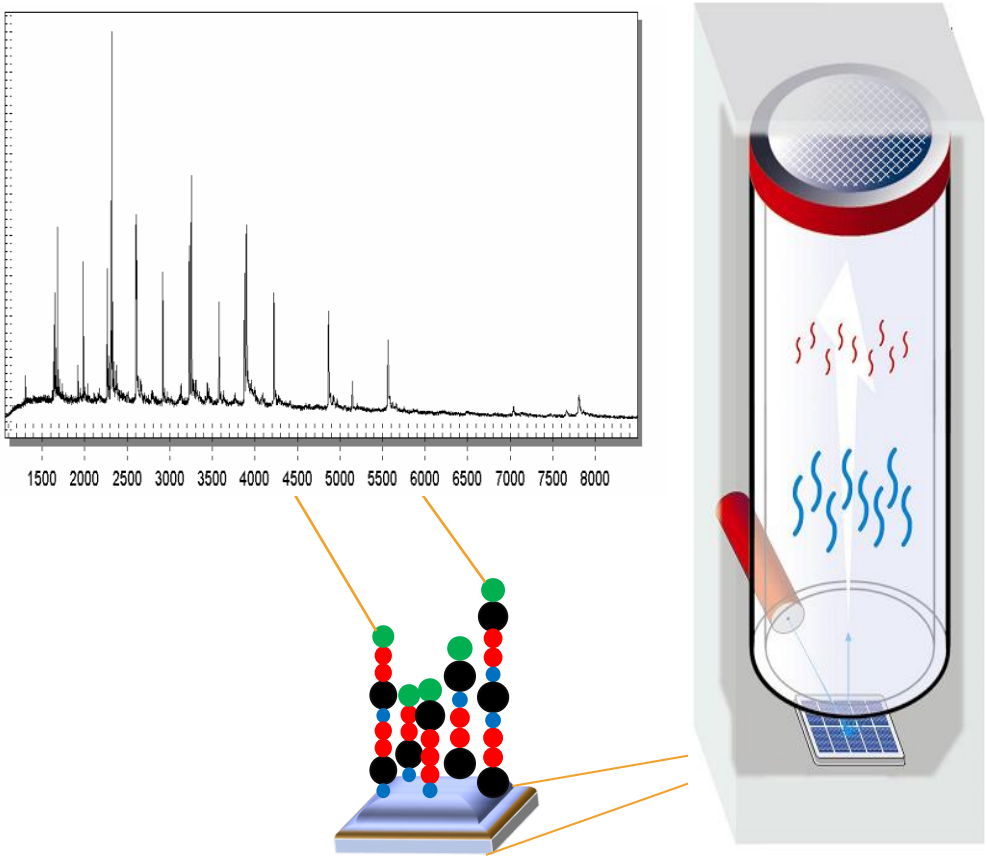
MASSARRAY™

Transcription Coupled T/C Specific RNaseA Cleavage



MALDI-TOF MS

Direct measurement of an intrinsic property of the NA molecule



Each base has defined molecular mass:

- A = 313.2 Da
- C = 289.2 Da
- G = 329.2 Da
- T = 304.2 Da

CGTTCGTTA

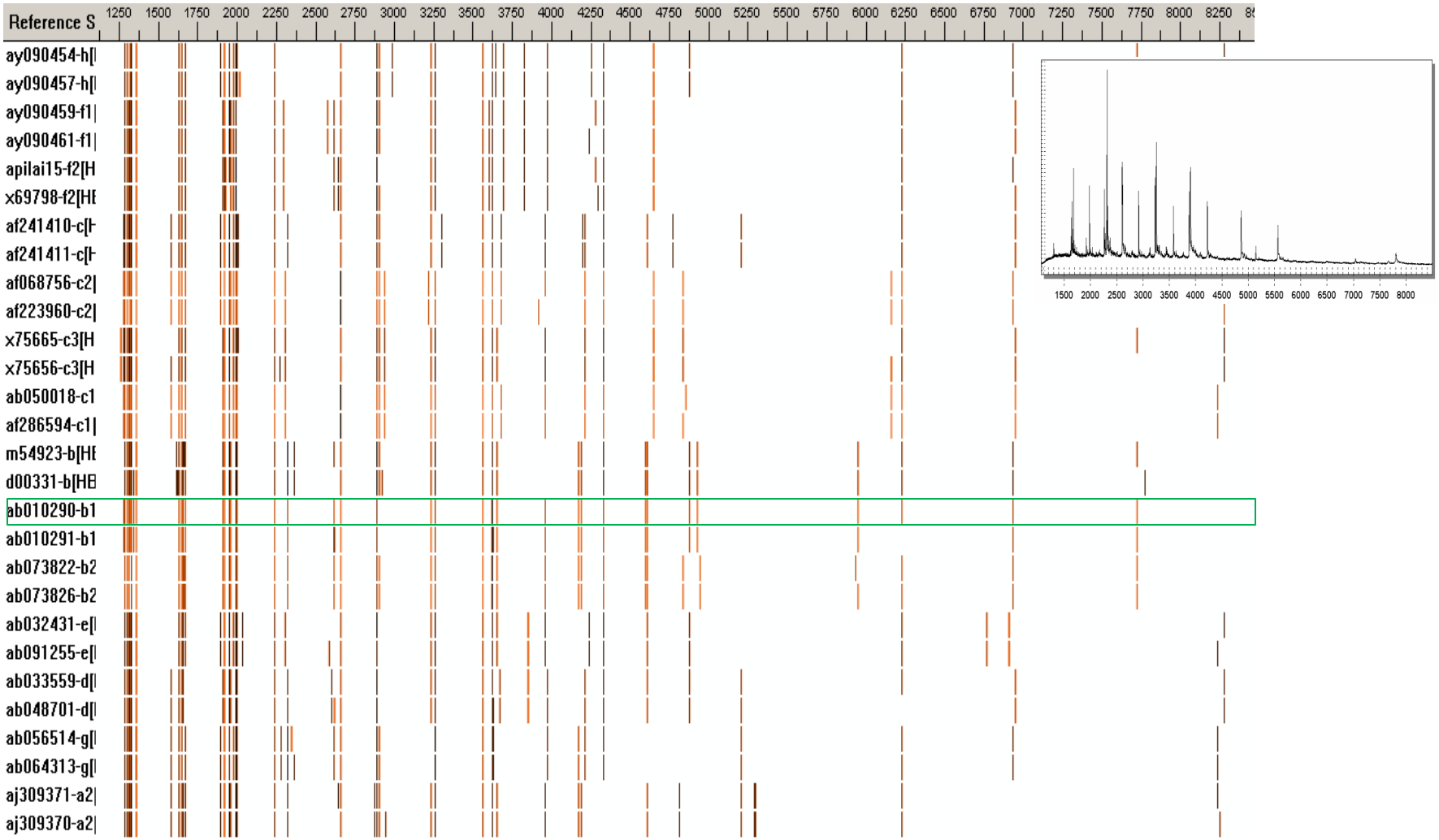
2C+4T+2G+1A=2816.8Da

GTTCGCTTGA

2C+4T+3G+1A=3121.0Da

ANALYSIS BY MASSARRAY™

Automatic *in silico* Pattern Matching Algorithms



DETECTION OF HCV TRANSMISSION

CURRENT MOLECULAR APPROACHES TO OUTBREAK INVESTIGATION

- **HCV genome organization**

- **Transmission event is identified by common genetic characteristics**
  - Genotyping (5'UTR, NS5B)
  - Consensus sequencing (NS5B, HVR1, NS5A)
  - EPLD followed by sequencing for quasispecies analysis

- **Phylogenetic analysis – current gold standard**

PROTOCOL INTEGRATION AND AUTOMATION

Extraction

Automation

Amplification

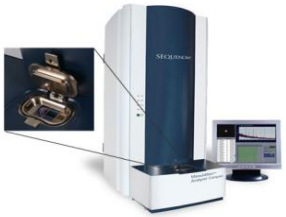
quasispecies separated by EPLD

Involved
Costly
Analysis
days

3130*xl* Genetic analyzer

384samples/32hr

quasispecies mixed



Simplified
Inexpensive
Analysis
hours

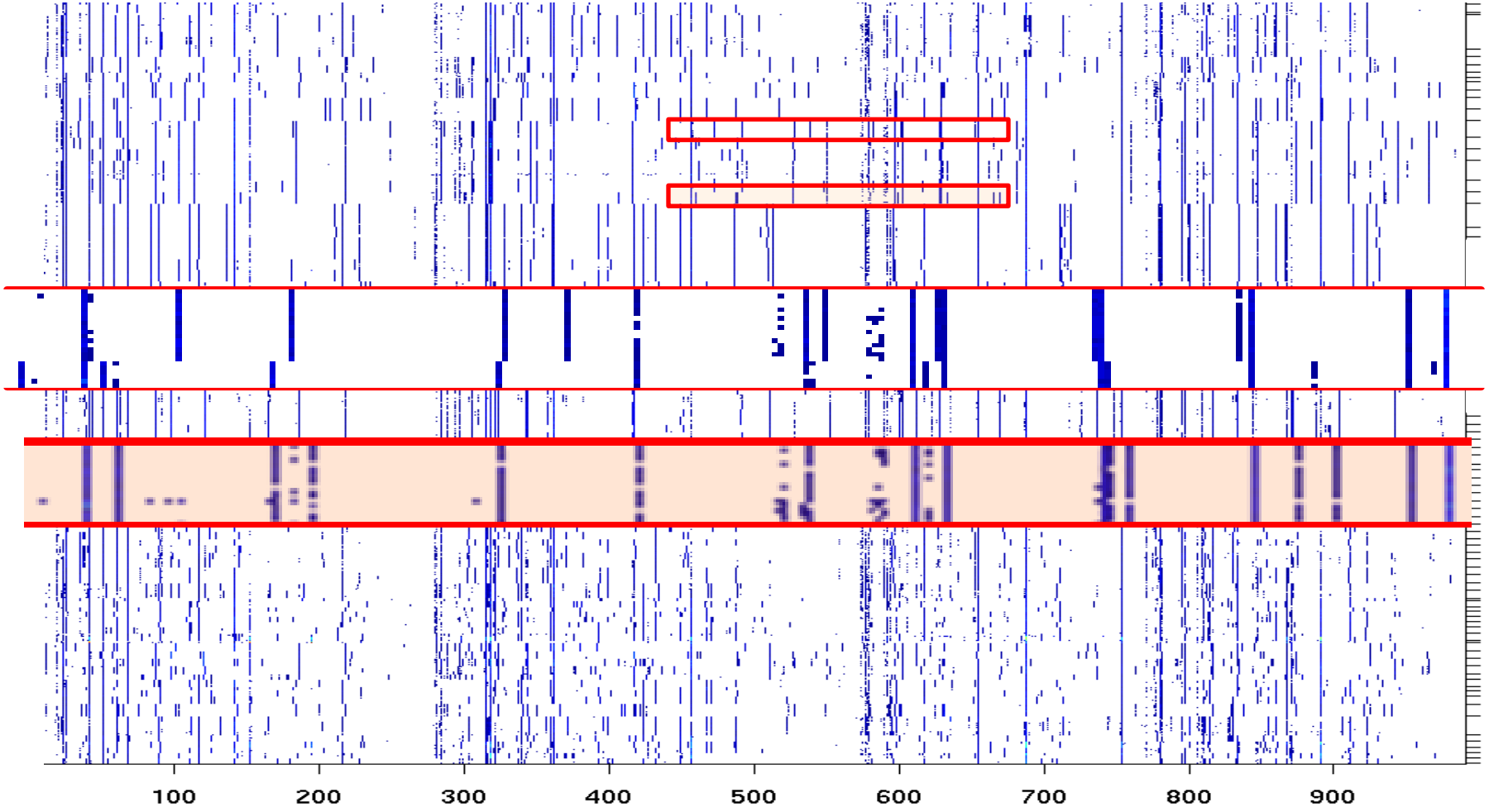
MALDI-ToF (MassArray)

384samples/4hr

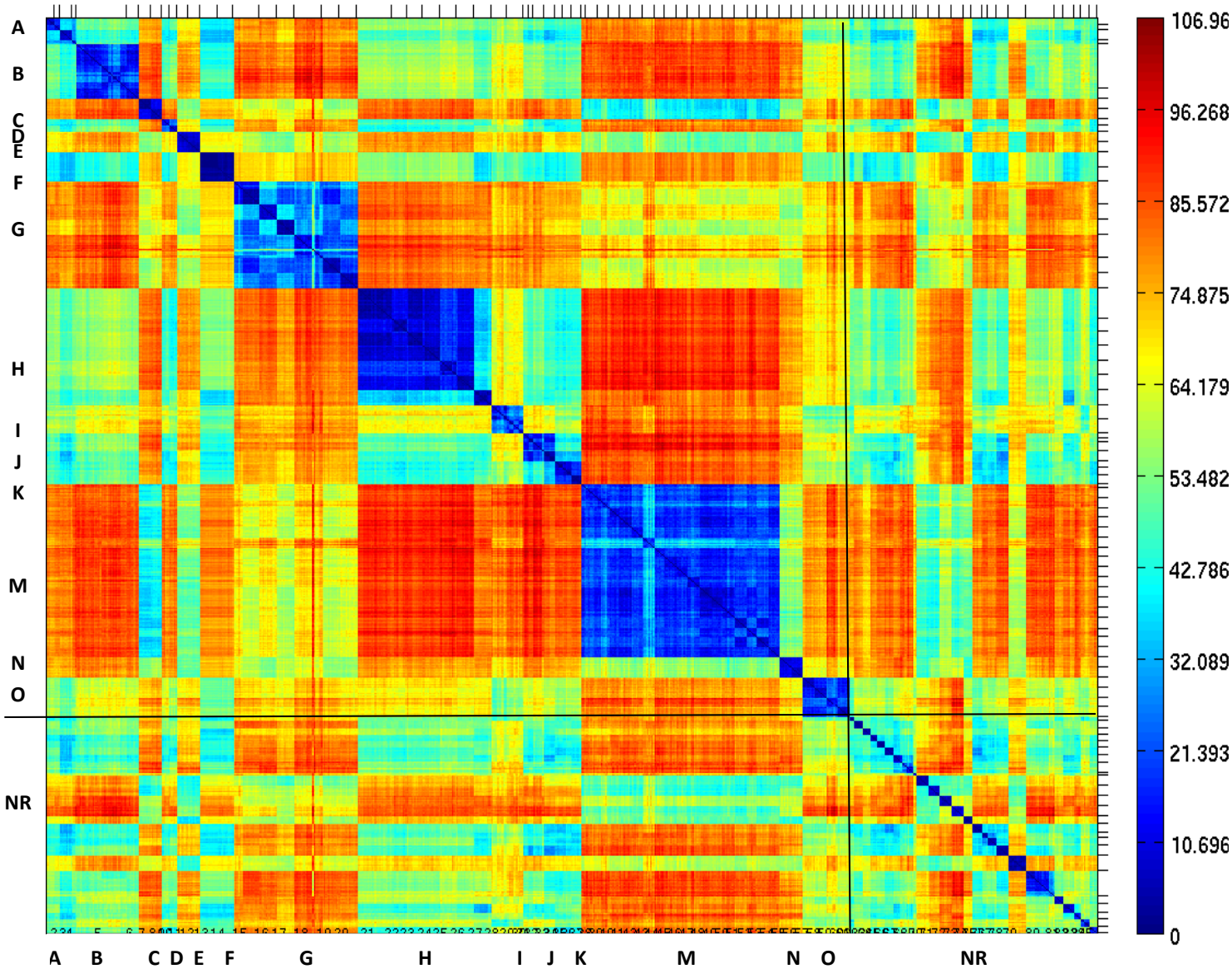
QUASISPECIES MASS FINGERPRINTS DATA

($N_{patients}$ = 85; $N_{samples}$ = 824)

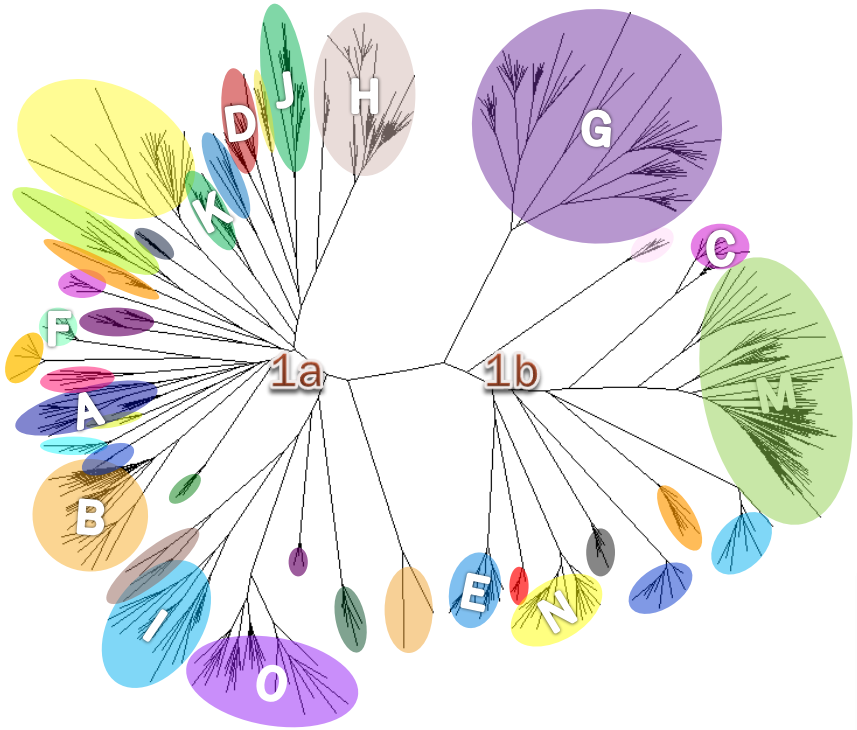
- Sera from 22 epidemiologically unrelated HCV cases and from 63 patients involved in 14 different outbreaks
- All previously confirmed by HVR1 quasispecies sequence analysis (n=50clones/case)



HEATMAP ($N_{PATIENTS}$= 85; $N_{SAMPLES}$= 824)



NEIGHBOR JOINING TREES (COMPARISON)

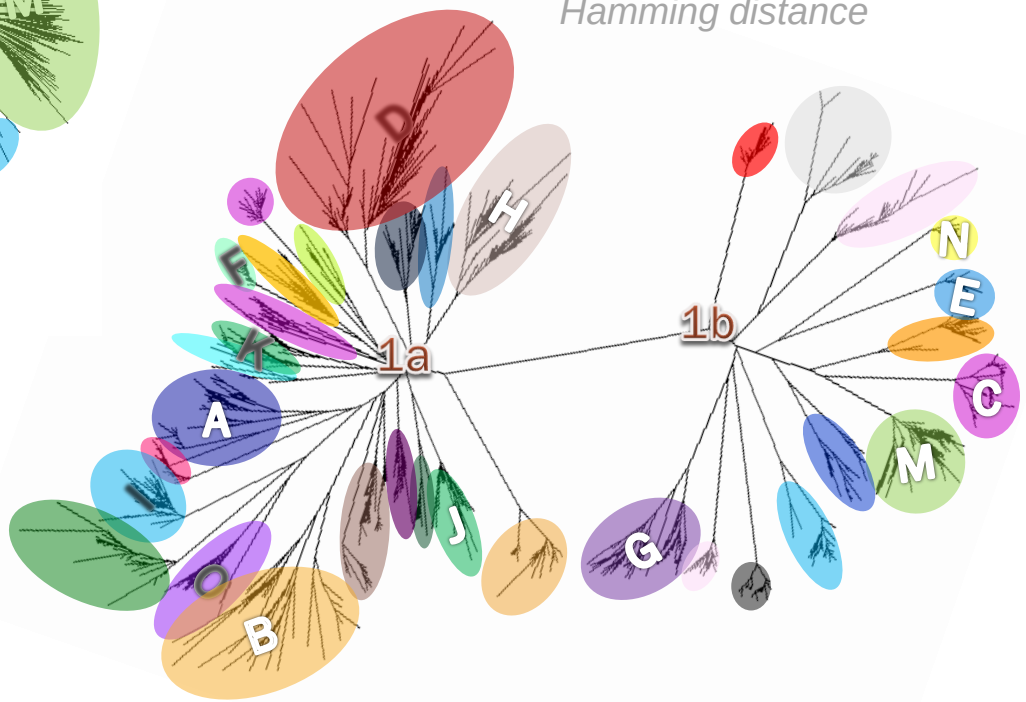


Mass NJ tree

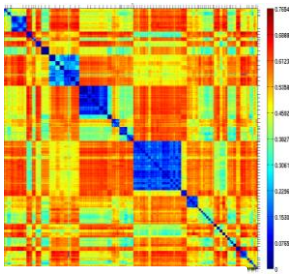
Entropy weighted Hamming distance

Phylogenetic NJ tree

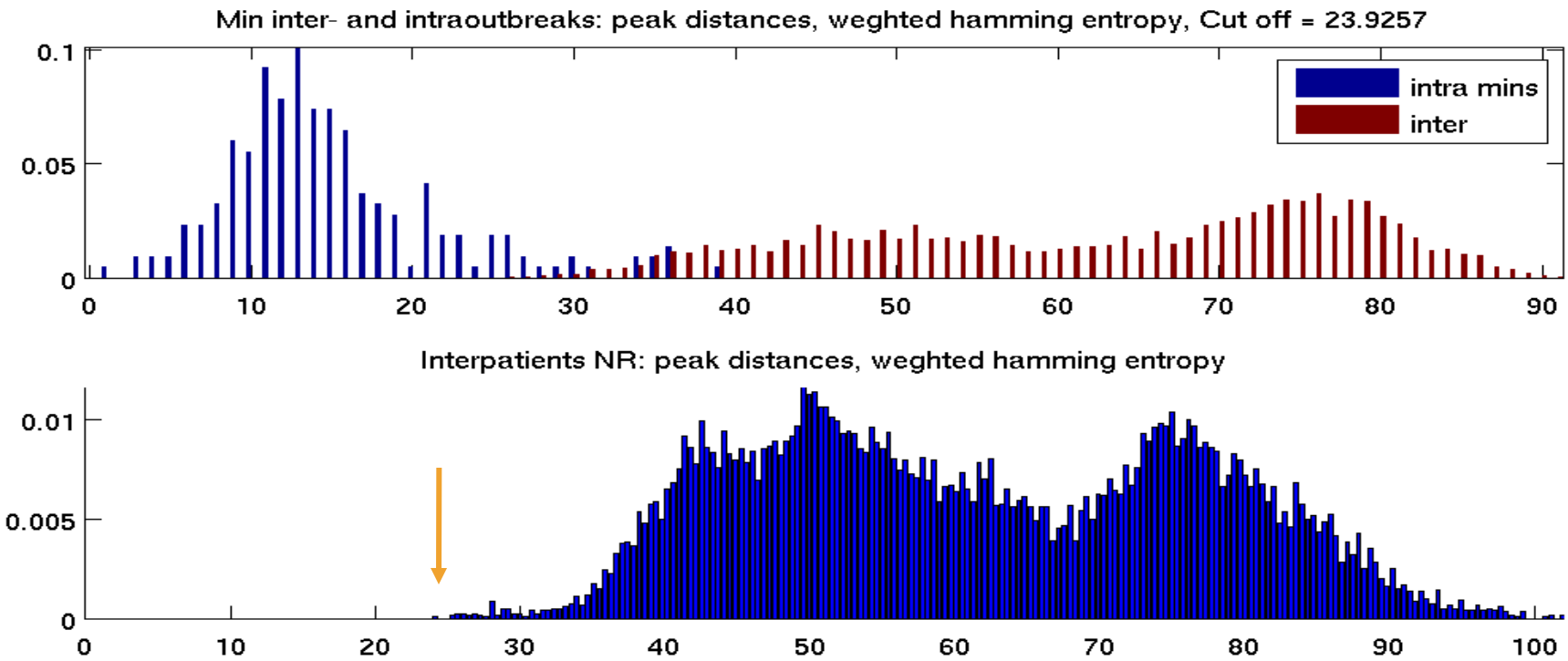
Hamming distance



DISTANCES BETWEEN UNRELATED CASES

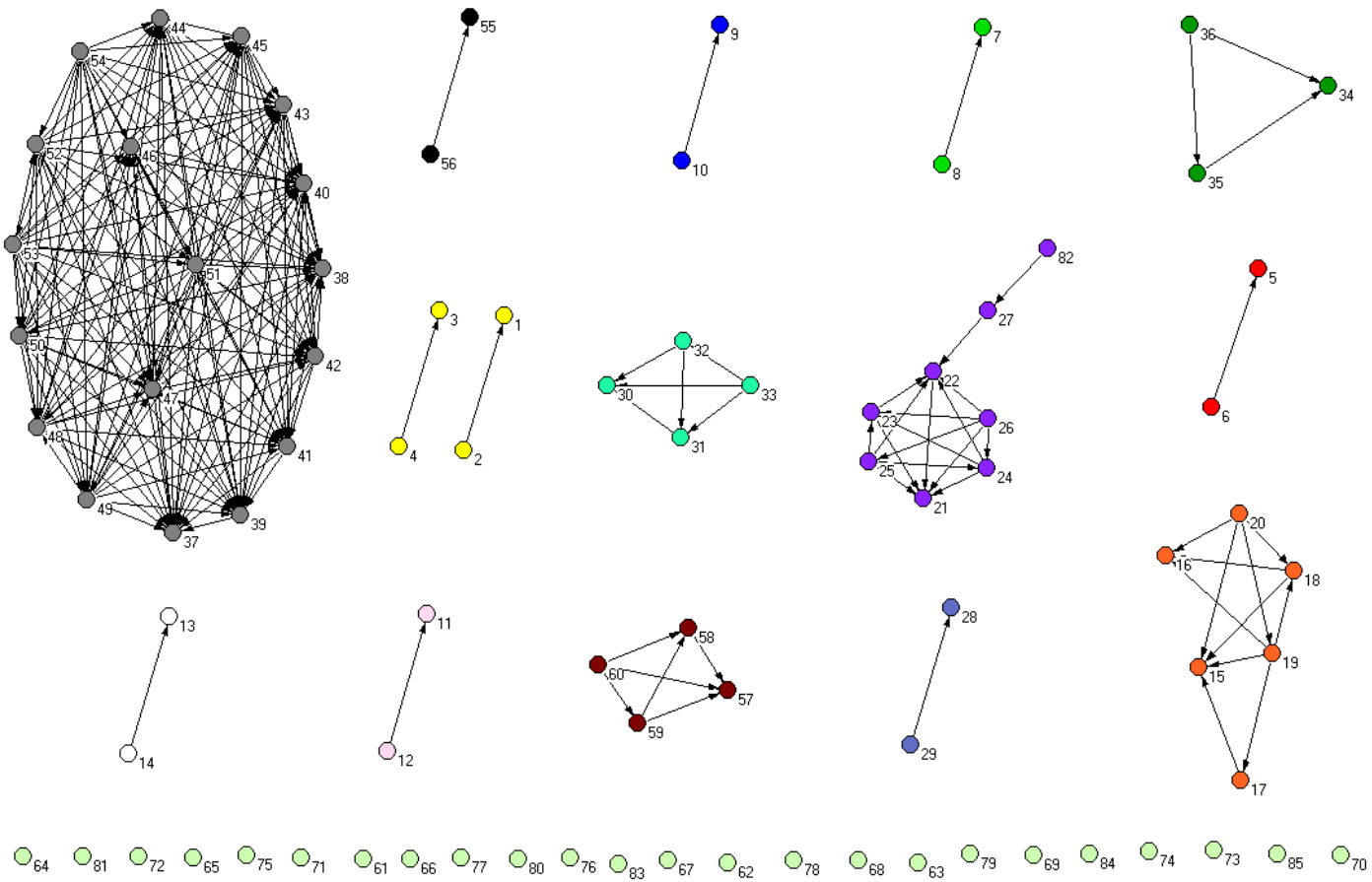


All distances between epidemiologically non-related cases
Minimum = 23.9659 = cutoff



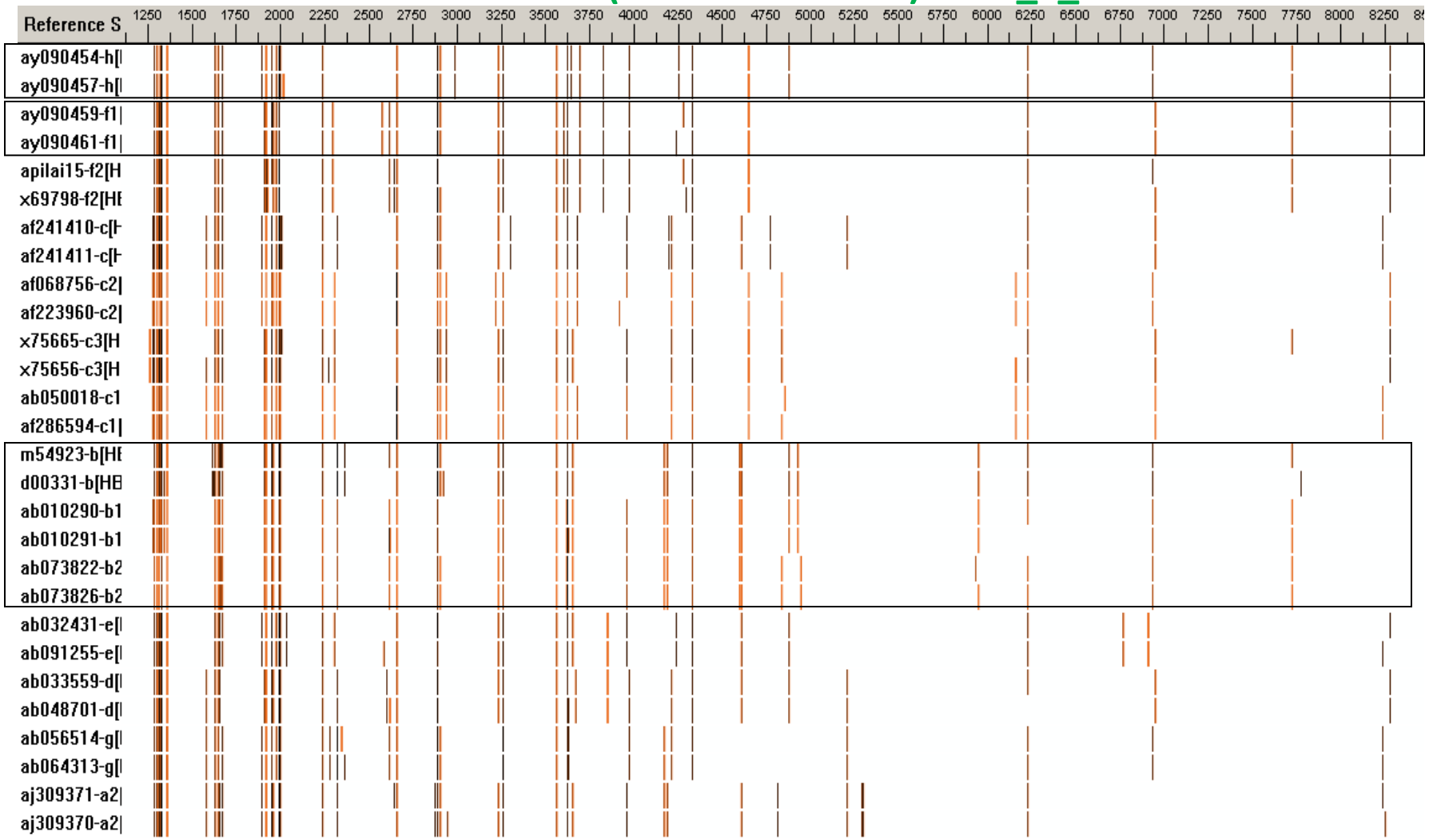
LINKAGE NETWORK

Streamlined Analysis for Public Health Application



GENOTYPING BY MASSARRAY™

MASS SPECTRUM FINGERPRINTS (T-reaction alone) HBV_S_153DB



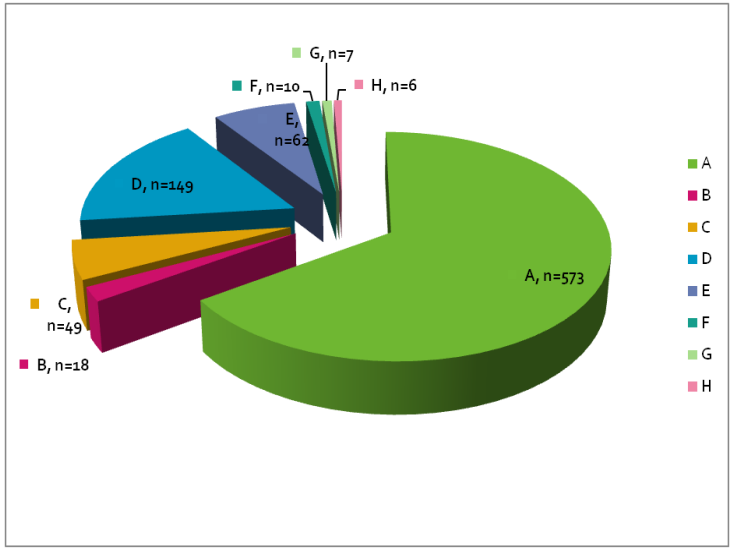
Reference set : 153 S gene 441 nt, including the a-determinant, sequences from GenBank representing all genotypes (genetic divergence 0.2-11.5)

HBV GENOTYPING BY MASSARRAY™

CONCORDANCE

Genotype	by Mass Fingerprint (ref DB HBV-S_153)	by Sequencing
A	573	573
B	18	18
C	49	49
D	149	149
E	62	62
F	10	10
G	7	7
H	6	6
Total	874	874

Genotype concordance = 100%



JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2010, p. 4161–4168 Vol. 48, No. 11

Robust Hepatitis B Virus Genotyping by Mass Spectrometry

L. Ganova-Raeva[1*], S. Ramachandran[1], C. Honisch[2], J. C. Forbi[1], X. Zhai[3] , and Y. Khudyakov[1]
[1]Division of Viral Hepatitis, Centers for Disease Control and Prevention, Atlanta, Georgia; [2]Sequenom, Inc., San Diego, California; and [3]Jiangsu Centers for Disease Control, Jiangsu, China

CONCLUSIONS

- Detection of HCV transmission by quasispecies mass fingerprints analysis
- Genotyping results are completely concordant with sequencing methods
- Significantly reduced the analysis time (10-fold)
- Significantly lowers the testing cost (12-fold)
- Allows for 10-fold increase of the throughput
- The approach is amenable to streamlined automated analysis and reporting
- The outcome is as accurate as the gold standard of sequence phylogeny

Thank you

Zoya Dimitrova

David Campo Randon

Sumathi Ramachandran

Yulin Lin

Guo-liang Xia

Joseph Forbi

Yury Khudyakov

Molecular Epidemiology and Bioinformatics Team
DVH, NCHHSTP, CDC, Atlanta, GA

Christiane Honisch

Sequenom, Inc., San Diego, CA