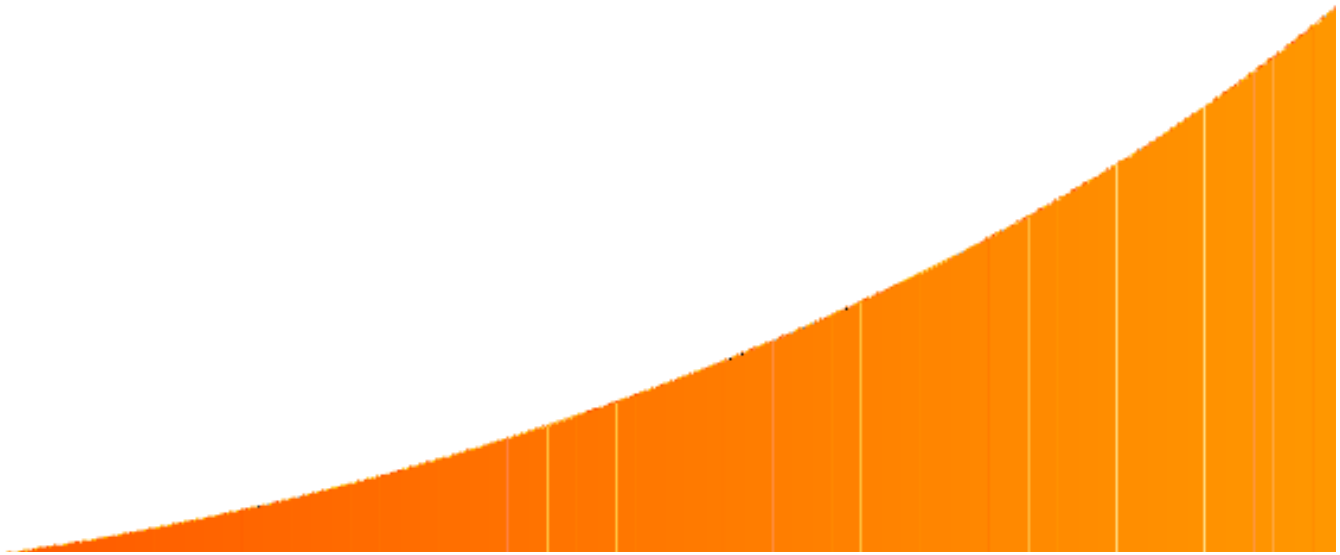
Future Perspectives in Public Health Microbiology



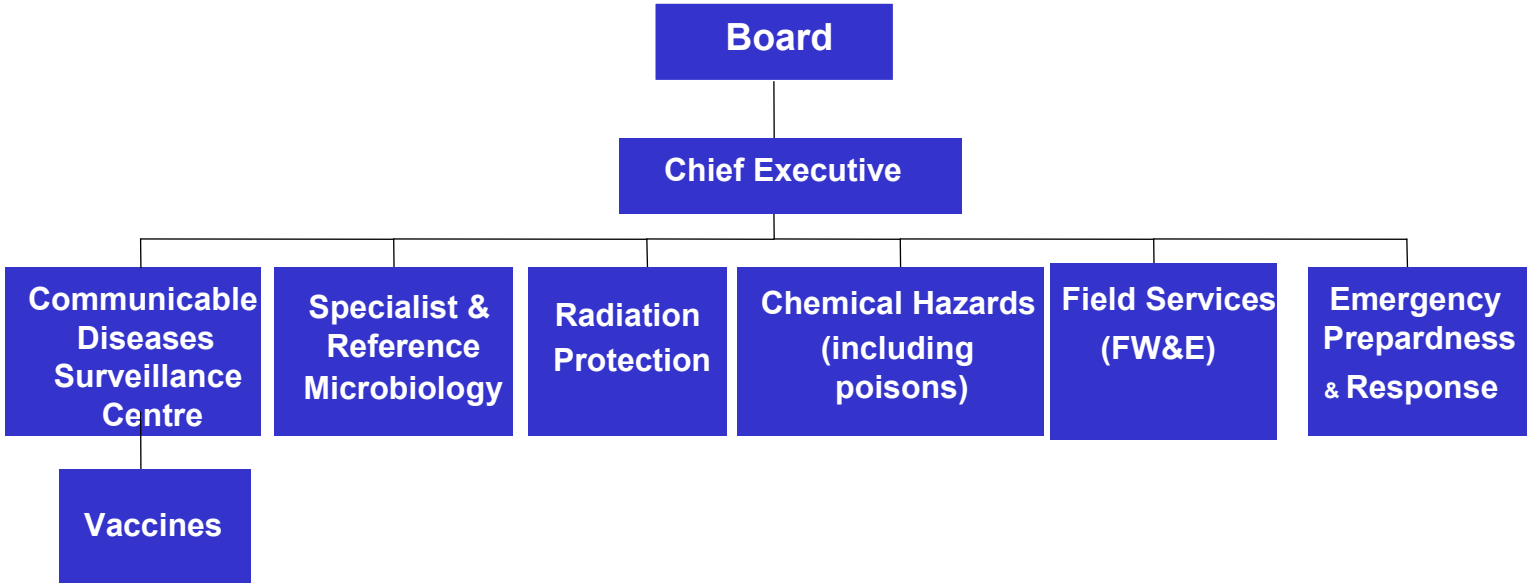
PHLS

31/3/03

Public Health Laboratory Service

- Reference
- Surveillance
- Diagnostics
- Food Water and Environmental

The New Health Protection Agency

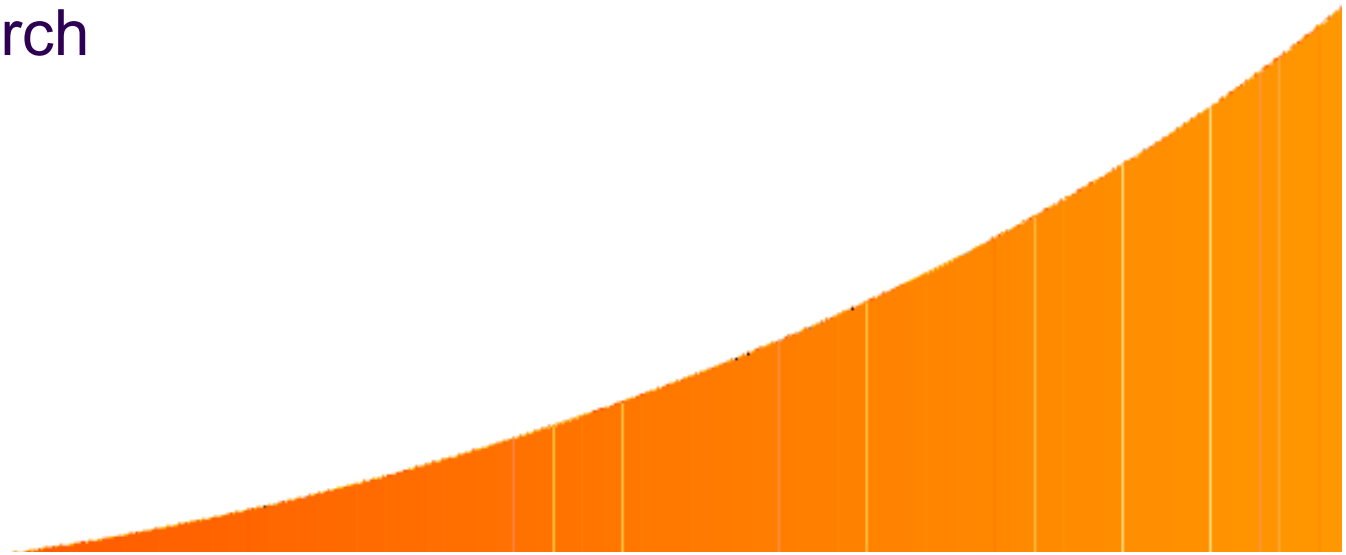


Key functions of the Agency

- Microbial epidemiology for diseases control & prevention
- Environmental Health and Protection
- Emergency Preparedness
- Reference and Specialist testing
- Expert advice

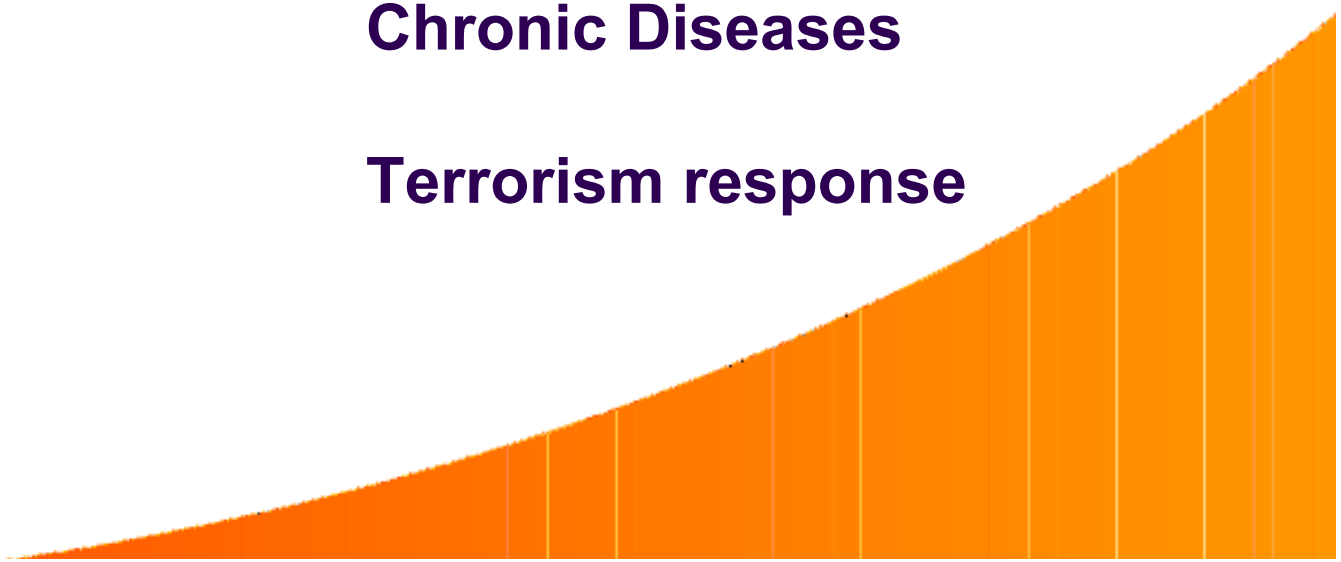
The Specialist and Reference Microbiology Division

- Processes over 200,000 specialist reference specimens per year
- Publishes over 150 papers per year
- Attracts over £2M pa in external grants to support research



CMO KEY PRIORITIES

- New and Emerging
- TB
- HCAI
- A^R
- Hepatitis
- Childhood Infections
- STI
- New Vaccines
- Chronic Diseases
- Terrorism response



The Genomics, Proteomics and Bioinformatics Unit, SRMD HPA

- To utilise accelerated genomic information and enhanced biotechnological methods to streamline the traditional approach used in classical molecular genetics
- Develop systems for identifying genetic variations and gene expression
- Explore proteome profiling as a pathogen discovery platform through motif matching, transcriptional output and antigen capture reactions

Computational Genomics

- Adopt appropriate biological annotation and information structure
- Build relationships on the basis of biological knowledge and expand these with microarray and proteomic data
- Establish data presentation, interrogation and analysis platforms of integrated systems

Developmental Objectives

- Reformat sequence-based typing into fast, high throughput systems
- Explore microbial genome sequences for VNTRs and SNP targets as tools for high resolution strain comparison
- Provide tools for molecular-based surveillance of high priority infections

- What is molecular typing?
- Why is it done?
- Technologies/techniques currently used/near future
- Examples (HIV-1, *Mycobacterium tuberculosis*, MLST,
- The future

Why detect polymorphisms?

- Identification
- Taxonomy
- Typing
- Molecular epidemiology
- Antibiotic resistance determination
- Toxicity/Pathogenicity determination

Molecular Typing

- Reproducibility
- Ability to discriminate between unrelated isolates
- Capability to be applied to all isolates

Types of polymorphisms

- Insertions
- Deletions
- SNPs (single nucleotide polymorphisms, or point mutations)
- Microsatellites – highly informative markers found in the genomes of most species consisting of tandem repeats of one to ten nucleotides
  - SSRs (short sequence repeats)
  - STRs (short tandem repeats)
  - VNTRs (variable number of tandem repeats)

Methods for polymorphism detection

Can be divided into two groups:

(1) Scanning methods that can discover previously unknown differences

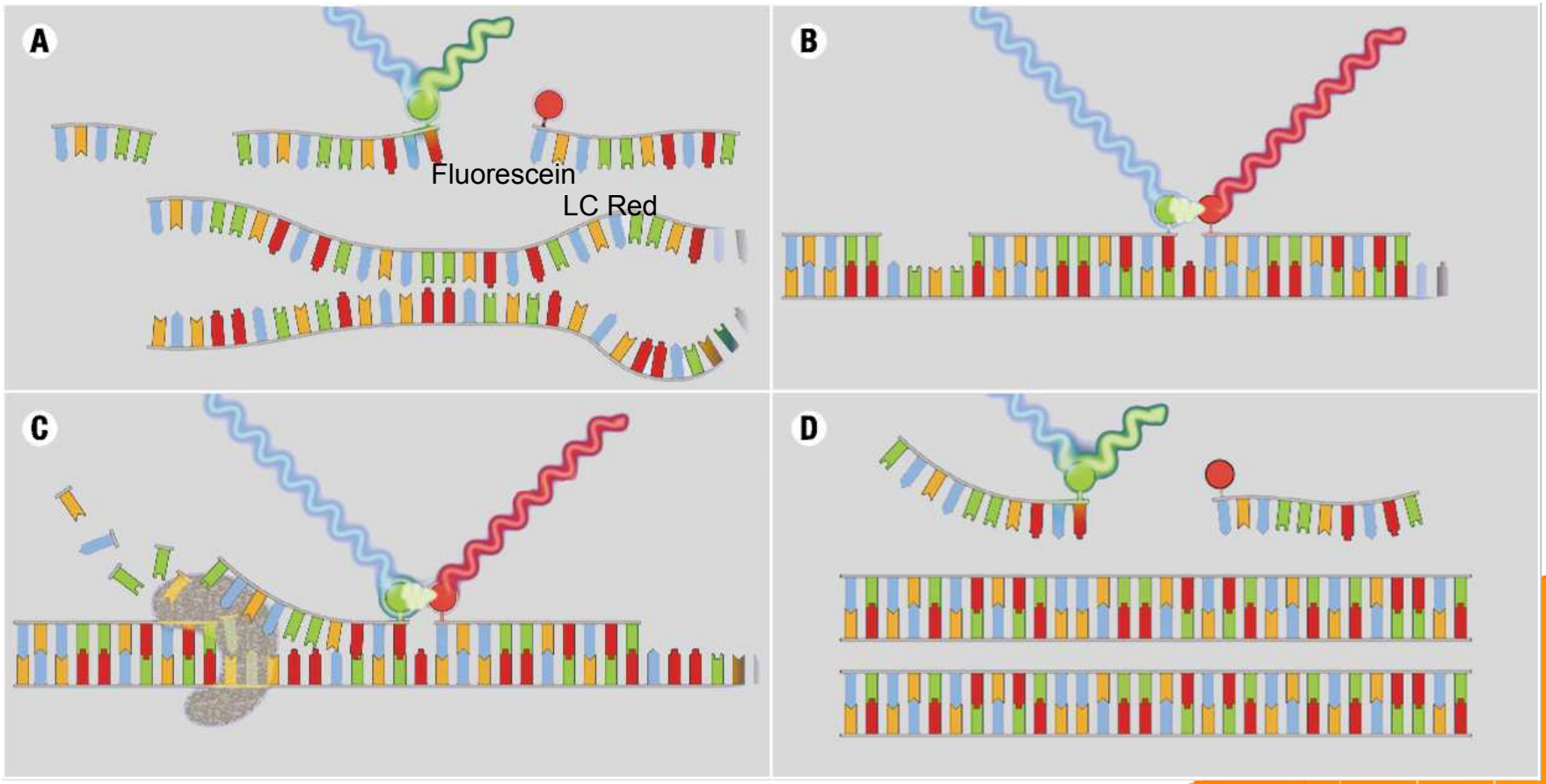
(2) Diagnostic methods designed to detect known polymorphisms

Examples of Technologies currently available

used in R&D, moving to routine

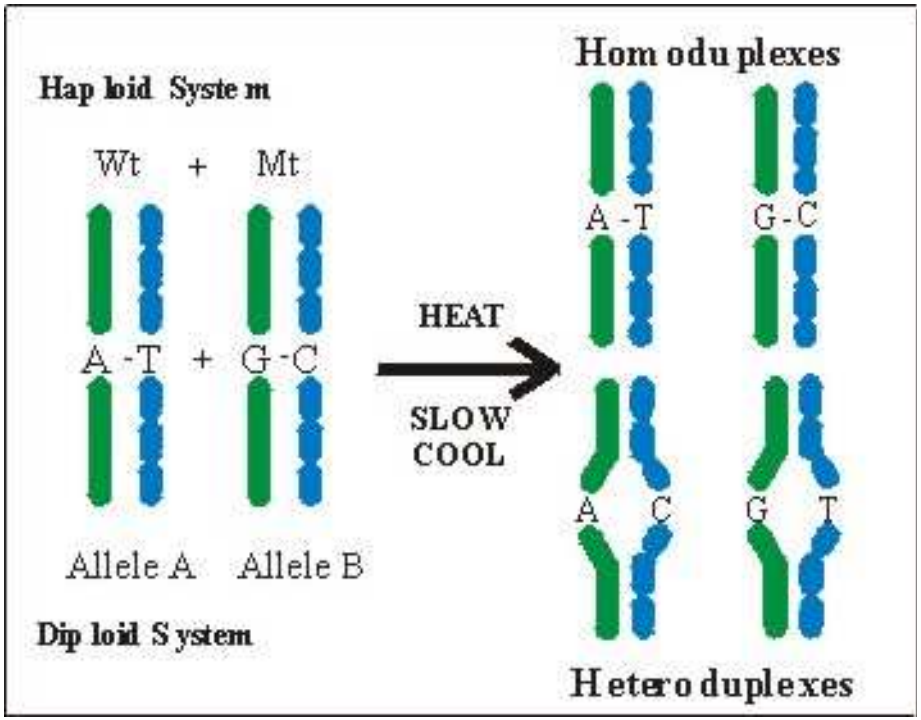
- 5’ nuclease assay (inc. MGBs)
- dHPLC
- capillary sequencing/ fragment analysis /SNP analysis
- pyrosequencing

LightCycler Hybridisation Probes



Picture courtesy of Roche Applied Science

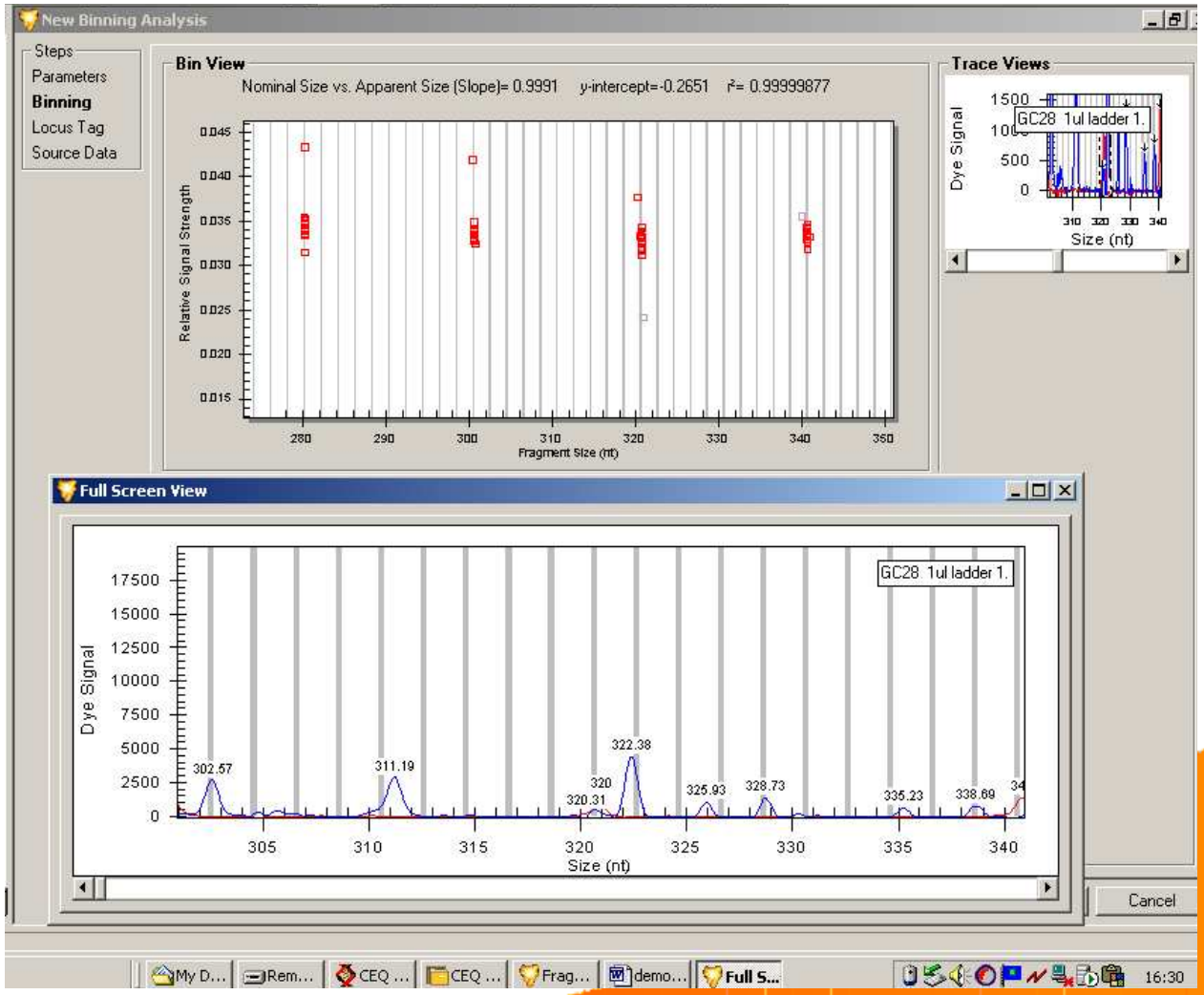
denaturing HPLC



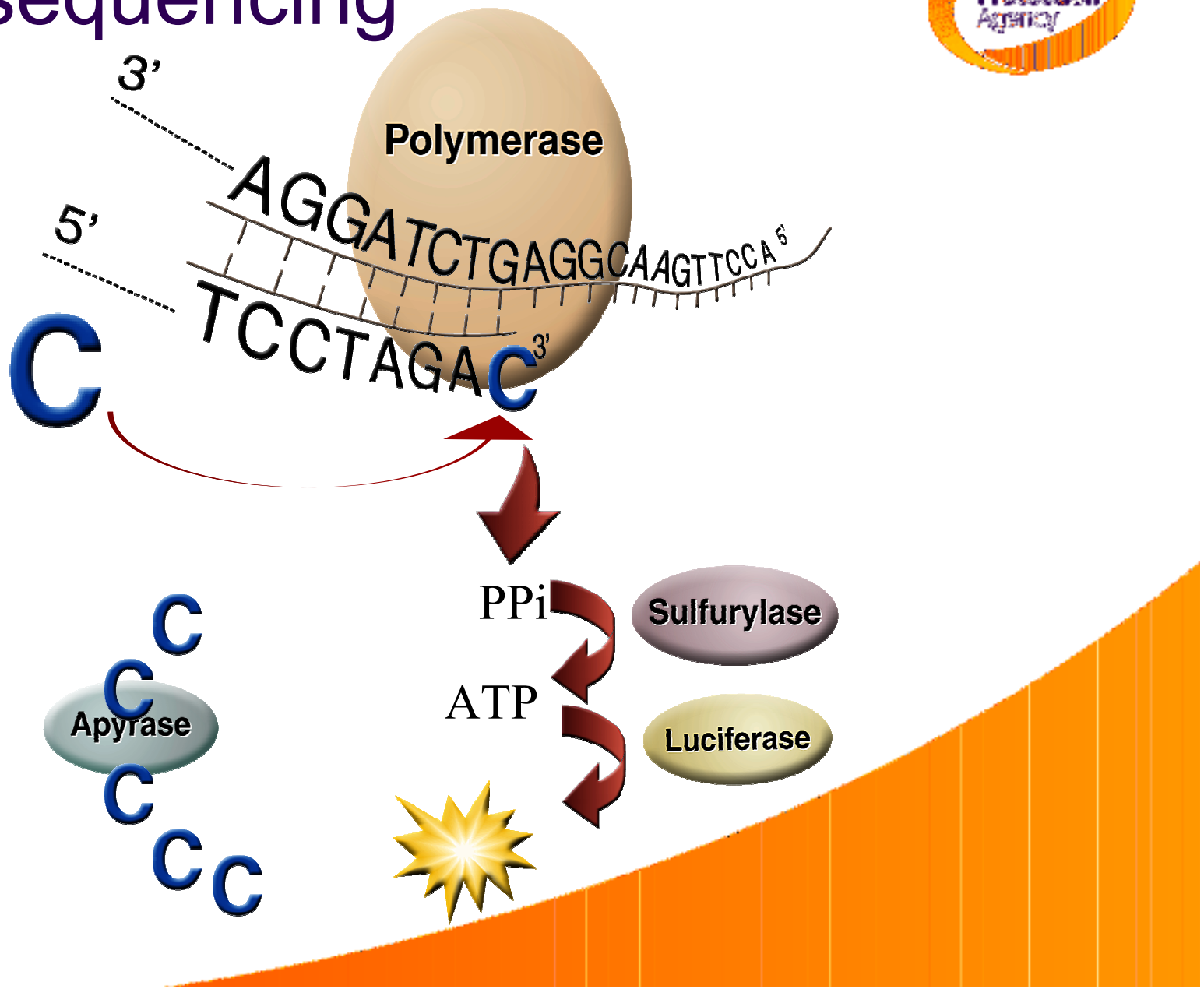
dHPLC identifies mutations and polymorphisms based on detection of heteroduplex formation between mismatched nucleotides in double stranded PCR amplified DNA.

When this mixed population is analyzed by HPLC under partially denaturing temperatures, the heteroduplexes elute from the column earlier than the homoduplexes because of their reduced Tm.

Capillary fragment analysis (inc. SNPs)

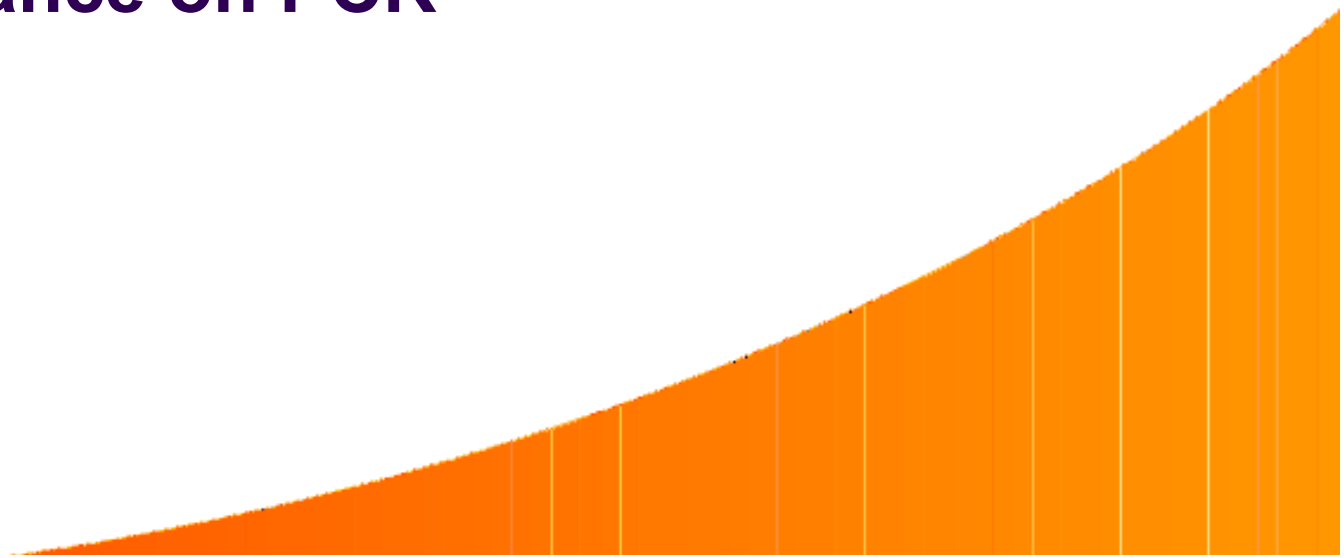


Pyrosequencing™

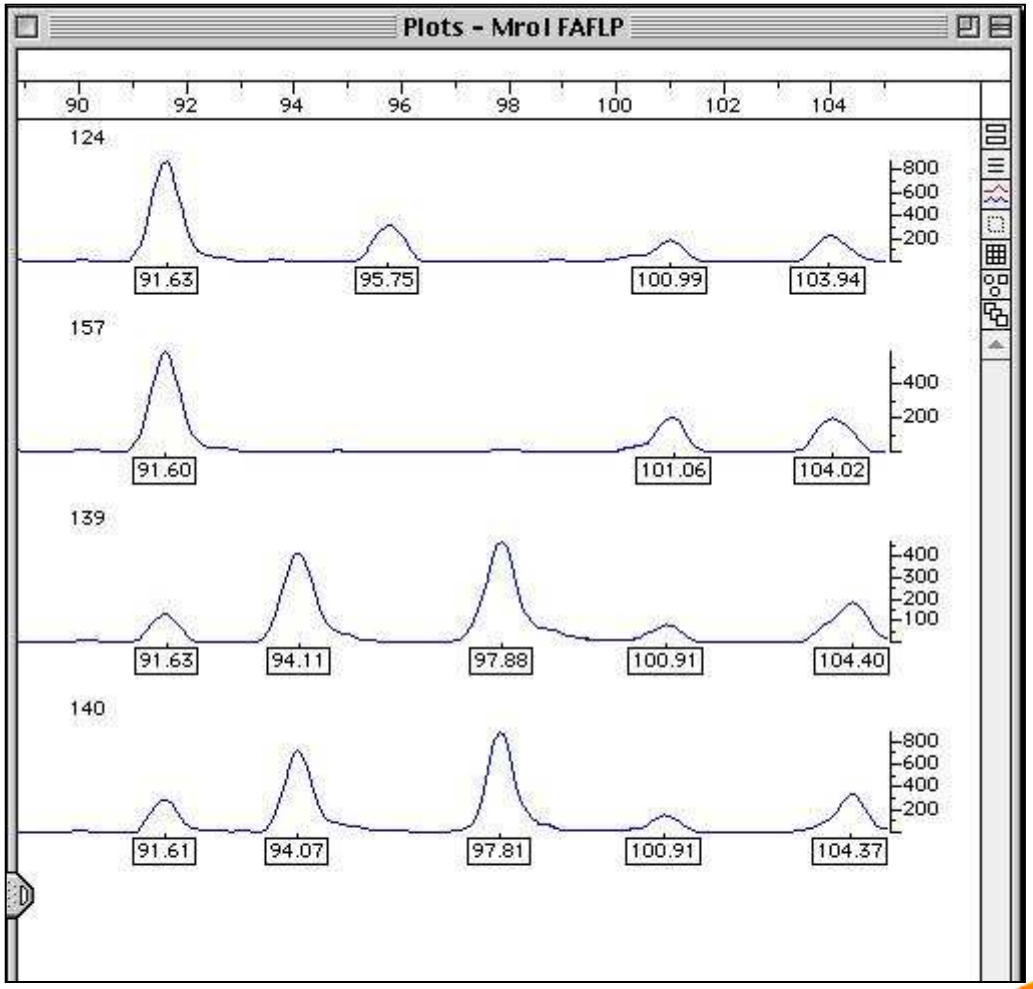


Limitations of Current Technologies

- Nucleic acid extraction
- Throughput?
- Reliance on PCR



*Mro*I FAFLP



- single-copy IS6110 isolates (124, 157)
- epidemiologically related isolates with multiple copy IS6110 (139, 140)

Multilocus Sequence Typing (MLST)

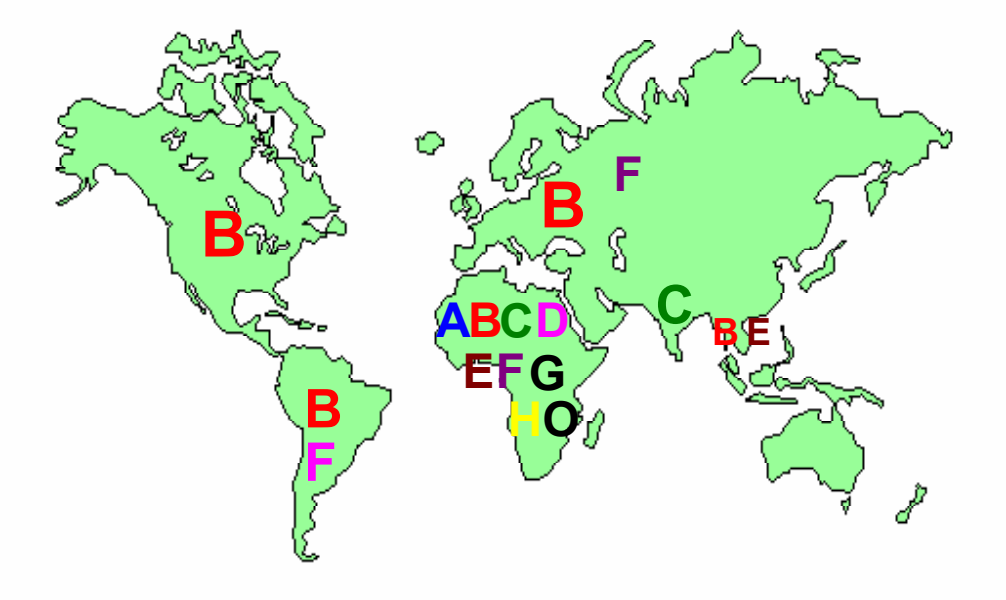
- Sequence both strands of 7 housekeeping gene fragments (450 bp) - *minute hand of molecular clock*
  - *SNPs*
- Hypervariable genes (e.g. antigen genes)

-*second hand of molecular clock*

  -*MAST*

-BIOINFORMATICS

Geographical distribution of HIV-1 subtypes



A - Central and East Africa

B - Americas, Europe, Thailand, Japan

C - Southern Africa and India

D - Central, East, and Southern Africa

E - Thailand, Japan, India

F - Romania, Brazil, Zaire

G - West Africa

H - West Africa, Taiwan

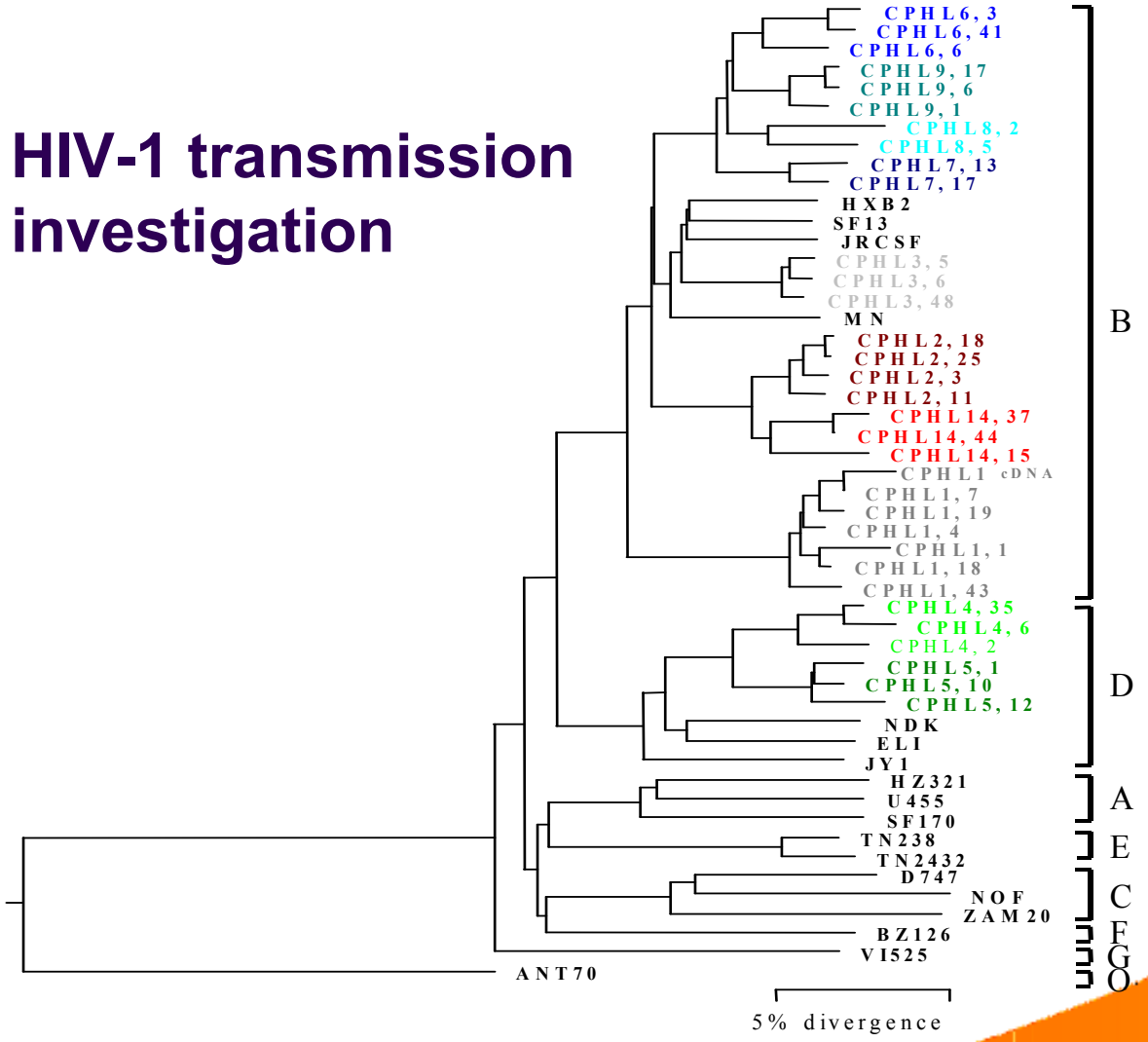
O - West Africa

HIV-1

Subtyping

- Different subtypes of HIV-1 may be associated with increased transmissibility/virulence in different subsets of the community (e.g. heterosexual or homosexual transmission).
- Approximately 25% of HIV-1 positive patients in the UK are infected with non-B subtypes. Notably this study showed that 40% and 61% of infections in heterosexual men and women respectively were non-B subtypes.
- 14% and 35% of the men and women respectively were of UK or European origin, suggesting that the spread of infection of non-B subtypes is mainly the result of heterosexual contact.
- Heterosexual contact is now the most common form of transmission in the U.K.

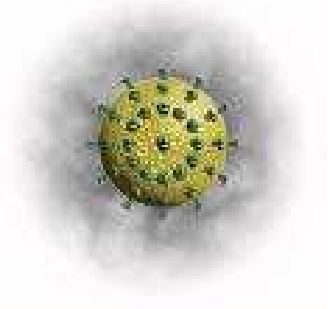
HIV-1 transmission investigation



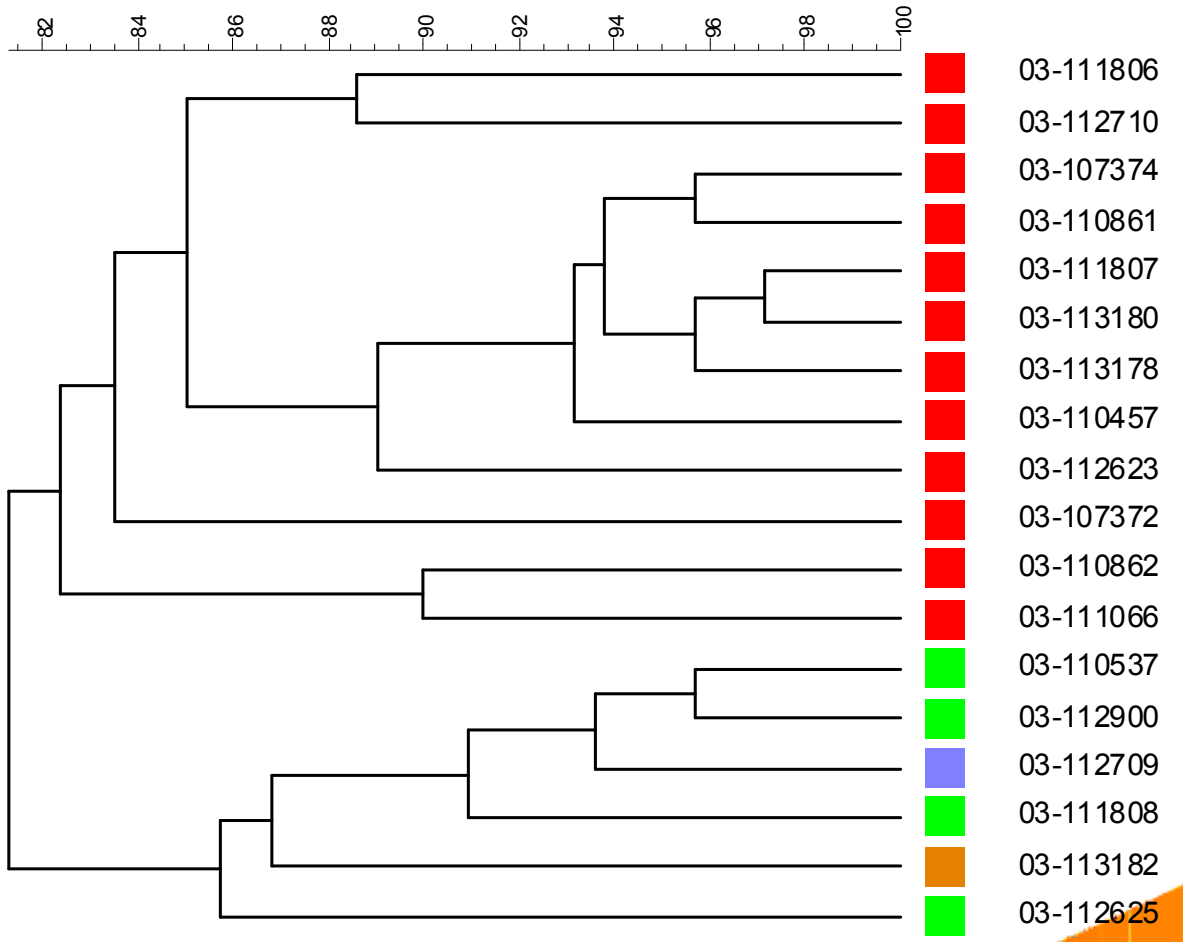
HIV-1

Drug resistance

- **Drug-resistant viral variants** emerge as a consequence of incomplete suppression of HIV-1 replication during treatment with antiretroviral drugs
- **Sequencing** to determine the resistance profiles has become important in the clinical management of HIV-1 patients
  - in the design of an initial therapeutic plan
  - in selecting an alternative drug regimen in the case of resistance emerging
- Viral resistance is a major issue affecting 30 to 50% of all individuals under combination therapy. Simple affordable assays with sufficient sensitivity for different genetic subtypes and low copy number samples are still lacking.



HIV-1 subtyping - Results



Antibiotic resistance

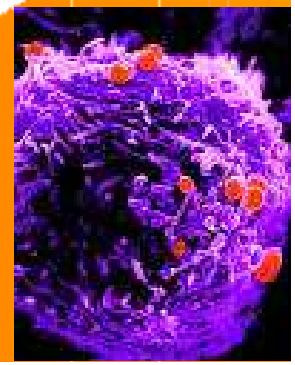
Neisseria gonorrhoeae

Salmonella typhimurium

Helicobacter pylori

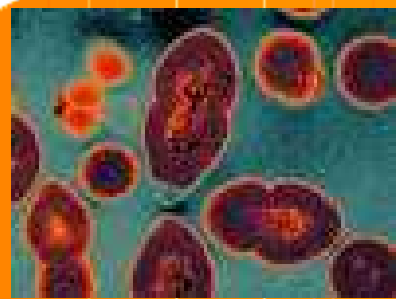
Enterococcus

Mycobacterium tuberculosis



Linezolid Resistance in Enterococci

- Pyrosequencing was used to detect and estimate the number of 23S rRNA genes with a G2576T mutation in 43 linezolid-resistant and -susceptible clinical isolates of enterococci.
- The method showed 100% concordance with PCR-RFLP for detetcing isolates homozygous for either G2576T or heterozygous for this mutation.
- Good correlation was found between linezolid MICs and the number of 23S rRNA gene copies carrying the mutation



Mycobacterium tuberculosis

Drug Resistance

- SNPs have been identified in a short region of the *rpo*B gene conferring resistance to rifampicin
- SNPs in other genes (including *inh*A, *Kat*G and *pnc*A) also confer resistance to other drugs used to combat tuberculosis and these include isoniazid and pyrazinamide.
- We investigated a blinded panel of 74 well-characterised Mtb isolates for drug resistance. Correlation between phenotypic testing and pyrosequencing was 100%. Eight out 74 strains were found to be resistant to isoniazid with a GSer315C mutation.

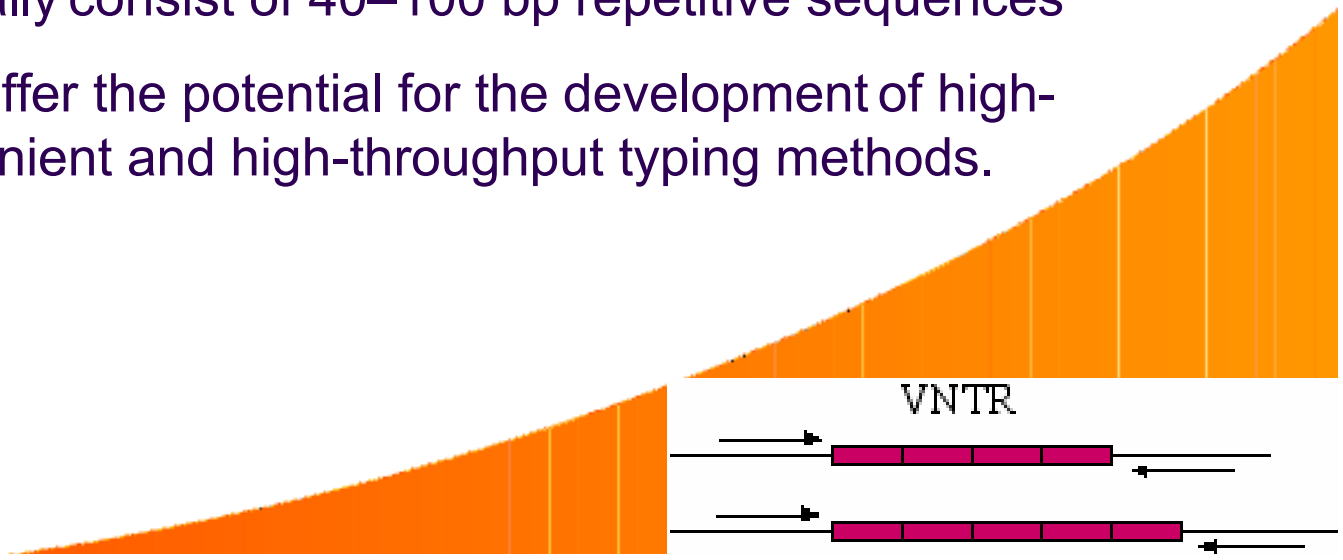
Mycobacterium tuberculosis

VNTRs

- The lack of a convenient high-resolution strain-typing method has held back the application of molecular epidemiology to the surveillance of *Mycobacterium tuberculosis*.
- The availability of whole genome sequences for strains of the *M. tuberculosis* complex, new PCR-based methods have been developed that target the variable-number tandem repeats (VNTRs, or minisatellites).

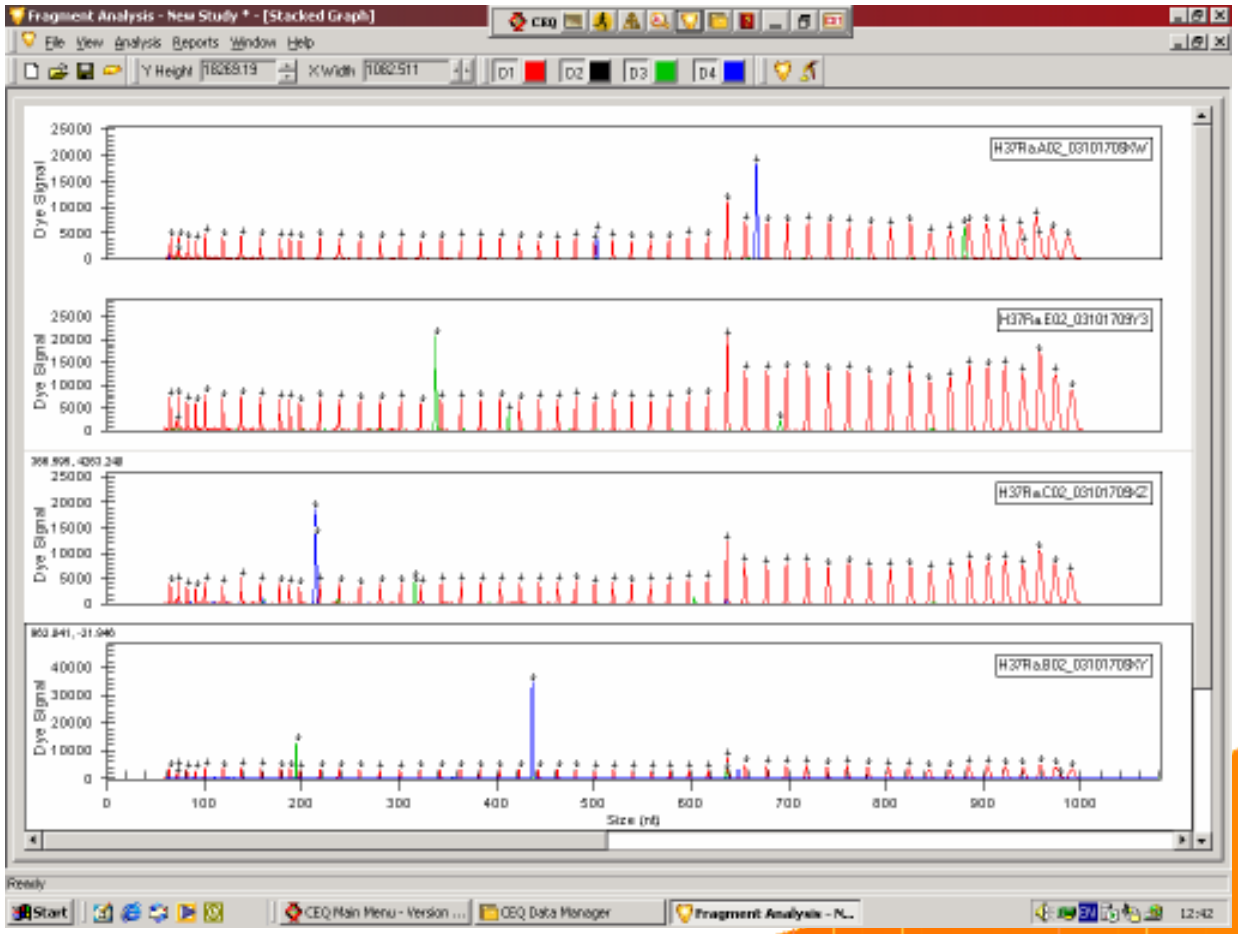
VNTRs/MIRUs

- Microsatellites are highly informative markers found in the genomes of most species and consist of tandem repeats of one to ten nucleotides.
- Tandem repeat loci, similar to eukaryotic minisatellites, have been identified in *M. tuberculosis*. These so-called variable-number tandem repeats (**VNTRs,** also called mycobacterial interspersed repetitive units, or **MIRUs**), often differ in copy number between isolates and usually consist of 40–100 bp repetitive sequences
- These targets offer the potential for the development of high-resolution, convenient and high-throughput typing methods.

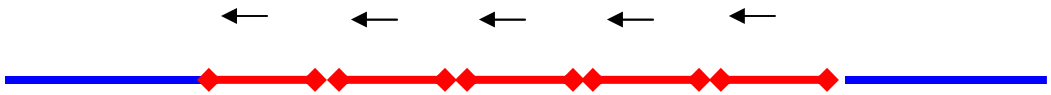


Current methods

The current ‘gold standard’ method is to amplify the VNTR by PCR, including flanking regions, and size on an agarose or acrylamide gel.



VNTR calculation using pyrosequencing

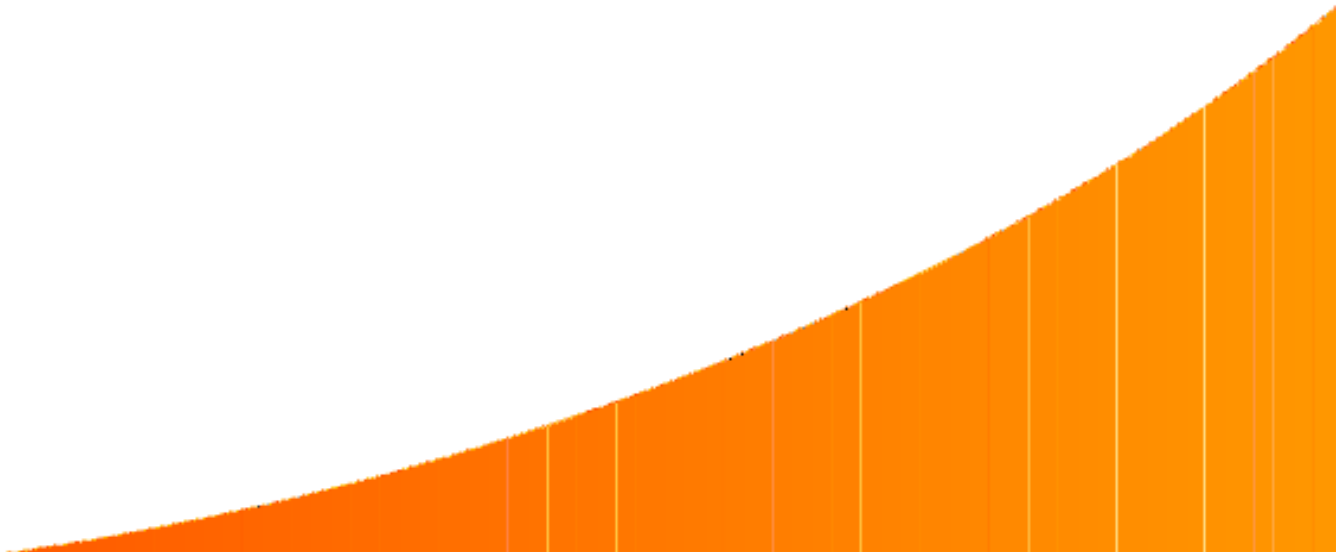


- First nucleotide dispensed will give a light signal corresponding to multiple incorporations along the repeat. This is proportional to the number of repeats present.
- Second nucleotide dispensed will give a signal corresponding to a single incorporation in the flanking region. This gives the ‘denominator’ signal strength, used to calculate the number of repeats present.

Advantages of VNTR/MIRU-typing

The key advantages of VNTR/MIRU-typing are:

- PCR-based
- Typing data produced are numerical and easily managed (portable)
- Not restricted to expensive equipment



H antigen typing of Salmonella

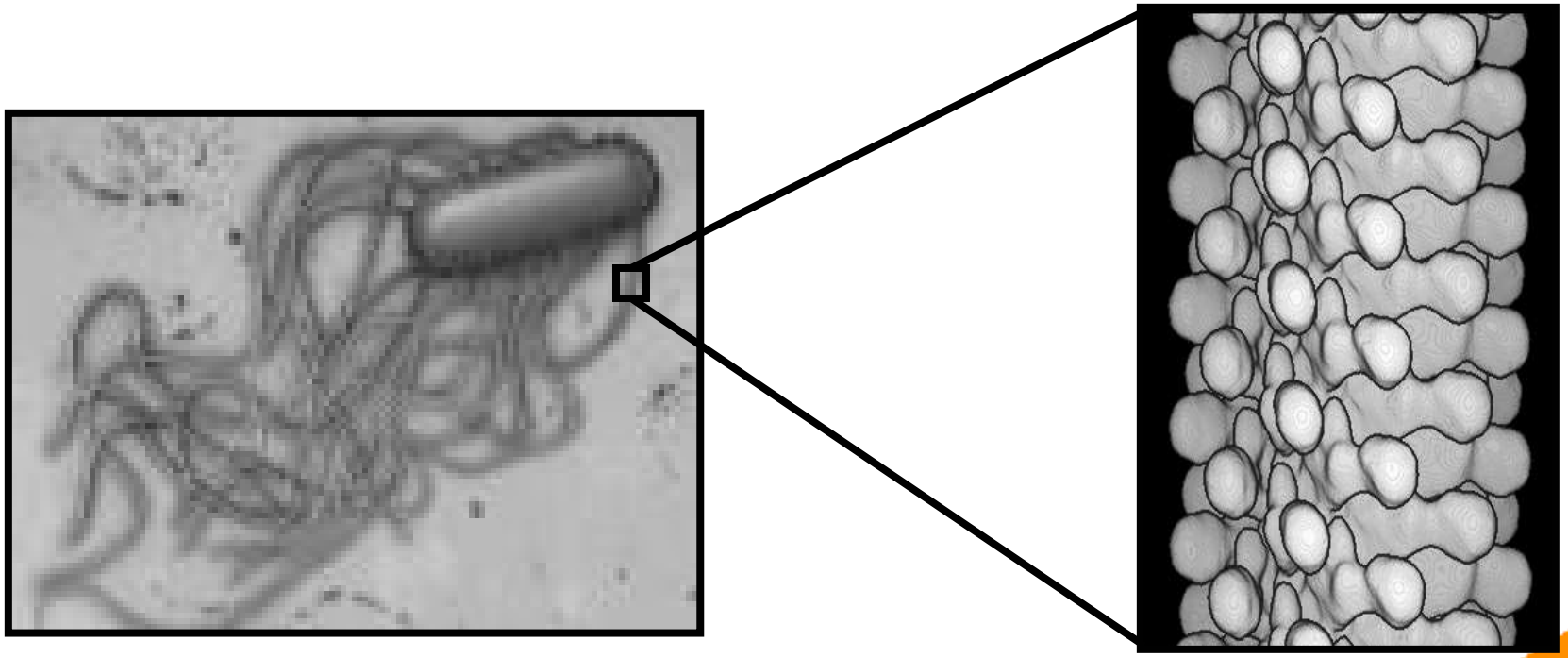
Kauffmann-White scheme

- Expression of antigens is determined by agglutination with specific antisera
- In accordance with this scheme, routine clinical laboratories classify *Salmonella* by their particular combination of flagellar (H) and somatic (O) antigens.
  - O antigens (60 have been distinguished)
  - H1 antigens (63 have been distinguished)
  - H2 antigens, not always present (37 have been distinguished)
- H-antigens are designated by letters of the alphabet (a, to z, z1, z2 etc.) and by Arabic numerals.

Convention for labelling *Salmonella* serotypes

	Antigenic formula		
Serotype	Somatic antigen	Phase 1 flagella	Phase 2 flagella
S. Enteritidis	1,9,12	g,m	-
S. Typhimurium	1,4,5,12	i	1,2
S. Virchow	6,7	r	1,2
S. Hadar	6,8	z10	e,n,x
S. Braenderup	6,7	e,h	e,n,z15
S. Infantis	6,7	r	1,5
S. Newport	6,8	e,h	1,2

The flagellum is composed of 20,000 flagellin subunits



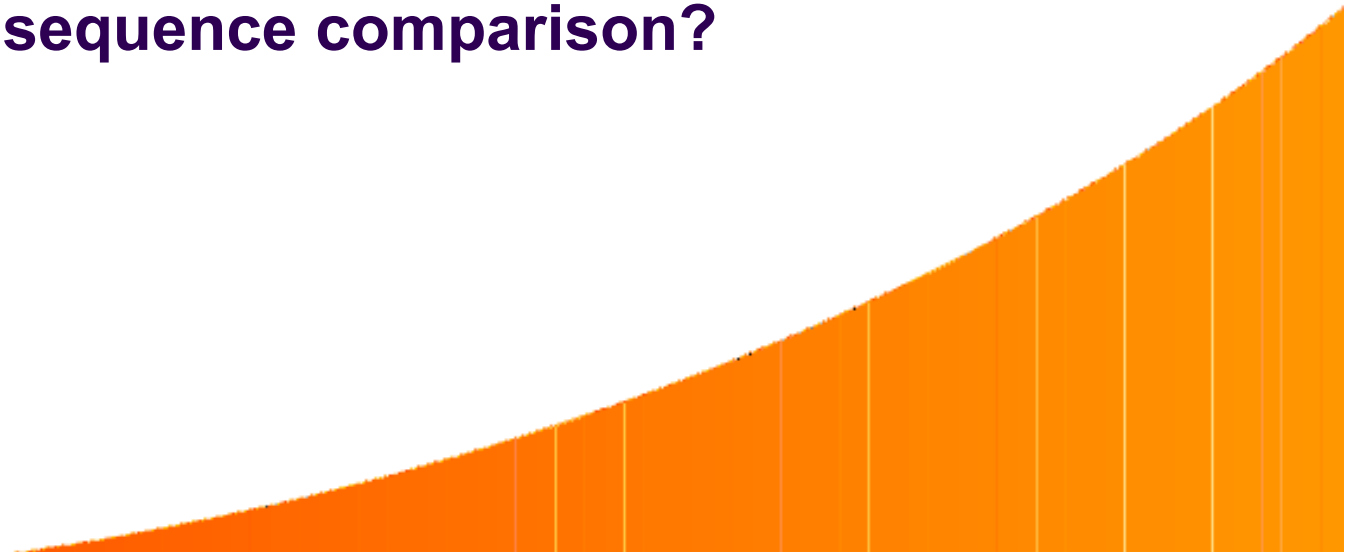
- Flagellin subunit is the antigenic determinant

Future?

Microarrays for everything?

- Identification
- Pathogenicity/virulence factors
- Host factors
- Drug resistance

Whole genome sequence comparison?



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