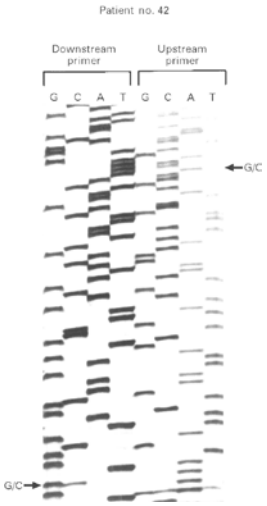
Community Reference Laboratory Antimicrobial Resistance

WHO Collaborating Centre for Antimicrobial Resistance in Foodborne Pathogens
www.antimicrobialresistance.dk/who

National Food Institute
Technical University of Denmark

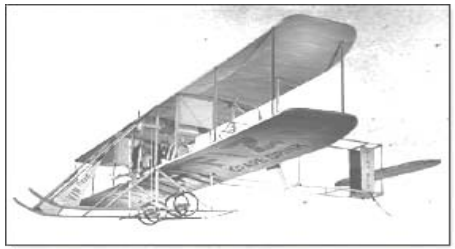
Detection of resistance genes and typing of bacteria based on whole genome sequencing (WGS)



Henrik Hasman
DTU -Food

Research group for bacterial genomics and antimicrobial resistance

- Reference laboratory for DK, EU and WHO
- Surveillance (Global detection and control)
- Various *ad hoc* research projects



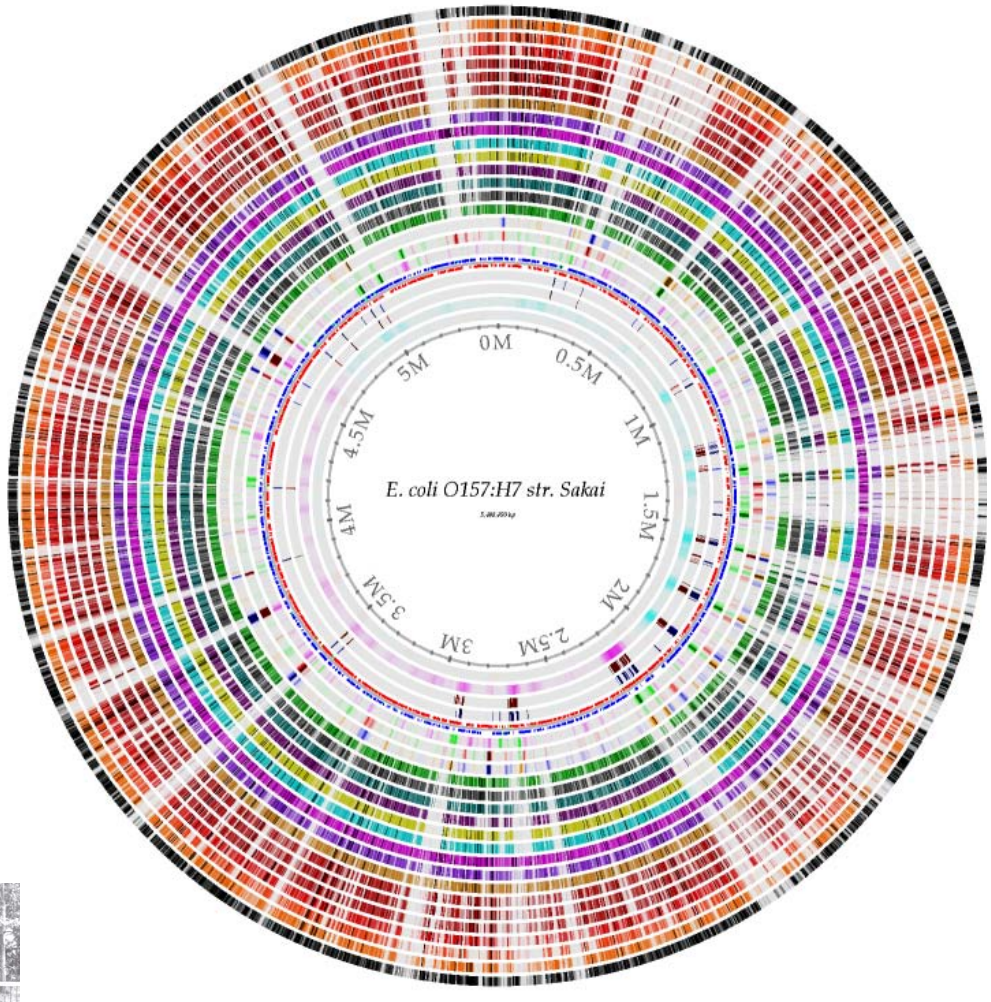
The Challenge

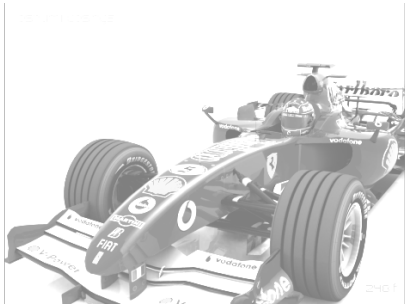
Is to:

- Continue to increase the power of surveillance and diagnostic using molecular tools
- Develop sequenced-based diagnostics that can be used as close to the patient as possible

Google maps

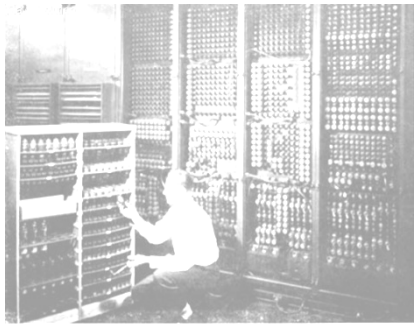
Center for Genomic Epidemiology (CGE)

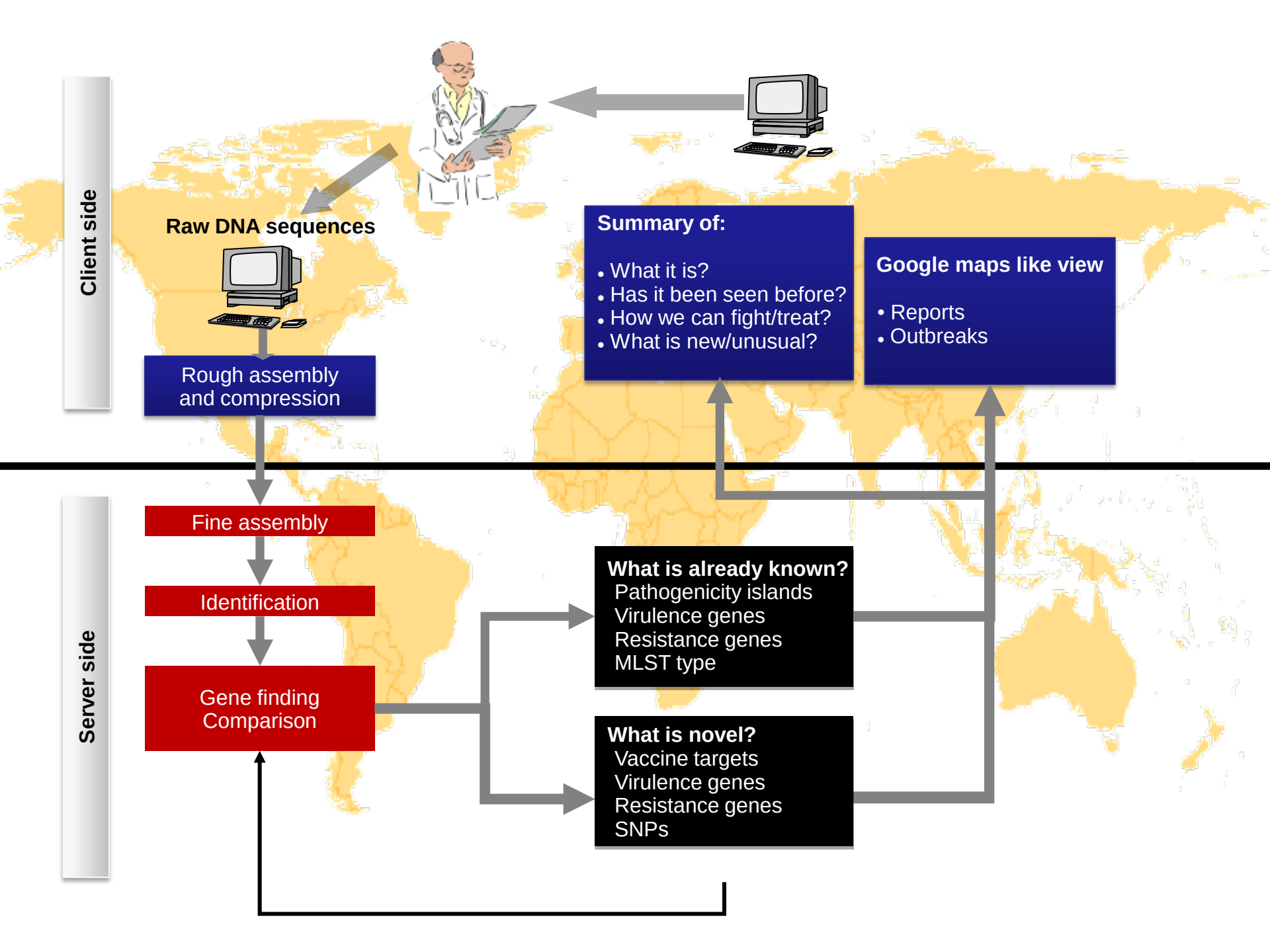


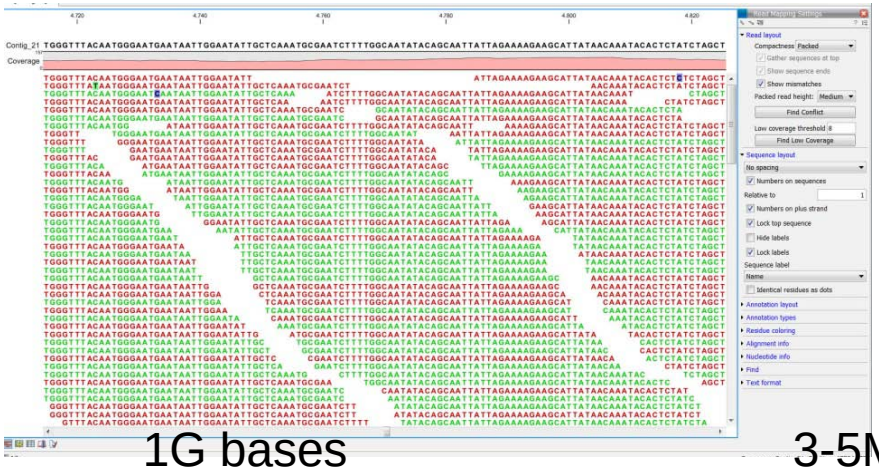


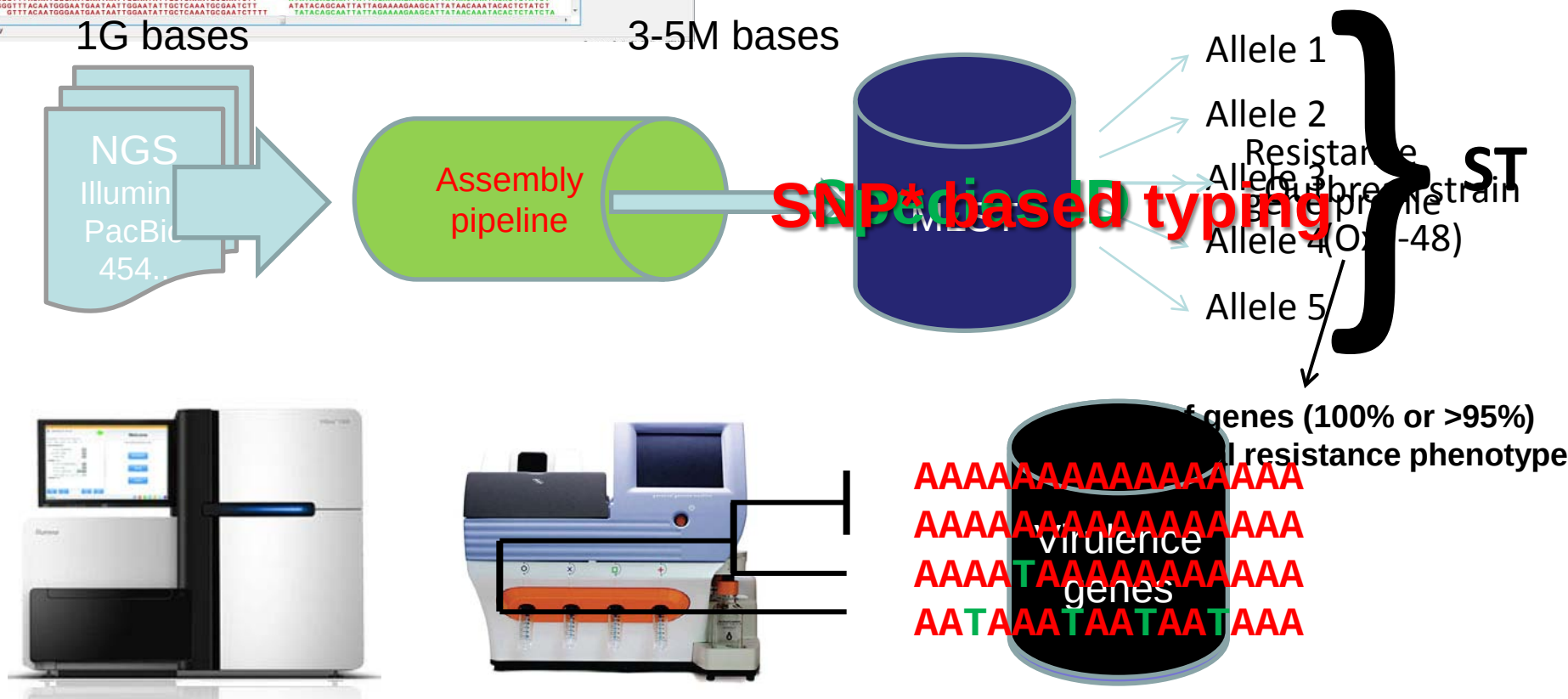
Purpose of CGE

- Provide a proof of concept of combining bioinformatics with both local diagnostics and global epidemiology in real-time




Client side
Raw DNA sequences
Rough assembly and compression
Summary of:
• What it is?
• Has it been seen before?
• How we can fight/treat?
• What is new/unusual?
Google maps like view
• Reports
• Outbreaks
Server side
Fine assembly
Identification
Gene finding Comparison
What is already known?
Pathogenicity islands
Virulence genes
Resistance genes
MLST type
What is novel?
Vaccine targets
Virulence genes
Resistance genes
SNPs

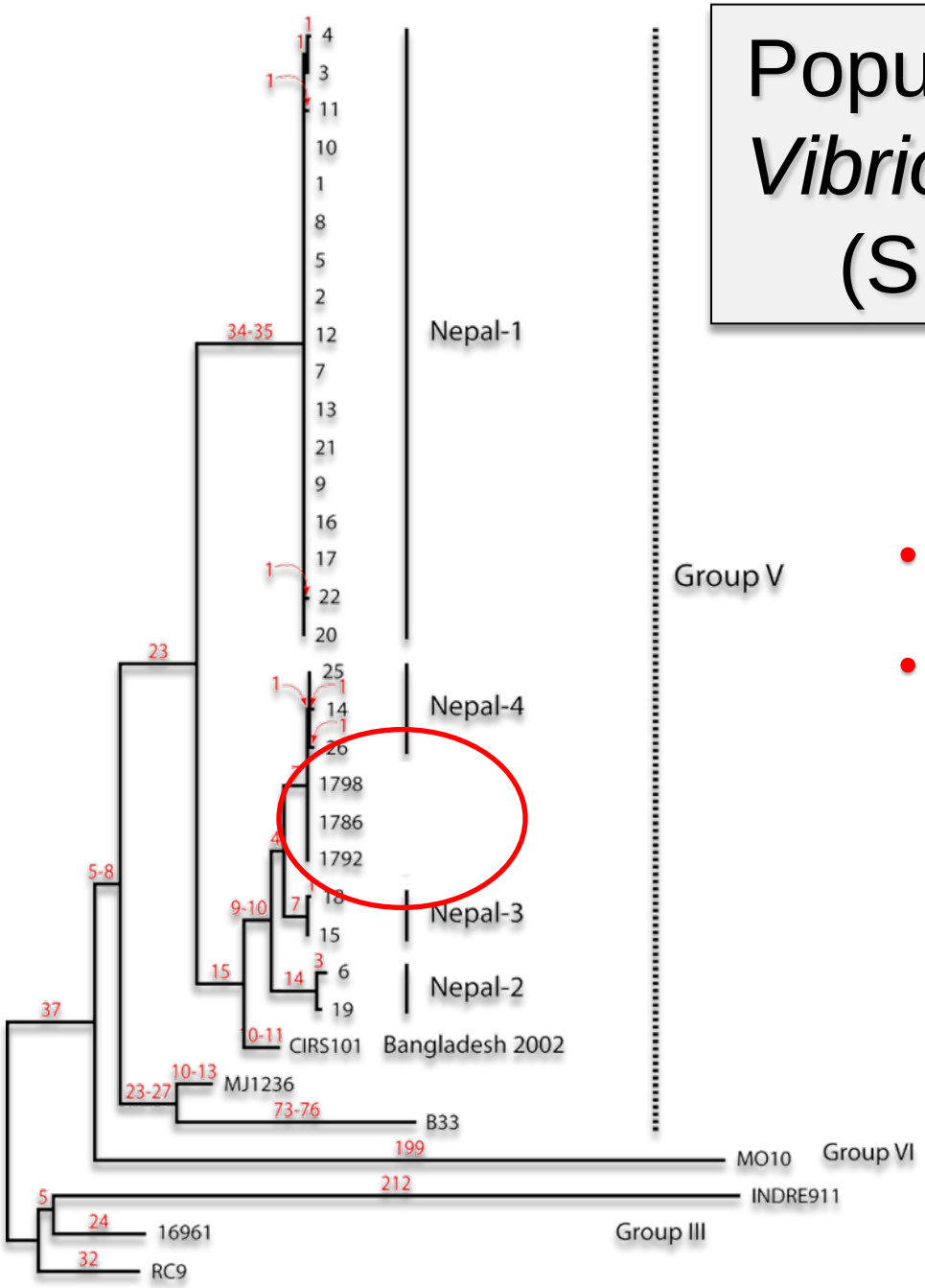




*SNP – Single Nucleotide Polymorphism (extreme MLST)

EPIDEMIOLOGY

Population Genetics of *Vibrio cholera* in Nepal (SNP-based tree)



- 24 *V. cholera* Genomes
- 2 major Nepalese Clades
  - Nepal-1 has low diversity

Rene S. Hendriksen, L. Price, P. Keim,
Geeta Shakya, Frank M. Aarestrup, & others
In press *(mBio)*

Danish Technical University - Copenhagen
TGen – Flagstaff, Arizona
National Public Health Laboratory, Katmandu

Examples – MLST and Resfinder

MLST (Multilocus Sequence Typing)

Instructions | Output format | Article abstract

Browse | Remove | Clear

Uploads

Total files: 0 (N/A).

Select MLST configuration

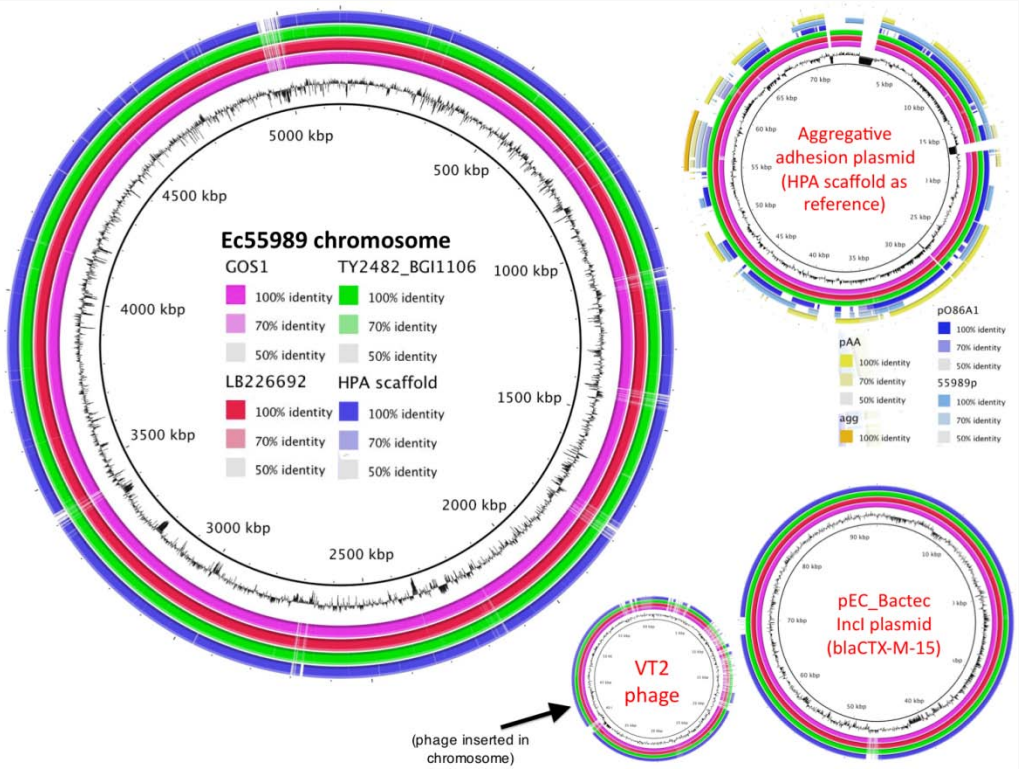
Escherichia coli#1

Select type of your reads

Assembled Genome/Contigs*

Submit | Clear fields

VTEC O104:H4 outbreak strain



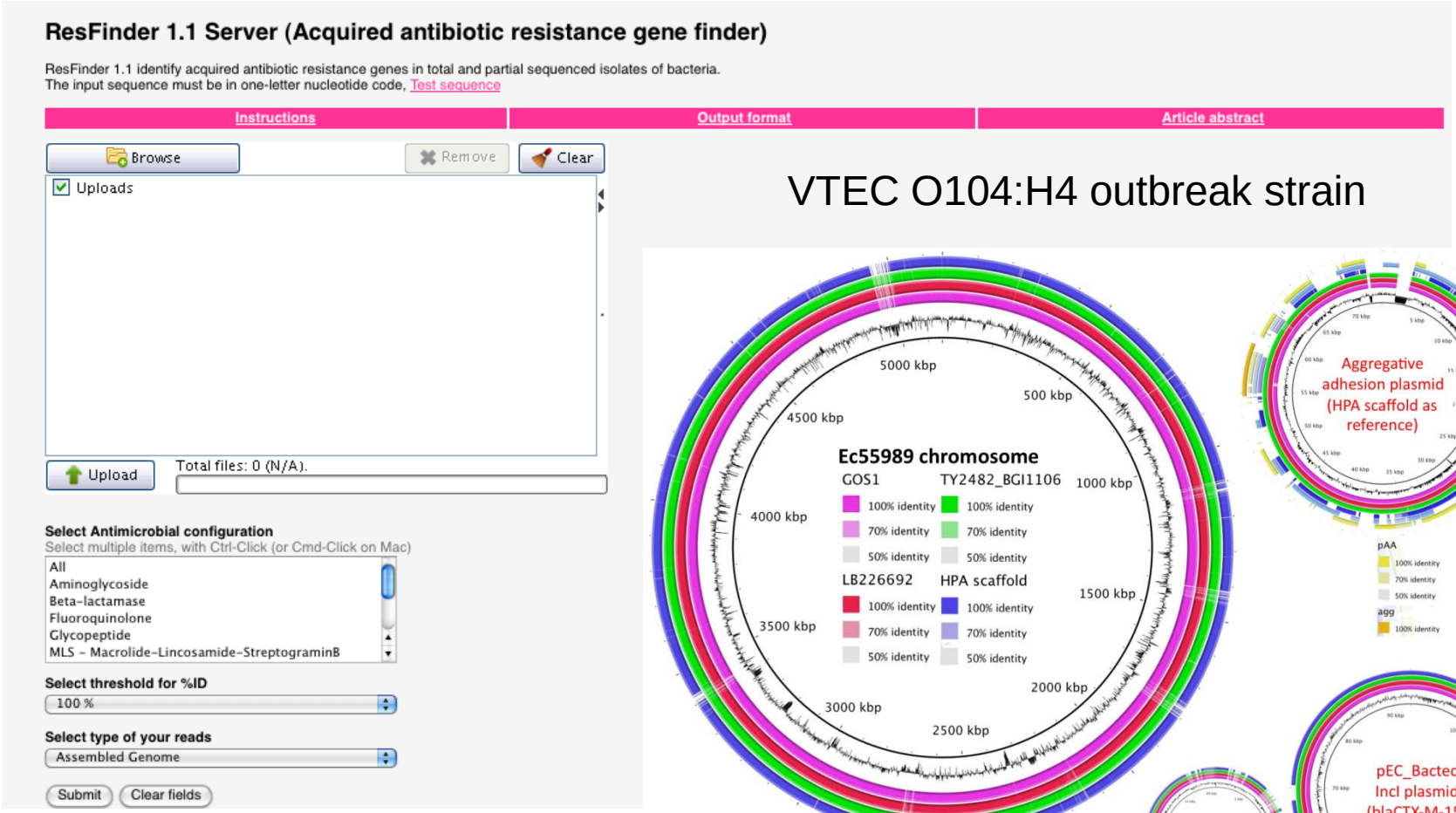
MLST Results

Sequence Type: *ST-678*

SETTINGS:
Organism: *Escherichia coli*
MLST Profile: *ecoli*
Genes in MLST Profile: *7*

Locus	%Identity	Allele Length/HSP Length	Gaps	Allele
adk	100%	536/536	0	*adk-6*
fumc	100%	469/469	0	*fumc-6*
gyrb	100%	460/460	0	*gyrb-5*
icd	100%	518/518	0	*icd-136*
mdh	100%	452/452	0	*mdh-9*
pura	100%	478/478	0	*pura-7*
reca	100%	510/510	0	*reca-7*

Examples – MLST and Resfinder



Results

ResFinder Results

Resistence gene	%Identity	Gene Length/HSP Length	Phenotype	Accession number
Aminoglycoside				
strA	100.00%	804/804	Aminoglycoside resistance Alternate name; aph(3")-Ib	AF321551
strB	100.00%	837/837	Aminoglycoside resistance Alternate name; aph(6)-Id	FJ474091
Beta-lactam				
C -M-15	100.00%	876/876	Beta-lactamase resistance Alternate name; UOE-1	DQ302097
Fluoroquinolone				
No resistence genes found.				
MLS - Macrolide-Lincosamide-StreptograminB				
No resistence genes found.				
Phenicol				
No resistence genes found.				
Sulphonamide				
sulI	100.00%	840/761	Sulphonemide resistance	AY224185
Tetracycline				
tet(A)	100.00%	1200/1200	Tetracycline resistance	AJ517790
Trimethoprim				
dfrA7	100.00%	474/474	Trimethoprim resistance	JF806498
Glycopeptide				
No resistence genes found.				

Antimicrobial susceptibility

Phenotypes vs genotypes

Phenotyping

Pro's:

Relatively fast

Easy

Cheep

Can detect new phenotypes

Con's:

Not always fast enough (hence empiric treatment)

Require that the bacterium can grow in the lab

Influenced by "the human factor" and by biological variation

No breakpoint consensus (CLSI vs EUCAST)

Antimicrobial susceptibility
Phenotypes vs genotypes

Genotyping

Pro's:

Can be used for many other purposes (e.g. outbreak investigation)

Can cover all relevant (known) resistance genes

Can detect genes with heterogeneous expression (e.g. *mecA*)

Can be combined with phenotypes to detect new genes

Can properly be used directly on clinical samples (sputum, blood, urine ect.)

Con's:

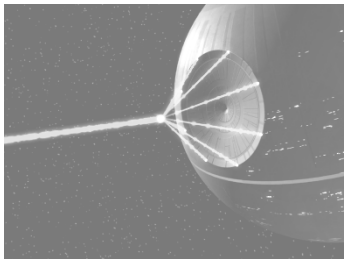
Still relatively expensive (~1000 Dkr/strain)

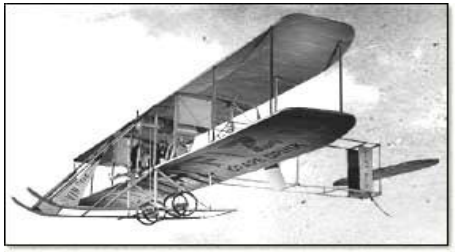
Require high level of skills and new equipment

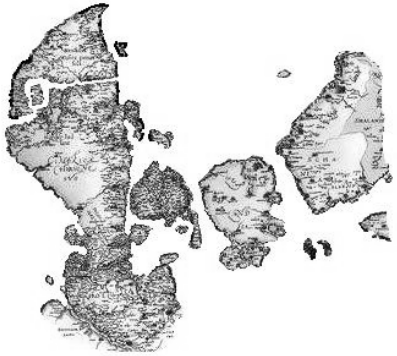
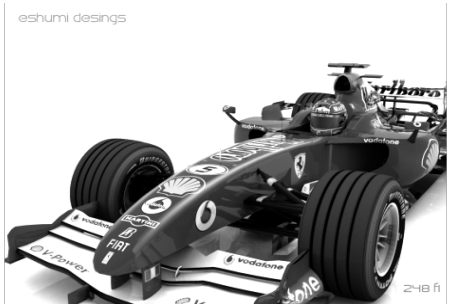
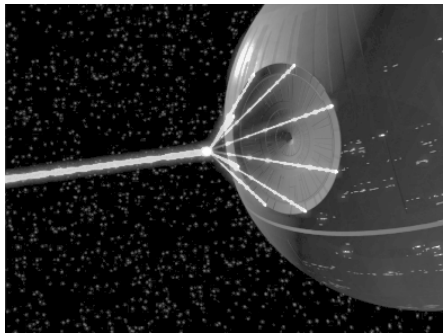
Does not detect new genes automatically

Require the ability to generate "plain language reports"

CGE







Thank you for your attention!