EpiBioTrain

Plasmid typing in outbreak analysis

Henrik Hasman

May 2023

Objectives

Specific objectives of this session:

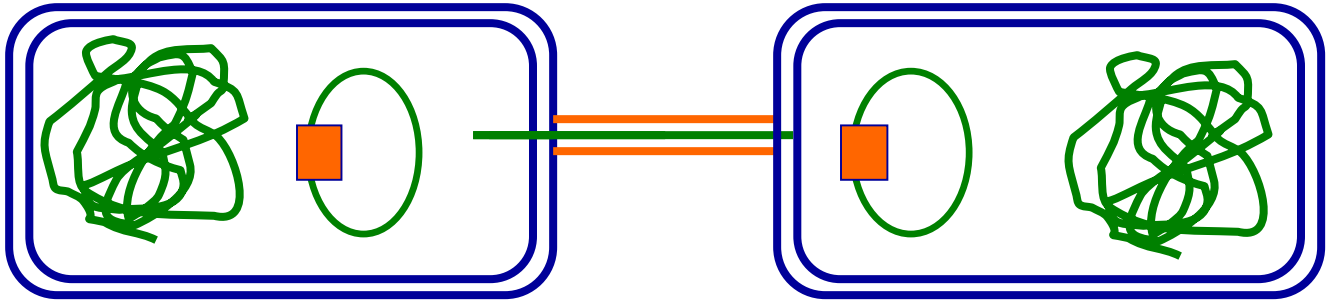
1. Learn about Plasmids as vehicles of AMR
2. Learn about Plasmid in Outbreak situations
3. Learn about Plasmid typing principles
4. Learn how to run PlasmidFinder and to interpret the results

Outline

This session consists of the following elements

1. Introduction to Plasmids (Presentation)
2. Explanation of PlasmidFinder output (Presentation)
3. Group exercise integrating PlasmidFinder and SNP analysis data

Conjugation

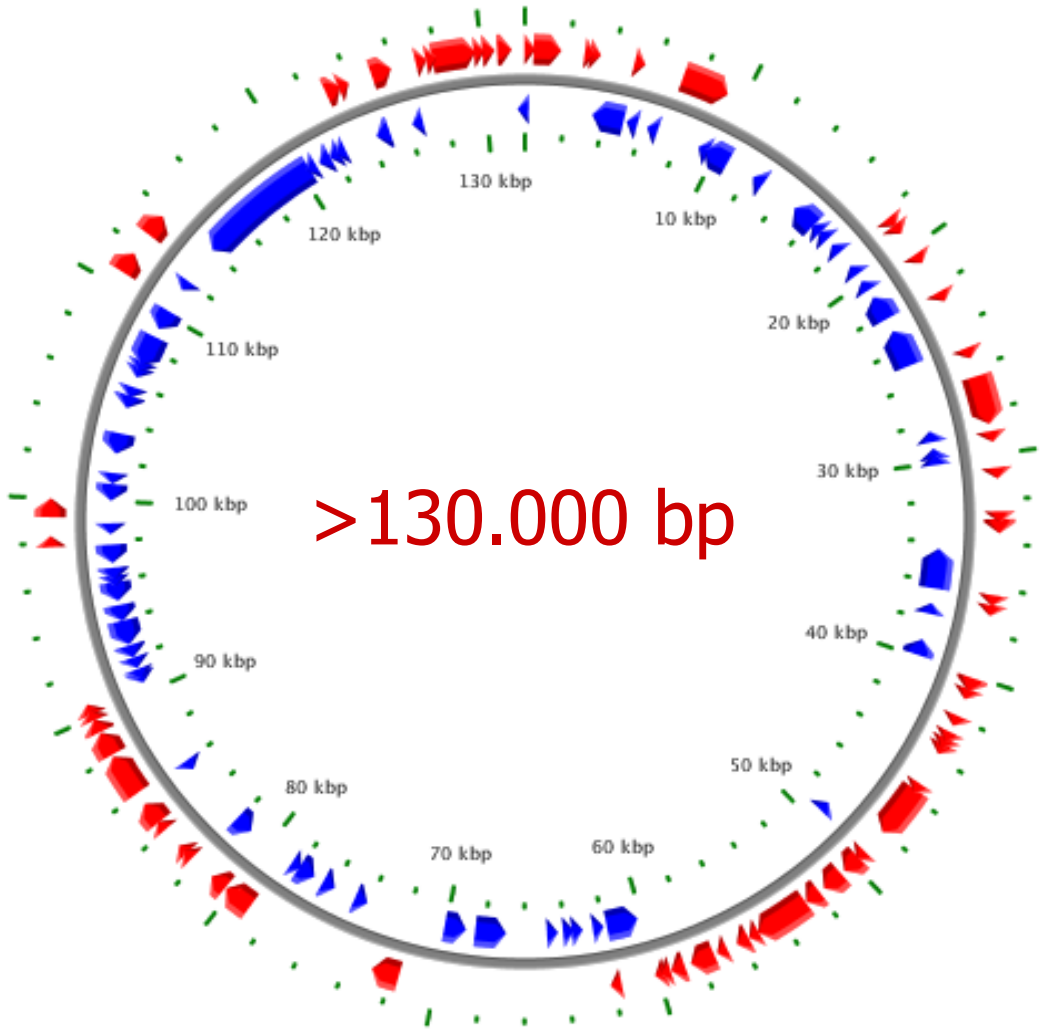


Plasmids

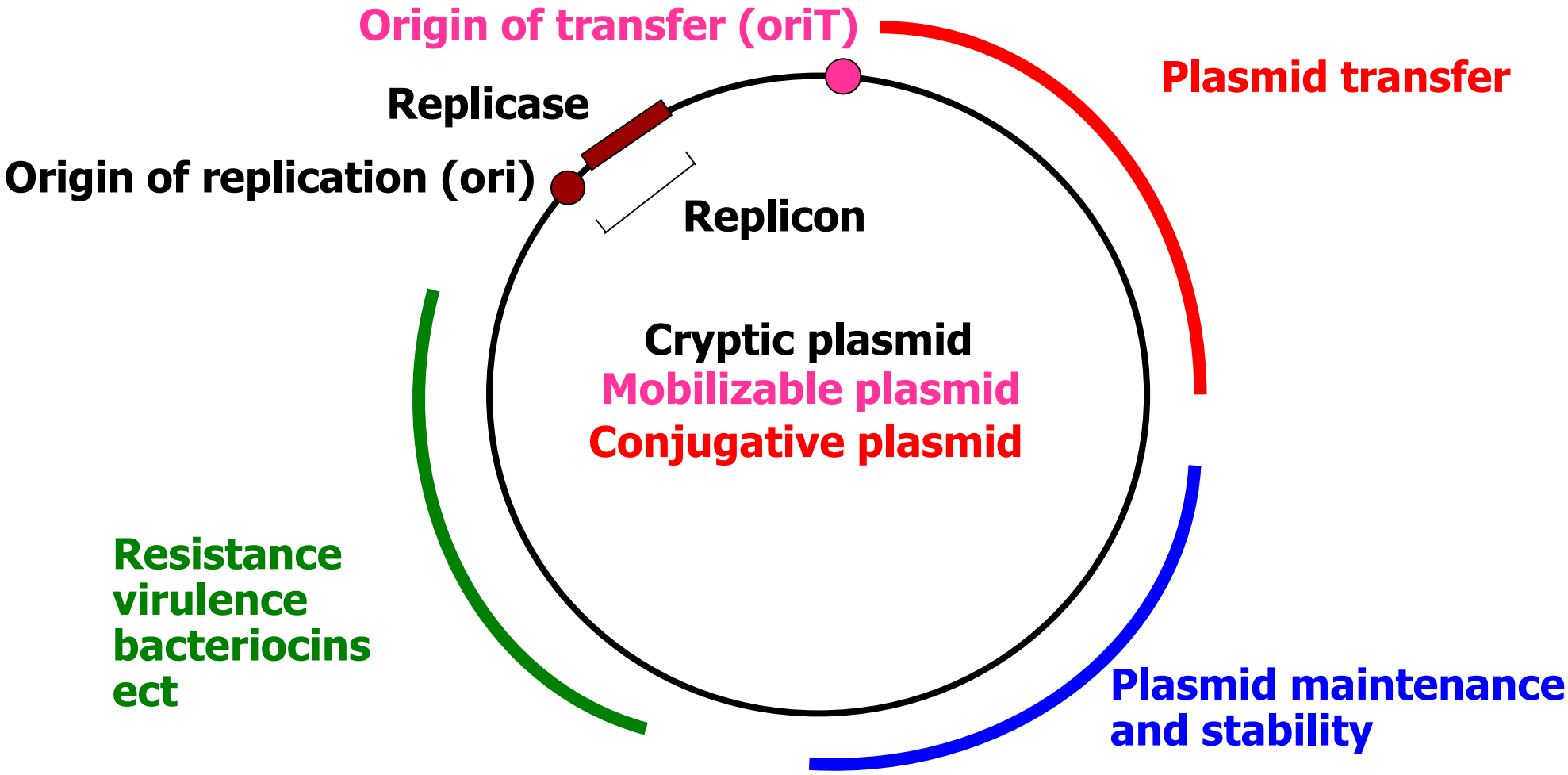
- **Plasmids:** genetic elements that replicate independently of the host chromosome.
- Thousands of different plasmids are known, almost all of which are dsDNA, most of which are super-coiled and circular, are vary in size from **<1-2,400 kbp**.
- Different plasmids are present in cells in a particular number of plasmid molecules per cell = **copy number**, which can vary from **1-100+**.
- Most plasmids in Gram negative bacteria replicate similar to the chromosome, although some replicate unidirectionally. Most plasmids in Gram positive bacteria replicate by the rolling circle mechanism similar to a phage.

Plasmid sizes

Pseudomonas syringae pv. phaseolicola 1448A large plasmid, c...

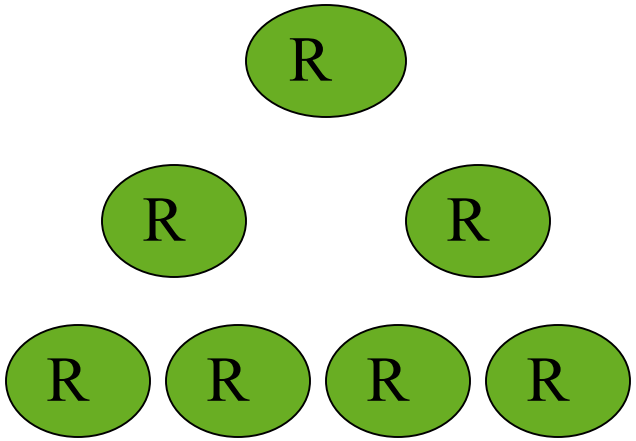


Plasmids – how are they designed?



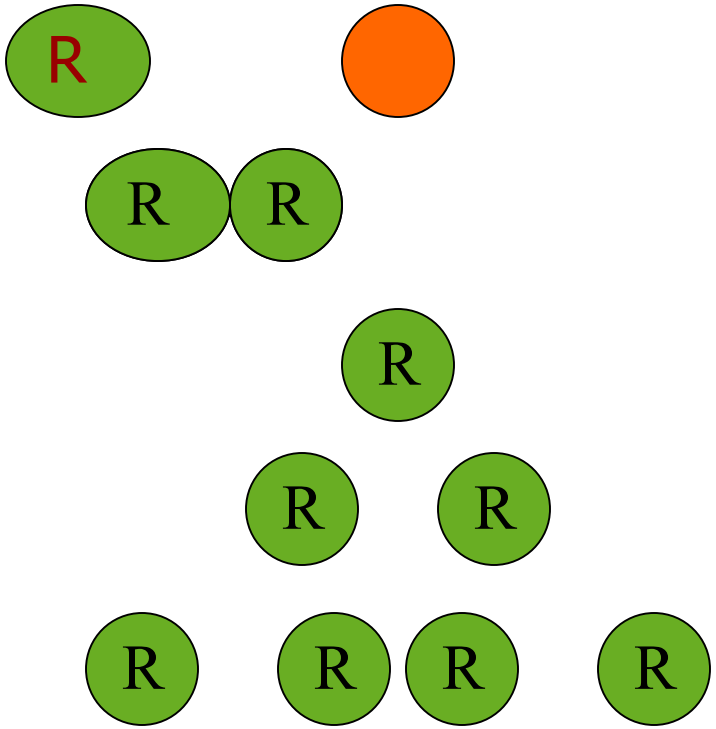
Spread of resistance

Vertical spread



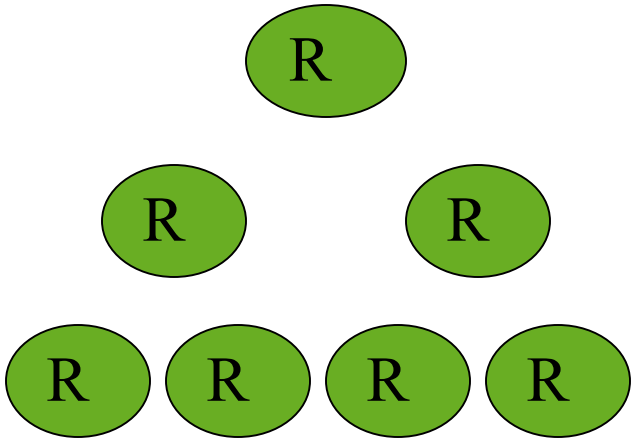
SNP / cgMLST

Horizontal spread

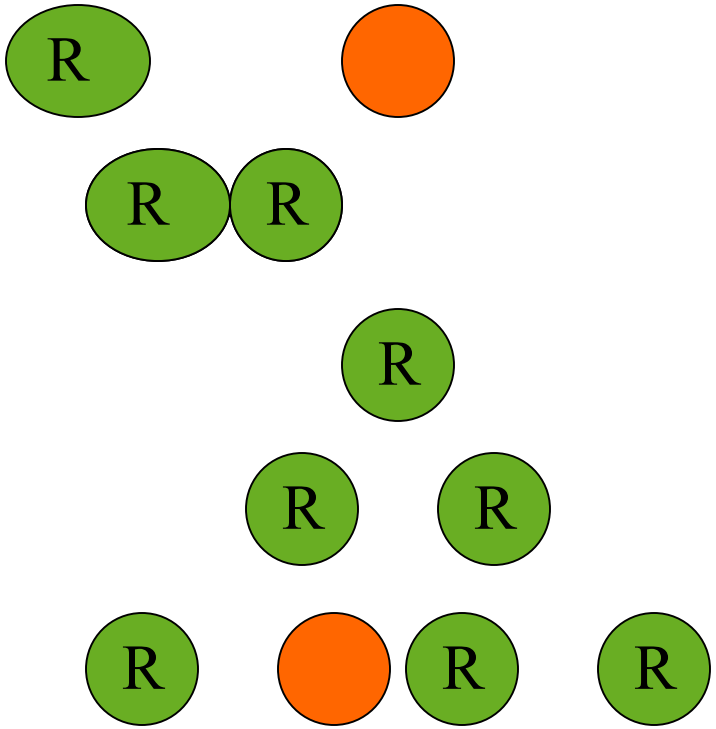


Plasmid can easily be gained and lost

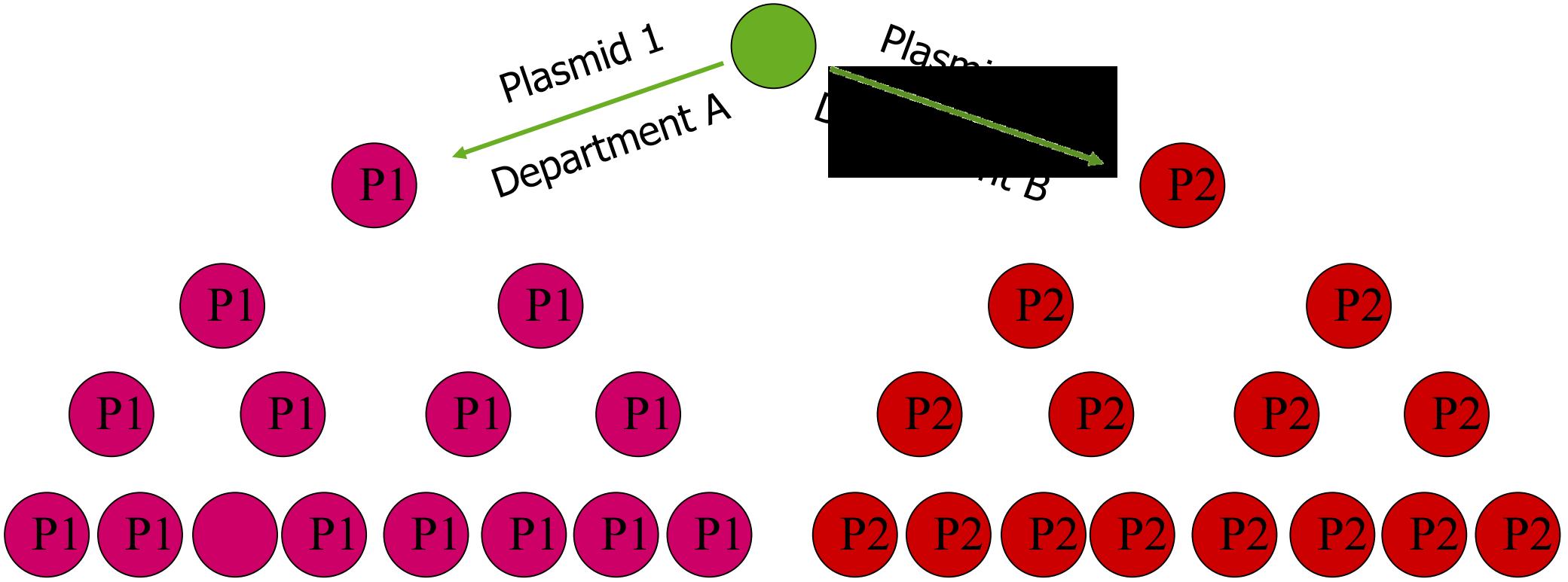
Vertical spread



Horizontal spread

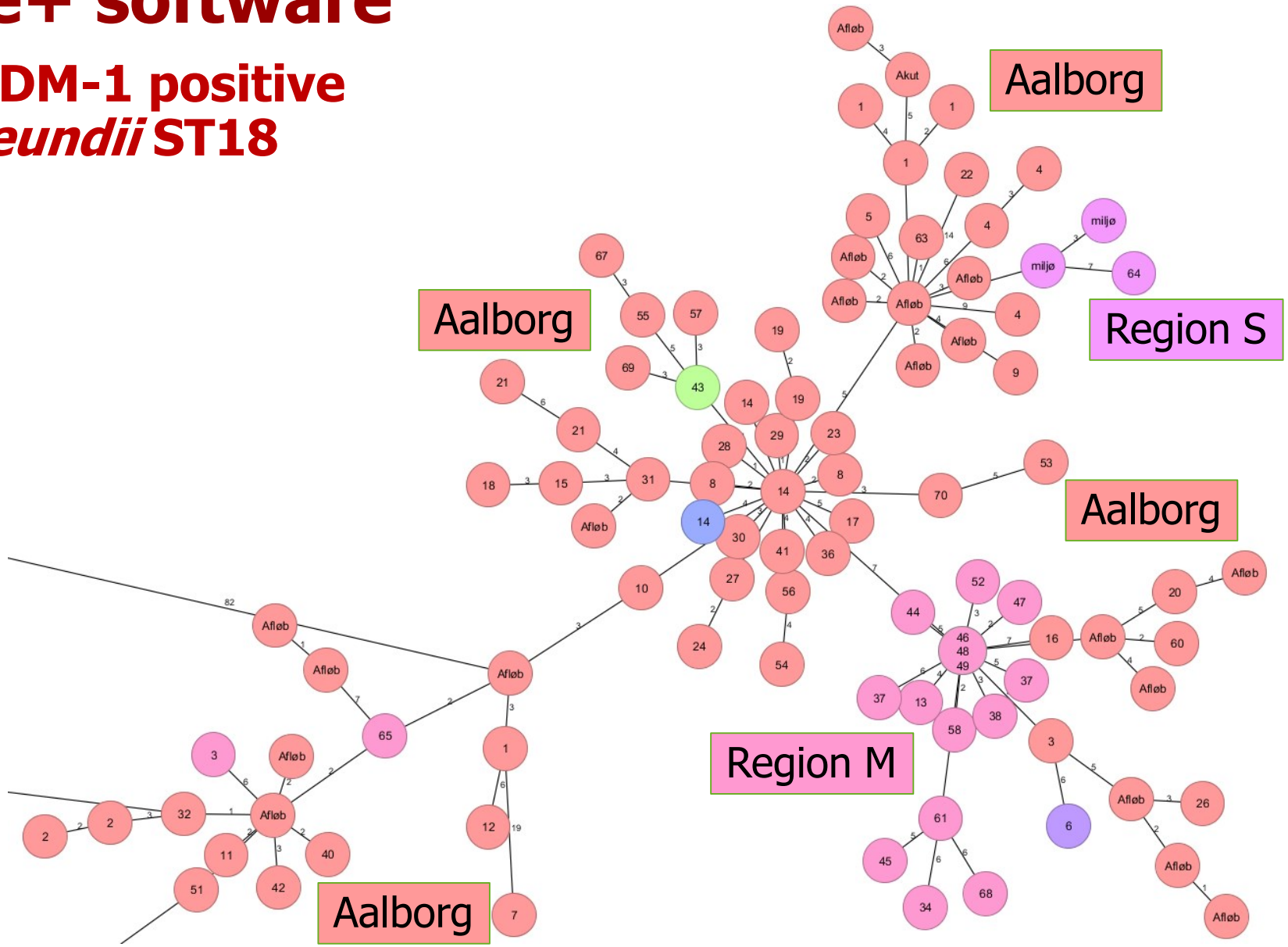


Plasmid can easily be gained and lost

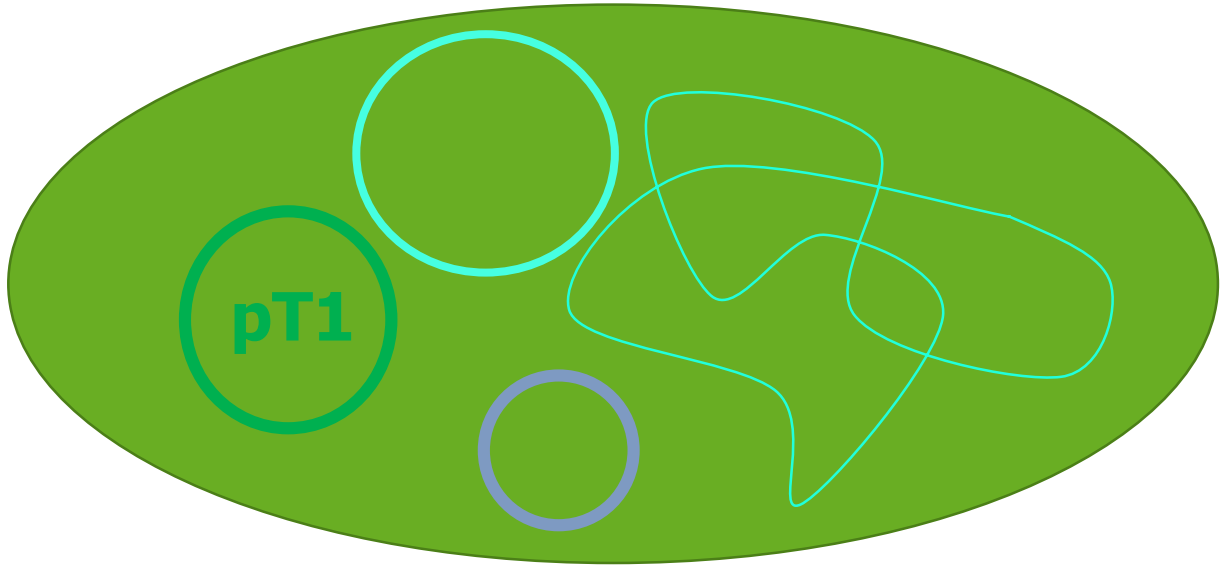


SeqSphere+ software

Outbreak of NDM-1 positive *Citrobacter freundii* ST18

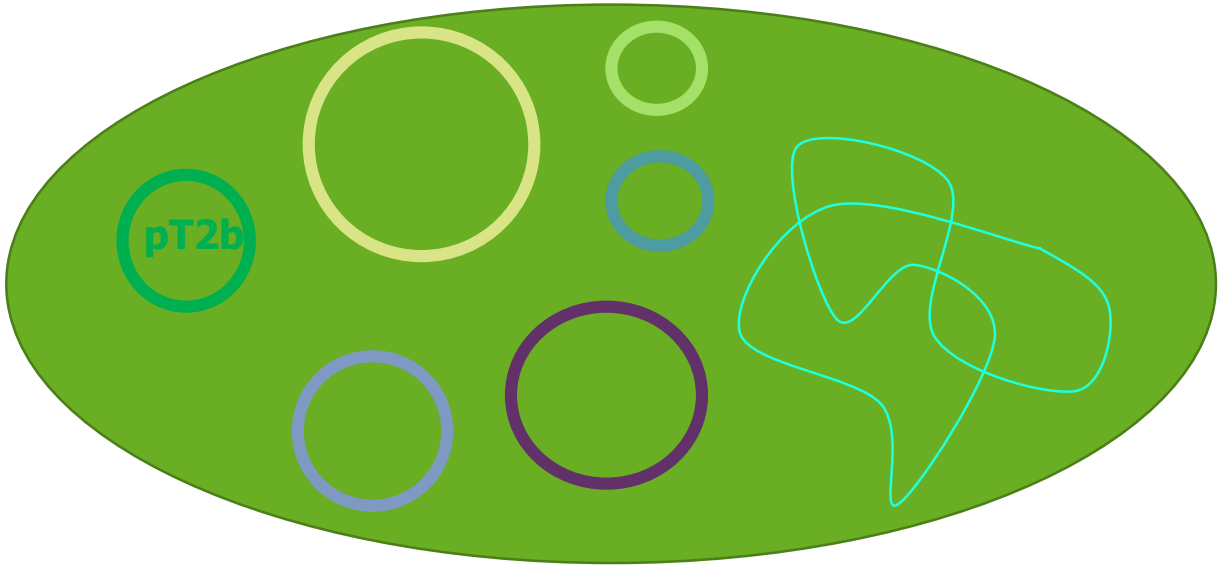


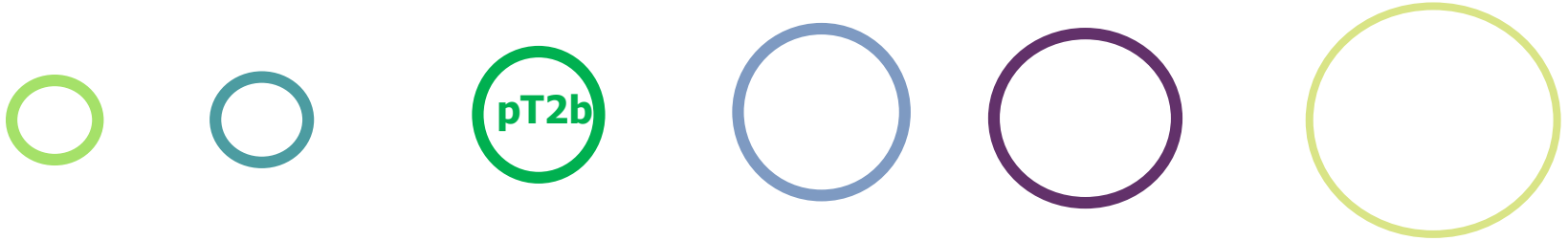
AMA332 (AMA1443) from Patient 1 - Hematology



Name	pAMA1443_4	**pT1**	pAMA1443_2
AMR(BL)		blaNDM-1	blaTEM-1b
Size	110 kb	**154 kb**	248 kb
Replicon	IncFIB(pHCM2)	IncA/C2	IncHI2/IncHI2A

CPO20190170 from drain on Surg. Gastro (2019)

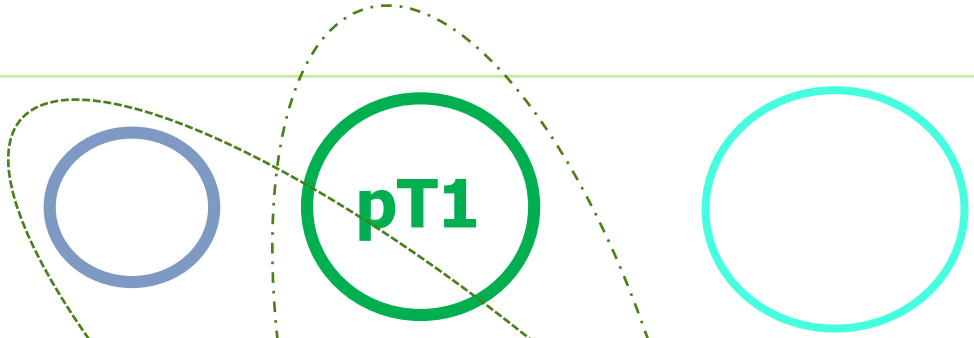




Name	pKloak_7	pKloak_6	**pT2b**	pKloak_4	pKloak_3	pKloak_2
AMR(BL)			blaNDM-1			
Size	57 kb	72 kb	**92 kb**	110 kb	144 kb	179 kb
Replicon	FII(Cf)	IncFIB(K)	IncA/C2	IncFIB(pHCM2)	IncF1A(HI1)	IncFII(S)+IncHIA(HI1)

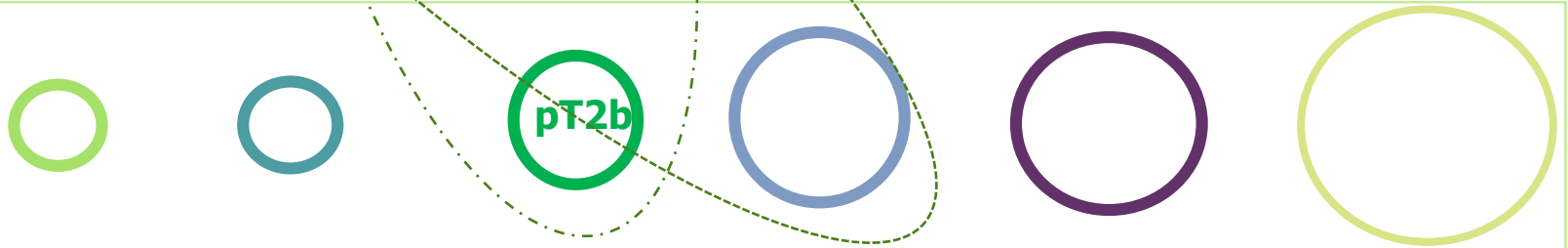
*Plasmid comparisons pT1 vs pT2b strains

AMA1443 (*Hametology*)



Name	pAMA1443_4	pT1	pAMA1443_2
AMR(BL)		blaNDM-1	blaTEM-1b
Size	110 kb	154 kb	248 kb
Replicon	IncFIB(pHCM2)	IncA/C2	IncHI2/IncHI2A

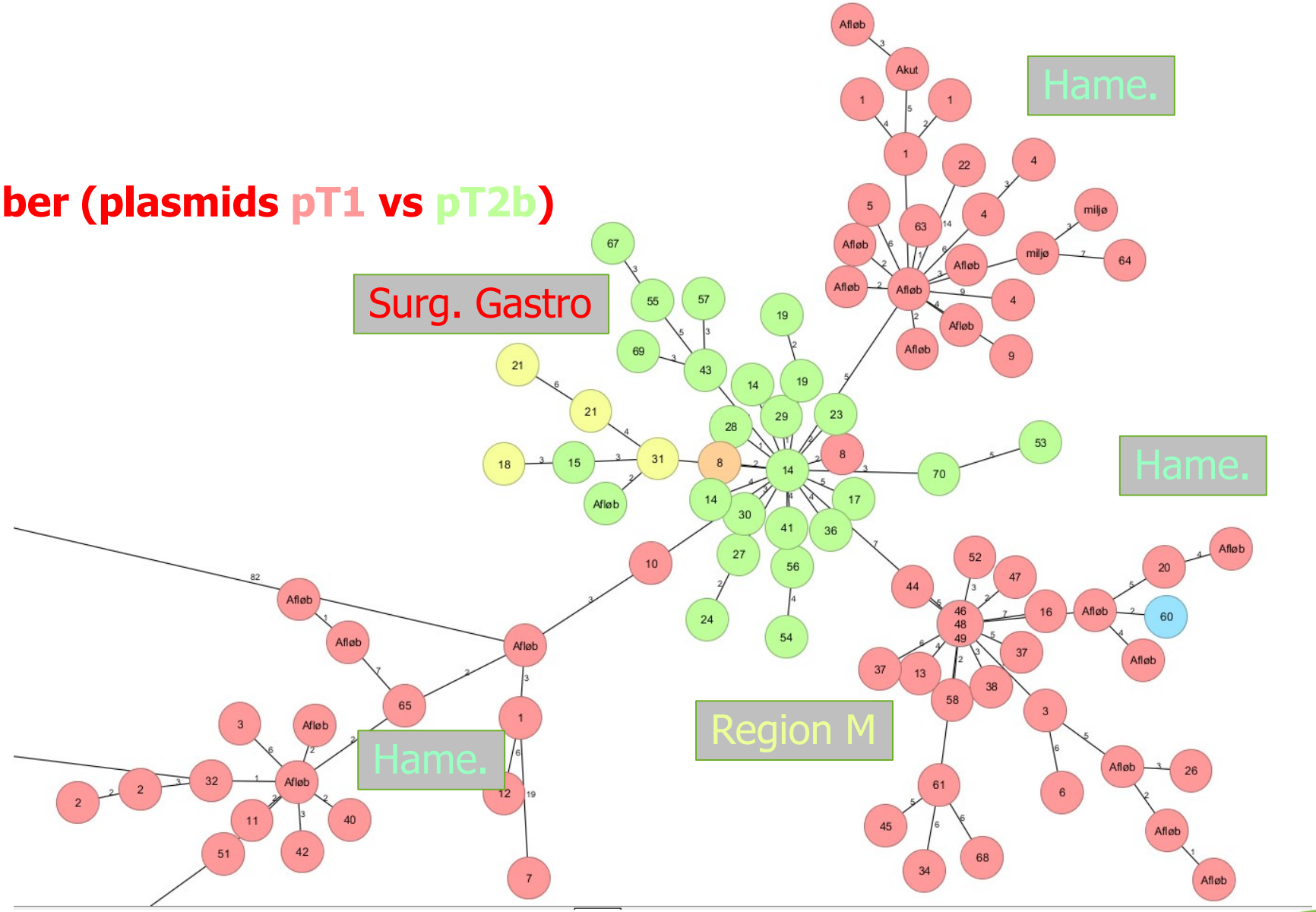
CPO20190170 (*Surg. Gastro*)



Name	pKloak_7	pKloak_6	pT2b	pKloak_4	pKloak_3	pKloak_2
AMR(BL)			blaNDM-1			
Size	57 kb	72 kb	92 kb	110 kb	144 kb	179 kb
Replicon	IncFII(Cf)	IncFIB(K)	IncA/C2	IncFIB(pHCM2)	IncF1A(HI1)	IncFII(S)+IncHIA(HI1)

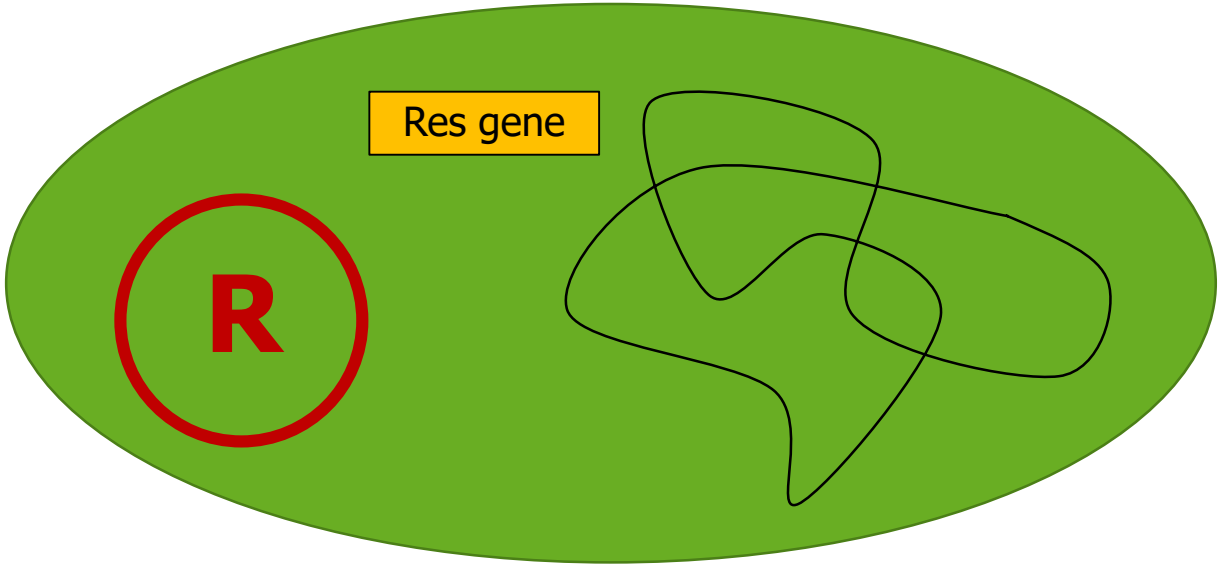
SeqSphere+ software – Aalborg departments

Patient-number (plasmids pT1 vs pT2b)

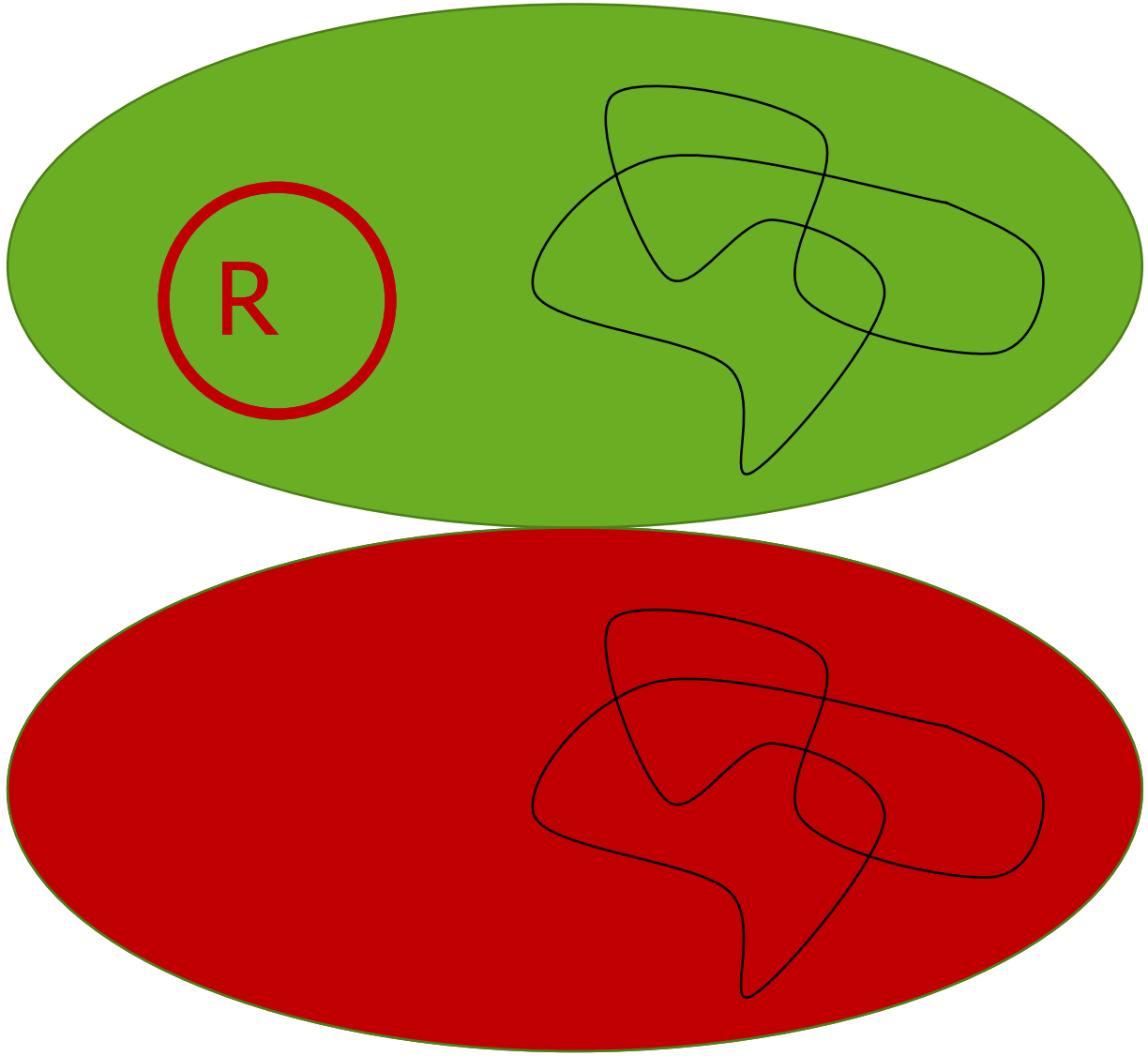


Plasmid typing

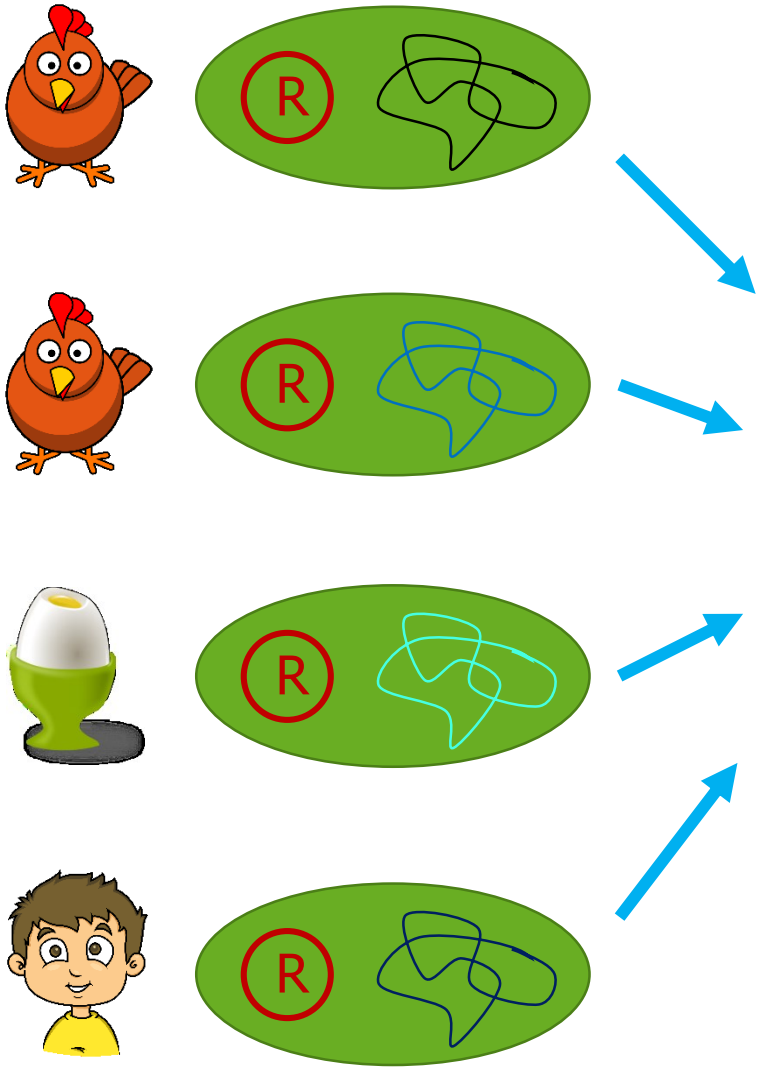
Plasmids can be mobilized



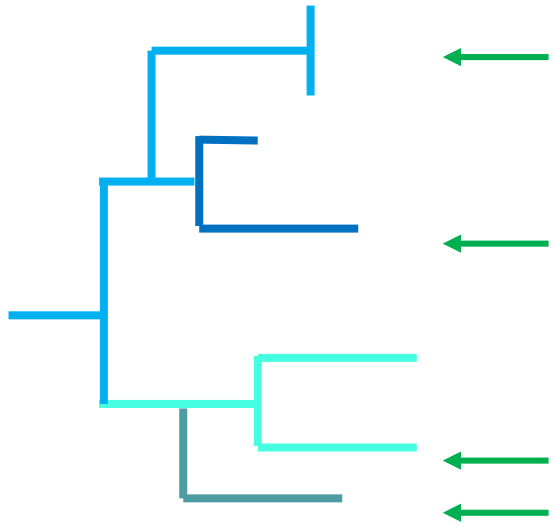
Mobilization



Plasmid epidemiology



SNP analysis



Plasmid typing

Resistance profile

- Plasmid size (uncut vs. linear)
- Restriction Fragment Length Polymorphism (RFLP)
- **Typing of conserved elements (replicons, *MOB* genes)**
- Complete sequencing

Replicon typing (Enterobacteriales only)

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Journal of Microbiological Methods 63 (2005) 219–228

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Identification of plasmids by PCR-based replicon typing

Alessandra Carattoli[a,*], Alessia Bertini[a], Laura Villa[a], Vincenzo Falbo[b], Katie L. Hopkins[c], E. John Threlfall[c]

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Known *inc* groups in *E. coli*

FIA	W
FIB	T
FIC	A/C
HI1	K
HI2	B/O
I1-Ig	X
L/M	Y
N	F
P	FIIA.

And more to come ……

BLAST PlasmidFinder database

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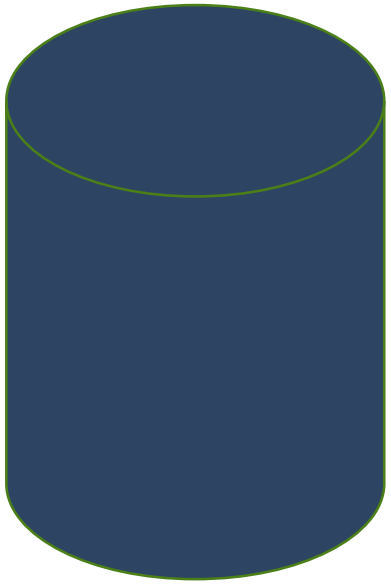


AAC Journals.ASM.org

In Silico Detection and Typing of Plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing

Alessandra Carattoli,[a] Ea Zankari,[b] Aurora García-Fernández,[a] Mette Voldby Larsen,[c] Ole Lund,[c] Laura Villa,[a] Frank Møller Aarestrup,[b] Henrik Hasman[b]

Department of Infectious, Parasitic and Immuno-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy[a]; Danish Technical University, National Food Institute, Division for Epidemiology and Microbial Genomics, Lyngby, Denmark[b]; Danish Technical University, Center for Biological Sequence Analysis, Department of Systems Biology, Lyngby, Denmark[c]

In the work presented here, we designed and developed two easy-to-use Web tools for *in silico* detection and characterization of whole-genome sequence (WGS) and whole-plasmid sequence data from members of the family *Enterobacteriaceae*. These tools will facilitate bacterial typing based on draft genomes of multidrug-resistant *Enterobacteriaceae* species by the rapid detection of known plasmid types. Replicon sequences from 559 fully sequenced plasmids associated with the family *Enterobacteriaceae* in the NCBI nucleotide database were collected to build a consensus database for integration into a Web tool called PlasmidFinder that can be used for replicon sequence analysis of raw, contig group, or completely assembled and closed plasmid sequencing data. The PlasmidFinder database currently consists of 116 replicon sequences that match with at least at 80% nucleotide identity all replicon sequences identified in the 559 fully sequenced plasmids. For plasmid multilocus sequence typing (pMLST) analysis, a database that is updated weekly was generated from www.pubmlst.org and integrated into a Web tool called pMLST. Both databases were evaluated using draft genomes from a collection of *Salmonella enterica* serovar Typhimurium isolates. PlasmidFinder identified a total of 103 replicons and between zero and five different plasmid replicons within each of 49 *S.* Typhimurium draft genomes tested. The pMLST Web tool was able to subtype genomic sequencing data of plasmids, revealing both known plasmid sequence types (STs) and new alleles and ST variants. In conclusion, testing of the two Web tools using both fully assembled plasmid sequences and WGS-generated draft genomes showed them to be able to detect a broad variety of plasmids that are often associated with antimicrobial resistance in clinically relevant bacterial pathogens.

Cut-off for large (>15 kb) plasmids: 95% ID, 90% Coverage

Cut-off for small (<15 kb) plasmids: 80% ID, 90% Coverage

I recommend that you always use the 80% ID cut-off…but remember to only report large plasmids, if they have %ID >95%

PlasmidFinder 2.1

Service | Instructions | Output | Article abstract | Citations

Software version: 2.0.1 (2020-07-01)
Database version: (2023-01-18)
Test sequence

The database is curated by:
Henrik Hasman and Alessandra Carattoli
(click to contact)

Select database

Gram Positive
Enterobacteriales

← 2 databases

Select threshold for minimum % identity

95 %

← Use 80%

Large plasmids > 95% ID
Small plasmids > 80% ID

Select minimum % coverage

60 %

← Use 90%

Select type of your reads

Only data from one single isolate should be uploaded. If raw sequencing reads are uploaded KMA will be used for mapping. KMA supports the following sequencing platforms: Illumina, Ion Torrent, Roche 454, SOLiD, Oxford Nanopore, and PacBio.

Assembled or Draft Genome/Contigs*

Choose File(s)

Name	Size	Progress	Status

Upload | Remove

Center for Genomic Epidemiology

Home | Services | Instructions | Output

PlasmidFinder-1.0 Server - Results

Input Files: *EC32_2011_70_39_2-illumina_pe_velvet1.1.04_kmer67_cov60_cut0.fna*

PlasmidFinder Results

SETTINGS:
Selected %ID

PlasmidFinder - Gram-neg

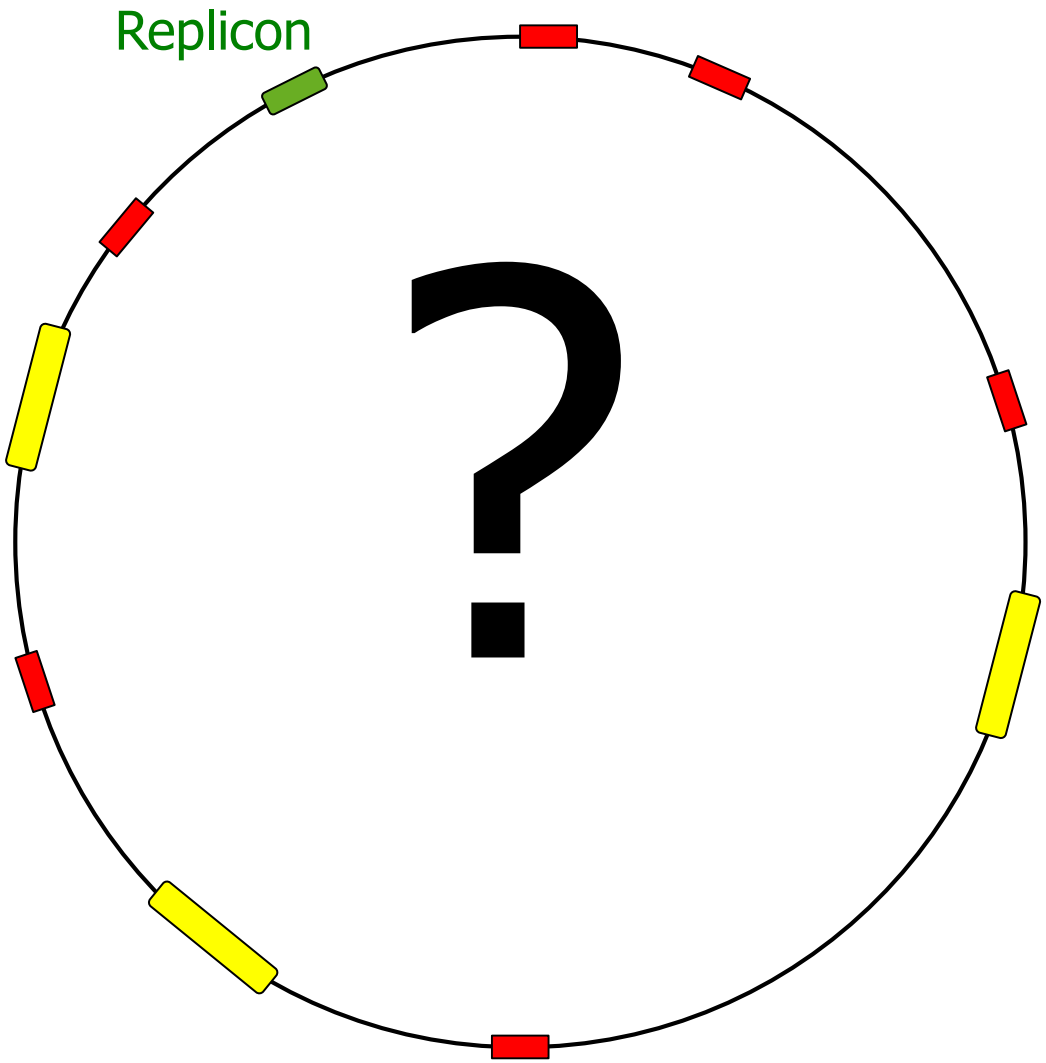
Plasmid	%Identity	Query/HSP leng	Contig	Position in contig	Note	Accession number
FIB	98.39%	682/682	NODE_37_length_8421_cov_88.829353	7176..7857		AP001918
FIC	96.67%	240/240	NODE_224_length_44811_cov_76.680367	2275..2514		AP001918
FII	97.31%	260/260	NODE_224_length_44811_cov_76.680367	2018..2276	pTUC100	AY091607
I1	100.00%	142/142	NODE_214_length_17795_cov_101.394043	1827..1968	(alpha)	AP005147

extended output

Input Files: *a_pe_velvet1.1.04_kmer67_cov60_cut0.fna*

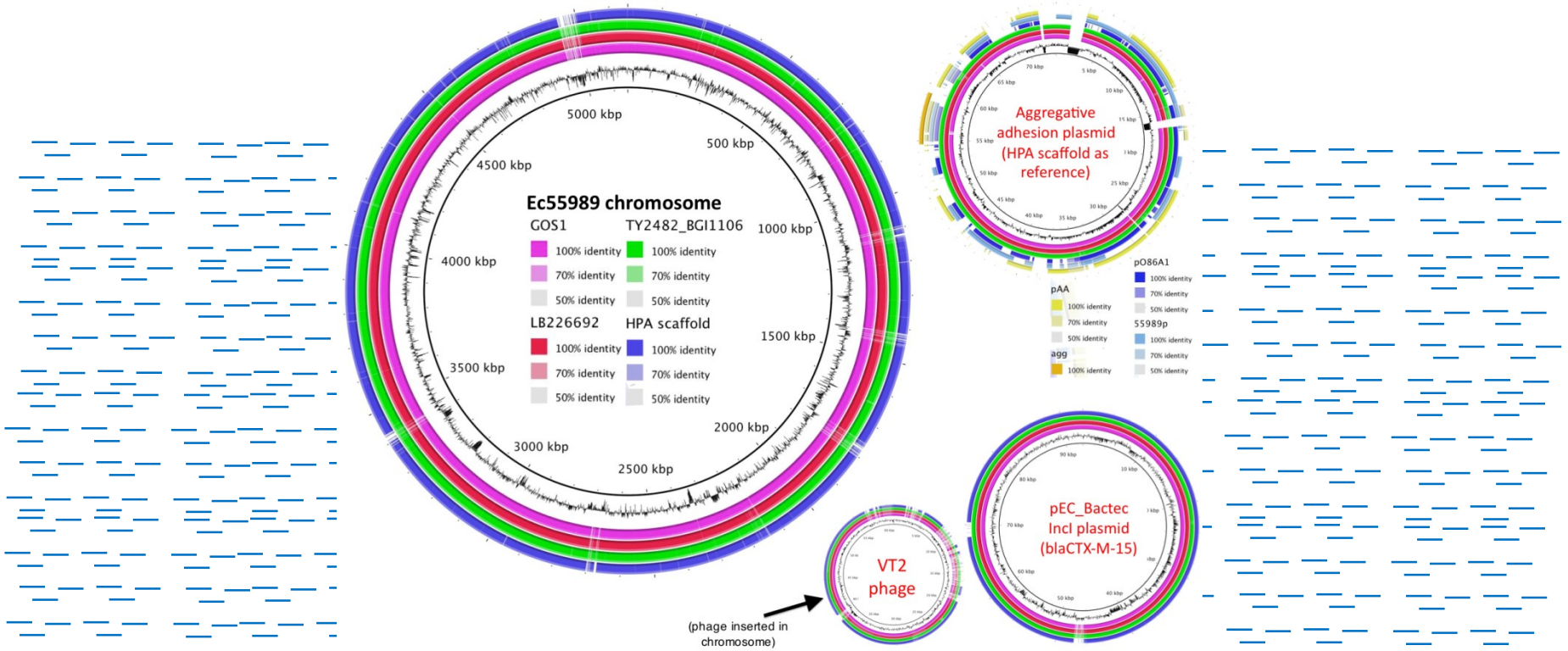
[redacted] | Scientific problems | Technical problems

Short reads vs. Long reads

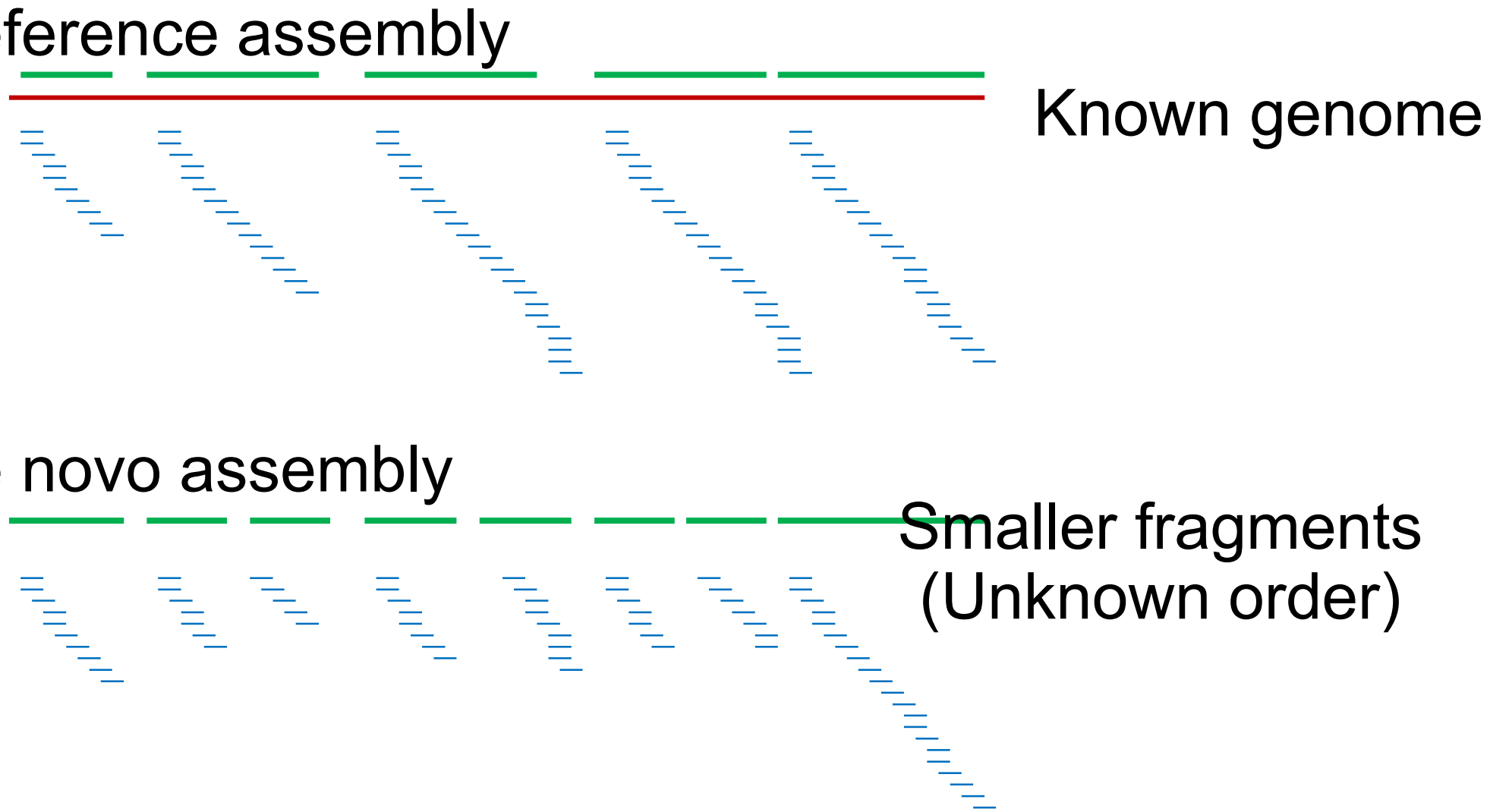


NGS output

Huge numbers of small fragments (35-500 bp)



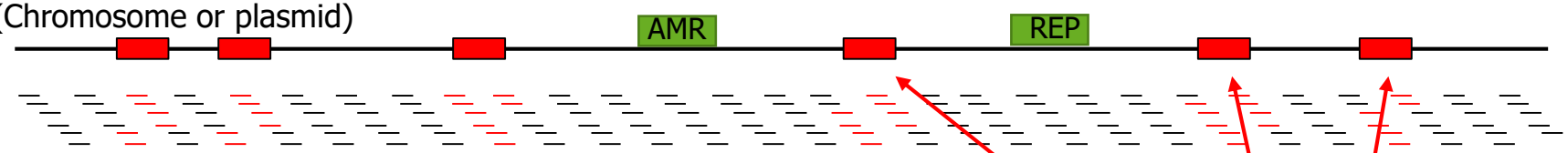
Reference vs. de novo assembly



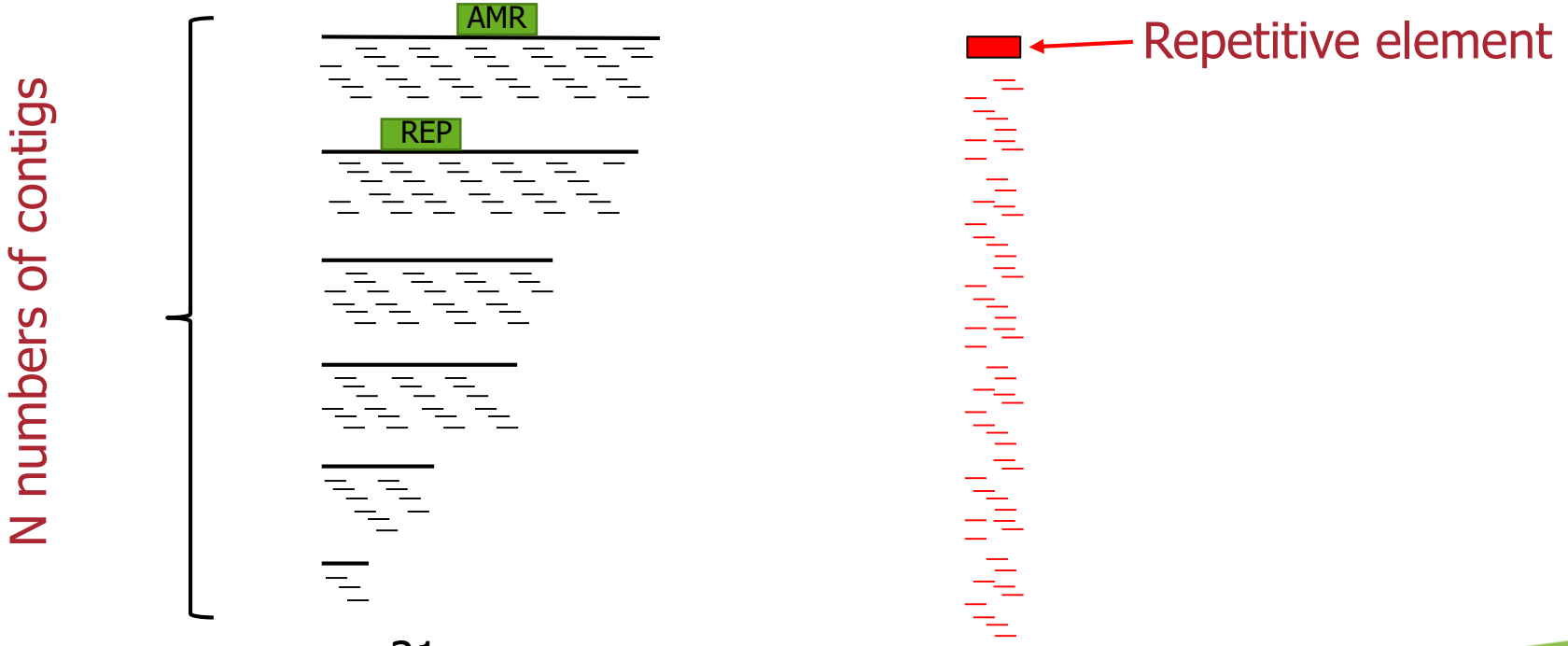
Illumina MiSeq system

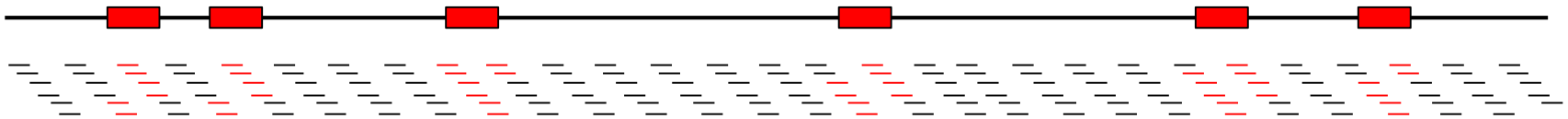
DNA

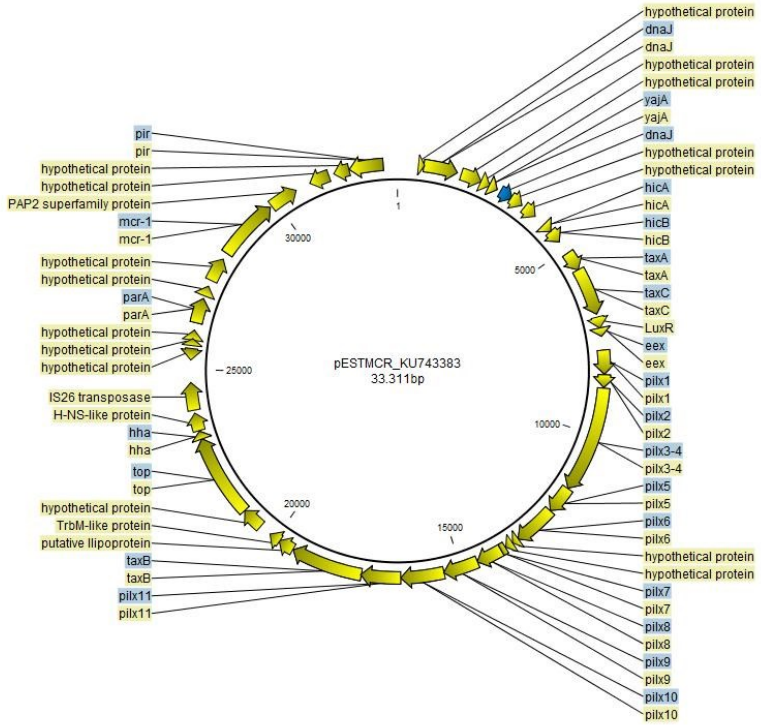
(Chromosome or plasmid)



De novo assembly






pESTMCR_KU743383
33.311bp
1
5000
10000
15000
20000
25000
30000
hypothetical protein
dnaJ
dnaJ
hypothetical protein
hypothetical protein
yajA
yajA
dnaJ
hypothetical protein
hypothetical protein
hicA
hicA
hicB
hicB
taxA
taxA
taxC
taxC
LuxR
eex
eex
pilx1
pilx1
pilx2
pilx2
pilx3-4
pilx3-4
pilx5
pilx5
pilx6
pilx6
hypothetical protein
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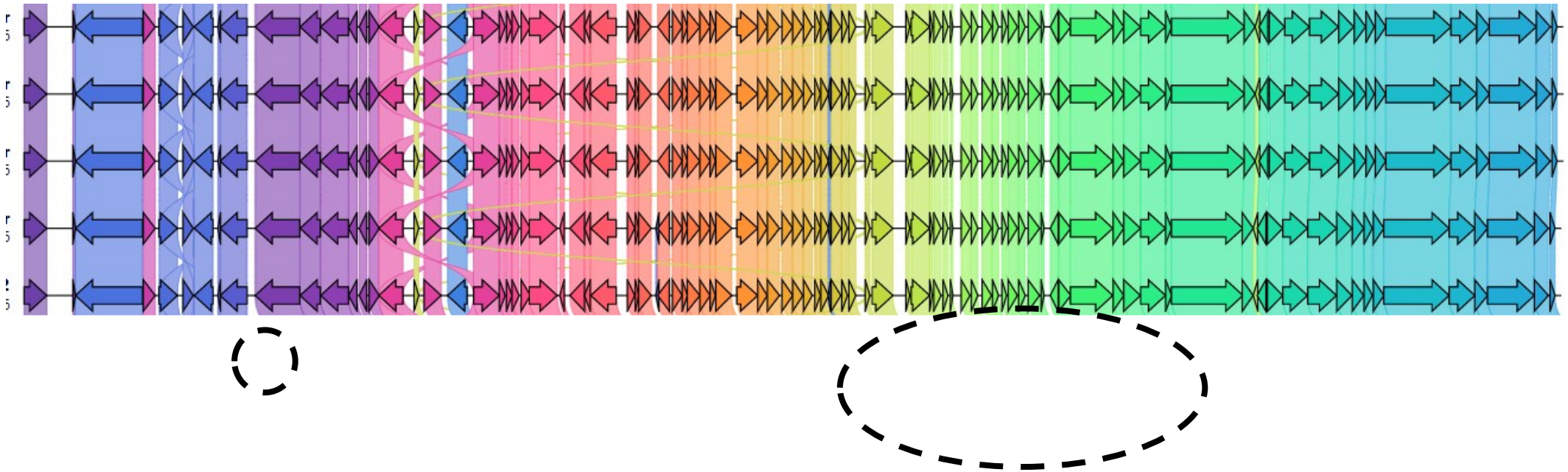
pilx9
pilx9
pilx10
pilx10
pir
pir
hypothetical protein
hypothetical protein
PAP2 superfamily protein
mcr-1
mcr-1
hypothetical protein
hypothetical protein
parA
parA
hypothetical protein
hypothetical protein
hypothetical protein
IS26 transposase
H-NS-like protein
hha
hha
top
top
hypothetical protein
TrbM-like protein
putative lipoprotein
taxB
taxB
pilx11
pilx11

Plasmid comparisons – Clinker tool



…BUT models (tools) for precise plasmid comparisons are lacking…

…as well as knowledge about the speed of plasmid recombination events.

In summary

List of learning points in this session:

In relation to Plasmids:

- Can be acquired or lost rapidly
- Some can be typed using PlasmidFinder

In relation to Plasmids in outbreak investigations:

- Analysing plasmid types may give added resolution in outbreak investigations
- But you have to be careful in your interpretations
- And consider to use long-read data if you want to combine AMR and plasmid analysis

Questions?

Thank’s for you attention

Acknowledgements

The creation of this training material was commissioned by ECDC to <Organisation1 (and organisation 2, if applicable)> with the direct involvement of <alphabetically ordered list of contributors>

The revision and update of this training material was commissioned by ECDC to <Organisation 3> with the direct involvement of <alphabetically ordered list of contributors>