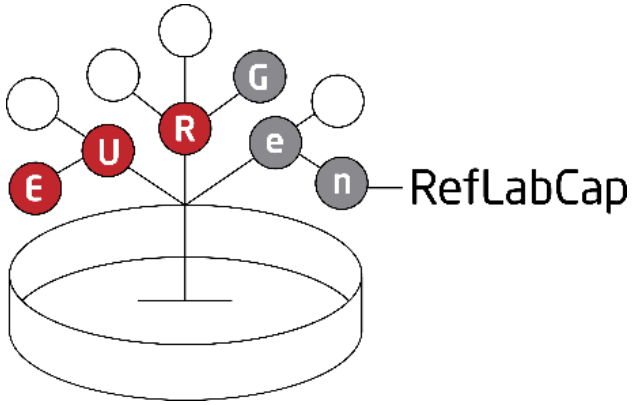
EURGen-RefLabCap

Technical training workshop # 1

Third day

Tuesday, 8 December 2022

09:00 – 15:30 CET



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Agenda

Third day (physical) – Thursday 8 December 2022, 9:00 - 15:30 CET

9:00 - 9:15: Re-cap and agenda for the day (Jette Sejer Kjeldgaard, DTU)

9:15 - 10:00: Discussion and conclusions of exercise about species identification and subtyping tools (Jette Sejer Kjeldgaard, DTU)

10:00 - 11:00: Exercise about bioinformatics tools for detection of antimicrobial resistance and mobile genetic elements (Markus Johansson, DTU)

11:00 - 11:15: Coffee break

11:15 - 12:00: Discussion and conclusions of exercise about antimicrobial resistance and mobile genetic elements (Markus Johansson, DTU)

12:00 - 12:30: Cluster methods (SNP-based and gene-by-gene) and tools, and the importance of metadata (Ana Rita Rebelo, DTU)

12:30 - 13:30: Lunch break

13:30 - 15:00: Exercise about cluster analysis (Ana Rita Rebelo and Jette Sejer Kjeldgaard, DTU)

15:00 - 15:30: Discussions, wrap-up, goodbye

[15:30: Bus transportation provided from DTU to the hotel]

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Jette Sejer Kjeldgaard
jetk@food.dtu.dk

Discussion and conclusions of exercise about species identification and subtyping tools

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Third day (physical) – Thursday 8 December 2022, 9:00 - 15:30 CET

[8:15: Bus transportation provided from the hotel to DTU]

9:00 - 9:15: Re-cap and agenda for the day (Jette Sejer Kjeldgaard, DTU)

9:15 - 10:00: Discussion and conclusions of exercise about species identification and serotyping tools (Jette Sejer Kjeldgaard, DTU)

10:00 - 11:00: Exercise about bioinformatics tools for detection of antimicrobial resistance and mobile genetic elements (Markus Johansson, DTU)

11:00 - 11:15: Coffee break

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Technical training workshop #1

7-8 December 2022

Jette Sejer Kjeldgaard, DTU

Exercises: tools for species identification and subtyping

What to do when we see discrepancy/inconclusive results?

- MLST's were fine and conclusive
- Serotype:

EURGEN_workshop_isolate_L	https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=6389198800007AFF423CC825	O36:H56 Low ID; 2 O-genes
EURGEN_workshop_isolate_M	https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=6389199500007B1FC7504B0D	O8:H21
EURGEN_workshop_isolate_N	https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=638919A700007BA6842D17CF	O15:H2 2 O-genes (wzx)

- Kmerfinder:

EURGEN_workshop_isolate_L	https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=638F2F3500005A020443E2FC	Escherichia mixed species
EURGEN_workshop_isolate_M	https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=638F2F4200005B4C49148683	Shigella + E. coli
EURGEN_workshop_isolate_N	https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=638F2F4A00005B791C3092ED	E. coli + Enterococcus

Check the sequence quality!

Quality control overview of 14 presumptive E. coli isolates sequenced by Illumina NextSeq

	QUALITY CONTROL REPORT										
ID FOR COURSE	Bases (MB)	Qual Bases (MB)	Qual bases %	Reads	Qual reads	Qual reads %	insert size	N50	No of contigs	longest contig	total bps in genome
EURGEN_ws_isl_A	663	562	84.73%	4713804	4285976	90.92%	207.85	78310	230	181289	5334643
EURGEN_ws_isl_B	568	409	71.99%	3867662	3140172	81.19%		75652	242	181289	5328985
EURGEN_ws_isl_C	679	493	72.70%	4711578	3827624	81.24%		73855	249	176983	5332638
EURGEN_ws_isl_D	582	414	71.14%	3975566	3199846	80.49%		73855	244	181289	5331209
EURGEN_ws_isl_E	258	185	71.59%	1758396	1419766	80.74%		71096	221	170559	5320663
EURGEN_ws_isl_F	391	283	72.45%	2704100	2198116	81.29%		71065	207	175874	5317604
EURGEN_ws_isl_G	383	307	80.34%	2592340	2301510	88.78%		180901	172	380185	5308247
EURGEN_ws_isl_H	335	266	79.30%	2286734	2013890	88.07%		203076	140	380185	5245654
EURGEN_ws_isl_I	833	714	85.63%	6210610	5648372	90.95%		203307	83	625654	5137317
EURGEN_ws_isl_J	590	527	89.33%	4032660	3756868	93.16%		251536	79	625838	5144977
EURGEN_ws_isl_K	777	642	82.71%	5374492	4818510	89.66%	231.64	217155	89	714995	5168842
EURGEN_ws_isl_L	275	231	84.05%	1976136	1797668	90.97%		226469	40	676641	4471461
EURGEN_ws_isl_M	145	121	84.04%	1061786	961774	90.58%		105253	143	306865	5040601
EURGEN_ws_isl_N	486	379	78.07%	3300292	2818786	85.41%		174166	222	350417	5448589

Overview of species determination of 14 presumptive E. coli isolates sequenced by Illumina NextSeq, including subtyping data (cgMLST, rMLST and MLST typing)

Course ID	rMLST output		KmerFinder output (simplified)			cgMLSTFinder output				MLST E. coli Scheme #1
Samples	Species	% of sup-port	% Query Coverage	% Template Coverage	Species	cgST	No of Alleles Found	Similarity	rMLST	MLST
EURGEN_ws_isl_A	E. coli	100%	92.11	96.49	E. coli	**36272**	2412	95.98	**1587**	**33**
EURGEN_ws_isl_B	E. coli	100%	92.15	96.46	E. coli	**36272**	2409	95.86	**1587**	**33**
EURGEN_ws_isl_C	E. coli	100%	92.14	96.49	E. coli	**36272**	2408	95.82	**1587**	**33**
EURGEN_ws_isl_D	E. coli	100%	92.14	96.49	E. coli	**36272**	2409	95.86	**1587**	**33**
EURGEN_ws_isl_E	E. coli	100%	92.14	96.51	E. coli	**36272**	2407	95.78	**1587**	**33**
EURGEN_ws_isl_F	E. coli	100%	92.15	96.46	E. coli	**36272**	2409	95.86	**1587**	**33**
EURGEN_ws_isl_G	E. coli	100%	91.17	95.71	E. coli	**9819**	2464	98.05	**14753**	**73**
EURGEN_ws_isl_H	E. coli	100%	92.3	95.74	E. coli	**36**	2464	98.05	**14753**	**73**
EURGEN_ws_isl_I	E. coli	100%	96.59	95.2	E. coli	**3785**	2421	96.34	**2153**	**968**
EURGEN_ws_isl_J	E. coli	100%	96.46	95.19	E. coli	**3785**	2423	96.42	**2153**	**968**
EURGEN_ws_isl_K	E. coli	100%	97.46	99.71	E. coli	**10134**	2466	98.13	**1503**	**131**
EURGEN_ws_isl_L	E. ruysiae	100%	31.85	26.22	E. coli	**55765**	2312	92.00	**64744**	**3568**
EURGEN_ws_isl_M	E. coli	98%	89.57	97.09	Shigella sonnei	**31173**	2350	93.51	**ND***	**155**
EURGEN_ws_isl_N	E. coli / Enterococcus faecalis	88% / 11%	88	93.91	E. coli	**41724**	2473	98.41	**2135**	**69**

ND*: no matching profile for rMLST

Isolate L

	QUALITY CONTROL REPORT										
ID FOR COURSE	Bases (MB)	Qual Bases (MB)	Qual bases %	Reads	Qual reads	Qual reads %	insert size	N50	No of contigs	longest contig	total bps in genome
EURGEN_ws_isl_L	275	231	84.05%	1976136	1797668	90.97%		226469	40	676641	4471461
EURGEN_ws_isl_M	145	121	84.04%	1061786	961774	90.58%		105253	143	306865	5040601
EURGEN_ws_isl_N	486	379	78.07%	3300292	2818786	85.41%		174166	222	350417	5448589

L:

N50 is OK

Longest contig is OK

# of contigs surprisingly low

total bases also low

DTU

KmerFinder-3.2 Server - Results

KmerFinder 3.2 results:

Template	Num	Score	Expected	Template_length	Query_Coverage	Template_Coverage	Depth	tot_query_Coverage	tot_template_Coverage	tot_depth	q_value	p_value
NZ_CP015229.1 Escherichia coli strain 06-00048 chromosome, complete genome	8022	46424	26	175299	31.85	26.22	0.25	31.85	26.22	0.25	46344.96	1.0e-26
NZ_CP056159.1 Escherichia marmotae strain RHBSTW-00814 chromosome, complete genome	1993	17722	35	160343	12.16	11.02	0.11	28.69	25.80	0.25	17616.45	1.0e-26
NZ_CP014111.1 Escherichia coli strain FDAARGOS_144 chromosome, complete genome	23581	4902	39	157299	3.36	3.09	0.03	29.11	26.68	0.27	4784.77	1.0e-26
NZ_CP099906.1 Escherichia albertii strain 121_1_EW_A chromosome, complete genome	13469	3864	39	154276	2.65	2.50	0.02	19.29	17.97	0.18	3748.19	1.0e-26
NZ_CP027579.1 Escherichia coli strain 2013C-4282 chromosome, complete genome	10045	2424	42	164029	1.66	1.48	0.01	28.17	24.77	0.25	2300.37	1.0e-26

EXTENDED OUTPUT

Input Files: *EURGEN_workshop_isolate_L.fa*

3 different *Escherichia* species?

? Contamination?

rMLST output

Predicted taxa

Rank	Taxon	Support	Taxonomy
SPECIES	Escherichia ruysiae	100%	*Proteobacteria* > *Gammaproteobacteria* > *Enterobacterales* > *Enterobacteriaceae* > *Escherichia* > *Escherichia ruysiae*

Ribosomal MLST

Matching profile

rST: 64744

genus: Escherichia/Shigella

species: Escherichia/Shigella sp.

A fourth *Escherichia* species? ? ?

Consult more analysis tools?

SpeciesFinder-2.0Server - Results

Species	Match	Confidence of result
Citrobacter freu		

Input Files: *EURGEN*

FAIL!

SpeciesFinder-2.0Server - Results

Species	Match	Confidence of result
r freundii	NGRE01000001	FAIL

SpeciesFinder-2.0Server *URGEN_workshop_isolate_A.fa*

Species
Citrobacter freundii

Input Files: *EURGEN_workshop_isolate_M.fa*

SpeciesFinder-2.0Server - Results

Species	Match	Confidence of result
Citrobacter freundii	NGRE01000001	FAIL

Input Files: *EURGEN_workshop_isolate_N.fa*

rMLST output

Predicted taxa

Rank	Taxon	Support	Taxonomy
SPECIES	Escherichia ruysiae	100%	*Proteobacteria* > *Gammaproteobacteria* > *Enterobacterales* > *Enterobacteriaceae* > *Escherichia* > *Escherichia ruysiae*

Ribosomal MLST

Matching profile

rST: 64744

genus: Escherichia/Shigella

species: Escherichia/Shigella sp.

A fourth *Escherichia* species? ? ?

Isolate L

- Conclusion?

How do you proceed with this isolate in the lab?

Can you use the sequence for e.g. resistance profiling?

Isolate M

	QUALITY CONTROL REPORT										
ID FOR COURSE	Bases (MB)	Qual Bases (MB)	Qual bases %	Reads	Qual reads	Qual reads %	insert size	N50	No of contigs	longest contig	total bps in genome
EURGEN_ws_isl_L	275	231	84.05%	1976136	1797668	90.97%		226469	40	676641	4471461
EURGEN_ws_isl_M	145	121	84.04%	1061786	961774	90.58%		105253	143	306865	5040601
EURGEN_ws_isl_N	486	379	78.07%	3300292	2818786	85.41%		174166	222	350417	5448589

M: QC looks fine!

KmerFinder: *Shigella sonnei*
rMLST: *E. coli*

MLST was conclusive
Serotype was conclusive

KmerFinder result

KmerFinder-3.2 Server - Results

KmerFinder 3.2 results:

Template	Num	Score	Expected	Template_length	Query_Coverage	Template_Coverage	Depth	tot_query_Coverage	tot_tem
NZ_CP055292.1 Shigella sonnei strain SE6-1 chromosome, complete genome	2810	149067	6	156657	89.57	97.09	0.95	89.57	97.09
NZ_CP010132.1 Escherichia coli strain C10, complete genome	26013	7645	62	165036	4.59	4.42	0.05	80.22	81.73
NZ_CP016546.1 Escherichia coli strain O177:H21 chromosome, complete genome	4209	3092	64	170275	1.86	1.85	0.02	80.70	81.35

EXTENDED OUTPUT

rMLST result

rMLST output:

Predicted taxa

Rank	Taxon	Support	Taxonomy
SPECIES	Escherichia coli	98%	*Proteobacteria* > *Gammaproteobacteria* > *Enterobacterales* > *Enterobacteriaceae* > *Escherichia* > *Escherichia coli*

No rMLST number obtained

Only exact matches are shown above. If a locus does not have an exact match, try querying specifically against that locus to find the closest match.

***Shigella spp.* & *Escherichia coli* are very similar!**

Mini review

Accurate differentiation of *Escherichia coli* and *Shigella* serogroups: challenges and strategies, https://doi.org/10.1016/j.nmni.2017.09.003

Isolate M

- Conclusion?

How do you proceed with this isolate in the lab?

Can you use the sequence for e.g. resistance profiling?

Isolate N

	QUALITY CONTROL REPORT										
ID FOR COURSE	Bases (MB)	Qual Bases (MB)	Qual bases %	Reads	Qual reads	Qual reads %	insert size	N50	No of contigs	longest contig	total bps in genome
EURGEN_ws_isl_L	275	231	84.05%	1976136	1797668	90.97%		226469	40	676641	4471461
EURGEN_ws_isl_M	145	121	84.04%	1061786	961774	90.58%		105253	143	306865	5040601
EURGEN_ws_isl_N	486	379	78.07%	3300292	2818786	85.41%		174166	222	350417	5448589

N:

QC is fine

A bit high on total bps?

KmerFinder Result

KmerFinder-3.2 Server - Results

KmerFinder 3.2 results:

Template	Num	Score	Expected	Template_length	Query_Coverage	Template_Coverage	Depth	tot_query_Coverage	tot_template_Coverage	tot_depth	q_value
NZ_CP066836.1 Escherichia coli strain Ec-FL1-1X chromosome, complete genome	19396	159441	6	171412	88.00	93.91	0.93	88.00	93.91	0.93	159420.57
NZ_AP022362.1 Escherichia coli strain E302 chromosome, complete genome	12294	4477	70	173594	2.47	2.34	0.03	53.05	55.76	0.55	4271.09
NZ_CP069996.1 Escherichia coli strain FDAARGOS_1300 chromosome, complete genome	1871	2784	70	172419	1.54	1.65	0.02	53.96	57.12	0.57	2579.04
NZ_CP090558.1 Enterococcus faecalis strain PCH555 chromosome, complete genome	2966	994	40	95697	0.55	1.05	0.01	0.56	1.07	0.01	879.44
NZ_CP035755.1 Escherichia coli E110019 extrachromosomal	16134	40	0	864	0.02	4.63	0.05	0.21	43.63	0.44	38.91

rMLST output

rMLST output:

Predicted taxa

Rank	Taxon	Support	Taxonomy
SPECIES	Escherichia coli	88%	*Proteobacteria > Gammaproteobacteria > Enterobacterales > Enterobacteriaceae > Escherichia > Escherichia coli*
SPECIES	Enterococcus faecalis	11%	*Firmicutes > Bacilli > Lactobacillales > Enterococcaceae > Enterococcus > Enterococcus faecalis*

Ribosomal MLST

Matching profile

rST: 2135
genus: Escherichia
species: Escherichia coli

Isolate N

- Conclusion?

How do you proceed with this isolate in the lab?

Can you use the sequence for e.g. resistance profiling?

ResFinder result

- Reliable?

ResFinder-4.1 Server - Results

Input Files: *EURGEN_workshop_isolate_N.fa*

Warning:
One or more resistance genes does not exist in the phenotype database. The Summary table does not take this into account.

escherichia coli complete

Antimicrobial	Class	WGS-predicted phenotype	Genetic background
amikacin	aminoglycoside	No resistance	
tigecycline	tetracycline	No resistance	
tobramycin	aminoglycoside	No resistance	
cefepime	beta-lactam	No resistance	
chloramphenicol	amphenicol	No resistance	
piperacillin+tazobactam	beta-lactam	No resistance	
cefoxitin	beta-lactam	No resistance	
ampicillin	beta-lactam	Resistant	blaTEM-1C (blaTEM-1C_FJ560503)
ampicillin+clavulanic acid	beta-lactam	No resistance	
cefotaxime	beta-lactam	No resistance	
ciprofloxacin	quinolone	No resistance	
colistin	polymyxin	No resistance	
sulfamethoxazole	folate pathway antagonist	Resistant	sul1 (sul1_U12338)
imipenem	beta-lactam	No resistance	
trimethoprim	folate pathway antagonist	Resistant	dfrA12 (dfrA12_AM040708)
nalidixic acid	quinolone	No resistance	
ertapenem	beta-lactam	No resistance	
tetracycline	tetracycline	Resistant	tet(B) (tet(B)_AF326777)
fosfomycin	fosfomycin	No resistance	
ceftazidime	beta-lactam	No resistance	
temocillin	beta-lactam	No resistance	
gentamicin	aminoglycoside	No resistance	
meropenem	beta-lactam	No resistance	
azithromycin	macrolide	Resistant	mph(A) (mph(A)_D16251)

Markus Johansson, DTU

Exercise about bioinformatics tools for detection of antimicrobial resistance and mobile genetic elements

Funded by the European Union

Detection of antibiotic resistance and estimation of resistance gene mobility

Markus Johansson
markjo@food.dtu.dk

Today

- Prediction of antibiotic resistance genes with ResFinder
  - ResFinder tool
  - ResFinder database
  - PointFinder database

- Estimation of resistance gene mobility using PlasmidFinder and MobileElementFinder
  - Introduction to Mobile Genetic Elements
  - PlasmidFinder tool
  - MobileElementFinder tool

About ResFinder v4.1

ResFinder tool

Detects presence of resistance genes and mutations and annotates expected phenotype

Two versions

- Online tool, https://cge.food.dtu.dk/services/ResFinder/
- Command line, https://bitbucket.org/genomicepidemiology/resfinder/src/master/

Input

- Raw reads or assembled contigs

Reference based prediction against the databases

- Resfinder db, https://bitbucket.org/genomicepidemiology/resfinder_db/src/master/
- Pointfinder db, https://bitbucket.org/genomicepidemiology/pointfinder_db/src/master/

ResfinderFinder database

- Resistance gene sequences grouped by per antibiotic class
- Phenotypic annotations in *phenotypes.txt*
- Description of genes in bacterial panels in *phenotype_panels.txt*
- Changes to the database in *history.txt*

Name	Size	Last commit	Message
.gitignore	11 B	2022-06-16	Update gitignore file
CHECK-entries.sh	2.33 KB	2019-01-23	CHECK-entries: make sure to escape regex chars
INSTALL.py	5.34 KB	2022-07-19	change str format to work with python version on server.
README.md	5.37 KB	2021-04-20	Added hsitory file to content overview
VERSION	6 B	2022-10-13	Add VERSION file
aminoglycoside.fsa	196.45 KB	2022-10-26	fix amr class of bleO
antibiotic_classes.txt	2.51 KB	2022-10-26	fix amr class of bleO
beta-lactam.fsa	1.78 MB	2022-05-03	Adds genes blaGMB-1 and tet(O/32/O)
colistin.fsa	89.96 KB	2022-10-24	remove mcr-9_1_NZ_NAAN01000063.1 from colistin.fsa
config	900 B	2022-09-06	Merge branch 'add_misc_class'
fosfomycin.fsa	18.68 KB	2021-03-11	added gar1,fosl1,erm50,qnrb89,catt,qnrb91,aac6,qnrb90,mcr126,mcr127
fusidicacid.fsa	1.96 KB	2019-02-20	Update fusidic acid db
glycopeptide.fsa	94.75 KB	2022-10-26	fix amr class of bleO
history.txt	39.89 KB	2022-10-24	remove mcr-9_1_NZ_NAAN01000063.1 from colistin.fsa
macrolide.fsa	170.44 KB	4 days ago	remove 5 duplicate genes (crf, aac(6')-Ib-cr)
misc.fsa	2.51 KB	2022-06-27	fix header and naming related to Misc and Ionophores
nitroimidazole.fsa	6.81 KB	2018-05-24	Reformat fosfomycin, fusidic acid and nitroimidazole db
notes.txt	88.39 KB	2022-10-26	fix amr class of bleO
oxazolidinone.fsa	42.76 KB	4 days ago	remove 5 duplicate genes (crf, aac(6')-Ib-cr)
phenicol.fsa	43.52 KB	2021-03-11	added gar1,fosl1,erm50,qnrb89,catt,qnrb91,aac6,qnrb90,mcr126,mcr127
phenotype_panels.txt	2.55 KB	2021-10-06	changed rifampin for rifampicin phenotype_panels
phenotypes.txt	501.73 KB	2022-10-26	fix amr class of bleO
pseudomonicacid.fsa	9.21 KB	2021-03-09	added aac(3)-IIa_6_CP023555, blaCMY-150_2_NG_060513, blaCARB-4_1_U14749, mupA_1_X75439, mupA_2_GU237136, mu...

ResFinder database – Phenotype annotations

Reference gene

Associated resistance and resistance class

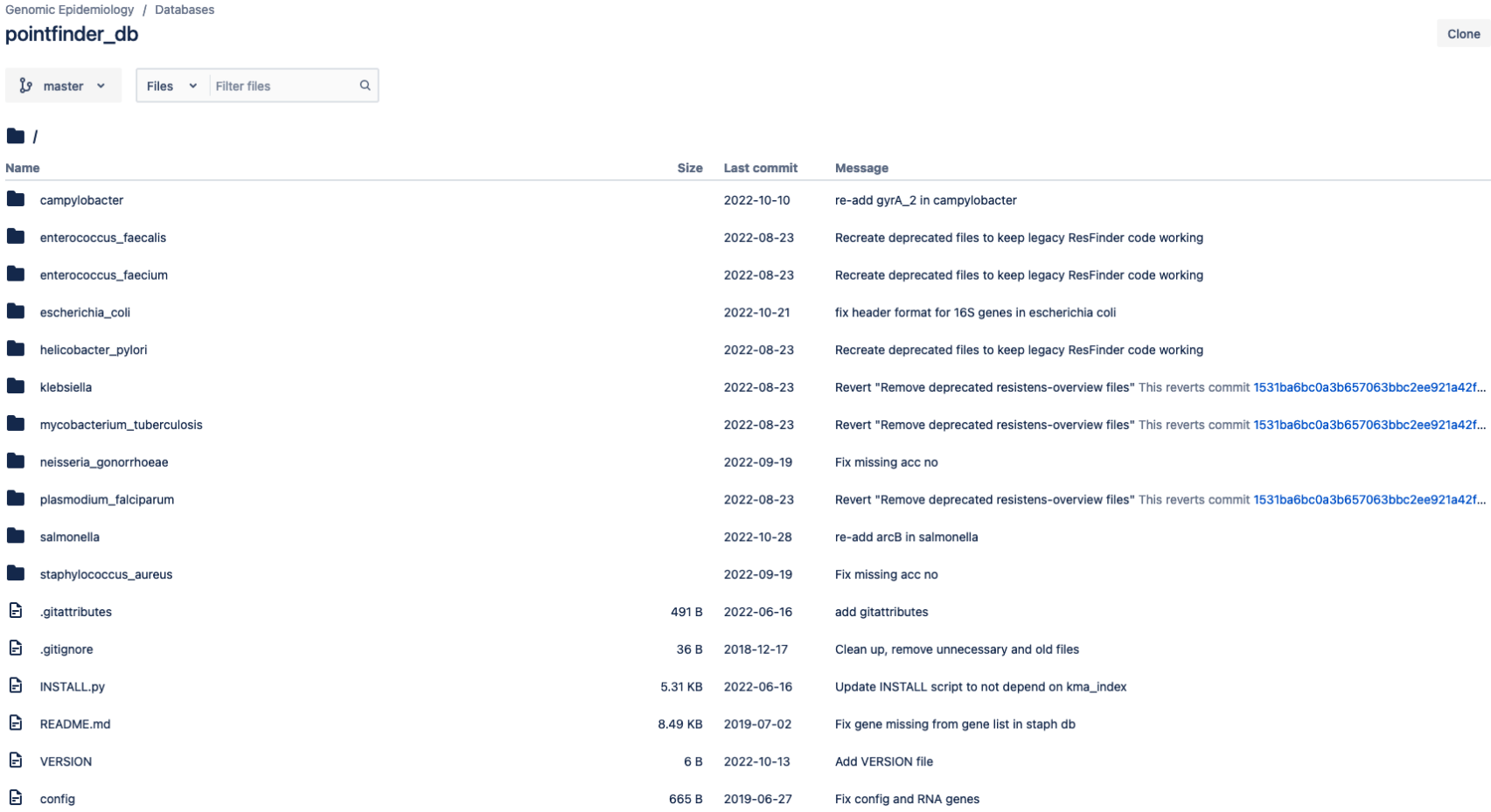
resfinder_db / **phenotypes.txt**

	Gene_accession no.	Class	Phenotype	PMID	Mechanism of resistance	Notes	Required_gene
2	ant(2'')-Ia_1_X04555	Aminoglycoside	Gentamicin, Tobramycin	3024112	Enzymatic modification	Alternative name aadB	
3	ant(2'')-Ia_10_HM367617	Aminoglycoside	Gentamicin, Tobramycin	21873033	Enzymatic modification		
4	ant(2'')-Ia_11_HM367620	Aminoglycoside	Gentamicin, Tobramycin	21873033	Enzymatic modification		
5	ant(2'')-Ia_12_HQ880250	Aminoglycoside	Gentamicin, Tobramycin	unpublished	Enzymatic modification		
6	ant(2'')-Ia_13_DQ176450	Aminoglycoside	Gentamicin, Tobramycin	16304199	Enzymatic modification		
7	ant(2'')-Ia_14_DQ266447	Aminoglycoside	Gentamicin, Tobramycin	unpublished	Enzymatic modification		
8	ant(2'')-Ia_15_EF205594	Aminoglycoside	Gentamicin, Tobramycin	unpublished	Enzymatic modification		
9	ant(2'')-Ia_16_HQ386848	Aminoglycoside	Gentamicin, Tobramycin	unpublished	Enzymatic modification		
10	ant(2'')-Ia_17_JTTZ0100034	Aminoglycoside	Gentamicin, Tobramycin	unpublished	Enzymatic modification		
11	ant(2'')-Ia_19_GQ466184	Aminoglycoside	Gentamicin, Tobramycin	unpublished	Enzymatic modification		
12	ant(2'')-Ia_2_JF826500	Aminoglycoside	Gentamicin, Tobramycin	22271862	Enzymatic modification		
13	ant(2'')-Ia_20_AY139599	Aminoglycoside	Gentamicin, Tobramycin	19719593	Enzymatic modification		
14	ant(2'')-Ia_3_X74412	Aminoglycoside	Gentamicin, Tobramycin	unpublished	Enzymatic modification		
15	ant(2'')-Ia_4_AF458082	Aminoglycoside	Gentamicin, Tobramycin	12384364	Enzymatic modification		
16	ant(2'')-Ia_5_AY139594	Aminoglycoside	Gentamicin, Tobramycin	19719593	Enzymatic modification		
17	ant(2'')-Ia_6_AJ871915	Aminoglycoside	Gentamicin, Tobramycin	unpublished	Enzymatic modification		
18	ant(2'')-Ia_7_DQ018384	Aminoglycoside	Gentamicin, Tobramycin	15837385	Enzymatic modification		
19	ant(2'')-Ia_8_AY920928	Aminoglycoside	Gentamicin, Tobramycin	16048994	Enzymatic modification		
20	ant(2'')-Ia_9_HM367610	Aminoglycoside	Gentamicin, Tobramycin	21873033	Enzymatic modification		

PointFinder database

Organized by bacteria

- Each bacteria contain gene reference sequences
- Phenotypic information in *phenotypes.txt*



Genomic Epidemiology / Databases

pointfinder_db

Clone

master | Files | Filter files

/

Name	Size	Last commit	Message
campylobacter		2022-10-10	re-add gyrA_2 in campylobacter
enterococcus_faecalis		2022-08-23	Recreate deprecated files to keep legacy ResFinder code working
enterococcus_faecium		2022-08-23	Recreate deprecated files to keep legacy ResFinder code working
escherichia_coli		2022-10-21	fix header format for 16S genes in escherichia coli
helicobacter_pylori		2022-08-23	Recreate deprecated files to keep legacy ResFinder code working
klebsiella		2022-08-23	Revert "Remove deprecated resistens-overview files" This reverts commit 1531ba6bc0a3b657063bbc2ee921a42f...
mycobacterium_tuberculosis		2022-08-23	Revert "Remove deprecated resistens-overview files" This reverts commit 1531ba6bc0a3b657063bbc2ee921a42f...
neisseria_gonorrhoeae		2022-09-19	Fix missing acc no
plasmodium_falciparum		2022-08-23	Revert "Remove deprecated resistens-overview files" This reverts commit 1531ba6bc0a3b657063bbc2ee921a42f...
salmonella		2022-10-28	re-add arcB in salmonella
staphylococcus_aureus		2022-09-19	Fix missing acc no
.gitattributes	491 B	2022-06-16	add gitattributes
.gitignore	36 B	2018-12-17	Clean up, remove unnecessary and old files
INSTALL.py	5.31 KB	2022-06-16	Update INSTALL script to not depend on kma_index
README.md	8.49 KB	2019-07-02	Fix gene missing from gene list in staph db
VERSION	6 B	2022-10-13	Add VERSION file
config	665 B	2019-06-27	Fix config and RNA genes

PointFinder database – Phenotype annotations

Reference gene, mutation, position, ref nucleotide

Associated resistance and resistance class

pointfinder_db / escherichia_coli / **phenotypes.txt**

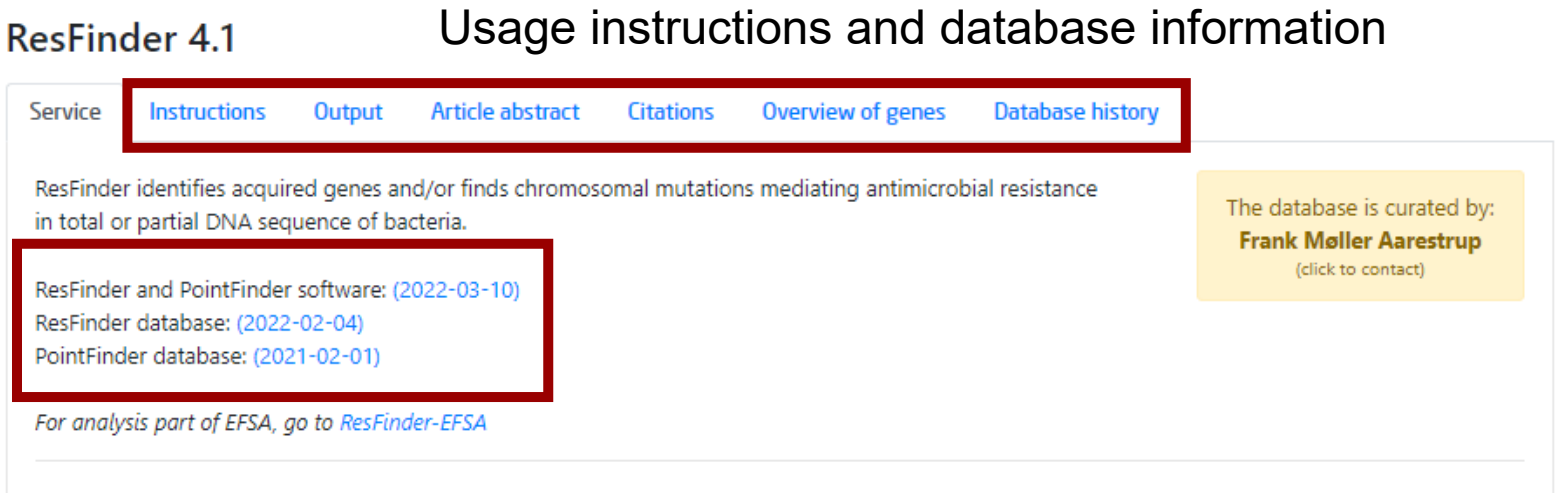
	#Gene_accession	TypeGene	Mutation	ID	Codon_pos	Ref_nuc	Ref_codon	Res_codon	Class	Phenotype	PMID	Mechanism of resistance	Notes	Required mutation	% abundance mutation required
2	gyrA_1_CP073768.1	DNA	ALA-51	51	GCC	A	V		Quinolone	Nalidixic acid	11451702	Target modification			
3	gyrA_1_CP073768.1	DNA	ALA-67	67	GCC	A	S		Quinolone	Nalidixic acid	2168148	Target modification			
4	gyrA_1_CP073768.1	DNA	GLY-81	81	GGT	G	C,D		Quinolone	Ciprofloxacin	2168148,8392306	Target modification	G81D mutation confers resistance to ciprofloxacin only. If G81D and D82G are present		
5	gyrA_1_CP073768.1	DNA	GLY-81	81	GGT	G	C,D		Quinolone	Nalidixic acid,Ciprofloxacin	2168148,8392306	Target modification	G81D mutation confers resistance to ciprofloxacin only. If G81D and		
6	gyrA_1_CP073768.1	DNA	ASP-82	82	GAC	D	G		Quinolone	Ciprofloxacin	8980760	Target modification	D82G mutation confers resistance to ciprofloxacin only. If D82G and G81D are present simulta		
7	gyrA_1_CP073768.1	DNA	ASP-82	82	GAC	D	G		Quinolone	Nalidixic acid,Ciprofloxacin	8980760	Target modification	D82G mutation confers resistance to ciprofloxacin only. If D82G and G81D are		
8	gyrA_1_CP073768.1	DNA	SER-83	83	TCG	S	L,W,A,V		Quinolone	Nalidixic acid,Ciprofloxacin	8891148,2168148,12654733,12654733	Target modification			
9	gyrA_1_CP073768.1	DNA	ALA-84	84	GCG	A	P,V		Quinolone	Nalidixic acid,Ciprofloxacin	11451702,7840592	Target modification	Unknown phenotype if A84P or A84V occur alone. Nalidixic acid ar		
10	gyrA_1_CP073768.1	DNA	ASP-87	87	GAC	D	N,G,Y,A,H,V		Quinolone	Nalidixic acid	12654733,12654733,12654733,22878251,12654733,1850972	Target modification	D87G or D87Y confer resistance to na		
11	gyrA_1_CP073768.1	DNA	ASP-87	87	GAC	D	N,G,Y,A,H,V		Quinolone	Nalidixic acid,Ciprofloxacin	12654733,12654733,12654733,22878251,12654733,1850972	Target modification	D87G or D87Y confer		
12	gyrA_1_CP073768.1	DNA	GLN-106	106	CAG	Q	H,R		Quinolone	Nalidixic acid,Ciprofloxacin	2168148,1850970	Target modification	Unknown phenotype if Q106H occurs alone. Nalidixic acid and ciproflo		
13	gyrA_1_CP073768.1	DNA	ALA-196	196	GCG	A	E		Quinolone	Nalidixic acid,Ciprofloxacin	14506034	Target modification	Unknown phenotype if A196E occurs alone. Nalidixic acid and ciprofloxaci		
14	gyrB_1_CP047010.1	DNA	ASP-426	426	GAC	D	N		Quinolone	Nalidixic acid,Ciprofloxacin	1656869	Target modification			
15	gyrB_1_CP047010.1	DNA	LYS-447	447	AAG	K	E		Quinolone	Nalidixic acid	1656869	Target modification			
16	parC_1_CP084529.1	DNA	ALA-56	56	GCC	A	T		Quinolone	Nalidixic acid,Ciprofloxacin	12654733	Target modification			

Using ResFinder

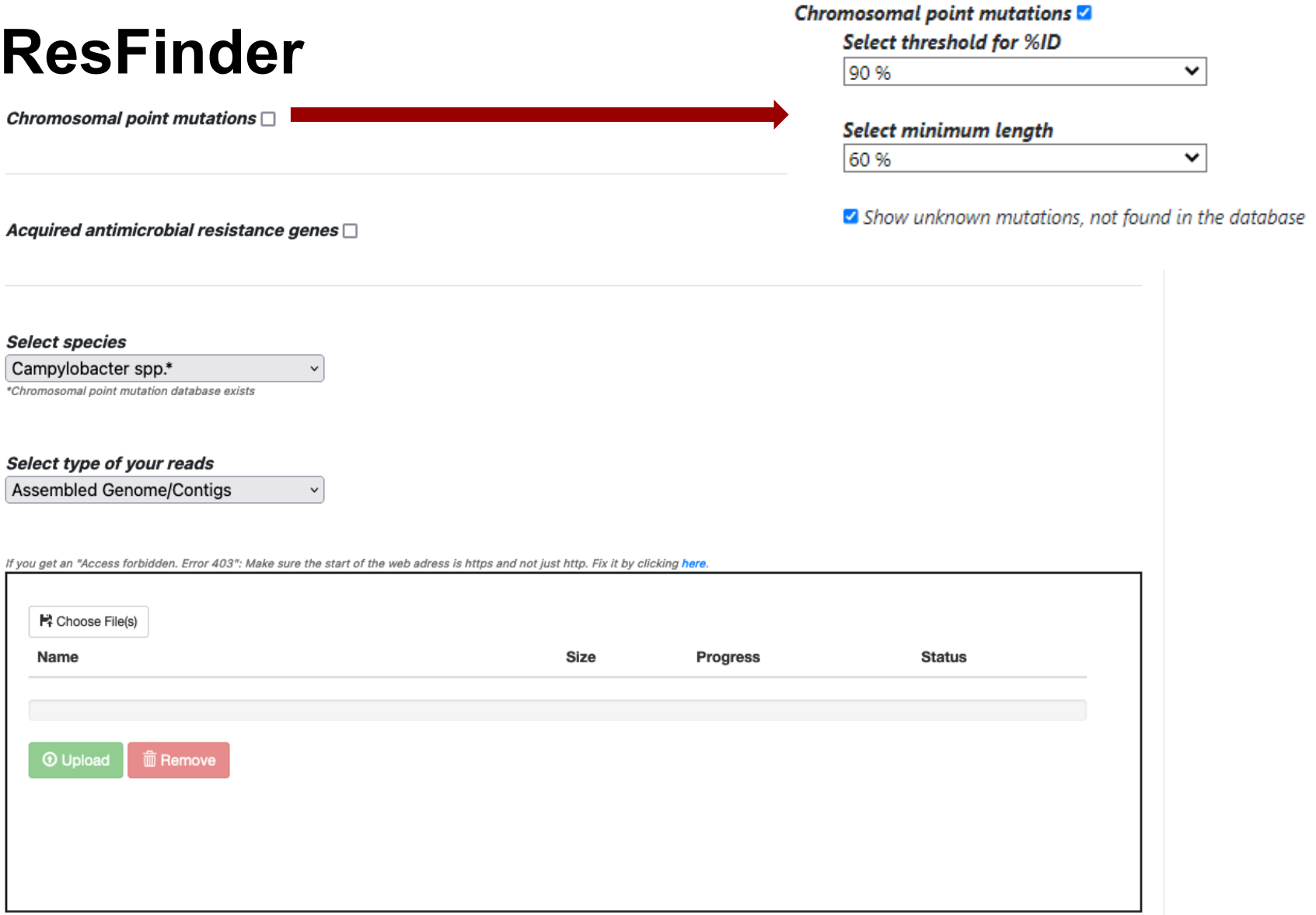
https://cge.food.dtu.dk/services/ResFinder/

Usage instructions and database information

Version information of tool and database



Use ResFinder

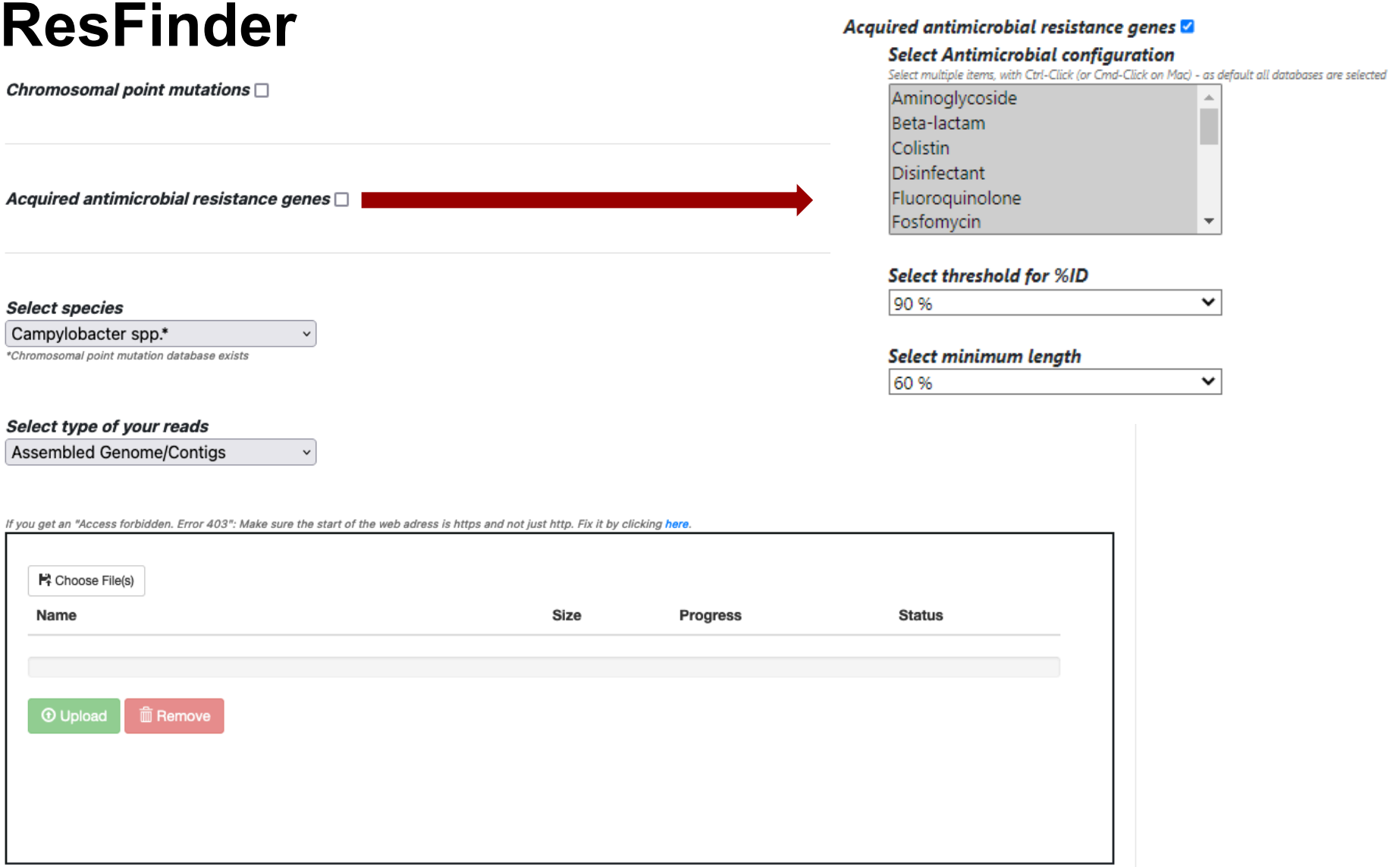


Use ResFinder

Selection of specie specific panel

Input data type

Upload a genome for analysis



ResFinder result – Summary table

Gene panel specific result

Other

escherichia coli complete

Antimicrobial	Class	WGS-predicted phenotype	Genetic background
amikacin	aminoglycoside	No resistance	
tigecycline	tetracycline	No resistance	
tobramycin	aminoglycoside	No resistance	
cefepime	beta-lactam	No resistance	
chloramphenicol	amphenicol	No resistance	
piperacillin+tazobactam	beta-lactam	No resistance	
cefoxitin	beta-lactam	No resistance	
ampicillin	beta-lactam	Resistant	blaTEM-1B (blaTEM-1B_AY458016)
ampicillin+clavulanic acid	beta-lactam	No resistance	
cefotaxime	beta-lactam	No resistance	
ciprofloxacin	quinolone	No resistance	
colistin	polymyxin	No resistance	
sulfamethoxazole	folate pathway antagonist	No resistance	
imipenem	beta-lactam	No resistance	
trimethoprim	folate pathway antagonist	No resistance	
nalidixic acid	quinolone	No resistance	
ertapenem	beta-lactam	No resistance	
tetracycline	tetracycline	Resistant	tet(B) (tet(B)_AF326777)
fosfomycin	fosfomycin	No resistance	
ceftazidime	beta-lactam	No resistance	
temocillin	beta-lactam	No resistance	
gentamicin	aminoglycoside	No resistance	
meropenem	beta-lactam	No resistance	
azithromycin	macrolide	No resistance	

Antimicrobial	Class	WGS-predicted phenotype	Genetic background
vancomycin	glycopeptide	No resistance	
mupirocin	pseudomonic acid	No resistance	
tobramycin	aminoglycoside	No resistance	
hygromycin	aminoglycoside	No resistance	
isepamicin	aminoglycoside	No resistance	
virginiamycin s	streptogramin b	No resistance	
hydrogen peroxide	peroxide	Resistant	sitABCD (sitABCD_AY598030)
butirosin	aminoglycoside	No resistance	
ampicillin	beta-lactam	Resistant	blaTEM-1B (blaTEM-1B_AY458016)
astromicin	aminoglycoside	No resistance	

ResFinder result – full result

Alignment quality

Positional information

Predicted phenotype

Resistance genes

Mutations

Peroxide									
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig	Phenotype	PMID	Accession no.	Notes
sitABCD	97.2246313964	3459/3459	1..3459	NODE_45_length_13731_cov_5.452293	7368..10817	hydrogen peroxide	16514154	AY598030	

Tetracycline									
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig	Phenotype	PMID	Accession no.	Notes
tet(B)	100.0	1206/1206	1..1206	NODE_64_length_4666_cov_3.854153	1334..2539	doxycycline,tetracycline,minocycline	11553538	AF326777	

No class defined					
Mutation	Nucleotide change	Amino acid change	Phenotype	PMID	Notes
pmrB:p.E123D	gaa -> gat	e -> d	Unknown phenotype	-	Phenotype not found in database
16S_rrsH;;16S_rrsH;;vnaf;;16S_rrsH;;jwbg;;16S_rrsH;;gelg;;16S_rrsH;;dymy:g.1093C>G	c -> g	-	Unknown phenotype	-	Phenotype not found in database
23S:g.1733G>T	g -> t	-	Unknown phenotype	-	Phenotype not found in database
folP:p.E73A	gaa -> gca	e -> a	Unknown phenotype	-	Phenotype not found in database

Download acquired AMR gene results:

Results as text | Hit in genome sequences | Resistance gene sequences | Results as tabseperated file

Download Chromosomal point mutation results:

Results as tabseperated file | Results as a text file

Selected %ID threshold for ResFinder: *90 %*

Selected minimum length for ResFinder: *60 %*

Selected %ID threshold for PointFinder: *90 %*

Selected minimum length for PointFinder: *60 %*

Inheritance of genes through Mobile Genetic Elements

Mobile Genetic Elements

- **Discrete genetic elements that promote its own mobility within and between DNA molecules**
- Divided into different types based on their properties
- Highly diverse, both within and between types
- Generally, provide genetic machinery for their own mobility
- Their transposition is generally repressed, often coupled to SOS response
- Transposition is sensitive to non-lethal concentrations of DNA damaging antibiotics
- Relatively poorly understood, likely many types that have not been discovered
- **Work together to mobilize and disseminate genes**
- Tend to be carried on one another

Overview of Mobile Genetic Elements

Within cell	**Modulating** • MITEs • Insertion Sequences	Gene inactivation, up-regulation & repression of gene expression
	Recruiting • Integrons	Recruits and store genes
	Gene transporter • Unit transposons • Composite transposons	Carries passenger genes
Between cell	**Conjugating** • Integrative Conjugative Elements (ICE) • Integrative Mobilizable Elements (IME) • Plasmids	Transpose genes between bacteria

Overview of Mobile Genetic Elements

Between cell	**Conjugating** • Plasmids • Integrative Conjugative Elements (ICE) • Integrative Mobilizable Elements (IME)	Transpose genes between bacteria

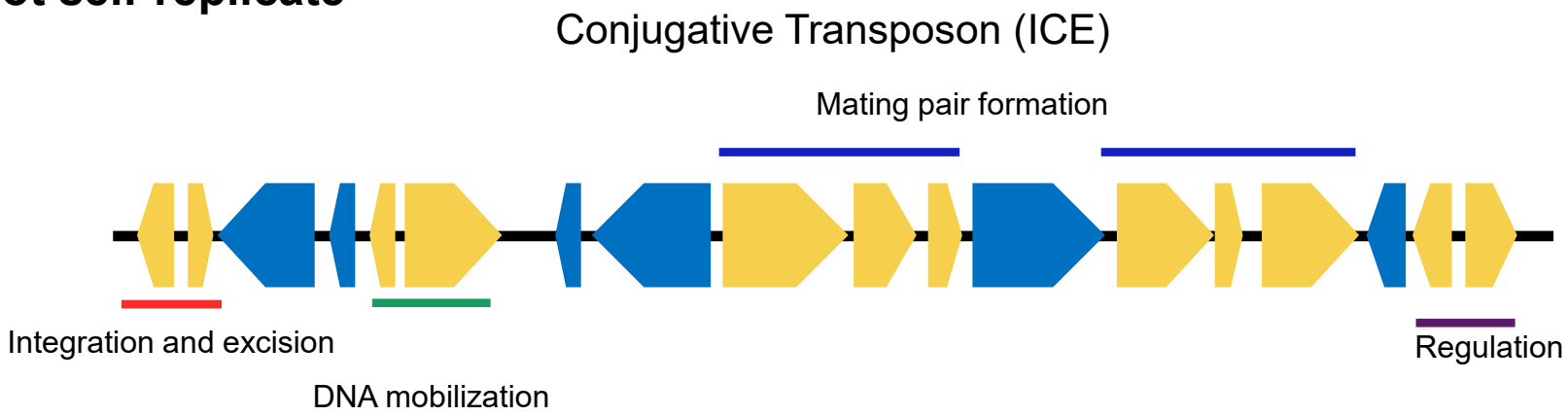
Conjugating Elements - Plasmids

Plasmids

- Circular
- Located in the cytosol
- Highly variable size
- Variable copy number, low copy & high copy
- Autonomous, can self replicate
- Carry accessory genes and other MGEs
- Traditionally classified into incompatibility groups

Integrative Conjugative Elements (ICE)

- Located on bacterial chromosome
- Has been grouped with Genomic Islands
- Backbone with core genes clustered according to function
- Carry accessory genes, often antibiotic resistance genes
- Can transfer between bacterial cell through **conjugation**
- **Cannot self-replicate***



ICE backbone

Integration

- Diverse mechanisms for integration
  - Lambda-phage like tyrosine integrase — similar to phages
  - DDE-type transposase — similar to Transposons / Insertion sequences
  - Serine recombinase — similar to Transposons / Insertion sequences

Conjugation

- Similar to plasmid machinery
- Regions similar to plasmid *oriT* and releases in several ICEs

Excision

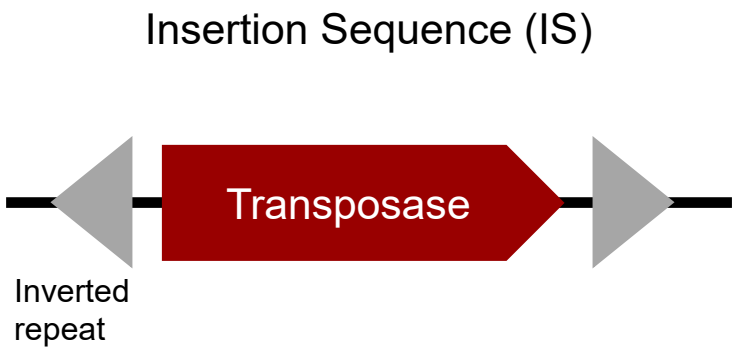
- Excision rate is influenced by environmental factors, eg growth phase

Overview of Mobile Genetic Elements

Within cell	**Modulating** • MITEs • Insertion Sequences	Gene inactivation, up-regulation & repression of gene expression
	Recruiting • Integrons	Recruits and store genes
	Gene transporter • Unit transposons • Composite transposons	Carries passenger genes

Insertion Sequence

- Small, ~ 1-3 kb
- Often bounded by special sequence motifs
  – IR or sub-terminal sequences
- **Don’t carry accessory genes**
- Intracellular mobility, within or between DNA molecules
- Transposition is catalyzed by transposase gene/s
- Insertion site and frequency, determined by transposase and host
- Insertion can leave characteristic Target Site Duplication sequences



IS modulation of gene expression

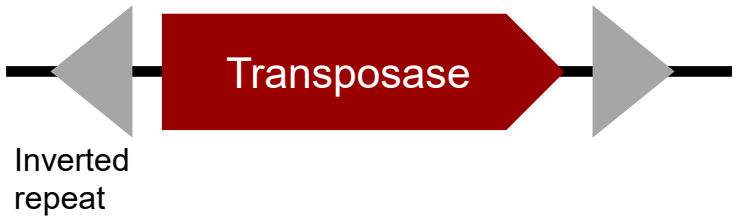
Up-regulation of gene expression

- Carrying outward directed full or partial promoter, ex ISEcp1
- Promoters can respond to environmental stimuli
- Influence DNA topology of nearby genes
- Read through transcription, transposase & nearby gene are co-transcribed

Gene inactivation and repression

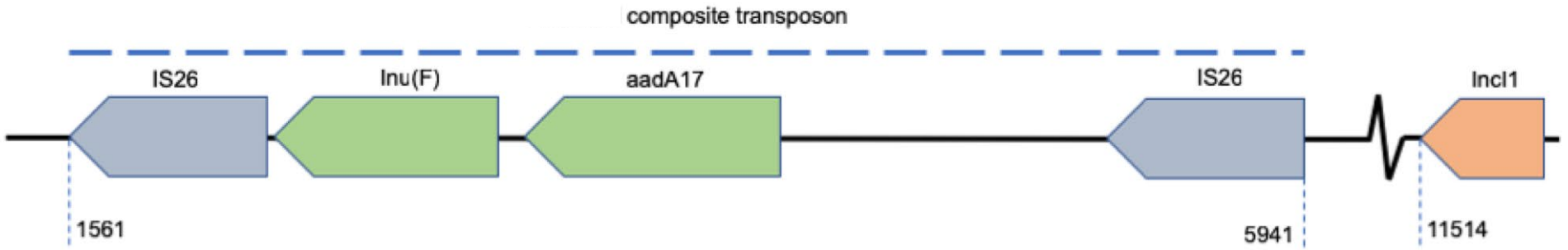
- Disruption of genes by insertion into gene or promoter region
- Carrying transcription termination, ex IS1 and IS2

Insertion Sequence (IS)



Composite transposons

- Consists of **two insertion sequences in proximity to one another**
- Transposase of one IS acts on IR of nearby MGE instead of itself
- Transpose the two IS with intermediary DNA as one unit
- Can act on non-autonomous elements (MITES etc.)
- Can act on surrogate sequences, random boundary sequences, partial IS etc
- Example Tn10, Tn9 and Tn5 transposons



Unit-transposon

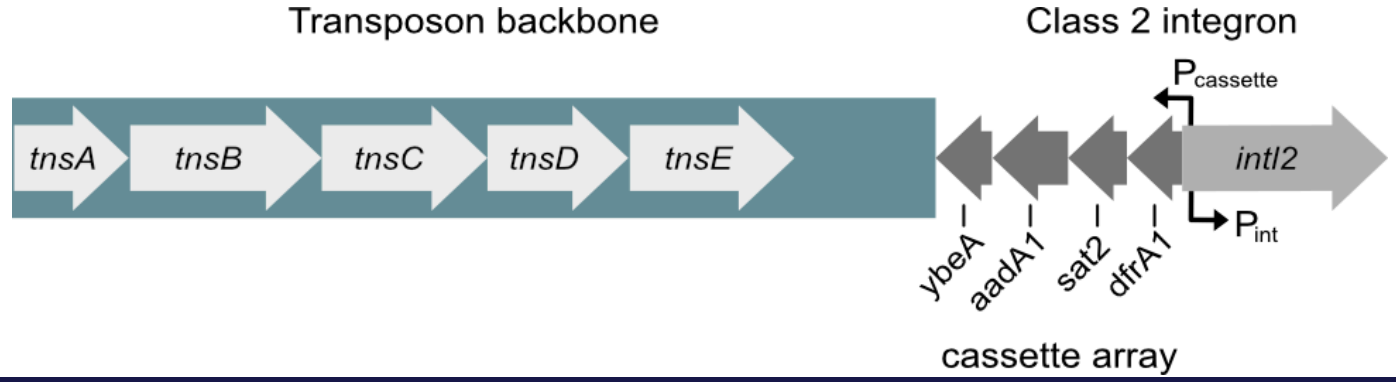
- Intra-molecular transposition
- Typically small, usually 3 - 10 kb long
- Highly diverse
- Carries accessory genes, many carries antibiotic resistance
- Central to driving the spread of antibiotic resistance
- Divided into families based on transposase and sequence similarity
- Genes are recruited by **homologous recombination** or by carry an **integron**
- Horizontal transposition, between cells, requires mobilization on plasmids

Tn3-family of transposons

- One of the largest and most diverse families
- Similar genetic layout to insertion sequences
- Many members carry ARGs, resistance to heavy metals
- Replicative transposition, “copy-and-paste” via a DDE-transposase
- Replicate by generating a cointegrate structure that is resolved by a TnpR – resolavese gene
- Prevent acquisition additional transposons of the same type. **Transposition immunity**

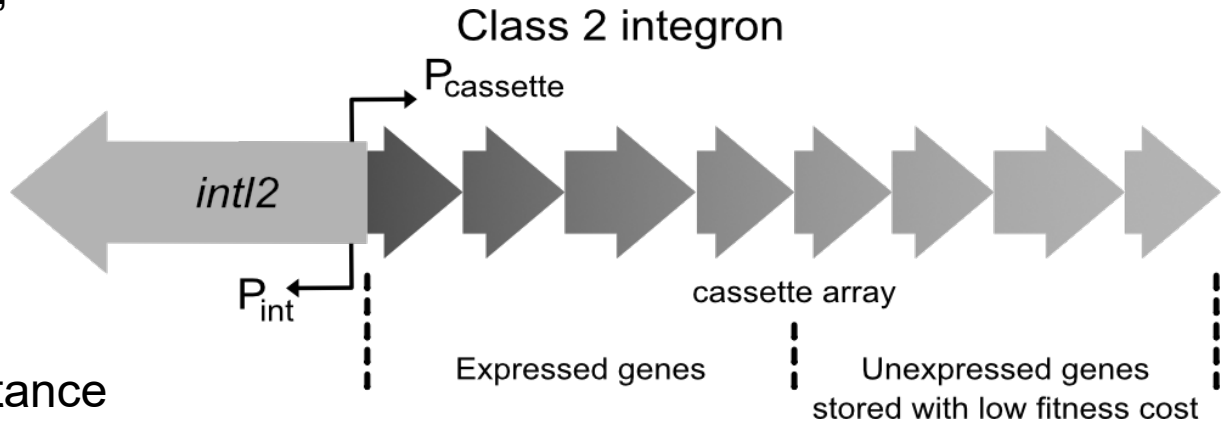
Tn7-family of transposons

- Transposes via “cut-and-paste”
- Special because of its level of control over its transposition
- Genes for transposition called *tnsA*, *tnsB*, *tnsC*, *tnsD* and *tnsE*
  - TnsA, TnsB – excises Tn7 from original site
  - TnsC captures target
  - TnsD directs chromosomal integration in gram negative bacteria
  - TnsE directs integration in plasmids by targeting the lagging strand
- Shows **target immunity**
- Carries a class 2 integron which carries aminoglycoside and trimethoprim resistance



Expression of cassettes & cassette shuffling

- Two part system, stable backbone and flexible gene cassettes
- Backbone contains a recombinase and two promoters, integrase and cassette directed
- Cassettes are small, often contains 1 gene
- **Do not contain promoter**
- Cannot be maintained in circular form



- Level of expression of a cassette is determined by distance to P_c promoter
- Enables harboring of genes at low fitness cost
- **cassette shuffling**, random excision of a cassette followed by its reintegration enables expression of gene
- Enables rapid adaptaion

Two types of integrons, chromosomal and mobile

Chromosomal Integrons (CI)

- Very large, can contain > 200 cassettes
- Multiple cassettes whose function are unknown
- Very old structures

Mobile Integrons (MI)

- Carries up to 8 cassettes
- Almost exclusively antibiotic and antiseptic resistance
- Exists in 22%-59% of all clinical isolates
- Mobilized on transposons → exposed to high diversity of cassettes
- Divided into 5 classes based on *intI* sequence

Interplay of Mobile Genetic Elements

- Integrons ”scans” recipient plasmids and exchange gene cassette
- Integrons shuffle gene cassettes

- IS transpose, expression of some genes might be modulated and form composite Tn
- Deletions due to IS transposition
- Transposons move genes to and from plasmids to chromosome

- DNA might be exchanged between similar regions through Homologous recombination

Estimating mobility of resistance genes

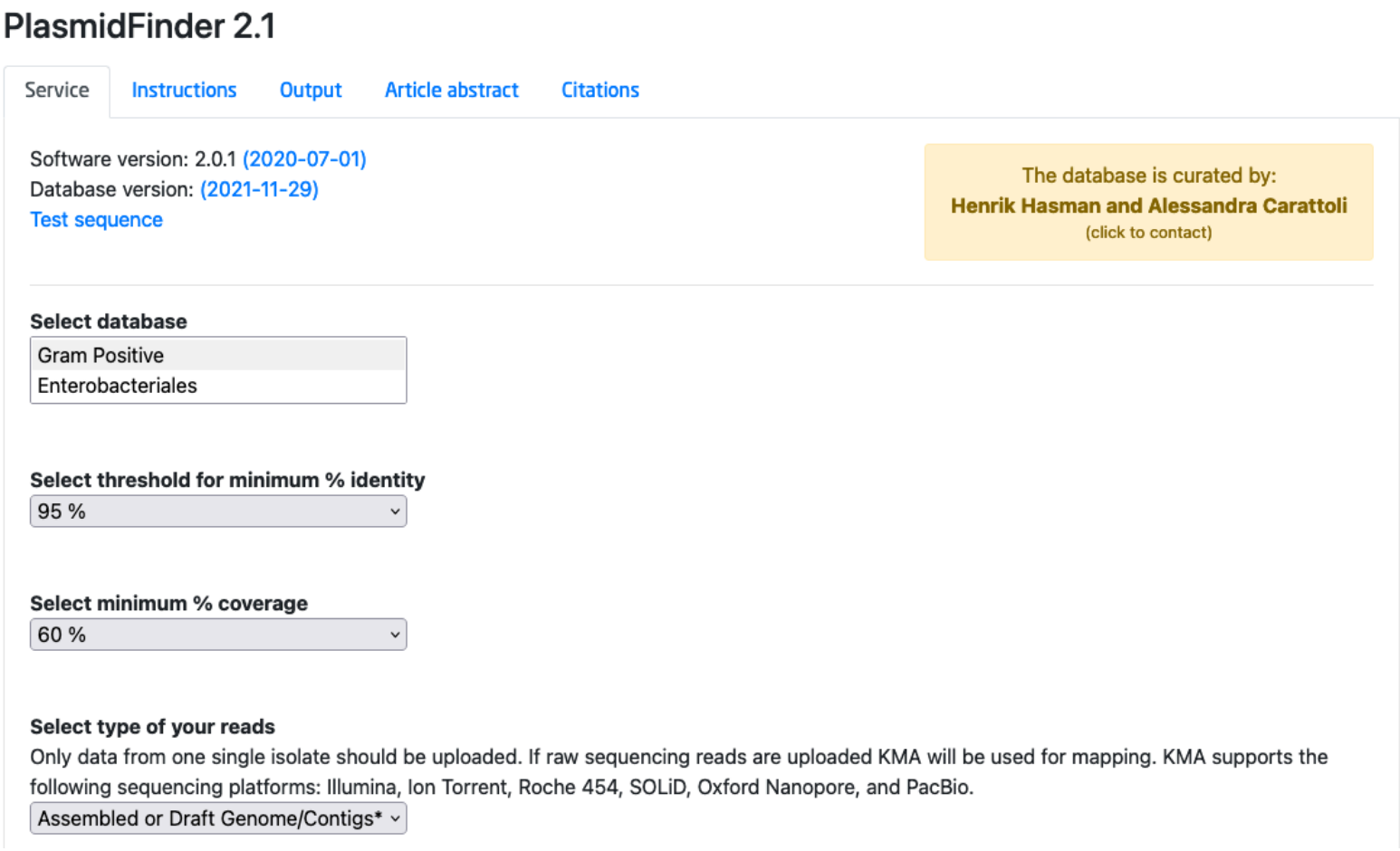
Importance of quality input data

- Large mobile elements are hard to assemble using short read data
- Tend to be fragmented into several contigs
- Can be difficulty to accurately determine if a gene is mobilized

PlasmidFinder

https://cge.food.dtu.dk/services/PlasmidFinder/

- Takes raw reads or assembled sequences as input
- Detects plasmid by mapping reads/ aligning to a database of replicon sequences
- Uses KMA for mapping
- Has database with gram-positive and enterobacteriales plasmid replicons
- User definable QC thresholds



PlasmindFinder result

PlasmidFinder-2.0 Server - Results

Organism(s): *Enterobacteriales*

Enterobacteriales						
Plasmid	Identity	Query / Template length	Contig	Position in contig	Note	Accession number
IncFIA	99.74	388 / 388	NODE_41_length_16959_cov_3.430430	9491..9878		AP001918
IncFIB(AP001918)	98.39	682 / 682	NODE_38_length_20127_cov_3.619100	1961..2642		AP001918
IncFII	100	261 / 261	NODE_37_length_22157_cov_4.960554	19236..19496		AY458016

extended output

Input Files: *DTU2018-646-PRJ1139-Escherichia-coli-1D-019_R1_001.fa*

Results as text | Results tsv | Hits in genome seqs | Plasmid sequences

MobileElement Finder

https://cge.food.dtu.dk/services/MobileElementFinder/

- **Input**: Assembled sequence data (contigs, scaffolds or genomes)*
- Detects MGEs based on sequence similarity to known elements
- Includes a curated database of ~4,400 MGEs

Webserver

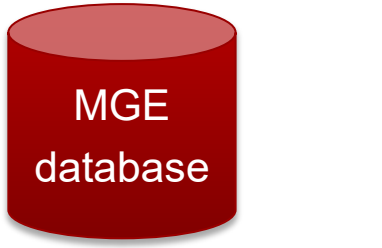
– Graphical user interface
– Annotates AMR genes, Virulence factors & Plasmid replicons (with default thresholds)
– User friendly but less flexible

Local installation

– Python package, hosted on PyPI, installed with pip
– Flexible with user customizable thresholds

* High assembly quality is very important

MobileElementFinder prediction method



Blast

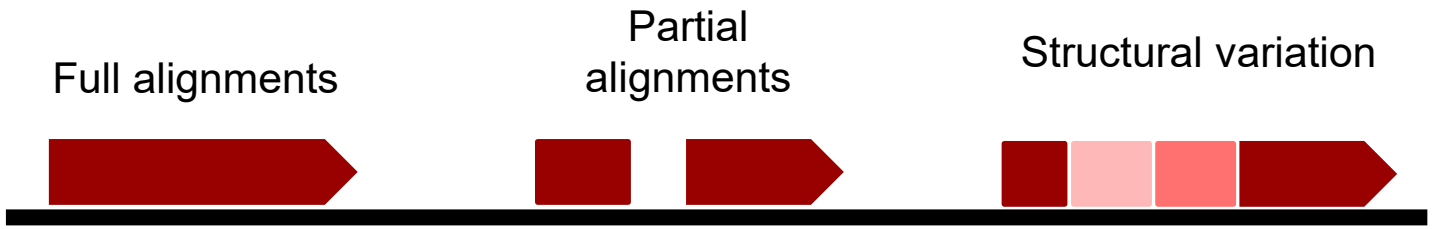
Assembled sequences

- Group HSPs that belong to the same MGE
- Remove overlapping MGE segments

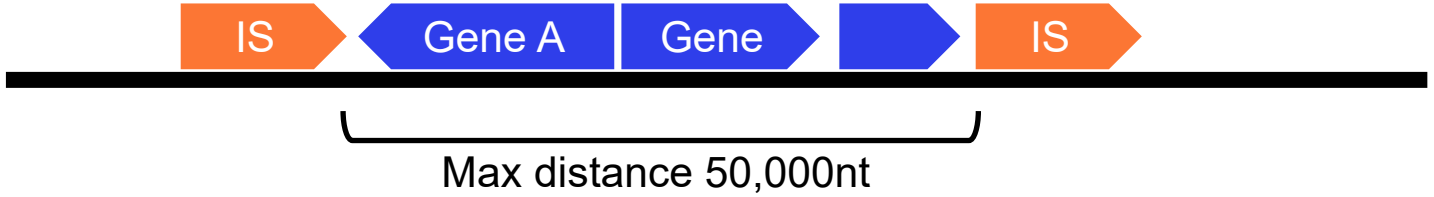
Final prediction based on

- Alignment coverage
- Sequence identity
- Level of truncation

For probable HSPs



Detection of putative composite transposons



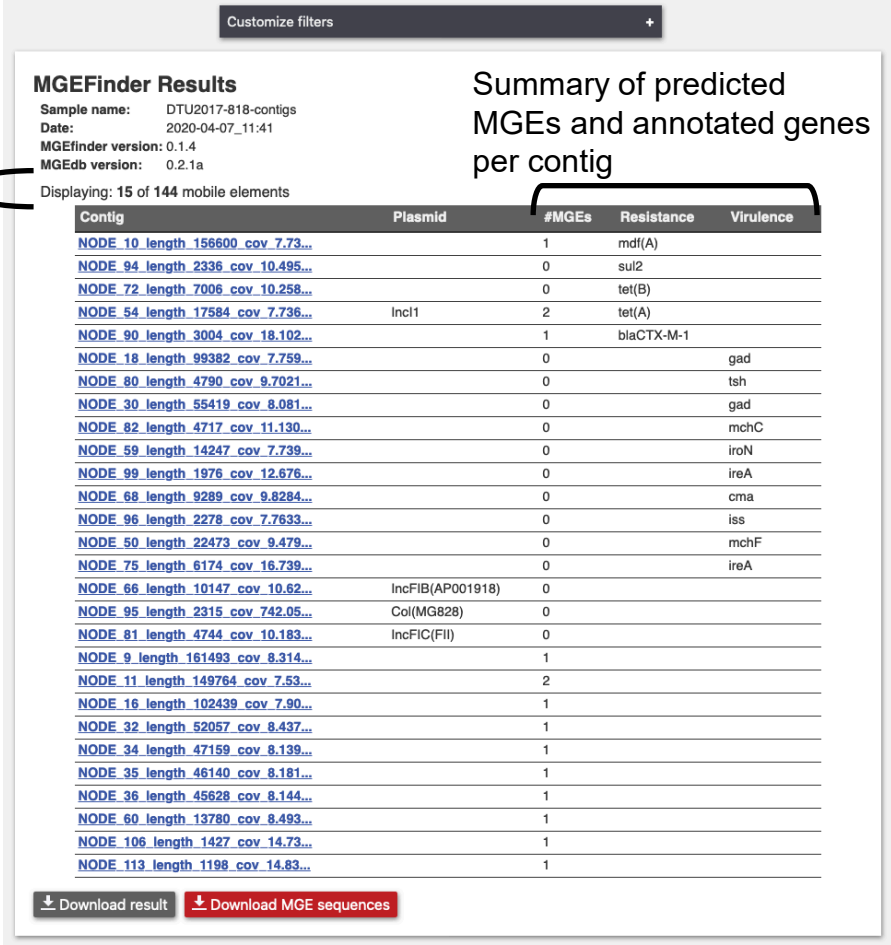
Output and filtering options

https://cge.food.dtu.dk/services/MobileElementFinder/

Result index

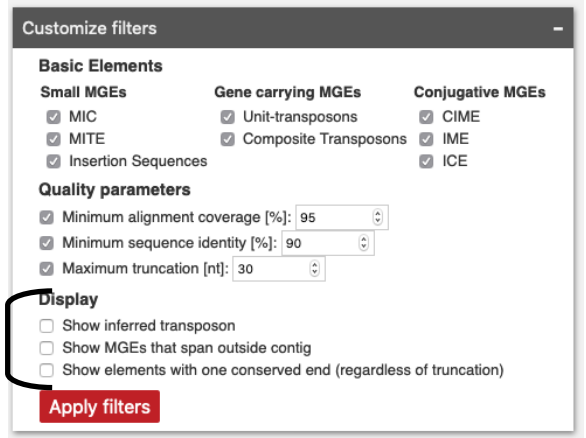
Num displayed MGEs with current filtering criteria of all possible

Summary of predicted MGEs and annotated genes per contig



- Show inferred composite transposon
- Show special cases regardless of other quality parameters

Customizable filters of results



- Filter on MGE types
- Set minimum quality parameters

Contig result

https://cge.food.dtu.dk/services/MobileElementFinder/

MobileElementFinder annotates the contigs with *ResFinder*, *PlasmidFinder* and *VirulenceFinder*.

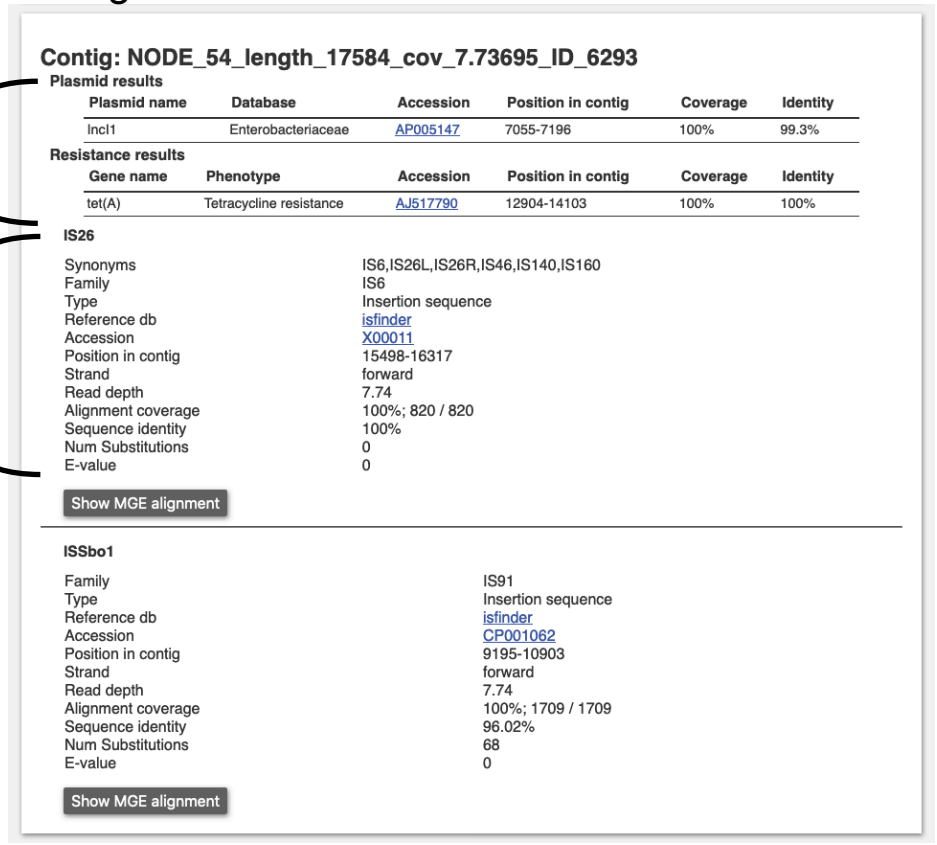
Note: You need to verify that predicted genes are relevant for your specie

Genes & plasmid replicons located on the same contig

Predicted MGEs with metadata and quality metrics

Genes carried on MGEs are displayed here

Contig result



Contig: NODE_54_length_17584_cov_7.73695_ID_6293

Plasmid results

Plasmid name	Database	Accession	Position in contig	Coverage	Identity
IncI1	Enterobacteriaceae	AP005147	7055-7196	100%	99.3%

Resistance results

Gene name	Phenotype	Accession	Position in contig	Coverage	Identity
tet(A)	Tetracycline resistance	AJ517790	12904-14103	100%	100%

IS26

Synonyms	IS6,IS26L,IS26R,IS46,IS140,IS160
Family	IS6
Type	Insertion sequence
Reference db	isfinder
Accession	X00011
Position in contig	15498-16317
Strand	forward
Read depth	7.74
Alignment coverage	100%; 820 / 820
Sequence identity	100%
Num Substitutions	0
E-value	0

Show MGE alignment

ISSbo1

Family	IS91
Type	Insertion sequence
Reference db	isfinder
Accession	CP001062
Position in contig	9195-10903
Strand	forward
Read depth	7.74
Alignment coverage	100%; 1709 / 1709
Sequence identity	96.02%
Num Substitutions	68
E-value	0

Show MGE alignment

Coffee break

Back at 11:15.

Funded by the European Union

Markus Johansson, DTU

Discussion and conclusions of exercise about antimicrobial resistance and mobile genetic elements

Funded by the European Union

Detection of antibiotic resistance genes and estimation of resistance gene mobility

Exercise

Goal of exercise

1. Download assembled example genomes

2. Submit the genomes for analysis to ResFinder and MobileElementFinder

3. Analyze the result,
   – Estimate the antimicrobial resistance profile for each genome
   – Find evidence of genes being mobilized

Download and submit genomes for analysis

1. Download example genomes
   https://sciencedata.dk/themes/deic_theme_oc7/apps/files_sharing/public.php?t=91ff6dc91c9e514c9b86d0c32e121b42&

2. Submit the genomes to ResFinder, PlasmidFinder and MobileElementFinder
   https://www.genomicepidemiology.org/services/

3. Fill in your email to get notification when analysis has finished
   Results are stored on CGE servers for a few days before they expire

Cached example results

ResFinder

- 1D-019 - https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=63904035000012237OCEE34F
- 1D-020 - https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=63909FBC0000641C2F1F51B4

PlasmidFinder

- 1D-019 - https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=6390401500001 1DDE875566C
- 1D-020 - https://cge.food.dtu.dk/cgi-bin/webface.fcgi?jobid=639196B700006AB9F5D36DF7

MobileElementFinder

- 1D-019 - https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=63903CA700000373966 1C6C1
- 1D-020 - https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=63909C9B000052291FA575DA

Discussion on how to analyze results

1. Evaluate sequencing quality
   – Verify that the sample is not contaminated (Not covered)
   – Verify assembly quality by comparing quality metrics to internal thresholds

2. Identify resistance genes, point mutations and their predicted phenotype

3. Evaluate if genes are mobilized

1. Evaluate sequencing quality

Sequence QC reports needs to be generated separately by your analysis pipeline.

FoodQC pipeline									
Sample id	Bases (MB)	Qual Bases (MB)	Qual bases %	Reads	Qual reads %	N50	No ctgs	Longest ctg	total bps
1D-019	383	307	80.34%	2592340	88.78%	180901	172	380185	5308247
1D-020	335	266	79.30%	2286734	88.07%	203076	140	380185	5245654
1D-164	275	231	84.05%	1976136	90.97%	226469	40	676641	4471461

Or other CGE tools such as KmerFinder and cgMLST finder

KmerFinder			
Sample id	**Query Coverage**	**Template coverage**	**Predicted specie**
1D-019	91.17%	95.71%	Escherichia coli
1D-020	92.3%	95.74%	Escherichia coli
1D-164	31.85%	26.22%	Escherichia coli

2. Identify resistance genes and point mutations

Sample Id	Resistance gene	Identity	Alignment Length / Gene Length	Contig	Phenotype (panel)	Phenotypes not in panel
1D-019	blaTEM-1B	100%	861 / 861	NODE_104_length_1377_cov_71.828000	Ampicillin	Amoxicillin Cephalothin Piperacillin Ticarcillin
1D-019	tet(B)	100%	1206 / 1206	NODE_64_length_4666_cov_3.854153	Tetracycline	Doxycycline Minocycline
1D-019	sitABCD	97.2%	3459 / 3459	NODE_45_length_13731_cov_5.452293		Hydrogen peroxide

Questions

- How can low sequence quality impact the result?
- Since the isolate carries a blaTEM-1B can we be certain that its resistant to Ampicillin?
- Can we be certain that the isolate is susceptible to Colistin?

3. Evaluate if genes are mobilized – Sample 1D-019

21 MGEs

Detected ARGs

- blaTEM-1B
- tet(B)

Detected Plasmid replicons

- IncFII
- IncFIB
- IncFIA

Questions

- Can we be sure that the genes are not mobilized?
- How can we improve analysis performance?

Displaying: **21** of **117** mobile elements

Contig	Plasmid	#MGEs	Resistance	Virulence
NODE_64_length_4666_cov_3.8541...		0	tet(B)	
NODE_45_length_13731_cov_5.452...		0	sitABCD	sitA
NODE_104_length_1377_cov_71.82...		0	blaTEM-1B	
NODE_27_length_54246_cov_5.894...		1		focI, mcmA, ...
NODE_35_length_26610_cov_6.707...		1		iha, sat, ...
NODE_19_length_107365_cov_6.12...		0		usp
NODE_37_length_22157_cov_4.960...	IncFII	0		traT
NODE_42_length_16798_cov_5.300...		0		mcbA
NODE_62_length_4985_cov_28.930...		0		cea
NODE_15_length_135151_cov_5.39...		0		tcpC
NODE_2_length_365325_cov_6.489...		2		kpsE, terC, ...
NODE_91_length_1653_cov_7.0019...		0		papA_F43
NODE_31_length_37413_cov_7.273...		2		papA_F14, ireA
NODE_8_length_206343_cov_6.251...		0		fimH
NODE_54_length_8895_cov_7.1178...		0		cnf1
NODE_17_length_122359_cov_5.71...		0		irp2, clbB, ...
NODE_4_length_260371_cov_6.074...		1		terC
NODE_99_length_1471_cov_7.4553...		0		tia
NODE_41_length_16959_cov_3.430...	IncFIA	0		
NODE_38_length_20127_cov_3.619...	IncFIB(AP001918)	1		

3. Evaluate if genes are mobilized – Sample 1D-020

Sample ID: 1D-020

Detected ARGs

- blaTEM-1B
- tet(B)

Detected Plasmid replicons

- IncFIB
- IncFIA

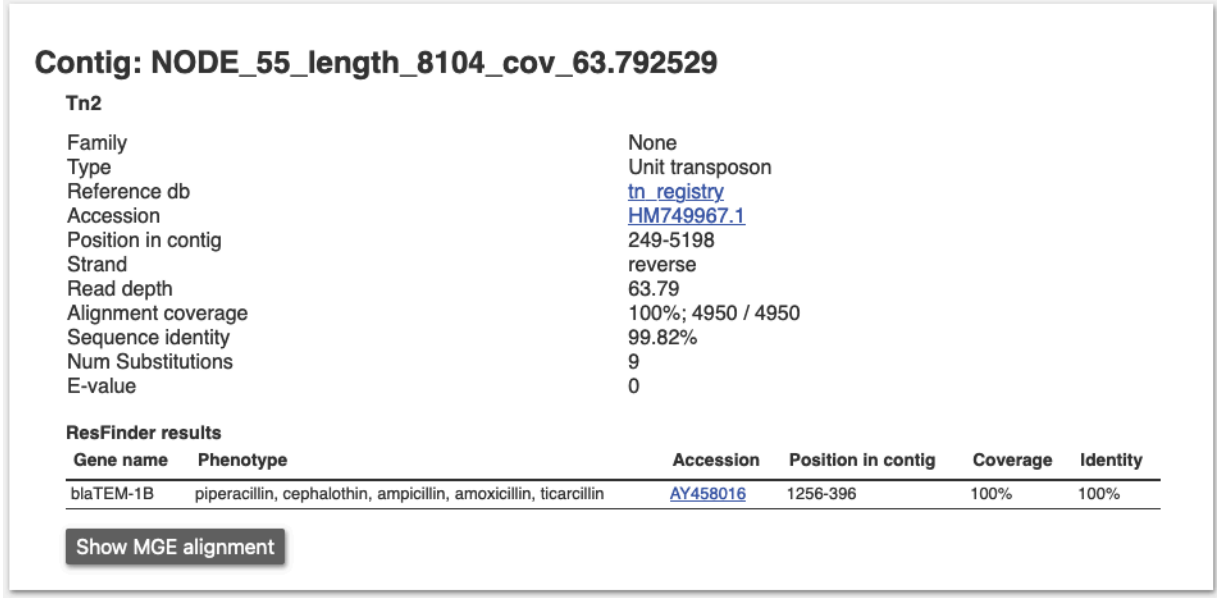
- Detected 22 MGEs
- Several Virulence factors

Displaying: **22** of **115** mobile elements

Contig	Plasmid	#MGEs	Resistance	Virulence
NODE_55_length_8104_cov_63.792...		1	blaTEM-1B	
NODE_41_length_16005_cov_4.217...		0	sitABCD	sitA
NODE_65_length_4650_cov_2.9204...		0	tet(B)	
NODE_52_length_9697_cov_6.0277...		0		hra
NODE_30_length_29839_cov_4.983...		1		mchF, focG, ...
NODE_14_length_153488_cov_5.77...		0		shiB, shiB
NODE_1_length_380185_cov_4.836...		2		yehB, yehA, ...
NODE_23_length_83121_cov_4.785...		0		fyuA, irp2
NODE_63_length_5010_cov_4.7022...		0		iss
NODE_4_length_299549_cov_5.204...		1		vat, fdeC, ...
NODE_53_length_8895_cov_5.5155...		0		cnf1
NODE_2_length_360414_cov_5.558...		1		kpsE, kpsMII, ...
NODE_84_length_1655_cov_6.0700...		0		papA_F43
NODE_102_length_1103_cov_11.99...		0		papA_F14
NODE_15_length_134815_cov_4.47...		0		tcpC
NODE_38_length_18820_cov_2.727...	IncFIB(AP001918)	0		
NODE_35_length_19982_cov_2.795...	IncFIA	0		
NODE_3_length_305714_cov_6.264...		1		

3. Evaluate if genes are mobilized – Sample 1D-020

- blaTEM-1B is predicted to be mobilized on a Tn2 unit transposon



Contig: NODE_55_length_8104_cov_63.792529

Tn2

Family	None
Type	Unit transposon
Reference db	tn_registry
Accession	HM749967.1
Position in contig	249-5198
Strand	reverse
Read depth	63.79
Alignment coverage	100%; 4950 / 4950
Sequence identity	99.82%
Num Substitutions	9
E-value	0

ResFinder results

Gene name	Phenotype	Accession	Position in contig	Coverage	Identity
blaTEM-1B	piperacillin, cephalothin, ampicillin, amoxicillin, ticarcillin	AY458016	1256-396	100%	100%

Show MGE alignment

3. Evaluate if genes are mobilized – Sample 1D-020

- A potential ISEc1 composite transposon
- Residing outside of the virulence factors
- One of the included insertion sequences as 127 nt truncation

Contig: NODE_1_length_380185_cov_4.836404

Virulence results

Gene name	VirulenceFinder db	Protien function	Accession	Position in contig	Coverage	Identity
yehB	Escherichia coli	Usher, YHD fimbriael cluster	CP042934	275730-278210	100%	96.57%
yehA	Escherichia coli	Outer membrane lipoprotein, YHD fimbriael cluster	CP042934	278226-279260	100%	97.58%
yehD	Escherichia coli	Major pilin subunit, YHD fimbriael cluster	CP042934	274417-274959	100%	95.03%
yehC	Escherichia coli	Chaperone, YHD fimbriael cluster	CP042934	275040-275714	100%	96.44%
yfcV	Escherichia coli	Fimbrial protein	UFZT01000001	20163-20729	100%	100%

cn_2586_ISEc1

Family	ISAs1
Type	Composite transposon
Reference db	isfinder
Accession	L02370
Position in contig	363087-365673
Strand	forward
Read depth	4.84
Truncation description	**5p truncation:** 127 nt
Prediction	Putative MGE
Alignment coverage of flanking MGEs	100%
Sequence identity of flanking MGEs	96.13%
Num Substitutions in flanking MGEs	50
E-value	0

Show MGE alignment

Thank you very much!

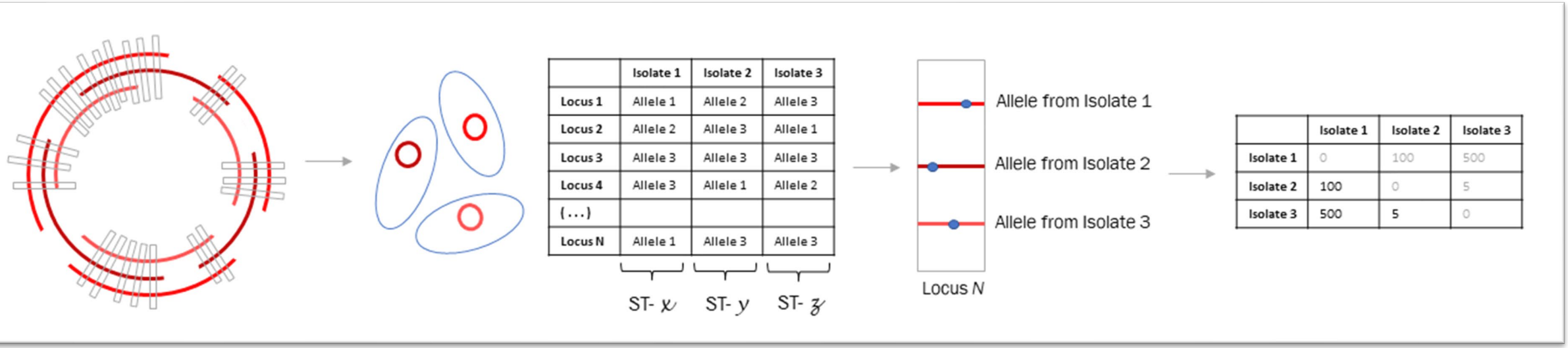
DTU Food
National Food
Institute

Ana Rita Rebelo
anrire@food.dtu.dk

Cluster methods and tools, and the importance of metadata

Funded by the European Union

Gene-by-gene vs. SNP-based analysis

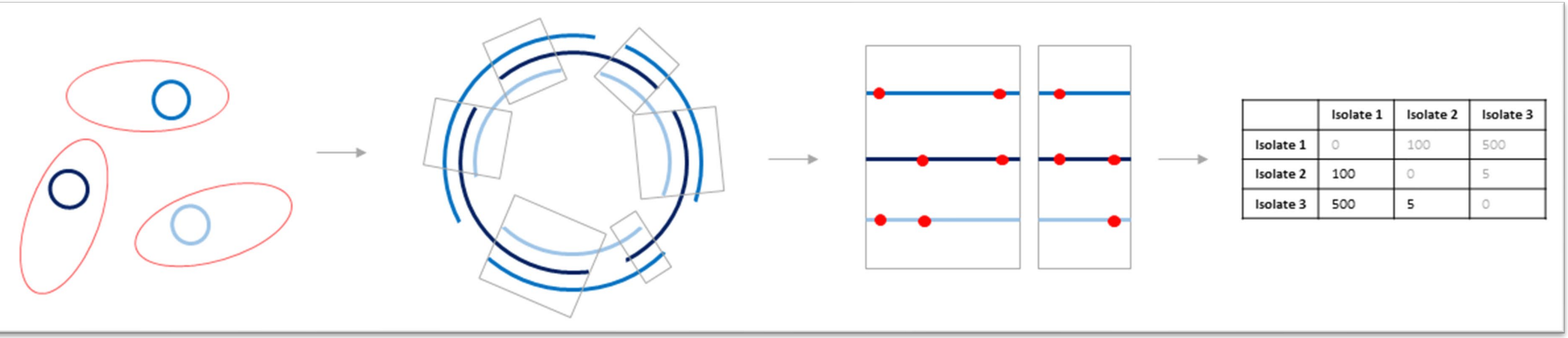


	Isolate 1	Isolate 2	Isolate 3
Locus 1	Allele 1	Allele 2	Allele 3
Locus 2	Allele 2	Allele 3	Allele 1
Locus 3	Allele 3	Allele 3	Allele 3
Locus 4	Allele 3	Allele 1	Allele 2
(...)			
Locus N	Allele 1	Allele 3	Allele 3

ST- x ST- y ST- z

Allele from Isolate 1
Allele from Isolate 2
Allele from Isolate 3
Locus N

	Isolate 1	Isolate 2	Isolate 3
Isolate 1	0	100	500
Isolate 2	100	0	5
Isolate 3	500	5	0



	Isolate 1	Isolate 2	Isolate 3
Isolate 1	0	100	500
Isolate 2	100	0	5
Isolate 3	500	5	0

Thresholds for genetic relatedness

Difficult to achieve harmonised agreement

EURGen-RefLabCap protocol:

- **SNP distance under 5** suggests relatedness of isolates, but slightly higher thresholds should not be discarded (e.g. up to 25 SNPs)
- Be aware of method limitations: at least 90% of each query genome should have been included in the alignment to create the distance matrix

- **Differences of 5 cgMLST alleles** suggest close genetic relatedness, but higher values should not be discarded (e.g. up to 10)

Thresholds for genetic relatedness

Other examples from literature:

SNP-based analysis: **up to 20** SNP distance

cgMLST: **up to 26** alleles difference

- Kluytmans-van den Bergh MFQ, Rossen JWA, Bruijning-Verhagen PCJ, et al. Whole-Genome Multilocus Sequence Typing of Extended-Spectrum-Beta-Lactamase-Producing Enterobacteriaceae. J Clin Microbiol 2016; 54: 2919–27.
- Jamin C, De Koster S, van Koeveringe S, et al. Harmonization of whole-genome sequencing for outbreak surveillance of Enterobacteriaceae and Enterococci. Microb Genomics 2021; 7: 000567.
- González-Escalona N, Kase JA. Virulence gene profiles and phylogeny of Shiga toxin-positive Escherichia coli strains isolated from FDA regulated foods during 2010-2017 Ibekwe AM, ed. PLoS One 2019; 14: e0214620.
- Gutiérrez S, Díaz L, Reyes-jara A, Yang X. Whole-Genome Phylogenetic Analysis Reveals a Wide Diversity of Non-O157 STEC Isolated From Ground Beef and Cattle Feces. 2021; 11: 1–12.
- Weber RE, Pietsch M, Frühauf A, et al. IS26-Mediated Transfer of blaNDM–1 as the Main Route of Resistance Transmission During a Polyclonal, Multispecies Outbreak in a German Hospital. Front Microbiol 2019; 10: 1–14.
- Jones G, Lefèvre S, Donguy M, Nisavanh A, Terpant G, Fougère E. Outbreak of Shiga toxin-producing Escherichia coli (STEC) O26 paediatric haemolytic uraemic syndrome (HUS) cases associated with the consumption of soft raw cow's milk cheeses, France, March to May 2019. Eurosurveillance 2019; 24.
- Zhang P, Essendoubi S, Keenliside J, et al. Genomic analysis of Shiga toxin-producing Escherichia coli O157:H7 from cattle and pork-production related environments. npj Sci Food 2021; 5.
- Dallman TJ, Greig DR, Gharbia SE, Jenkins C. Phylogenetic structure of Shiga toxin-producing Escherichia coli O157: H7 from sub-lineage to SNPs. Microb Genomics 2021; 7.
- Bush SJ, Foster D, Eyre DW, et al. Genomic diversity affects the accuracy of bacterial single-nucleotide polymorphism-calling pipelines. Gigascience 2020; 9: 1–21.
- Yoshimura D, Kajitani R, Gotoh Y, et al. Evaluation of SNP calling methods for closely related bacterial isolates and a novel high-accuracy pipeline: BactSNP. Microb genomics 2019; 5: 1–8.
- Jenkins C, Dallman TJ, Launders N, et al. Public health investigation of two outbreaks of shiga toxin-producing Escherichia coli O157 associated with consumption of watercress. Appl Environ Microbiol 2015; 81: 3946–52.
- Rowell S, King C, Jenkins C, et al. An outbreak of Shiga toxin-producing Escherichia coli serogroup O157 linked to a lamb-feeding event. Epidemiol Infect 2016; 144: 2494–500.
- Ludden C, Coll F, Gouliouris T, et al. Defining nosocomial transmission of Escherichia coli and antimicrobial resistance genes: a genomic surveillance study. The Lancet Microbe 2021; 2: e472–80.
- Roer L, Hansen F, Frølund Thomsen MC, et al. WGS-based surveillance of third-generation cephalosporin-resistant Escherichia coli from bloodstream infections in Denmark. J Antimicrob Chemother 2017; 72: 1922–9.
- Dallman TJ, Byrne L, Ashton PM, et al. Whole-Genome Sequencing for National Surveillance of Shiga Toxin-Producing Escherichia coli O157. Clin Infect Dis 2015; 61: 305–12.
- Joensen KG, Scheutz F, Lund O, et al. Real-Time Whole-Genome Sequencing for Routine Typing, Surveillance, and Outbreak Detection of Verotoxigenic Escherichia coli. J Clin Microbiol 2014; 52: 1501–10.

Tools for analysis

23 September and 10 October. Virtual multidisciplinary training to resolve a CCRE outbreak scenario using simulation exercises. (2 hours each)

Multidisciplinary training workshop engaging a mix of national microbiology experts and health care epidemiology experts.

The training will include exercises on WGS data analysis, including sequence quality control, *in silico* typing and detection of antimicrobial resistance genes as well as cluster analysis. The training will be organised as virtual outbreak exercise to practise and test the capability to use WGS-data in outbreak investigations and interpreting these in relation to the epidemiological investigations.

The first workshop will be divided into two online sessions of approximately two hours. The first session will set the scene, introduce phylogenetic analysis and explain the exercise dataset and setup. After the first session, sequences and exercise material will be shared with the participants and they will have approximately two weeks to work with the exercise. During the second session, a walk-through of the exercise will be presented, and participants can ask questions, show their own results and participate in discussion with other participants and exercise coordinators.

Exercise overview

Presentations
Intro and CSIPhylogeny by Jette Sejer Kjeldgaard
SNP vs cgMLST by Henrik Hasman
MinION typing by Henrik Hasman

Exercise guidelines and questions
Exercise guidelines
Exercise questions
Exercise metadata

https://www.eurgen-reflabcap.eu/activities-and-events/courses-and-workshops

Questions?

The importance of metadata

James B Pettengill, Austin Markell, Amanda Conrad, Heather A Carleton, Jennifer Beal, Hugh Rand, Steven Musser, Eric W Brown, Marc W Allard, Jasmine Huffman, Stic Harris, Matt Wise, Annie Locas, **A multinational listeriosis outbreak and the importance of sharing genomic data**, The Lancet Microbe, Volume 1, Issue 6, 2020, Pages e233-e234, https://doi.org/10.1016/S2666-5247(20)30122-1.

" By the end of 2019, cluster PDS000011550 in the NCBI L monocytogenes Pathogen Detection database contained 36 clinical isolates with a distinct genetic pattern. The associated illnesses, which included four deaths and six pregnancy-associated infections, began in 2016 and a suspected food vehicle could not be identified from available food exposure histories."

" In February, 2020, the Canadian Food Inspection Agency (CIFA) (…)"

" In late February to early March, 2020, US state and federal partners (…)"

" In the middle of March, 2020, US public health officials (…)"

" In late March, 2020, further testing by the CFIA (…)"

" In April, 2020, as part of the US domestic outbreak investigation (…)"

The importance of metadata

James B Pettengill, Jennifer Beal, Maria Balkey, Marc Allard, Hugh Rand, Ruth Timme, **Interpretative Labor and the Bane of Nonstandardized Metadata in Public Health Surveillance and Food Safety**, Clinical Infectious Diseases, Volume 73, Issue 8, 15 October 2021, Pages 1537–1539, https://doi.org/10.1093/cid/ciab615

"The 2020 international outbreak of Listeriosis monocytogenes in enoki mushrooms [9] provides a real-life example that illustrates the interpretive labor incurred due to nonstandardized and vague metadata. Public health authorities had been investigating clinical listeriosis cases that were clustered together based on their sequencing results for several years. In early 2020, a sample was uploaded to the public database with isolation source simply as "food." The interpretive labor to more fully describe these isolates required multiple subsequent discussions with the submitting agency to obtain more specific metadata that eventually revealed they were from "enoki mushrooms." The more specific information was later made public. The delay in obtaining this information hindered the epidemiologic investigation as additional time was required to identify enoki mushrooms as the food that was causing human illness"

The importance of metadata

James B Pettengill, Jennifer Beal, Maria Balkey, Marc Allard, Hugh Rand, Ruth Timme, **Interpretative Labor and the Bane of Nonstandardized Metadata in Public Health Surveillance and Food Safety**, Clinical Infectious Diseases, Volume 73, Issue 8, 15 October 2021, Pages 1537–1539, https://doi.org/10.1093/cid/ciab615

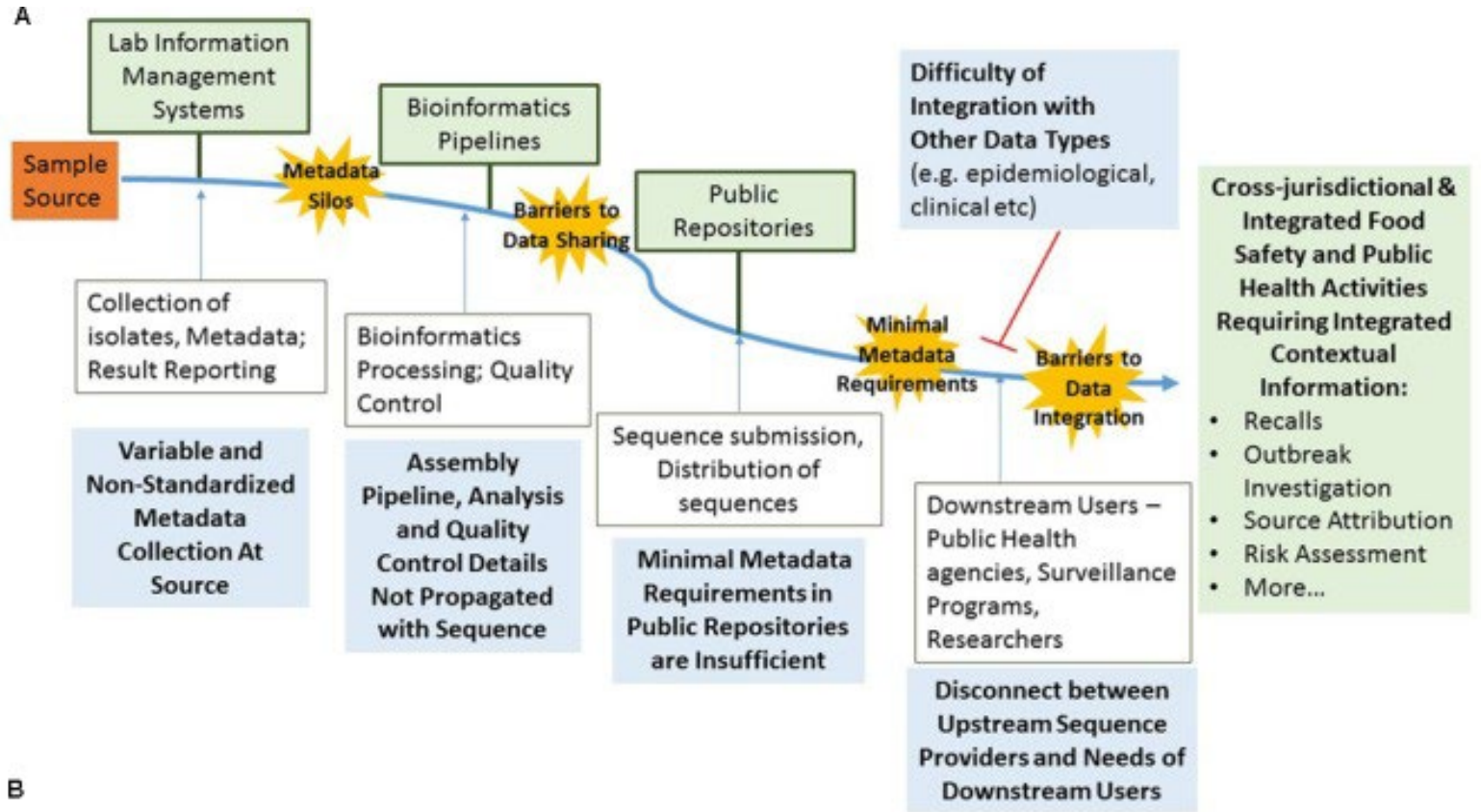
Table 1.

Number of Samples and Percent of Total Samples Missing a Value for 4 Metadata Attributes for Each High-Level Epidemiological Type Category in the National Center for Biotechnology Information Pathogen Detection Database as of 12 March 2021

Taxon	Epidemiological Type	Isolation Source	Collected By	Collection Location	Collection Date
Listeria	Food/Environmental/Other	431 (4.9%)	1316 (14.9%)	1821 (20.6%)	408 (4.6%)
	Clinical	3742 (42.3%)	1915 (21.6%)	789 (8.9%)	4120 (46.5%)
Salmonella	Food/Environmental/Other	1180 (0.6%)	1537 (0.8%)	2066 (1.1%)	691 (0.4%)
	Clinical	129 372 (67.2%)	135 032 (70.1%)	20 212 (10.5%)	3718 (1.9%)
Escherichia coli and *Shigella*	Food/Environmental/Other	212 (0.3%)	1153 (1.6%)	2114 (3%)	1038 (1.5%)
	Clinical	1019 (1.4%)	40 037 (56.6%)	43 139 (60.9%)	9768 (13.8%)

The importance of metadata

Griffiths E, Dooley D, Graham M, Van Domselaar G, Brinkman FSL, Hsiao WWL. **Context Is Everything: Harmonization of Critical Food Microbiology Descriptors and Metadata for Improved Food Safety and Surveillance**. Front Microbiol. 2017 Jun 26;8:1068. doi: 10.3389/fmicb.2017.01068.



"Figure 1. The political and technological barriers to propagating contextual information with genomics sequences. (…)"

The importance of metadata

Black A, MacCannell DR, Sibley TR, Bedford T. Ten recommendations for supporting open pathogen genomic analysis in public health. Nat Med. 2020 Jun;26(6):832-841. doi: 10.1038/s41591-020-0935-z.

ISO 23418:2022

Microbiology of the food chain — Whole genome sequencing for typing and genomic characterization of bacteria — General requirements and guidance

What to do?

Wilkinson MD, Dumontier M, Aalbersberg IJ, et al. **The FAIR Guiding Principles for scientific data management and stewardship.** Sci Data. 2016 Mar 15;3:160018. doi: 10.1038/sdata.2016.18.

Timme RE, Wolfgang WJ, Balkey M, et al. **Optimizing open data to support one health: best practices to ensure interoperability of genomic data from bacterial pathogens**. One Health Outlook. 2020;2(1):20. doi: 10.1186/s42522-020-00026-3.

Etc., etc.

The importance of metadata

ISO 23418:2022

Section 8 – Metadata

8.4 - Metadata associated with sample collection
8.5 - Metadata associated with the isolate
8.6 - Metadata associated with the sequence

Minimum fields that should be recorded for adequate traceability of isolates and sequencing results:

- Improved quality control over time
- Easier to detect problems in methods that are being used
- Reduced time for outbreak investigation
- Easier comparison of national and international data
- …

Lunch break

Back at 13:30.

Funded by the European Union

Ana Rita Rebelo and Jette Sejer Kjeldgaard
anrire@food.dtu.dk / jetk@food.dtu.dk

Exercise about cluster analysis

Funded by the European Union

Explanation

1) All clinically relevant bacterial isolates were collected from all hospitals in a region in one day
2) All isolates were sequenced using Illumina NextSeq 500

Can't use "oldest" strategy to select reference for phylogenetic analysis

3) Bioinformatics analysis was performed including:
   - Species identification with rMLST and KmerFinder
   - Prediction of MLST with MLST tool
   - Detection of antimicrobial resistance genes and point mutations with ResFinder
   - Detection of plasmid replicons with PlasmidFinder

4) All *E. coli* isolates were subjected to cgMLST analysis
5) A tree was constructed from those cgMLST results
6) Some clusters were identified with genetic distances under 15 alleles of difference

"proof-of-concept", based on literature finding >5 alleles difference on real-life outbreaks

7) Within-cluster SNP distances were calculated with CSIPhylogeny

for each analysis the reference was the best-quality isolate within that cgMLST cluster (almost guaranteed to be more closely related than other publicly available genomes)

Exercise

- Evaluate the results from gene-by-gene cluster analysis and from SNP-based phylogenetic analysis in the clusters.

- Discuss and conclude about the results:
  - Are the results of good enough quality to be used? Why or why not?
  - Are the isolates related within the clusters?
  - What can you conclude from the metadata?
  - How would you proceed next?

Exercise

Clusters	Pair of isolates	Source	Hospital	cgMLST allele difference (Nr.)	SNP difference (Nr.)	Valid positions in reference (%)	MLST	ARGs and PMs	Plasmid replicons
Cluster 12	isolate_G	Urine	F1	8	111	99.8	ST-73	bla_{TEM-1B} tet(B)	IncFIA, IncFIB(AP001918) IncFII (only isolate_G)
	isolate_H*	Blood	F1						

Clusters	Pair of isolates	Source	Hospital	cgMLST allele difference (Nr.)	SNP difference (Nr.)	Valid positions in reference (%)	MLST	ARGs and PMs	Plasmid replicons
Cluster 13	isolate_I	Urine	F9	8	2	99.8	ST-968	None	ColRNAI Col156
	isolate_J*	Blood	F9						

Exercise

Clusters	Pair of isolates	Source	Hospital	cgMLST allele difference (Nr.)	SNP difference (Nr.)	Valid positions in reference (%)	MLST	ARGs and PMs	Plasmid replicons
Cluster 3	isolate_C	Stool	F5	7	0	98.7	ST-33	bla_{TEM-1B} tet(B)	Col(MP18) Col156, Col(MG828) IncB/O/K/Z IncFIA IncFII/IncFII(pCoo)
	isolate_B	Blood	F5						
	isolate_F*	Stool	F5	8	0				
	isolate_B	Blood	F5						
	isolate_F	Stool	F5	9	1				
	isolate_C	Stool	F5						
	isolate_A	Blood	F3	10	0				
	isolate_B	Blood	F5						
	isolate_D	Stool	F3	11	1				
	isolate_C	Stool	F5						
	isolate_D	Stool	F3	12	1				
	isolate_B	Blood	F5						
	isolate_E	Stool	F5	12	0				
	isolate_F	Stool	F5						
	isolate_A	Blood	F3	12	1				
	isolate_F	Stool	F5						
	isolate_A	Blood	F3	13	0				
	isolate_C	Stool	F5						
	isolate_E	Stool	F5	14	0				
	isolate_B	Blood	F5						
	isolate_A	Blood	F3	16	1				
	isolate_D	Stool	F3						
	isolate_A	Blood	F3	16	0				
	isolate_E	Stool	F5						
	isolate_D	Stool	F3	18	1				
	isolate_F	Stool	F5						
	isolate_C	Stool	F5	19	0				
	isolate_E	Stool	F5						
	isolate_D	Stool	F3	20	1				
	isolate_E	Stool	F5						

Discussion

Clusters	Pair of isolates	Source	Hospital	cgMLST allele difference (Nr.)	SNP difference (Nr.)	Valid positions in reference (%)	MLST	ARGs and PMs	Plasmid replicons
Cluster 12	isolate_G	Urine	F1	8	111	99.8	ST-73	bla_{TEM-1B} tet(B)	IncFIA, IncFIB(AP001918) IncFII (only isolate_G)
	isolate_H*	Blood	F1						

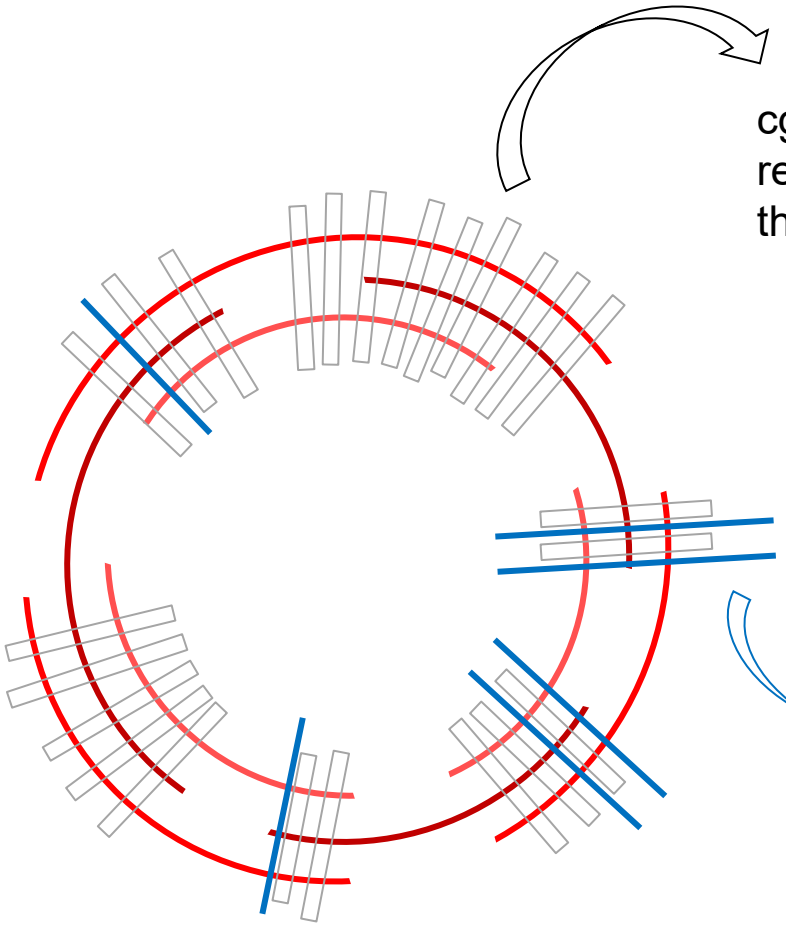
Seems like the isolates are not that closely related after all

Good quality

No discrepancies

Discrepancy

Discussion



cgMLST allele differences restricted to the loci included in the scheme

SNP distributed in regions outside of the loci included in the scheme

SNP > cgMLST diff.

Discussion

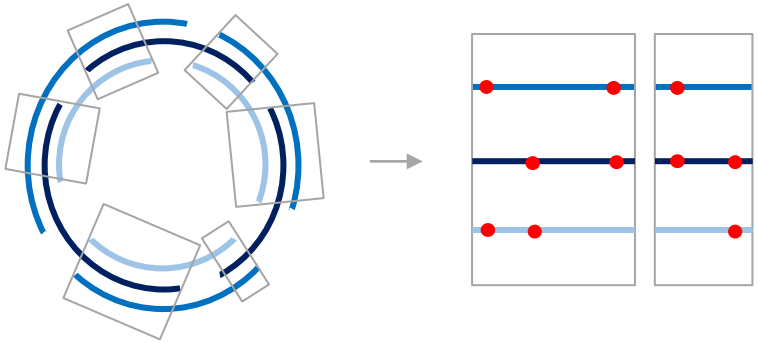
Clusters	Pair of isolates	Source	Hospital	cgMLST allele difference (Nr.)	SNP difference (Nr.)	Valid positions in reference (%)	MLST	ARGs and PMs	Plasmid replicons
Cluster 13	isolate_I	Urine	F9	8	2	99.8	ST-968	None	ColRNAI Col156
	isolate_J*	Blood	F9						

How can SNP be lower than cgMLST differences?

Good quality

No discrepancies

Discussion



Only the sections that align

…	C	T	A	G	C	T	T	A	G	G	…
…	C	T	A	G	C	T	C	A	G	G	…

1 SNP

…	C	T	A	G	C	T	T	A	G	G	…
…	C	T	A	G	C	T	-	A	G	G	…

…	C	T	A	G	C	T
…	C	T	A	G	C	T

A	G	G	…
A	G	G	…

0 SNP

Same thing for insertions

SNPs don't include indels!

Discussion

Clusters	Pair of isolates	Source	Hospital	cgMLST allele difference (Nr.)	SNP difference (Nr.)	Valid positions in reference (%)	MLST	ARGs and PMs	Plasmid replicons
	isolate_C	Stool	F5	7	0				
	isolate_B	Blood	F5						
	isolate_F*	Stool	F5	8	0				
	isolate_B	Blood	F5						
	isolate_F			9	1				
	isolate_C								
	isolate_A			10	0				
	isolate_B								
	isolate_D			11	1				
	isolate_C								
	isolate_D			12	1				
	isolate_B								
	isolate_E			12	0				
	isolate_F								
Cluster 3	isolate_A			12	1	98.7	ST-33	bla_{TEM-1B} tet(B)	Col(MP18) Col156, Col(MG828) IncB/O/K/Z IncFIA IncFII/IncFII(pCoo)
	isolate_F								
	isolate_A			13	0				
	isolate_C								
	isolate_E			14	0				
	isolate_B								
	isolate_A			16	1				
	isolate_D								
	isolate_A	Blood	F3	16	0				
	isolate_E	Stool	F5						
	isolate_D	Stool	F3	18	1				
	isolate_F	Stool	F5						
	isolate_C	Stool	F5	19	0				
	isolate_E	Stool	F5						
	isolate_D	Stool	F3	20	1				
	isolate_E	Stool	F5						

Not enough metadata:

No actionable conclusions

Very closely related

Good quality

No discrepancies

Ana Rita Rebelo
anrire@food.dtu.dk

Questions and wrapping up the day

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Please complete the feedback survey about this workshop

https://ec.europa.eu/eusurvey/runner/EURGen-RefLabCap_technical_training_workshop_2022

EURGen-RefLabCap@food.dtu.dk

Thank you on behalf of the EURGen-RefLabCap team

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