HADEA SERVICE CONTRACT 20197409

Provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples

FWD AMR·
RefLabCap

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Detection of antimicrobial resistance genes and prediction of phenotypic resistance by the ResFinder tool

Agenda

15:00 – 15:20: Introduction
- Rationale for WGS
- Current European guidance
- WGS-based analysis of bacteria

15:20 – 15:35: The ResFinder tool
- Background
- Example

15:35 – 17:00: Exercise
- Explanation (15:35 – 15:45)
- Retrieve and submit the genomes (15:45 – 16:00)
- Analysis of results (16:00 – 16:30)
- Discussion (16:30 – 17:00)

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Detection of antimicrobial resistance genes and prediction of phenotypic resistance by the ResFinder tool

Introduction

Rationale – Why WGS?

Advantages

- Only one protocol
- Very large amount of data
- Higher discriminatory power
- Harmonised and automatic analysis
- Direct comparison
- Ease of storage
- Retroactive screening

Why now?

- Increase in sequencing accuracy
- Decrease in cost
- Coordinated efforts throughout Europe

Rationale – What should we be able to do?

AMR surveillance of human *Salmonella* and *Campylobacter* infections, including tailored technology transfer for integration of WGS to national AMR surveillance and outbreak investigation

Subtyping
Phylogenetic analysis
Integration of epidemiological data

Taxonomic analysis
Detection of AMR determinants

Current European Union and international guidance

2016: “ECDC Expert opinion on whole genome sequencing for public health surveillance”
2016: “ECDC roadmap for integration of molecular and genomic typing into European-level surveillance and epidemic preparedness”
2019: “ECDC strategic framework for the integration of molecular and genomic typing into European surveillance and multi-country outbreak investigations”

2019: EFSA “Whole genome sequencing and metagenomics for outbreak investigation, source attribution and risk assessment of food-borne microorganisms”
2019: “EFSA and ECDC technical report on the collection and analysis of whole genome sequencing data from food-borne pathogens and other relevant microorganisms isolated from human, animal, food, feed and food/feed environmental samples in the joint ECDC-EFSA molecular typing database”
2021: “EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain”

2018: WHO “Landscape paper on whole genome sequencing for foodborne disease surveillance”
2020: WHO “Global Antimicrobial Resistance and Use Surveillance System (GLASS) document on whole-genome sequencing for surveillance of antimicrobial resistance”

TBA: ISO/DIS 23418 standard “Microbiology of the food chain – Whole genome sequencing for typing and genomic characterization of foodborne bacteria – General requirements and guidance”

Current European Union and international guidance

- Recommendations are similar regardless of setting (e.g. PH vs. food)
  - Importance of harmonization
  - Importance of data management infrastructures

- Several networks focused on training
  - Many training materials available
  - Potential to share and compare results with other national laboratories/other areas

- Standardization on the way
  - Almost: agreement on QC parameters
  - Almost: ISO standard
  - FWD AMR-RefLabCap + EURGen-RefLabCap

….too many options !

Tool	Reference database	Description and output
Tools for taxonomic analysis and typing		
KmerFinder [150,151]	KmerFinder	Provides hits of the query genome against whole reference genomes, the respective % of identity and % of coverage
SILVA [152]	SILVA	Collection of 16S rRNA genes, also possible to perform phylogenetic analysis and obtain phylogenetic trees
MLST [153]	PubMLST	MLST schemes, provides the sequence type
rMLST [126]	rMLST	rMLST schemes, provides the predicted species and respective allelic support metric
SerotypeFinder [154]	SerotypeFinder	Serotype, specific for E. coli
SeqSero [155]	SeqSero	Serotype, specific for Salmonella sp.
PneumoCaT [156]	PneumoCaT	Serotype, specific for S. pneumoniae
Tools for phylogenetic analysis		
cgMLST [140]	cgMLST	cgMLST schemes, available for few selected species. It can be used exclusively for typing but also clustering
CSIPhylogeny [144]	NA	SNP-based phylogenetic analysis, provides tree constructed through FastTree
Evergreen [157]	NCBI RefSeq	SNP-based phylogenetic tree integrating public genomes
Tools for detection of antimicrobial resistance determinants		
ResFinder [129]	ResFinder, PointFinder	Provides hits against reference ARGs and PMs and the respective % of identity and % of coverage, position in genome and predicted phenotype
KmerResistance [150,151]	KmerResistance	Provides hits of the query genome against reference genomes, as well as the detected ARGs and respective % of identity and % of
CARD/RGI [158]		are possible and the service is highly
AMRFinder [159]; AMRFinderPlus [160]		
ARIBA [161]	others defined by user	
Tools for detection of virulence factors		
VirulenceFinder [162]	VirulenceFinder	Provides hits against reference VFs
Victors [131]	Victors	Provides hits against reference VFs
Tools for detection and analysis of mobile genetic elements		
PlasmidFinder [163]	PlasmidFinder	Provides hits against reference plasmids and respective % of identity and % of coverage
Platon [164]	Platon	Provides hits against reference plasmids and respective % of identity and % of coverage, as well as relevant genes
pMLST [153]	PubMLST	Plasmid typing schemes
MobileElementFinder [135]	MobileElementFinder	Provides type and reference sequences of MGEs, respective % of identity and % of coverage, as well as associated ARGs and VFs
Pipelines for extensive analyses		
NCBI Pathogen Detection	NCBI DBs	Detects ARGs and VFs, provides SNP-based phylogenetic analysis
Pathogenwatch [165]	Pathogenwatch, tools' DBs	Performs taxonomic analysis, determines MLST and cgMLST and provides cgMLST-based phylogenetic clustering
BIGSdb [166]	PubMLST BIGSdb	Performs annotation and taxonomic analysis, detects ARGs and plasmids, determines MLST, rMLST and cgMLST, provides phylogenetic and spatio-phylogenetic analysis
PATRIC [167]	PATRIC, but also includes others such as CARD, NDARO and VFDV	Performs assemblies, quality control, annotation and taxonomic analysis, detects ARGs and performs phenotype prediction, detects VFs and MGEs, provides phylogenetic analysis, variation analysis and genome alignments

This is just a subset….

Current European Union and international guidance

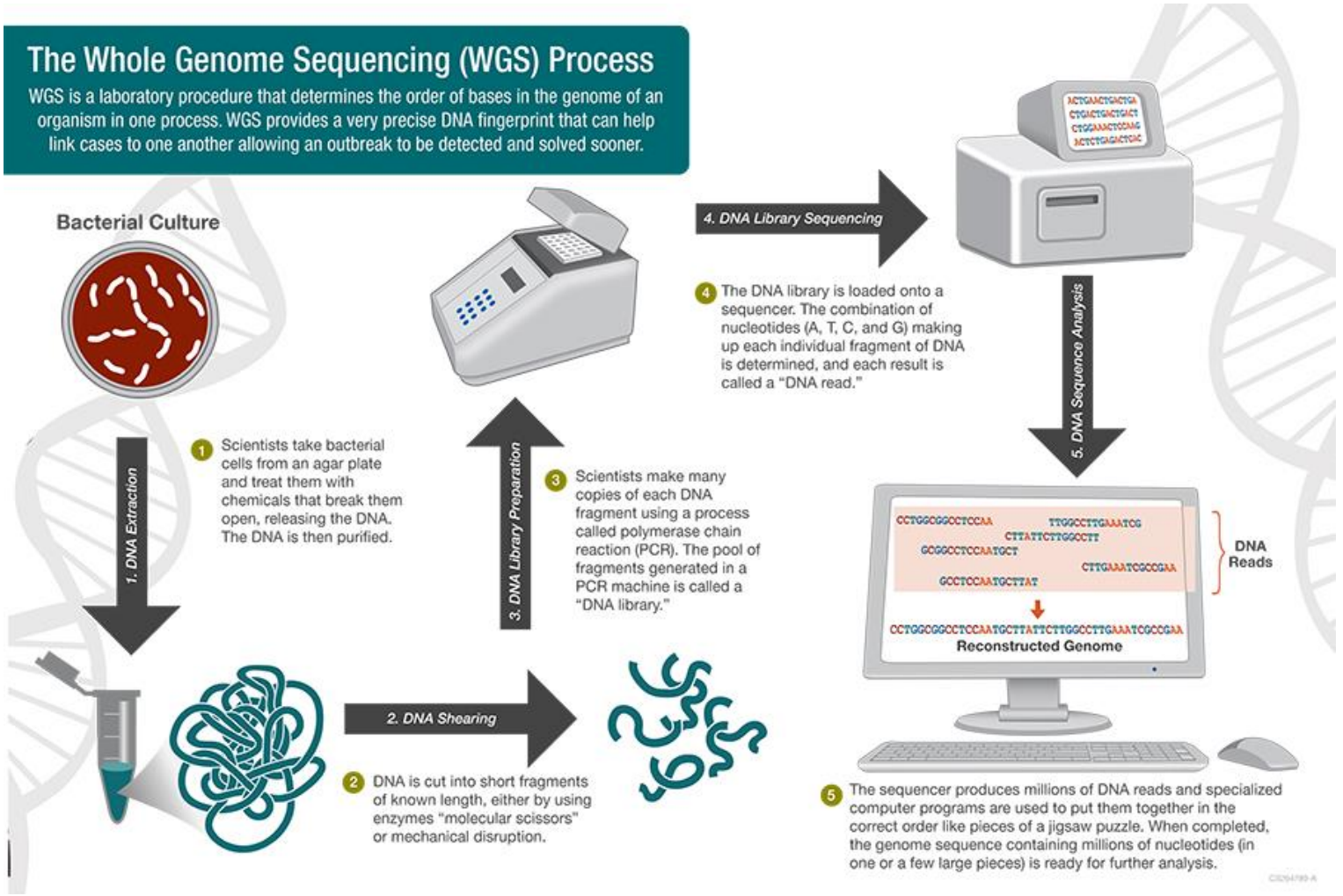
Agreement on necessary requirements:

- prediction of clinically and epidemiologically relevant microbial phenotypes

antigenic profile, AMR and virulence, including identification of determinants encoded in the accessory genome and mobile genetic elements

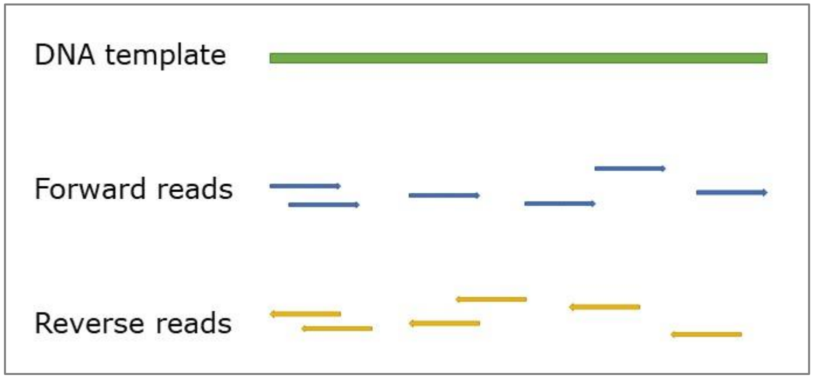
- phylogenetic analysis
- well defined QC parameters
- integration of sequence data with epidemiological and clinical data
- database for the collection and analysis of WGS data + proper management

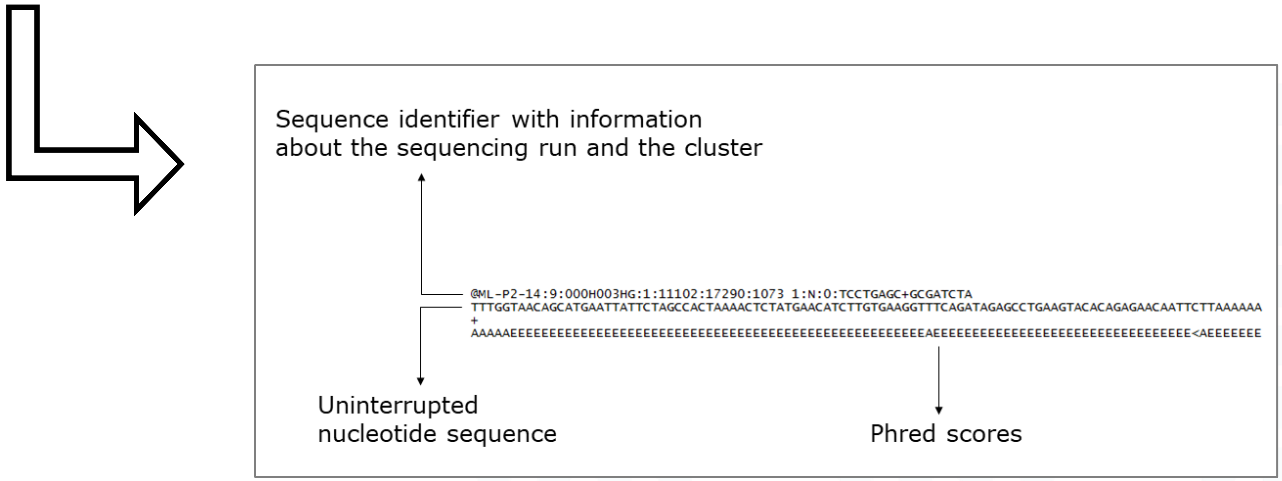
WGS-based analysis of bacteria - Overview



https://www.cdc.gov/pulsenet/pathogens/protocol-images.html#wgs

WGS-based analysis of bacteria – How it works





WGS-based analysis of bacteria – How it works

Raw data in *.fastq* format

```
@ML-P2-14:9:000H003HG:1:11102:17290:1073 1:N:0:TCCTGAGC+GCGATCTA
TTTGGTAACAGCATGAATTATTCTAGCCACTAAAACTCTATGAACATCTTGTGAAGGTTTCAGATAGAGCCTGAAGTACACAGAGAACAATTCTTAAAAAA
+
AAAAAEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEAEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE<AEEEEEEE
```

QC
Consensus
Assembly

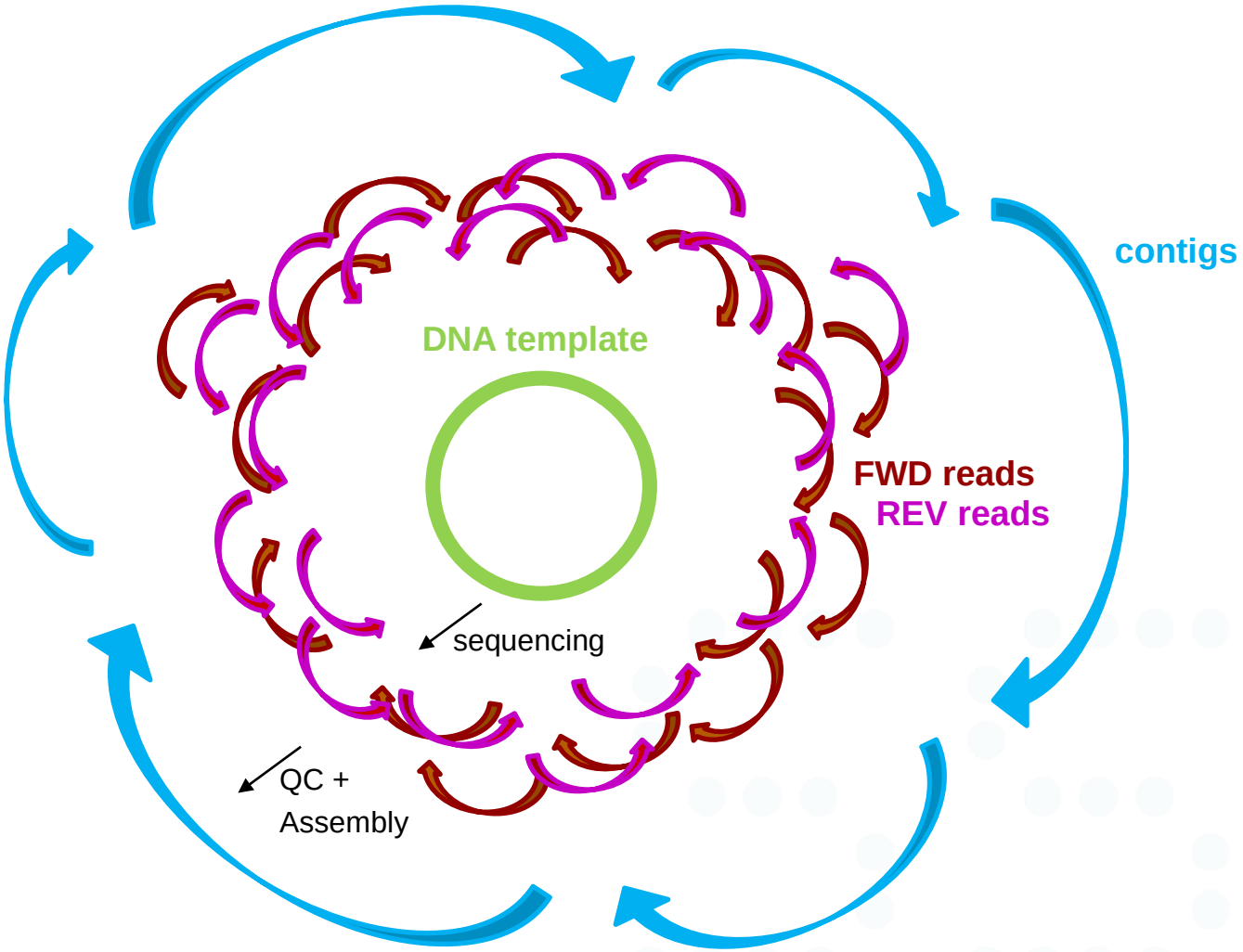
Assembled data in *.fasta* format

```
>NODE_1_length_665560_cov_2.979749
TGGCCGTGAAAGAAAGCAATCAGCGATGGTGCTCTGACGGGTTCGAGTTCTGCTGTGATA
ACGGAGAGAGACTGCGTGTCACGTTCGCGCTGGACTGCTGTGATCGTGAGGCACTGCACT
GGGCGGTCACTACCGGCGGCTTCAACAGTGAAACAGTACAGGACGTCATGCTGGGAGCGG
```

Compared with reference databases

What genes from the database are present in this genome?

WGS-based analysis of bacteria – How it works



WGS-based analysis of bacteria – Requirements

- Expertise on DNA extraction methods
- Expertise on library preparation methods

Not too technically demanding
Ideally a dedicated room

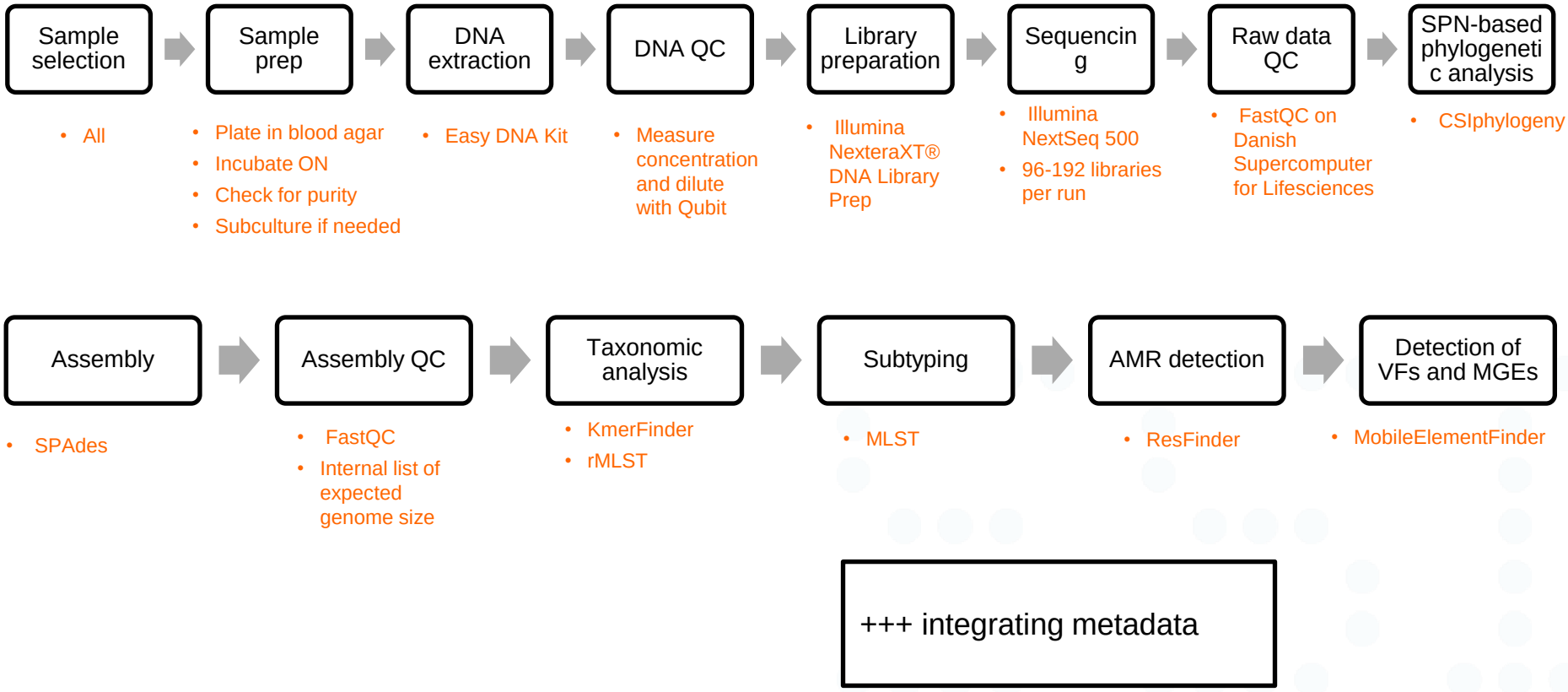
- Access to sequencing platform
- Access and expertise on bioinformatics tools

Main challenges: cost, implementation

- Data management infrastructure

Main challenges: cost, compatibility

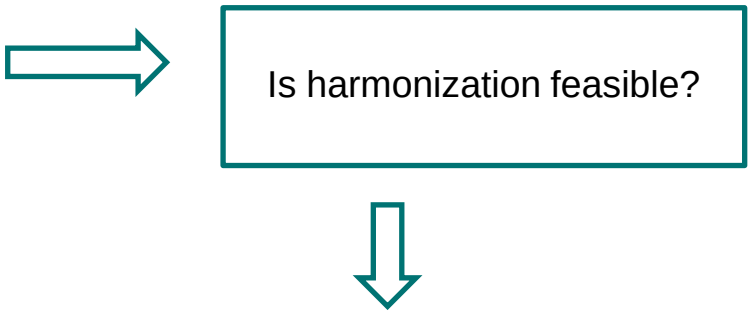
Example: a complete WGS workflow



Very important! – Quality control

Many different:

- DNA extraction kits
- Sequencing platforms
- Bioinformatics approaches
- Bioinformatics tools



Well defined set of QC parameters

- For the raw data
  - *E.g. nr. and length of raw reads, depth of coverage*
- For the assembled genomes
  - *E.g. N50, nr. of contigs, genome size*
- For the performance of the tools
  - *E.g. accurately detect PMs and ARGs in sets of benchmarking data*

Very important! – Quality control

Raw data QC	Assembled data QC
Number of reads Should be as high as possible. No assessed cut-off exist, but enough to obtain the desired coverage of the organism genome. **Average read length** Should correspond to that expected from the sequencing platform and kit. Illumina MiSeq avg read length = 300 bps Illumina NextSeq avg read length = 150 bps **Coverage** Should as a minimum be 25x, and preferably even higher (e.g. 50x).	**Size of assembled genome** *Salmonella*: 4.4 Mb - 5.8 Mb *Campylobacter*: 1.5 Mb - 1.9 Mb **Total number of contigs** Should be less than 500. *Campylobacter* will typically be assembled into less than 100 contigs and *Salmonella* to less than 300 contigs. **N50** Should be over 15.000 - 30.000 bp

$$Coverage = Number\ of\ reads\ x\ \frac{Read\ length}{Genome\ size}$$

Very important! – Quality control

Troubleshooting poor QC values

Usually poor **raw data** QC indicates:
- Inadequate DNA extraction
- Inadequate library preparation

Usually poor **assembly** QC indicates:
- Inadequate DNA extraction
- Contaminations

Re-sequence or re-extract?

Evaluation of QC becomes easier with experience + understanding the biochemical principles of the protocols.

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The ResFinder tool

Explanation – Online tool and databases

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

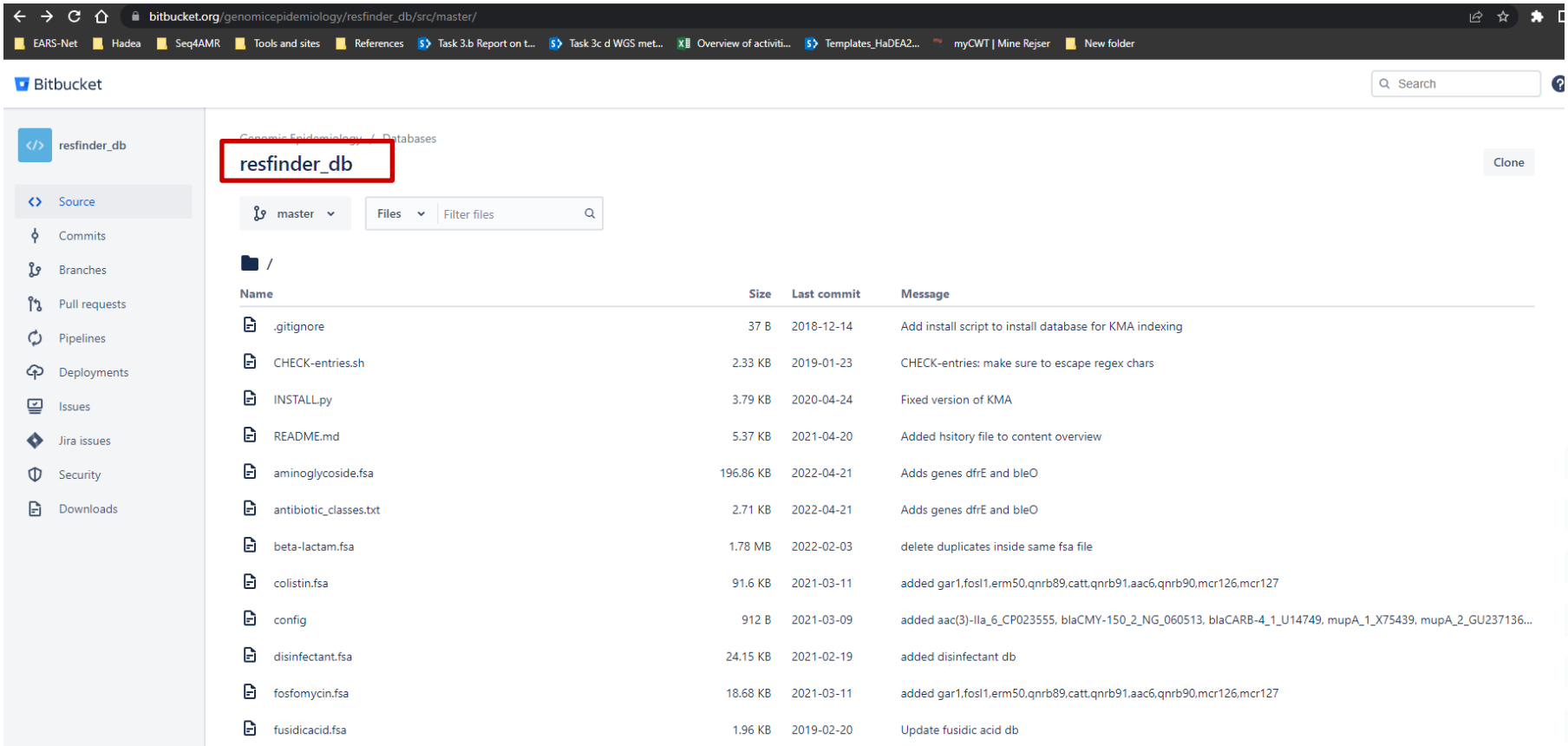
Databases with antimicrobial resistance genes and chromosomal point mutations

ResFinder database **PointFinder database**

https://bitbucket.org/genomicepidemiology/resfinder_db/src/master/

https://bitbucket.org/genomicepidemiology/pointfinder_db/src/master/

Explanation – Databases



Explanation – Databases

phenotype_panels.txt	2.55 KB	2021-10-06
phenotypes.txt	502.55 KB	2022-04-21

Genomic Epidemiology / Databases / resfinder_db

phenotypes.txt

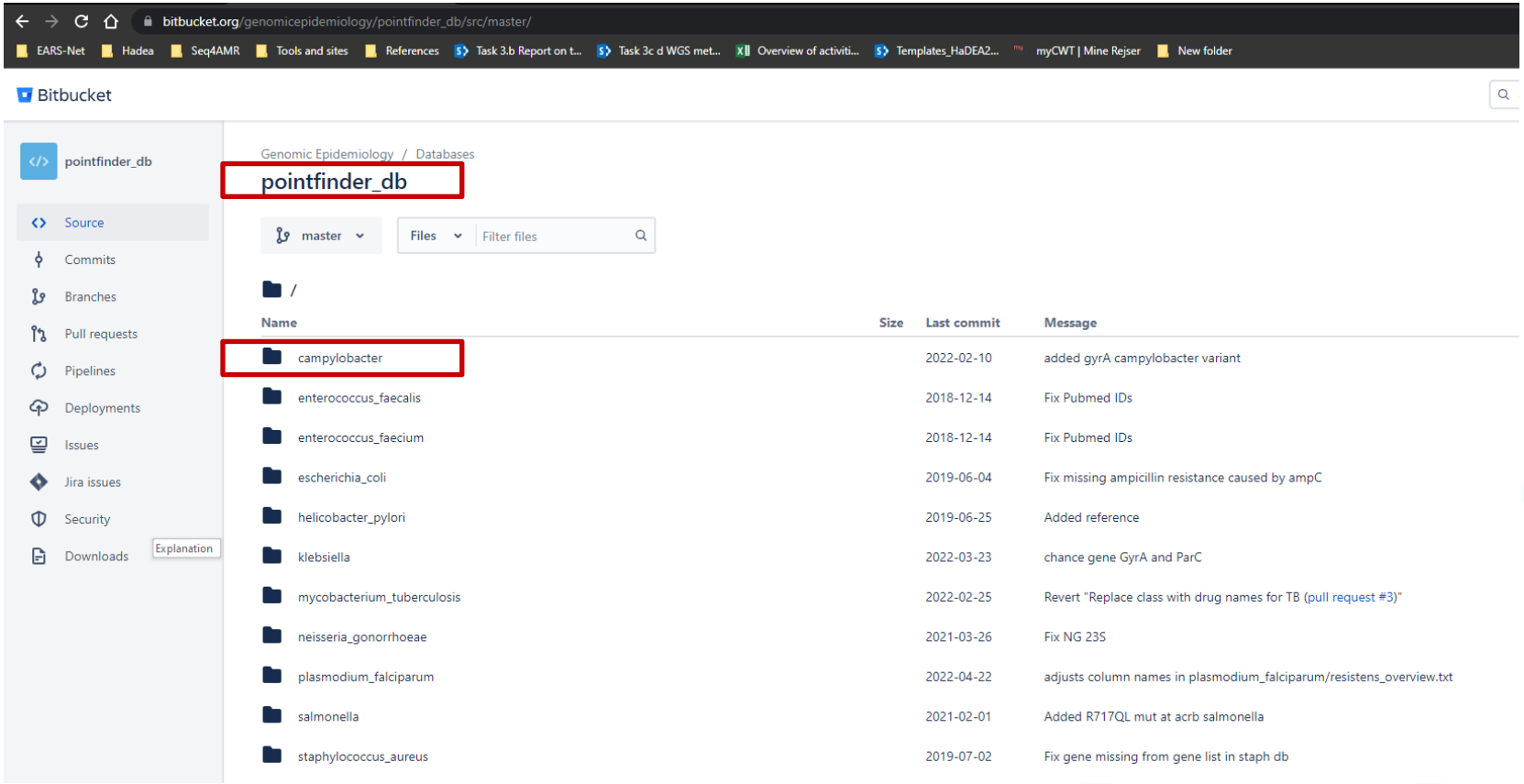
Pull requests Check out

Source master d504dca Full commit

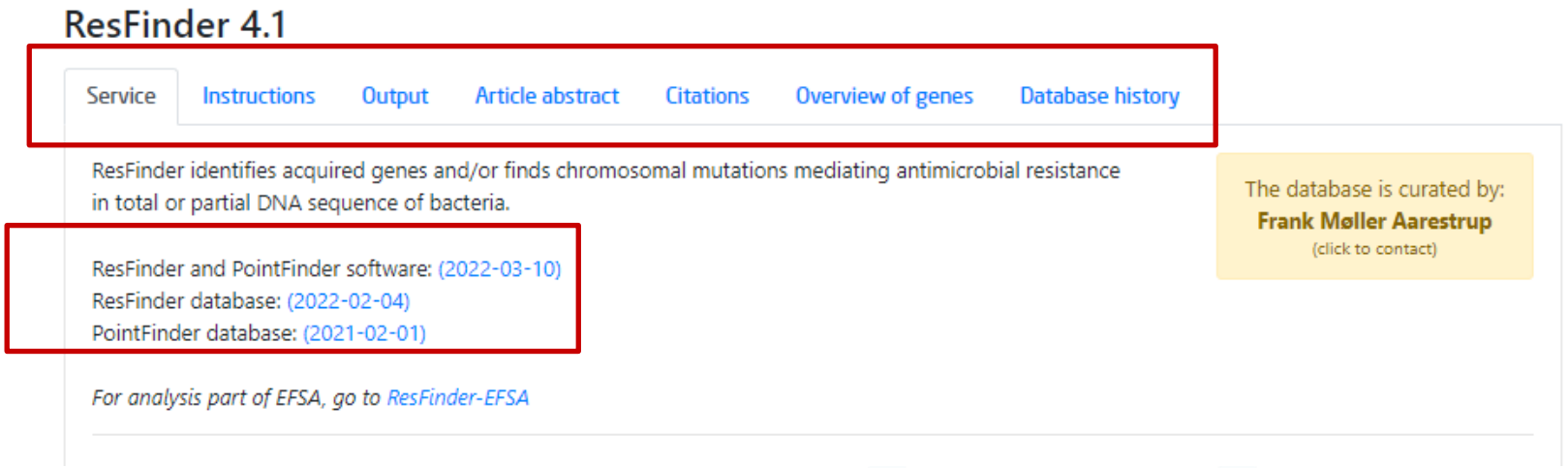
resfinder_db / phenotypes.txt Edit

```
Gene_accession no.  Class   Phenotype   PMID    Mechanism of resistance Notes   Required_gene
ant(2'')-Ia_1_X04555    Aminoglycoside  Gentamicin, Tobramycin  3024112 Enzymatic modification  Alternative name aadB
ant(2'')-Ia_10_HM367617 Aminoglycoside  Gentamicin, Tobramycin  21873033    Enzymatic modification
ant(2'')-Ia_11_HM367620 Aminoglycoside  Gentamicin, Tobramycin  21873033    Enzymatic modification
ant(2'')-Ia_12_HQ880250 Aminoglycoside  Gentamicin, Tobramycin  unpublished Enzymatic modification
ant(2'')-Ia_13_DQ176450 Aminoglycoside  Gentamicin, Tobramycin  16304199    Enzymatic modification
ant(2'')-Ia_14_DQ266447 Aminoglycoside  Gentamicin, Tobramycin  unpublished Enzymatic modification
ant(2'')-Ia_15_EF205594 Aminoglycoside  Gentamicin, Tobramycin  unpublished Enzymatic modification
ant(2'')-Ia_16_HQ386848 Aminoglycoside  Gentamicin, Tobramycin  unpublished Enzymatic modification
ant(2'')-Ia_17_JTTZ01000034 Aminoglycoside  Gentamicin, Tobramycin  unpublished Enzymatic modification
ant(2'')-Ia_19_GQ466184 Aminoglycoside  Gentamicin, Tobramycin  unpublished Enzymatic modification
ant(2'')-Ia_2_JF826500  Aminoglycoside  Gentamicin, Tobramycin  22271862    Enzymatic modification
ant(2'')-Ia_20_AY139599 Aminoglycoside  Gentamicin, Tobramycin  19719593    Enzymatic modification
ant(2'')-Ia_3_X74412    Aminoglycoside  Gentamicin, Tobramycin  unpublished Enzymatic modification
ant(2'')-Ia_4_AF458082  Aminoglycoside  Gentamicin, Tobramycin  12384364    Enzymatic modification
ant(2'')-Ia_5_AY139594  Aminoglycoside  Gentamicin, Tobramycin  19719593    Enzymatic modification
ant(2'')-Ia_6_AJ871915  Aminoglycoside  Gentamicin, Tobramycin  unpublished Enzymatic modification
ant(2'')-Ia_7_DQ018384  Aminoglycoside  Gentamicin, Tobramycin  15837385    Enzymatic modification
ant(2'')-Ia_8_AY920928  Aminoglycoside  Gentamicin, Tobramycin  16048994    Enzymatic modification
ant(2'')-Ia_9_HM367610  Aminoglycoside  Gentamicin, Tobramycin  21873033    Enzymatic modification
ant(3'')-Ia_1_X02340    Aminoglycoside  Streptomycin    8385262 Enzymatic modification  Alternative name aadA, aad(3'')(9), aadA1, aadA1a
ant(3'')-Ii-aac(6')-IId_1_AF453998  Aminoglycoside  Gentamicin, Streptomycin, Tobramycin, Spectinomycin, Amikacin   11959575,20833577   Enzymatic modification  Alternative name ant(3'')-Ih-aac(6')-Iid
ant(4')-Ib_1_AJ506108   Aminoglycoside  Amikacin, Tobramycin, Isepamicin, Dibekacin 12654668    Enzymatic modification  Alternative name aadA2
ant(4')-IIa_1_M98270    Aminoglycoside  Amikacin, Tobramycin, Isepamicin    8385262 Enzymatic modification
ant(4')-IIb_1_AY114142  Aminoglycoside  Amikacin, Tobramycin, Isepamicin    12709326    Enzymatic modification
```

Explanation – Databases



Explanation – Online tool



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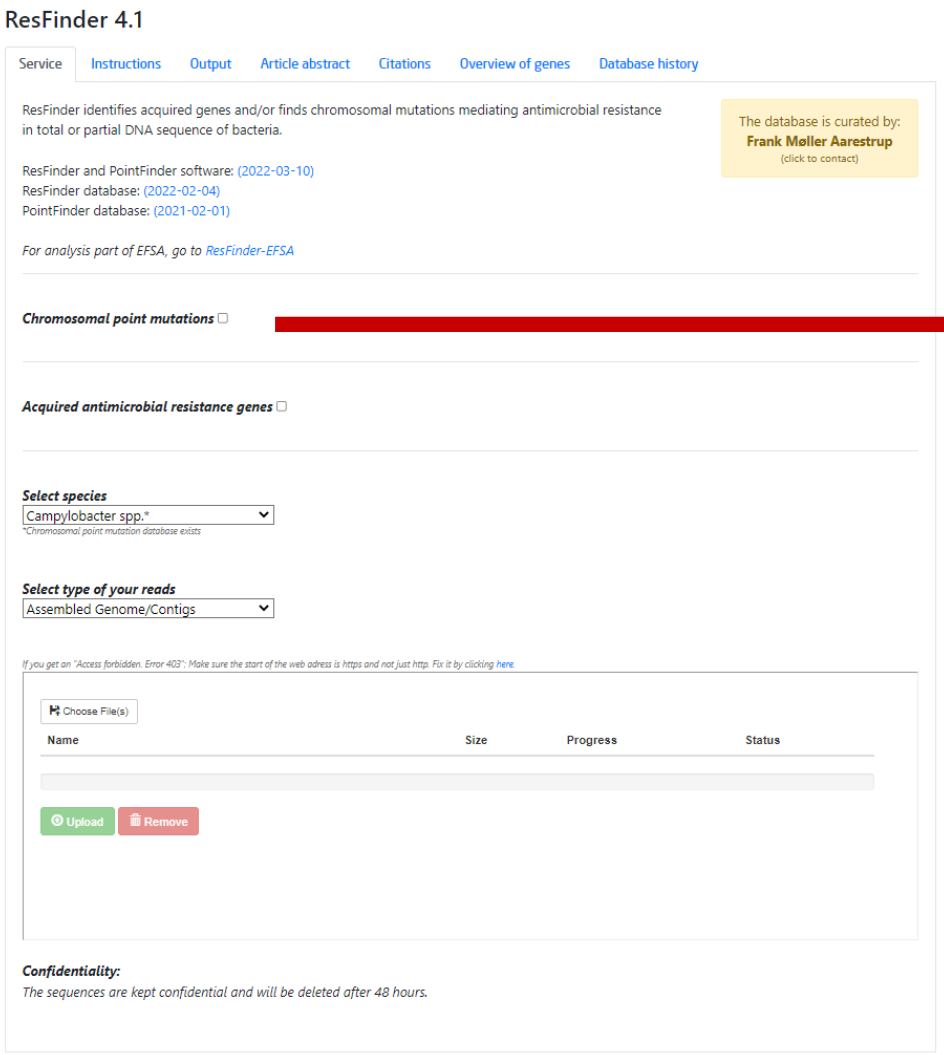

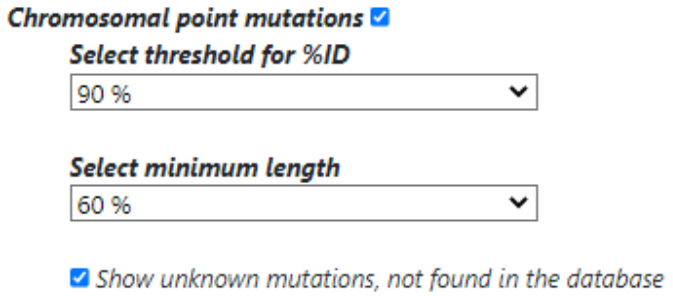

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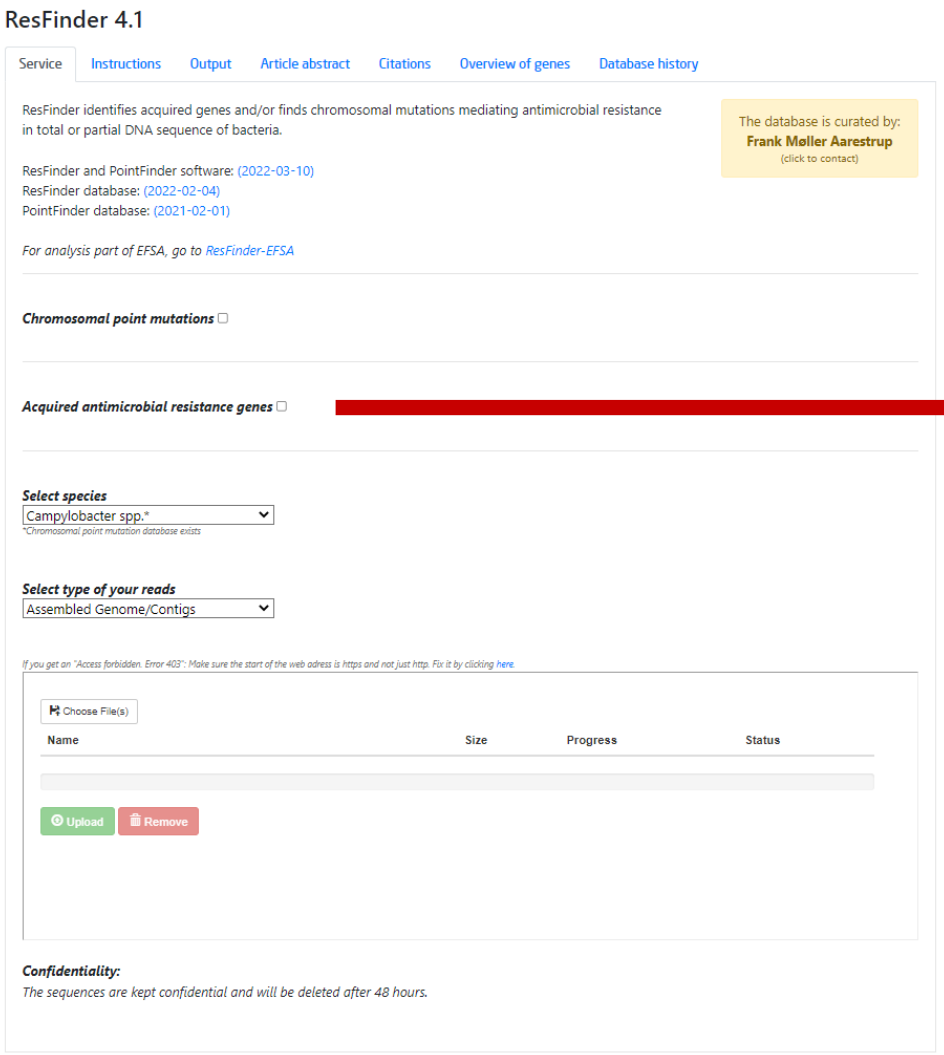
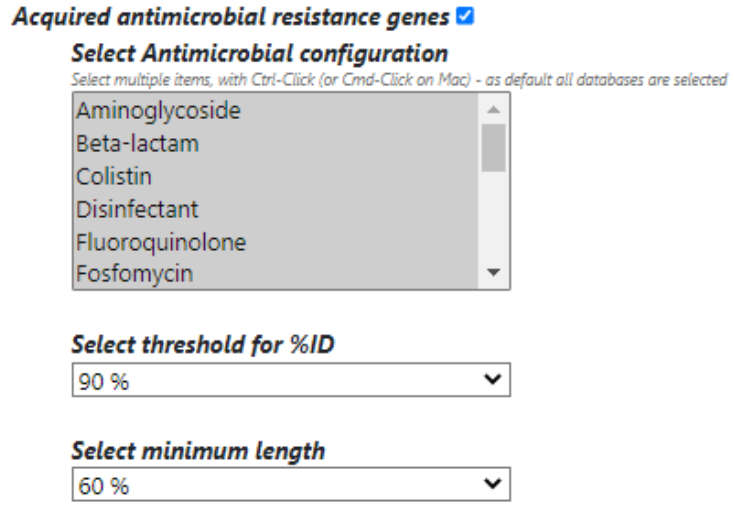
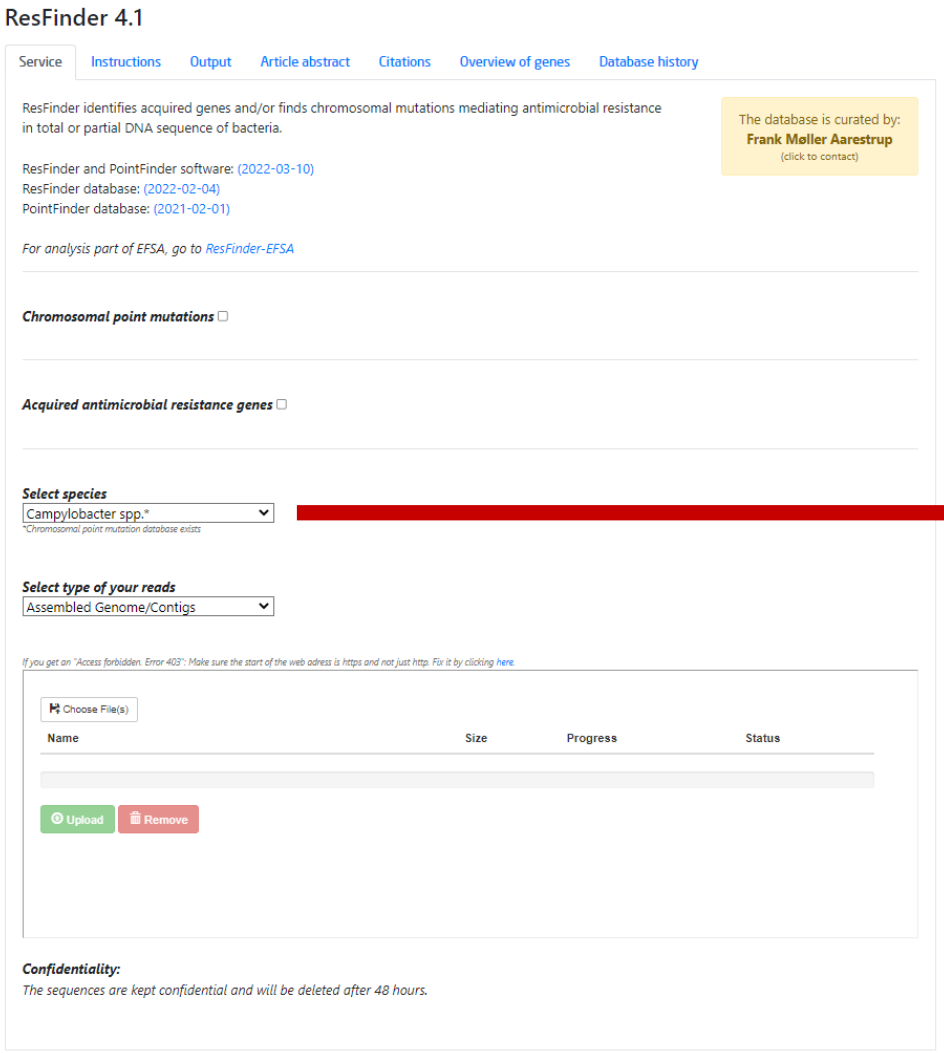
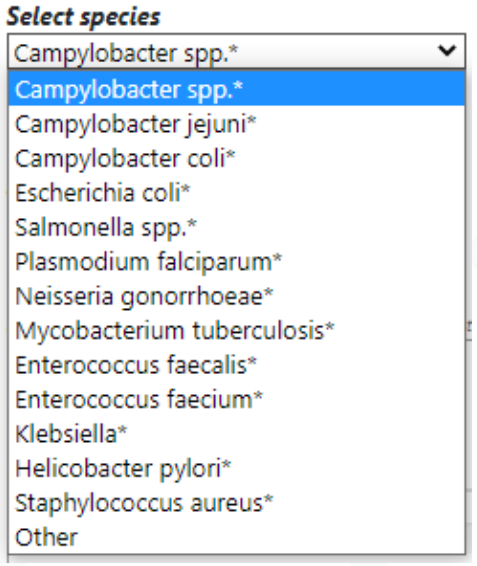




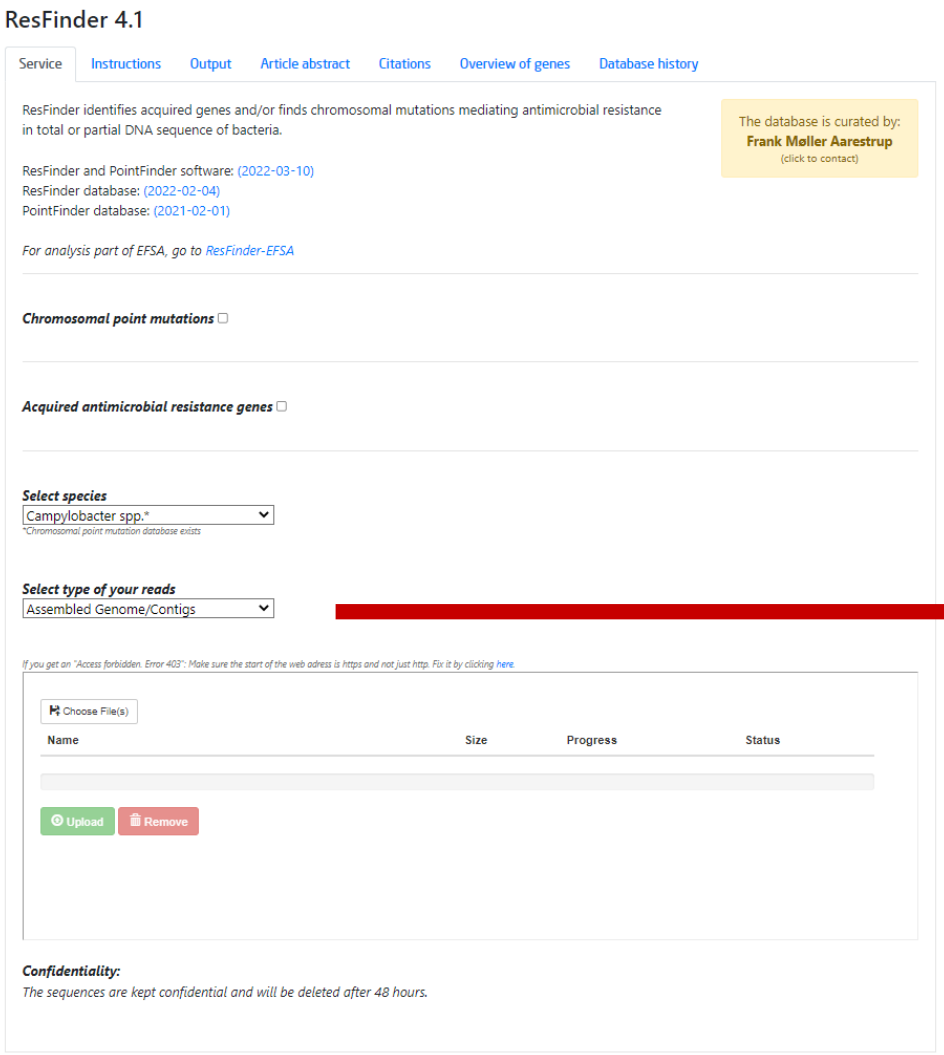
Explanation – Online tool

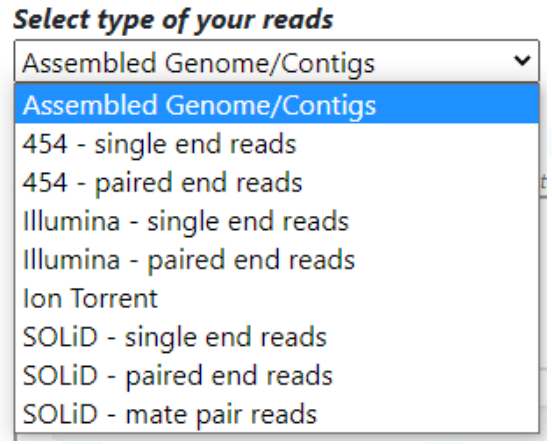




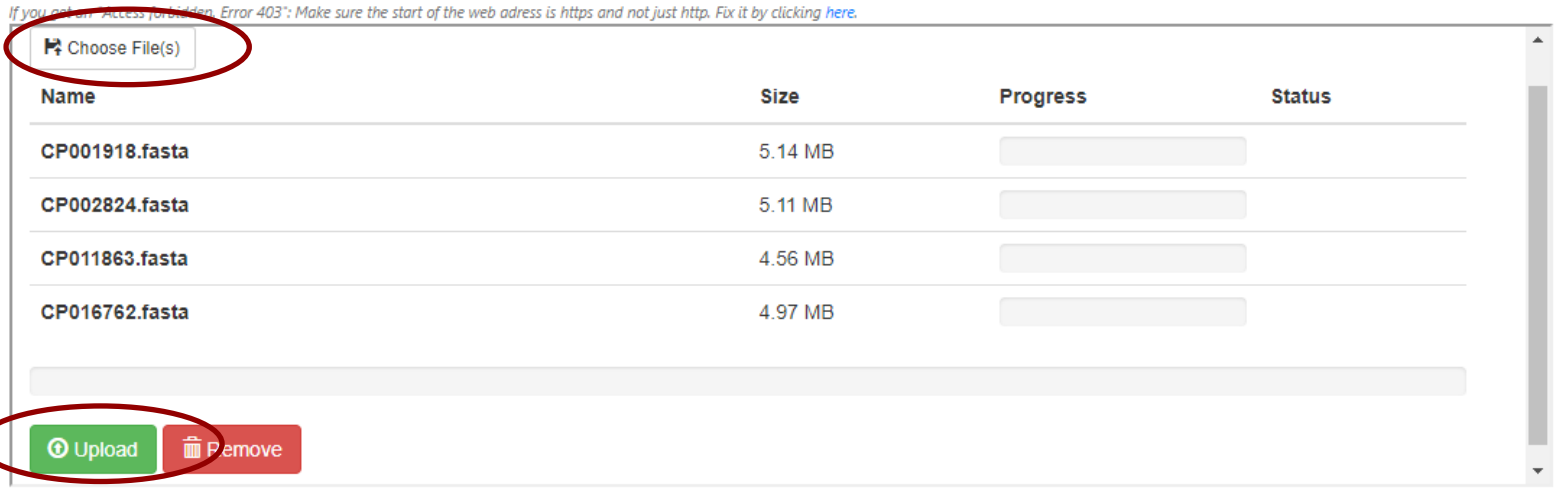
**Chromosomal point mutation database exists*

Explanation – Online tool





Explanation – Online tool



Confidentiality:

The sequences are kept confidential and will be deleted after 48 hours.

Explanation – Online tool

Center for Genomic Epidemiology

Your job has been queued

We are currently receiving a lot of job submissions, and there are no free computing slots available at the moment.
Your job will be processed as soon as a slot becomes available...

You can wait here to watch the progress of your job, or fill in the form below to get notified by email upon job completion.

Email address: Notify me via email

Thank you for your patience.

This page will update itself automatically.

Example – Output

escherichia coli | complete

Antimicrobial	Class	WGS-predicted phenotype	Genetic background
amikacin	aminoglycoside	Resistant	aac(6')-Ib-cr (aac(6')-Ib-cr_DQ303918)
tigecycline	tetracycline	No resistance	
tobramycin	aminoglycoside	Resistant	aac(6')-Ib-cr (aac(6')-Ib-cr_DQ303918)
cefepime	beta-lactam	Resistant	blaCTX-M-15 (blaCTX-M-15_AY044436), blaOXA-1 (blaOXA-1_HQ170510)
chloramphenicol	amphenicol	Resistant	catB3 (catB3_U13880), catB3 (catB3_AJ009818)
piperacillin+tazobactam	beta-lactam	Resistant	blaOXA-1 (blaOXA-1_HQ170510)
cefoxitin	beta-lactam	No resistance	
ampicillin	beta-lactam	Resistant	blaCTX-M-15 (blaCTX-M-15_AY044436), blaOXA-1 (blaOXA-1_HQ170510)
ampicillin+clavulanic acid	beta-lactam	Resistant	blaOXA-1 (blaOXA-1_HQ170510)
cefotaxime	beta-lactam	Resistant	blaCTX-M-15 (blaCTX-M-15_AY044436)
ciprofloxacin	quinolone	Resistant	aac(6')-Ib-cr (aac(6')-Ib-cr_DQ303918) gyrA (p.S83L)
colistin	polymyxin	No resistance	
sulfamethoxazole	folate pathway antagonist	Resistant	sul1 (sul1_U12338)
imipenem	beta-lactam	No resistance	
trimethoprim	folate pathway antagonist	Resistant	dfrA17 (dfrA17_FJ460238)
nalidixic acid	quinolone	Resistant	gyrA (p.S83L), gyrA (p.D87N)
ertapenem	beta-lactam	No resistance	
tetracycline	tetracycline	No resistance	
fosfomycin	fosfomycin	No resistance	
ceftazidime	beta-lactam	Resistant	blaCTX-M-15 (blaCTX-M-15_AY044436)
temocillin	beta-lactam	No resistance	
gentamicin	aminoglycoside	No resistance	
meropenem	beta-lactam	No resistance	
azithromycin	macrolide	Resistant	mph(A) (mph(A)_D16251)

Example – Output

Antimicrobial	Class	WGS-predicted phenotype	Genetic background
vancomycin	glycopeptide	No resistance	
mupirocin	pseudomonic acid	No resistance	
tobramycin	aminoglycoside	Resistant	aac(6')-Ib-cr (aac(6')-Ib-cr_DQ303918)
virginiamycin m	streptogramin a	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	blaCTX-M-15 (blaCTX-M-15_AY044436), blaOXA-1 (blaOXA-1_HQ170510)
astromicin	aminoglycoside	No resistance	
lividomycin	aminoglycoside	No resistance	
sulfamethoxazole	folate pathway antagonist	Resistant	sul1 (sul1_U12338)
temocillin	beta-lactam	No resistance	
g418	aminoglycoside	No resistance	
trimethoprim	folate pathway antagonist	Resistant	dfrA17 (dfrA17_FJ460238)
oleandomycin	macrolide	No resistance	
florfenicol	amphenicol	No resistance	
clindamycin	lincosamide	No resistance	
quinupristin	streptogramin b	No resistance	
ceftriaxone	beta-lactam	Resistant	blaCTX-M-15 (blaCTX-M-15_AY044436)
cephalothin	beta-lactam	No resistance	
hygromycin	aminoglycoside	No resistance	
spectinomycin	aminocyclitol	Resistant	aadA5 (aadA5_AF137361)
piperacillin+clavulanic acid	beta-lactam	No resistance	
paromomycin	aminoglycoside	No resistance	
fluoroquinolone	quinolone	Resistant	aac(6')-Ib-cr (aac(6')-Ib-cr_DQ303918)
amoxicillin+clavulanic acid	beta-lactam	Resistant	blaOXA-1 (blaOXA-1_HQ170510)
teicoplanin	glycopeptide	No resistance	
tiamulin	pleuromutilin	No resistance	
ribostamycin	aminoglycoside	No resistance	
erythromycin	macrolide	Resistant	mph(A) (mph(A)_D16251)
kanamycin	aminoglycoside	No resistance	
gentamicin	aminoglycoside	No resistance	
amikacin	aminoglycoside	Resistant	aac(6')-Ib-cr (aac(6')-Ib-cr_DQ303918)
tigecycline	tetracycline	No resistance	

Example – Output

Folate pathway antagonist									
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig	Phenotype	PMID	Accession no.	Notes
dfrA17	100.0	474/474	1..474	NODE_42_length_11333_cov_5.776905	8717..9190	trimethoprim	19573249	FJ460238	
sul1	100.0	840/840	1..840	NODE_42_length_11333_cov_5.776905	6412..7251	sulfamethoxazole	unpublished	U12338	Purine synthesis

Aminoglycoside									
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig	Phenotype	PMID	Accession no.	Notes
aac(6')-Ib-cr	100.0	600/600	1..600	NODE_69_length_2438_cov_6.118563	173..772	ciprofloxacin	unpublished	DQ303918	MIC of ciprofloxacin does not always increase above ECOFF PMID 16369542
aadA5	100.0	789/789	1..789	NODE_42_length_11333_cov_5.776905	7798..8586	spectinomycin,streptomycin	10673049	AF137361	

Example – Output

Quinolone									
Resistance gene	**Identity**	**Alignment Length/Gene Length**	**Position in reference**	**Contig or Depth**	**Position in contig**	**Phenotype**	**PMID**	**Accession no.**	**Notes**
aac(6')-Ib-cr	100.0	600/600	1..600	NODE_69_length_2438_cov_6.118563	173..772	ciprofloxacin	unpublished	DQ303918	MIC of ciprofloxacin does not always increase above ECOFF PMID 16369542

Mutation	**Nucleotide change**	**Amino acid change**	**Phenotype**	**PMID**	**Notes**
parC:p.S80I	agc -> att	s -> i	nalidixic acid,ciprofloxacin	8851598, 8851598, 21856834-20638608, 8524852, 25631675, 25631675, 25631675	Unknown phenotype if each mutation occurs alone. Nalidixic acid and ciprofloxacin resistance when associated with gyrA mutations
parE:p.I529L	att -> ctt	i -> l	nalidixic acid,ciprofloxacin	14506034	Unknown phenotype if I529L occurs alone. Nalidixic acid and ciprofloxacin resistance when associated with gyrA mutations.
parC:p.E84V	gaa -> gta	e -> v	nalidixic acid,ciprofloxacin	12654733, 8524852, 12654733, 20638608, 20638608	Unknown phenotype if each mutation occurs alone. Nalidixic acid and ciprofloxacin resistance when associated with gyrA mutations
gyrA:p.D87N	gac -> aac	d -> n	nalidixic acid,ciprofloxacin	12654733, 12654733, 12654733, 22878251, 12654733, 1850972	D87G or D87Y confer resistance to nalidixic acid only, if occurring alone. Unknown phenotype if D87H occurs alone
gyrA:p.S83L	tcg -> ttg	s -> l	nalidixic acid,ciprofloxacin	8891148, 2168148, 12654733, 12654733	

Example – Output

No class defined					
Mutation	**Nucleotide change**	**Amino acid change**	**Phenotype**	**PMID**	**Notes**
23S;;23S;;urgw:g.547_548insA	ins -> a	-	Unknown phenotype	-	Phenotype not found in database
23S;;23S;;urgw:g.1171G>A	g -> a	-	Unknown phenotype	-	Phenotype not found in database
23S;;23S;;urgw:g.2794C>T	c -> t	-	Unknown phenotype	-	Phenotype not found in database
parC:p.A471G	gcc -> ggc	a -> g	Unknown phenotype	-	Phenotype not found in database
23S;;23S;;urgw:g.1870C>T	c -> t	-	Unknown phenotype	-	Phenotype not found in database
23S;;23S;;urgw:g.2215C>G	c -> g	-	Unknown phenotype	-	Phenotype not found in database
23S;;23S;;urgw:g.2211A>T	a -> t	-	Unknown phenotype	-	Phenotype not found in database
23S;;23S;;urgw:g.541_541del	del -> a	-	Unknown phenotype	-	Phenotype not found in database
folP:p.I38L	ata -> tta	i -> l	Unknown phenotype	-	Phenotype not found in database
gyrB:p.A618T	gct -> acc	a -> t	Unknown phenotype	-	Phenotype not found in database
23S;;23S;;urgw:g.1870_1871insG	ins -> g	-	Unknown phenotype	-	Phenotype not found in database
23S;;23S;;urgw:g	c -> t		Unknown		Phenotype not found in database

Example – Output

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 %*

Ana Rita Rebelo
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Detection of antimicrobial resistance genes and prediction of phenotypic resistance by the ResFinder tool

Exercise

Objectives

- Recover 3 genomes
- Submit the genomes to the ResFinder online tool
- Analyse the output
- **Predict AMR phenotypes for each genome**

Recover the genomes

- Go to link:

https://sciencedata.dk/themes/deic_theme_oc7/apps/files_sharing/public.php?t=491f60d77a14f1679ed0fb4426719b40&

- Download the files to your computer

Submit the genomes

- Upload each genome to the online ResFinder tool:

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

- Provide your email address so that you can close the internet browser safely

Results may take a while…

Get the results

- The results from the webtool expire after a few days:

https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=6285FD90000018269B6B1C21

https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=6285FDA4000018855FDDFD55

https://cge.food.dtu.dk//cgi-bin/webface.fcgi?jobid=6285FDB0000018C50E23D92D

Get the results

- Get the permanent results files
- Go to link:

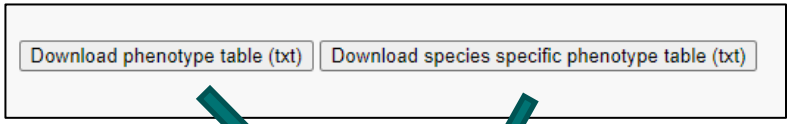
https://sciencedata.dk/themes/deic_theme_oc7/apps/files_sharing/public.php?t=a31061b7d93e5e2c6402b88a93ec0f8b&

- Download the results files to your computer

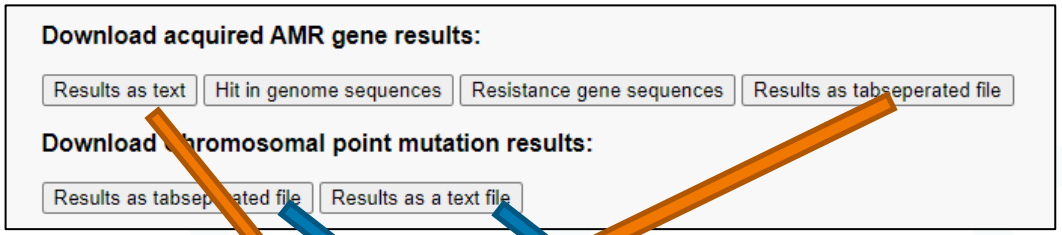
Get the results

- Important to know how to work with the downloaded outputs
- Non-assigned PMs only in the ”pheno_table_full”

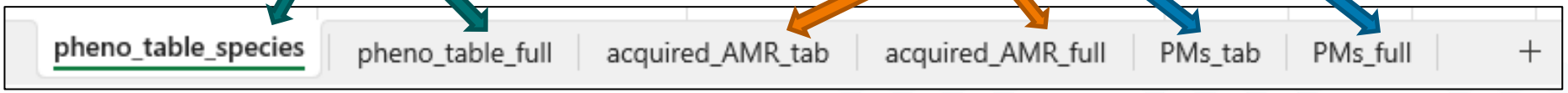
After the first table in the webtool:



At the end of the page in the webtool:



In the excel files:



Analyse the results

Analyse the output for each genome:

1) Is the quality of the genomes good enough?

- If not, what QC parameters are not up to standard and how would you proceed to improve quality?

2) What ARGs or PMs can you see?

- Is the quality of each hit good enough to predict a phenotype? If not, how would you proceed to improve results?

3) Which resistance phenotypes would you report?

Discussion

Quality control

Calculated afterwards (not part of FoodQC output)

Sample	Bases (MB)	Qual Bases (MB)	Qual bases %	Reads	Qual reads	Qual reads %	N50	No ctgs	longest	total bps	Coverage (MiSeq)
EQA_AST.C21.0001_R1_001.fastq.gz	600	551	91.75%	4612410	4264016	92.45%	161093	30	347014	1747414	732
EQA_AST.C21.0002_R1_001.fastq.gz	516	477	92.47%	3935002	3671928	93.31%	130467	54	310670	1762310	625
EQA_AST.C21.0003_R1_001.fastq.gz	584	541	92.52%	4391772	4115616	93.71%	131228	63	288888	1835754	673
EQA_AST.C21.0004_R1_001.fastq.gz	533	493	92.51%	4046804	3781610	93.45%	178096	32	332914	1699491	668
EQA_AST.C21.0005_R1_001.fastq.gz	530	474	89.35%	4114474	3761460	91.42%	171315	29	373263	1745805	646

What if “No ctgs” was higher or lower?

What if “Coverage” was higher or lower?

What if “total bps” was higher or lower?

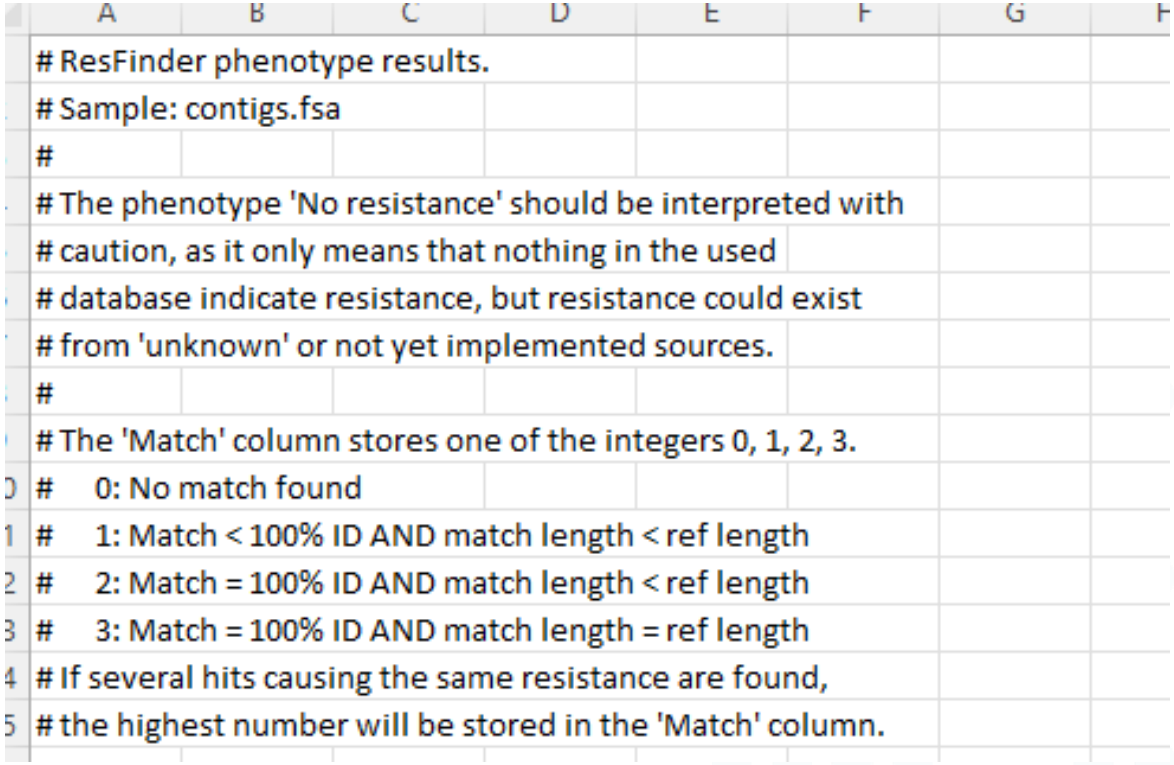
Phenotypes as determined in the laboratory

VS.

In silico antibiograms and predicted phenotypes

EQA_AST.C21.0002

pheno_table_species



	A	B	C	D	E	F	G	H
	# ResFinder phenotype results.							
	# Sample: contigs.fsa							
	#							
	# The phenotype 'No resistance' should be interpreted with							
	# caution, as it only means that nothing in the used							
	# database indicate resistance, but resistance could exist							
	# from 'unknown' or not yet implemented sources.							
	#							
	# The 'Match' column stores one of the integers 0, 1, 2, 3.							
10	# 0: No match found							
11	# 1: Match < 100% ID AND match length < ref length							
12	# 2: Match = 100% ID AND match length < ref length							
13	# 3: Match = 100% ID AND match length = ref length							
14	# If several hits causing the same resistance are found,							
15	# the highest number will be stored in the 'Match' column.							

EQA_AST.C21.0002

pheno_table_species

17	# Antimicrobial	Class	W	tch	Genetic background
18	ampicillin	beta-lactam	Re	2	blaOXA-61 (blaOXA-61_AY587956)
19					

EQA_AST.C21.0002

acquired_AMR_tab

Resistance gene	Identity	Alignment Length/Gene Length	Coverage	Position in reference	Contig	Position in contig	Phenotype	Accession no.
tet(O)	99.90	1920/1920	100.0	1..1920	NODE_12_length_46574_cov_28.972722	20074..21993	Tetracycline resistance	M18896
cat	100.00	624/624	100.0	1..624	NODE_11_length_67822_cov_36.449088	39168..39791	Phenicol resistance	M35190
blaOXA-489	99.87	774/774	100.0	1..774	NODE_11_length_67822_cov_36.449088	46265..47038	Warning: gene is missing from Notes file. Please infc	CP013733
blaOXA-451	99.87	774/774	100.0	1..774	NODE_11_length_67822_cov_36.449088	46265..47038	Warning: gene is missing from Notes file. Please infc	KR061504
blaOXA-450	99.87	774/774	100.0	1..774	NODE_11_length_67822_cov_36.449088	46265..47038	Warning: gene is missing from Notes file. Please infc	KR061502
blaOXA-61	99.87	774/774	100.0	1..774	NODE_11_length_67822_cov_36.449088	46265..47038	Beta-lactam resistance Alternate name; Cam-1	AY587956
blaOXA-453	99.87	774/774	100.0	1..774	NODE_11_length_67822_cov_36.449088	46265..47038	Warning: gene is missing from Notes file. Please infc	KR061507
blaOXA-452	99.87	774/774	100.0	1..774	NODE_11_length_67822_cov_36.449088	46265..47038	Warning: gene is missing from Notes file. Please infc	KR061505
blaOXA-193	99.87	774/774	100.0	1..774	NODE_11_length_67822_cov_36.449088	46265..47038	Beta-lactam resistance	CP013032
aph(2")-If	100.00	894/894	100.0	1..894	NODE_11_length_67822_cov_36.449088	45081..45974	Aminoglycoside resistance	AY701528
aph(3')-III	100.00	795/795	100.0	1..795	NODE_11_length_67822_cov_36.449088	40156..40950	Aminoglycoside resistance	M26832

PMs_tab

Mutation	Nucleotide change	Amino acid change	Resistance	PMID
gyrA p.T86I	ACA -> ATA	T -> I	Nalidixic acid, Ciprofloxacin	11266291

Chloramphenicol	Ciprofloxacin	Erythromycin	Gentamicin	Tetracycline
64	>32	2	>16	>64
ECOFF			ECOFF	

EQA_AST.C21.0003

acquired_AMR_tab

Resistance gene	Identity	Alignment Length/Gene Length	Coverage	Position in reference	Contig	Position in contig	Phenotype	Accession no.
aph(3')-III	100.00	795/795	100.0	1..795	NODE_43_length_1623_cov_115.727941	662..1456	Aminoglycoside resistance	M26832
aph(2'')-If	100.00	894/894	100.0	1..894	NODE_27_length_4611_cov_53.453390	196..1089	Aminoglycoside resistance	AY701528
tet(O)	99.58	1920/1920	100.0	1..1920	NODE_10_length_81949_cov_38.331109	682..2601	Tetracycline resistance	M18896
blaOXA-450	99.87	774/774	100.0	1..774	NODE_4_length_153021_cov_31.504232	35953..36726	Warning: gene is missing from Notes file. Please inform cu	KR061502
blaOXA-193	99.87	774/774	100.0	1..774	NODE_4_length_153021_cov_31.504232	35953..36726	Beta-lactam resistance	CP013032
blaOXA-61	99.87	774/774	100.0	1..774	NODE_4_length_153021_cov_31.504232	35953..36726	Beta-lactam resistance Alternate name; Cam-1	AY587956
blaOXA-452	99.87	774/774	100.0	1..774	NODE_4_length_153021_cov_31.504232	35953..36726	Warning: gene is missing from Notes file. Please inform cu	KR061505
blaOXA-489	99.87	774/774	100.0	1..774	NODE_4_length_153021_cov_31.504232	35953..36726	Warning: gene is missing from Notes file. Please inform cu	CP013733
blaOXA-453	99.87	774/774	100.0	1..774	NODE_4_length_153021_cov_31.504232	35953..36726	Warning: gene is missing from Notes file. Please inform cu	KR061507
blaOXA-451	99.87	774/774	100.0	1..774	NODE_4_length_153021_cov_31.504232	35953..36726	Warning: gene is missing from Notes file. Please inform cu	KR061504

PMs_tab

	Mutation	Nucleotide change	Amino acid change	Resistance	PMID
2	23S r.2075A>G	A -> G	RNA mutations	Azithromycin, Erythromycin, Clindamycin, Telithromycin	16713726
3	gyrA p.T86I	ACA -> ATA	T -> I	Nalidixic acid, Ciprofloxacin	11266291

Chloramphenicol	Ciprofloxacin	Erythromycin	Gentamicin	Tetracycline
8	32	256	16	>64
ECOFF			ECOFF	

EQA_AST.C21.0005

acquired_AMR_tab

	Resistance gene	Identity	Alignment Length/Gene Length	Coverage	Position in reference	Contig	Position in contig	Phenotype	Accession no.
2	blaOXA-489	100.00	774/774	100.0	1..774	NODE_5_length_163265_cov_28.845321	40922..41695	Warning: gene is missing from Notes fi	CP013733
3	tet(O)	99.48	1920/1920	99.95	1..1920	NODE_12_length_40044_cov_17.085077	23693..25611	Tetracycline resistance	Y07780
4	aph(3'')-III	100.00	795/795	100.0	1..795	NODE_12_length_40044_cov_17.085077	20170..20964	Aminoglycoside resistance	M26832
5									

PMs_tab

Mutation	Nucleotide change	Amino acid change	Resistance	PMID
rpsL p.K88R	AAA -> AGA	K -> R	Streptomycin	20370506
23S r.2075A>G	A -> G	RNA mutations	Azithromycin, Erythromycin, Clindamycin, Telithromycin	16713726

Chloramphenicol	Ciprofloxacin	Erythromycin	Gentamicin	Tetracycline
4	≤0.125	128	≤0.25	≤0.5
ECOFF			ECOFF	

Thank you on behalf of the FWD AMR-RefLabCap team

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