

EQA3-WGS-AMR PROTOCOL FOR IDENTIFICATION OF AMR GENES AND POINT MUTATIONS
UPLOAD OF READ FILES TO AN FTP SERVER

This document contains the protocol to follow when reporting genes and point mutations from your sequenced samples. Even though you may have used ResFinder before, please make sure to read and follow this protocol.

The final page (page 5) contains the protocol for upload of read files to our ftp server.

1. Open the website <http://genepi.food.dtu.dk/resfinder>
2. **Select ResFinder version 4.4.2**, even though there is a newer version available on the website.
3. Leave the default thresholds for chromosomal point mutations and acquired antimicrobial resistance genes (90% ID and 60% minimum length)
4. Do not select any additional options for chromosomal point mutations
5. Do not select the Disinfectant option
6. In the box "Species and input data type", select the relevant species:

<i>Salmonella</i>	<i>Campylobacter</i> (please select the species that you identified)
<p>Species and input data type:</p> <p>Select species</p> <p>Salmonella spp.*</p>	<p>Species and input data type:</p> <p>Select species</p> <p>Campylobacter jejuni*</p> <p>Species and input data type:</p> <p>Select species</p> <p>Campylobacter coli*</p>

7. Select the relevant FASTQ data type:

Non Nanopore reads (f. ex. Illumina)	Nanopore reads
<p>Select input type</p> <p>FASTQ (Non-nanopore Reads)</p>	<p>Select input type</p> <p>FASTQ (Nanopore Reads)</p>

8. Enter your email address and select the read files you want to upload (one file at a time) by pressing the button "Gennemse..." or "Vælg fil" in this window (depending on the browser, the text in the buttons might be different):

<p>Upload and submit job:</p> <p>Email (Get email, when finished - Optional):</p> <p>Enter your email address...</p> <p>Files (The sum of uploaded file sizes cannot exceed 1 gb):</p> <p>Gennemse... Ingen fil valgt.</p> <p>Gennemse... Ingen fil valgt.</p> <p>Submit Job</p>	<p>Upload and submit job:</p> <p>Email (Get email, when finished - Optional):</p> <p>Enter your email address...</p> <p>Files (The sum of uploaded file sizes cannot exceed 1 gb):</p> <p>Vælg fil Der er ikke valgt nogen fil</p> <p>Vælg fil Der er ikke valgt nogen fil</p> <p>Submit Job</p>
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For paired-end Illumina reads, one pair of read files (R1 and R2) can be uploaded at a time (example below):

Upload and submit job:

Email (Get email, when finished - Optional):

malm@ssi.dk

Files (The sum of uploaded file sizes cannot exceed 1 gb):

Gennemse... EQA3-C24-01_S28_L..._R1_001.fastq.gz

Gennemse... EQA3-C24-01_S28_L..._R2_001.fastq.gz

Submit Job

For Nanopore reads, a read file representing one isolate should be uploaded at a time (example):

Upload and submit job:

Email (Get email, when finished - Optional):

Enter your email address...

Files (The sum of uploaded file sizes cannot exceed 1 gb):

Gennemse... 2302H1257.trim.q...Mbp500.fastq.gz

Gennemse... Ingen fil valgt.

Submit Job

9. Press Submit Job and wait. After a while you will see the following:



Your job is running. Please wait...

Session ID: r7en5rg16c2eyqm15oy9xa03x8uwd516

If you stay on this website, the results will eventually appear. Simultaneously, you will receive an email with a link to your results (see point 10).

If you now press on the DTU logo in the upper left corner, you will go back to the main ResFinder website and be able to submit the next sequences.



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10. When the job on ResFinder is finished, you will receive the following email (example):



Dear user,

We are pleased to inform you that your task has been successfully completed on the ResFinder 4.5.0 server. You can now access the outcome by clicking on the provided link below:

<http://genepi.food.dtu.dk/resfinder/job/nm3nt5l7q49zkqx1ybgdiuy9ejlgvtxp>

Should you require any assistance or have any inquiries, please don't hesitate to reach out to CGE support at food-cgehelp@dtu.dk.

Best regards,
CGE Webserver

11. Upon clicking on the link, you will open a website with your results. In order to check which isolate the results are for, **scroll to the bottom of the website**, where you can see the Input Parameters and the name of your sample (example):

Input Parameters

Input File 1: EQA3-C24-01_S28_L555_R1_001.fastq.gz

Input File 2: EQA3-C24-01_S28_L555_R2_001.fastq.gz

Chromosomal point mutations

Threshold for ID: 90.0 %

Minimum length: 60.0 %

Show unknown mutations: False

Ignore premature stop codons: False

Ignore frameshift indels: False

Acquired antimicrobial resistance genes

Threshold for ID: 90.0 %

Minimum length: 60.0 %

Species and input data type

Selected species: Campylobacter

12. The results page is divided into the following parts:

- a. Phenotypes
- b. Acquired AMR gene hits
- c. Chromosomal mutations mediating AMR
- d. Acquired disinfectant resistance hits
- e. Downloads
- f. Input parameters

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13. Here’s an example of an output (please only look at species-relevant site: “salmonella” or “campylobacter” and not “All”):

Phenotypes

salmonella All

Antimicrobial	Class	WGS-predicted phenotype	Genetic background
gentamicin	aminoglycoside	Resistant	aac(3)-IIa;4;L22613
tobramycin	aminoglycoside	Resistant	aac(6)-Iaa;1;NC_003197
amikacin	aminoglycoside	Resistant	aac(6)-Iaa;1;NC_003197
ciprofloxacin	quinolone	Resistant	qnrS1;1;AB187515
nalidixic acid	quinolone	No resistance	
ampicillin	beta-lactam	Resistant	blaTEM-1B;1;AY458016

Acquired AMR gene hits

Hide

Resistance gene	Identity	Alignment length/gene length	Position in reference	Contig or depth	Position in contig	Phenotype	PMID	Accession no.	Notes
aac(6)-Iaa	98.63%	438 /	1...439	[tobramycin], [amikacin]	11677609	NC_003197	Chromosomal, Cryptic gene in Salmonella sp.
aph(6)-Id	100.00%	837 /	1...838	[streptomycin]	2653965	M28829	Alternative name strB
aph(3)-Ia	99.88%	816 /	1...817	[kanamycin], [neomycin]	17485180	EF015636	
ant(3)-Ia	98.66%	974 /	1...975	[streptomycin]	8385262	X02340	Alternative name aadA, aad(3)(9), aadA1, aadA1a
aadA2	100.00%	792 /	1...793	[streptomycin], [spectinomycin]	22511964	JQ364967	

The colours mean the following:

- Green: 100% identity over 100% of the length
- Light green: <100% identity over 100% of the length
- Grey: <100% identity over <100% of the length

14. Scroll down to the “Downloads” section and download and save the following three files, circled in red, for all samples:

Downloads

Table downloads

Download phenotypetable (txt) Download species specific phenotype table (txt)

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 text file

15. You are now ready to report your results in the reporting scheme:

- From the file with acquired AMR gene results, report all the genes in that file.
- From the file with Chromosomal point mutations, report all the point mutations in that file.
- From the phenotype table (txt) file – report the Resistant WGS-predicted phenotype **for the antimicrobials included in the predicted phenotype question in the reporting scheme.**

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Upload of reads to an ftp server for participant

You are asked to upload the reads that you obtained in your laboratory, so that the EQA provider can perform QC analysis on them. Please name the reads so that it is clear which DNA sample they represent. Each participant has a separate folder for upload that the other participants cannot access.

1. Click on the personal link below:
The personal link for upload for each laboratory can be found in the protocol sent to you in an email on the 10th of April.
2. You will see the following:
(.....)
3. Drag and Drop your reads or click on “Choose Files” and select your read files. Please do not compress your reads (for example, by zipping them).
4. When you see this message in the window, your upload is complete and you can close the website:
(.....)

Thank you!