**Determining the presence or absence of nucleotide sequences.**

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**Introduction**

NCBI provides a useful BLAST feature which allows the user to screen a public database for entries which contain smaller nucleotide sequences and/or are highly similar to the genome of interest. But when interested in multiple strains or a novel genome or plasmid, this tool is slow and tedious. To address this problem, here we have created a script which will screen multiple loaded sequences for a sequence of interest.

To demonstrate its functionality, five plasmids from classic Cystic Fibrosis pathogens were chosen at random to be screened for the antimicrobial resistance gene (AMR), *ampR.* For context, Cystic Fibrosis (CF) is a genetic condition caused by mutations of the CF transmembrane conductance regulator (CFTR) gene. Common CF pathogens include *Staphylococcus aureus* (in younger patients), *Pseudomonas* *aeruginosa*, *Achromobacter* spp., *Stenotrophomonas* *maltophilia*, and those defined in the *Burkholderia* *cepacia* complex (Blanchard, A. C. and Waters, V. J., 2019). Various antimicrobial strategies are reported to be effective against appropriate target species in individuals with CF however with the rapid increase in the number of antibiotic resistant strains, antibiotic agents are being rendered relatively ineffective (Chmiel, J. F., et al., 2014)). AMR genes encode for mechanisms which allow the microorganism to deal with antimicrobial agents. *ampR* is a transcriptional regulator of a gene set that confers β-lactam resistance (Kuga A., et al., 2000).

Briefly, the python script loads pre-saved FASTA files into the workspace using SeqIO from Biopython, extracts and assigns the nucleotide sequence from SeqRecord to a usable variable, and finally loops over a dictionary of these sequences and aligns them with the gene of interest using the module ‘Align’ from Biopython. Ultimately, the output will inform the user whether the AMR gene of interest is present in the sequences loaded.

**Methods**

This script was written in the JupyterLab 3.2.1 application supported by Anaconda Navigator. Here, we have written this alignment

*Preparing FASTA files*

To use this alignment script, the sequences of interest must be prepared and accessible to the JupyterLab file directory as FASTA files. The script written used the files as detailed in Table 1.

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| --- | --- | --- |
| **Table** **1** |  |  |
| NCBI Accession Number | Details | URL |
| X03274 | *Pseudomonas* *aeruginosa* plasmid pMG7 | <https://www.ncbi.nlm.nih.gov/nuccore/X03274.1?report=fasta> |
| NC\_005564.1 | *Staphylococcus* *aureus* plasmid pS194 | <https://www.ncbi.nlm.nih.gov/nuccore/X03274.1?report=fasta> |
| NZ\_KJ588780 | *Achromobacter* *xylosoxidans* strain A22732 plasmid PA22732-IMP | <https://www.ncbi.nlm.nih.gov/nuccore/NZ_KJ588780.1?report=fasta> |
| NC\_010464.1 | *Stenotrophomonas* *maltophilia* plasmid pSM76 | <https://www.ncbi.nlm.nih.gov/nuccore/NC_010464.1?report=fasta> |
| NZ\_LR890526.1 | *Burkholderia* *cepacia* 4Asc2280433 | <https://www.ncbi.nlm.nih.gov/nuccore/NZ_LR890526.1?report=fasta> |

*Loading prepared FASTA files and extracting sequence information*

SeqIO from Biopython is used to import the prepared FASTA files. Nucleotide sequences were extracted and assigned to sequence variable name.

*Alignment*

The Align module was imported from BioPython in order to use the PairwiseAligner() submodule. The alignment type to be conducted is a local alignment and this is set using the .mode function within PairwiseAligner(). The alignment method being used is checked using .algorithm. The default setting for the alignment conducted by PairwiseAligner() is to award the alignments one point for a match, and zero points for both gaps and mismatches. Here, we changed the gap score with a penalty of -0.1 using .gap\_score. Briefly, the script involves the creation of a dictionary of the test sequences which are aligned to the gene of interest using a loop. The output returns the user with a statement informing which sequences contain the gene and which do not. More details are annotated on the script itself.

The following websites were used as references for the creation of this script: [Dictionaries - Beginning Python (milliams.com)](https://milliams.com/courses/beginning_python/Dictionaries.html), [Using functions - Intermediate Python (milliams.com)](https://milliams.com/courses/intermediate_python/Using%20functions.html), [Functions - Intermediate Python (milliams.com)](https://milliams.com/courses/intermediate_python/Functions.html), [Biopython Tutorial and Cookbook](http://biopython.org/DIST/docs/tutorial/Tutorial.html#sec101)

**Results**

To demonstrate the functionality of the code written, four commonly occurring CF pathogen plasmids (Table 1) were chosen at random from the NCBI database and screened for a well characterised AMR gene known as ampR. A control was also included to compare the alignment scores of our test alignments against. Within the methodology, there is a variable stringency and the user can determine how similar the test alignment scores must be to the control score for the gene to be present. In our script, two stringencies (low, 60% and high, 95%) were tested. At the lower stringency threshold, all plasmids were found to include the AMR gene of interest. At the higher stringency, the absence of *ampR* *Staphylococcus* *aureus* plasmid pS194 was determined. It should be noted that this alignment method is not the same as that used by the NCBI blastn tool and so alignment scores can differ.

**Conclusion**

At the higher stringency threshold, all pathogen plasmids contain the *ampR* gene except *S. aureus* plasmid pS194 This script can be used to determine the absence and presence of a gene of interest in bacterial nucleotide sequences such as plasmids.

**References**

Blanchard, A. C., & Waters, V. J. (2019) ‘Cystic Fibrosis: Advances in Understanding and Treatment: Microbiology of Cystic Fibrosis Airway Disease’, *Seminars in Respiratory and Critical Care Medicine*, 40(6), pp. 727. https://doi.org/10.1055/S-0039-1698464

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