Recap Exercise 4

Center for Genomic Epidemiology

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RUCS 1.0

Rapid Identification of PCR Primers Pairs for Unique Core Sequences

Service Information: Show

Entry point: Find Unique Core Sequences

Choose the entry point you want to use. (See instructions for more information)

Reference*: Choose File No file chosen

The reference file to which the k-mers should be mapped.
*Optional: if left blank, the algorithm uses one of the positive samples as reference.

k-mer size: 20

The k-mer size defines the size of the oligo nucleotides. Small values decrease specificity, and large values increase specificity. A balance is needed for optimal results.

Read length: 250

This option helps the program ignore insignificant contigs which can cause noise. Default is 250, which corresponds to all contigs below 500 bp are ignored as noise.

Upload Intructions:

1. Create 2 folders called "positives" and "negatives" (No caps allowed, names must be exact!)
2. Add the draft genomes you wish to target in the "positives" folder (FASTA only!)
3. Add the draft genomes you do NOT wish to target in the "negatives" folder (FASTA only!)
4. Zip the two directories, and upload them

Notice: The name(s) of the zip file(s) are irrelevant as long as the extension is '.zip'.

For more help and examples, see the instruction page.

WARNING: To avoid exceeding the maximum runtime, our recommendation is to keep below 50 genomes per submission.

Isolate File

Name	Size	Progress	Status

Upload Remove

Prepare the data for RUCS:

Create a directory/folder called “positives” and add all positive draft genomes in fasta format (not zipped)

Create a directory/folder called “negatives" and add all negative draft genomes in fasta format (not zipped)

Select both folders and create a zip file (on Mac you right click and select “Compress 2 items”)

The zipped file is what you upload as “Isolate File”

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Sequence Analysis				
	Sequences	Size in bases	Seqs >200	Size >200
Reference	311	5286589	311	5286589
Core Sequences	40479	3919025	4453	1568854
Unique Core Sequences	286	32033	27	17953

Downloads:

UCS Dissected Scaffolds | UCS Contigs | CS Contigs | Statistics

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CITATION

For publication of results, please cite:

- Martin Christen Frølund Thomsen, Henrik Hasman, Henrik Westh, Hülya Kaya, Ole Lund; RUCS: rapid identification of PCR primers for unique core sequences, *Bioinformatics*, **2017**, btx526, https://doi.org/10.1093/bioinformatics/btx526

Acquired antimicrobial resistance gene - Results

Aminoglycoside

No resistance genes found.

Beta-lactam

No resistance genes found.

Colistin

No resistance genes found.

Fluoroquinolone

No resistance genes found.

Fosfomycin

No resistance genes found.

Fusidic Acid

No resistance genes found.

Glycopeptide

No resistance genes found.

MLS - Macrolide, Lincosamide and Streptogramin B

No resistance genes found.

Nitroimidazole

No resistance genes found.

Oxazolidinone

No resistance genes found.

Phenicol

No resistance genes found.

Rifampicin

No resistance genes found.

Sulphonamide

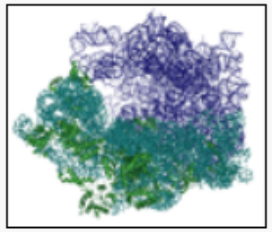
No resistance genes found.

Tetracycline

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
tet(B)	100.00	1206/1206	21_249_0	666..1871	Tetracycline resistance	AP000342

Searching for Open Reading Frames

Virtual Ribosome - version 2.0



The Virtual Ribosome is a comprehensive tool for translating DNA sequences to the corresponding peptide sequences.

Besides being a strong translation tool in it's own right (with an integrated ORF finder, support for all translation tables defined by the NCBI taxonomy group, and a number of options regarding START and STOP codons), the Virtual Ribosome can work directly on files containing annotation of gene structure. This makes it easy to map various aspect of Intron/Exon structure onto the translated sequence.

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Paste in DNA sequences in FASTA, GenBank or TAB format

Upload DNA sequences in FASTA, GenBank or TAB format

Choose File unique_core_…s.contig.fa

View example DNA files

Submit query Clear fields

Instructions: Basic usage - Paste in or upload one or more DNA sequences in FASTA (sequence only), GenBank (CDS sections are processed) or TAB (sequence and intron/exon annotation) format and hit submit. The Virtual Ribosome will then translate the DNA sequences using the standard genetic code (by default). ***Options can be customized in the section below.***

The longest peptide sequence hits to known tetracycline genes

```
>21_249_0 position=623_rframe-2_ORF
MIELFYHSLSVIEKSEMNSSTKIALVITLLDAMGIGLIMPVLPTLLREFIASEDIANHFG
VLLALYALMQVIFAPWLGKMSDRFGRRPVLLLSLIGASLDYLLLAFSSALWMLYLGRLLS
GITGATGAVAASVIADTTSASQRVKWFGWLGASFGLGLIAGPIIGGFAGEISPHSPFFIA
ALLNIVTFLVVMFWFRETKNTRDNTDTEVGVETQSNSVYITLFKTMPILLIIYFSAQLIG
QIPATVWVLFTENRFGWNSMMVGFSLAGLGLLHSVFQAFVAGRIATKWGEKTAVLLGFIA
DSSAFAFLAFISEGWLVFPVLILLAGGGIALPALQGVMSIQTKSHQQGALQGLLVSLTNA
TGVIGPLLFAVIYNHSLPIWDGWIWIIGLAFYCIIILLSMTFMLTPQAQGSKQETSA
```

Sequences producing significant alignments:

Select: All None Selected:0

Alignments | Download | GenPept | Graphics | Distance tree of results | Multiple alignment

Description	Max score	Total score	Query cover	E value	Ident	Accession
TetA [Type 88 trypsin release phage display vector f88TR1]	824	824	100%	0.0	100%	ADN93463.1
tetracycline resistance protein [Acinetobacter baumannii]	822	822	100%	0.0	99%	AFH57202.1
tetracycline efflux MFS transporter Tet(B) [Escherichia coli]	822	822	100%	0.0	99%	WP_012600019.1
transporter, major facilitator family protein [Acinetobacter baumannii UH6107]	821	821	100%	0.0	99%	ETQ92486.1
TetA-B [uncultured bacterium]	820	820	99%	0.0	99%	AMP51267.1
TetA-B [uncultured bacterium]	820	820	100%	0.0	99%	AMP46861.1
TetA [Escherichia coli]	801	801	97%	0.0	99%	AUO60067.1