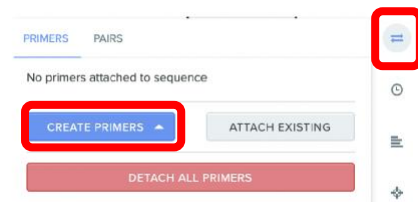


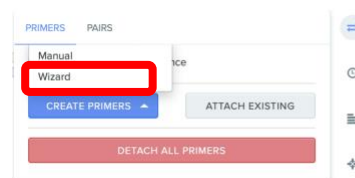
Help Exercise 2

First select the AMP coding sequence (click on the annotation in the plasmid map)

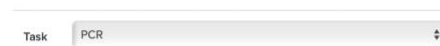
Create primers



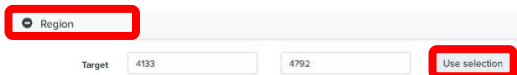
select 'wizard'



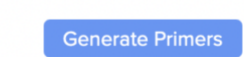
Select 'PCR' (do NOT select 'sequencing'!)



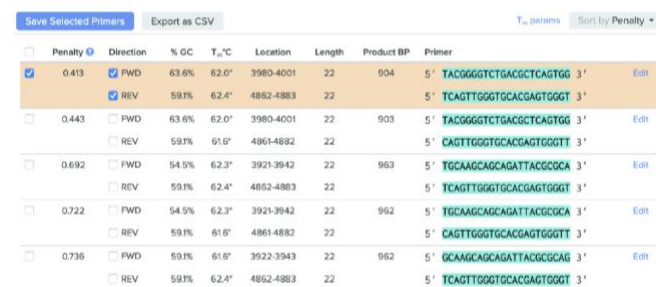
Define target region (=AmpR) in plasmid (numbering might be different in your plasmid!)



'Generate primers' (top right)



Select one suitable primer pair and save + attach it.



Penalty	Direction	% GC	T _m °C	Location	Length	Product BP	Primer
0.413	PWD	63.6%	62.0°	3980-4001	22	904	5' TACGGGGTCTGACGCTCAGTGG 3'
	REV	59.1%	62.4°	4862-4883	22		5' TCAGTTGGGTGCACGAGTGGGT 3'
0.443	PWD	63.6%	62.0°	3980-4001	22	903	5' TACGGGGTCTGACGCTCAGTGG 3'
	REV	59.1%	61.6°	4861-4882	22		5' CAGTTGGGTGCACGAGTGGGT 3'
0.692	PWD	54.5%	62.3°	3921-3942	22	963	5' TGCAGCAGCAGATTACGCCCA 3'
	REV	59.1%	62.4°	4862-4883	22		5' TCAGTTGGGTGCACGAGTGGGT 3'
0.722	PWD	54.5%	62.3°	3921-3942	22	962	5' TGCAGCAGCAGATTACGCCCA 3'
	REV	59.1%	61.6°	4861-4882	22		5' CAGTTGGGTGCACGAGTGGGT 3'
0.736	PWD	59.1%	61.6°	3922-3943	22	962	5' GCAGCAGCAGATTACGCCGAG 3'
	REV	59.1%	62.4°	4862-4883	22		5' TCAGTTGGGTGCACGAGTGGGT 3'

Help Exercise 3

Open the recombinant plasmid from last semester (size: 6609 bp)
On the top right select 'alignments'

The screenshot shows a bioinformatics tool interface. At the top, there are buttons: 'Create', 'Analyze', 'Copy', 'Create PDF', and a search icon. Below these, there are two DNA sequences. The first sequence is: TAGCTCCTAAGGGATTGGAGGGTGCAGGTCTCTCCACCCAATGAAAACGT
ATCGAGGATTCCTAAACCTCCCACGTCAGAGAGGTGGGTACTTTTGCA
Below the sequence, there is a protein sequence: L A P K G F G G V Q V S P P N E N V. The second sequence is: CATTAAACCACATGTGTGGCGCAGGCAATCCTGCAGGAACAAGCAGTACCTGT
GTAATTGGGTACACACCGCGTCCGTTAGGACGTCCTTGTTCGTCATGGACA
Below the sequence, there is a protein sequence: I N H M C G A G N P A G T S S T C. On the right side, there is a sidebar with icons. The 'Alignments' icon, which is a blue document with a list, is highlighted with a red box.

Create new alignment> upload (drag + drop) BOTH .ab1 files with chromatogram for forward and reverse sequencing primer
Use standard parameters (Auto MAFFT)> create alignment

The screenshot shows the 'Create DNA / RNA alignment' form. At the top, there are two steps: '1 Choose input' and '2 Define parameters'. Under 'Define parameters', there are three options: 'Pairwise', 'Multisequence', and 'Consensus'. The 'Multisequence' option is highlighted with a red box. Below these options, there is a text box that says: 'Multisequence Alignment - The results will be attached as a single alignment on the template sequence. [Show details](#)'. Under 'Template(s)', there is a button with a plus sign and a dropdown menu. Under 'Non-template sequence(s)', there are two input fields: 'Sequence1_pAmy2-His (...)' and 'Sequence2_pAmy2-His ...'. Below these, there is a section 'Choose an alignment program.' with a dropdown menu showing 'Auto (MAFFT)' and a 'Show parameters' button. The 'Auto (MAFFT)' option is highlighted with a red box. At the bottom right, there are two buttons: 'Back' and 'Create Alignment'. The 'Create Alignment' button is highlighted with a red box.

Help Exercise 4

copy/ paste *Sequence1.fasta* file in text box:

BLAST® » blastn suite

Standard Nucleotide BLAST

blastn blastp blastx tblastn tblastx

BLASTN programs search nucleotide databases using a nucleotide query sequence.

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [Clear](#)

Query subrange [?](#)

From

To

Or, upload file no file selected [?](#)

Job Title

Enter a descriptive title for your BLAST search [?](#)

☐ Align two or more sequences [?](#)

Choose Search Set

Database ☒ Standard databases (nr etc.): ☐ rRNA/ITS databases ☐ Genomic + transcript databases ☐ Betacoronavirus

☒ Experimental databases [Try experimental taxonomic nt databases](#) [Download](#)

[For more info see What are taxonomic nt databases?](#)

[?](#)

Organism [Optional](#)

☐ exclude [Add organism](#)

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown [?](#)

Exclude [Optional](#)

☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to [Optional](#)

☐ Sequences from type material

Entrez Query [Optional](#)

[YouTube](#) [Create custom database](#)

Program Selection

Optimize for ☒ Highly similar sequences (megablast) ☐ More dissimilar sequences (discontiguous megablast) ☐ Somewhat similar sequences (blastn)

Choose a BLAST algorithm [?](#)

Search database nt using Megablast (Optimize for highly similar sequences)

☐ Show results in a new window

Help Exercise 5

a) copy/ paste *Sequence2.fasta* file in text box (settings same as exercise 4)

b) copy/ paste *Sequence2.fasta* file in text box, change settings to RefSeq Select RNA sequences (refseq_select)

BLAST® » blastn suite

Standard Nucleotide BLAST

blastn blastp blastx tblastn tblastx

BLASTN programs search nucleotide databases using a nucleotide query sequence.

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [Clear](#)

Query subrange [?](#)

From

To

Or, upload file no file selected [?](#)

Job Title

Enter a descriptive title for your BLAST search [?](#)

☐ Align two or more sequences [?](#)

Choose Search Set

Database ☒ Standard databases (nr etc.): ☐ rRNA/ITS databases ☐ Genomic + transcript databases ☐ Betacoronavirus

☒ Experimental databases [Try experimental taxonomic nt databases](#) [Download](#)

[For more info see What are taxonomic nt databases?](#)

[?](#)

Organism [Optional](#)

☐ exclude [Add organism](#)

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown [?](#)

Exclude [Optional](#)

☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to [Optional](#)

☐ Sequences from type material

Entrez Query [Optional](#)

[YouTube](#) [Create custom database](#)

Program Selection

Optimize for ☒ Highly similar sequences (megablast) ☐ More dissimilar sequences (discontiguous megablast) ☐ Somewhat similar sequences (blastn)

Choose a BLAST algorithm [?](#)

Search database RefSeq Select RNA sequences (refseq_select) using Megablast (Optimize for highly similar sequences)

☐ Show results in a new window