

Help Exercise 2

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

Create primers

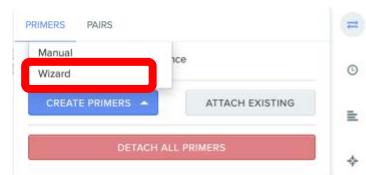


PRIMERS PAIRS

No primers attached to sequence

CREATE PRIMERS ATTACH EXISTING DETACH ALL PRIMERS

select 'wizard'



PRIMERS PAIRS

Manual Wizard

CREATE PRIMERS ATTACH EXISTING DETACH ALL PRIMERS

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

Task PCR

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



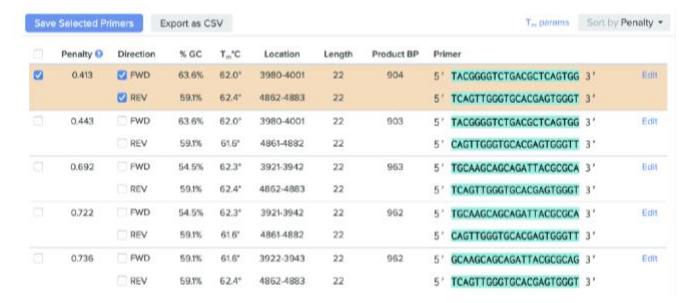
Region

Target 4133 4792 Use selection

'Generate primers' (top right)

Generate Primers

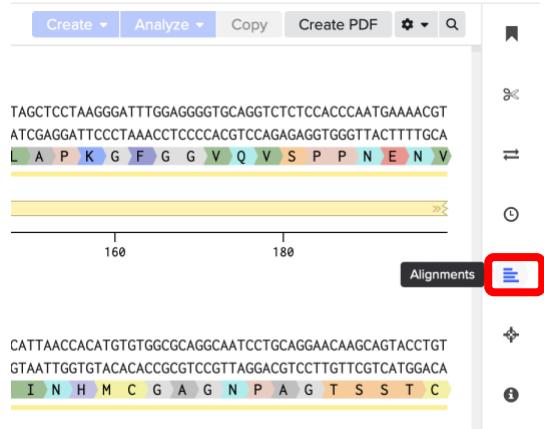
Select one suitable primer pair and save + attach it.



Penalty	Direction	% GC	T _m °C	Location	Length	Product BP	Primer	T _m primers	Sort by Penalty
0.413	<input checked="" type="checkbox"/> FWD	63.6%	62.0°	3980-4001	22	904	5' TACGGGGTCTGACGCTCAGTGG 3'		<input type="checkbox"/>
	<input checked="" type="checkbox"/> REV	59.1%	62.4°	4862-4883	22		5' TCAGTTGGTGCACGAGTGGGT 3'		<input type="checkbox"/>
0.443	<input type="checkbox"/> FWD	63.6%	62.0°	3980-4001	22	903	5' TACGGGGTCTGAGCCTCAGTGG 3'		<input type="checkbox"/>
	<input type="checkbox"/> REV	59.1%	61.6°	4861-4882	22		5' CAGTTGGTGCACGAGTGGGT 3'		<input type="checkbox"/>
0.692	<input type="checkbox"/> FWD	54.5%	62.3°	3921-3942	22	963	5' TGCAAGCACCAATTACCCCA 3'		<input type="checkbox"/>
	<input type="checkbox"/> REV	59.1%	62.4°	4862-4883	22		5' TCAGTTGGTGCACGAGTGGGT 3'		<input type="checkbox"/>
0.722	<input type="checkbox"/> FWD	54.5%	62.3°	3921-3942	22	962	5' TGCAAGCACCAATTACCCCA 3'		<input type="checkbox"/>
	<input type="checkbox"/> REV	59.1%	61.6°	4861-4882	22		5' CAGTTGGTGCACGAGTGGGT 3'		<input type="checkbox"/>
0.736	<input type="checkbox"/> FWD	59.1%	61.6°	3922-3943	22	962	5' GCAGCCAGCAGATTACCCCA 3'		<input type="checkbox"/>
	<input type="checkbox"/> REV	59.1%	62.4°	4862-4883	22		5' TCAGTTGGTGCACGAGTGGGT 3'		<input type="checkbox"/>

Help Exercise 3

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



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

Help Exercise 4

copy/ paste Sequence1.fasta file in text box:

BLAST® > blastn suite

Standard Nucleotide BLAST

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) 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

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

Organism

Optional Enter organism name or id—completions will be suggested exclude Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

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

Limit to Sequences from type material

Entrez Query Enter an Entrez query to limit search

Program Selection

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

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

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) 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

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

Organism

Optional Enter organism name or id—completions will be suggested exclude Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

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

Limit to Sequences from type material

Entrez Query Enter an Entrez query to limit search

Program Selection

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

Search database RefSeq Select RNA sequences (refseq_select) using Megablast (Optimize for highly similar sequences) Show results in a new window