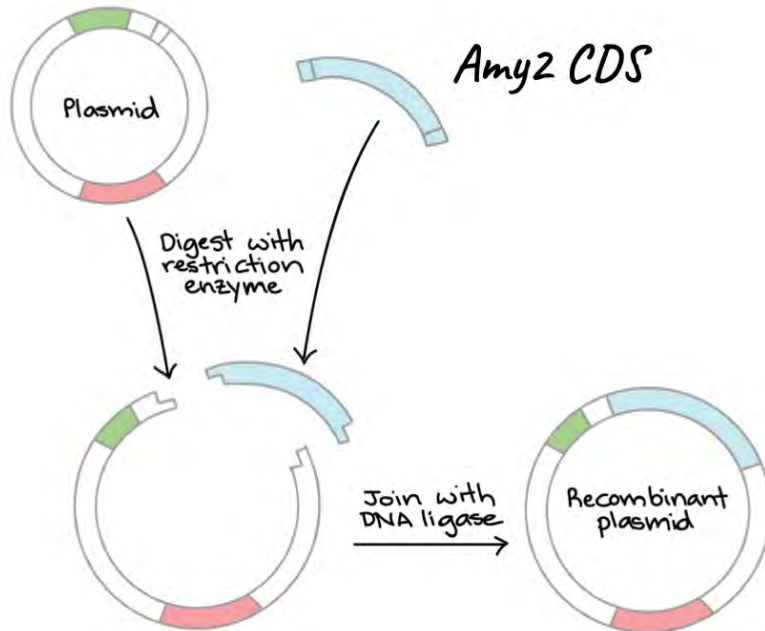




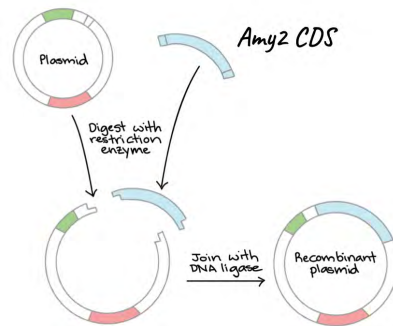
Benchling Exercises lab3 Virtual Cloning

Dr. Alexandra Bezler
BIO-203

Virtual Step-by-Step Cloning Procedure

Same procedure as done in lab sessions 3 + 4 :

- 1) Digest plasmid backbone
- 2) Digest PCR product (insert)
- 3) Ligate insert + linearized plasmid (will be used in **lab 6**)



In the lab you do all steps individually and keep intermediate products, this allows you to go back - one step at a time- in case of problems/ mistakes. This saves time compared to starting from scratch.

This Benchling exercise is designed in the same way- one step, one file.

Benchling provides an 'Assembly Wizard' for cloning without intermediate steps. We are not using it for the exercise- it is for advanced users!

Basic Benchmarking Functions

Tools Used in Lab3

explore all buttons to see what they are used for

Account

project

Molecular Biology

annotate

digest

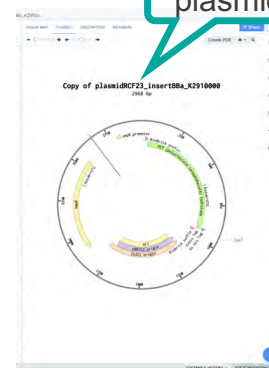
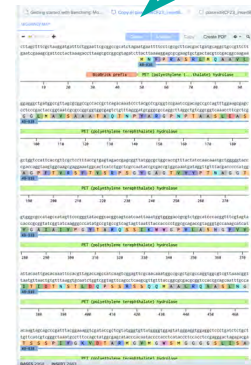
primer

info

split
workspace

The screenshot displays the Benchling Molecular Biology workspace. On the left, a sidebar shows a project named '01_BIO-203_2022_AB_files' with a sub-project 'plasmidRCF23_insertBba_K2910000'. The main workspace is divided into two panels. The left panel shows the 'Sequence' view of the plasmid, displaying the DNA sequence with various features highlighted, including the 'BlaS1' gene, 'ori' (origin of replication), and 'amp^r' (ampicillin resistance gene). The right panel shows the 'Map' view, which is a circular plasmid map with the same features. The 'Map' view includes a scale from 0 to 1000 bp and a 'Split workspace' button at the bottom right.

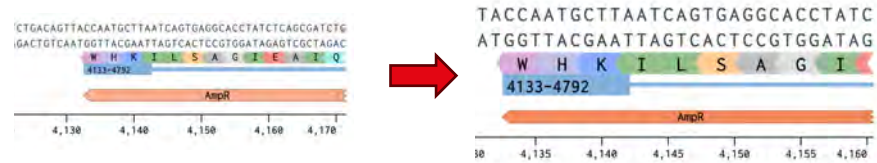
To Change the Window Layout



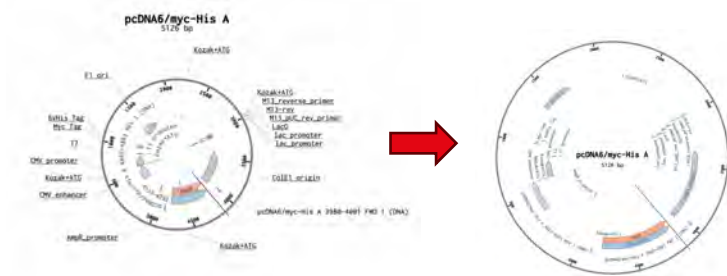
Explore Useful Display Functions

use two fingers to zoom/ rotate

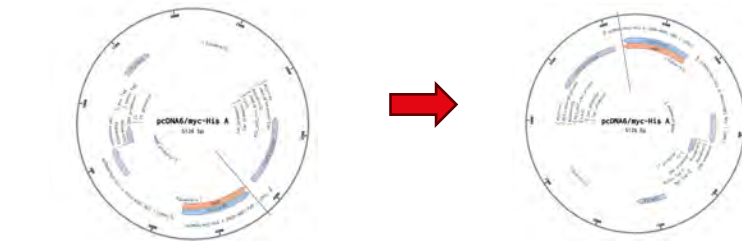
- zoom in/ out sequence



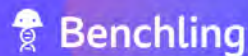
- zoom in/ out plasmid



- rotate map



Benchling Help : How to + Videos

[Go to Benchling](#)

Advice and answers from the Benchling Team



Benchling basics

Get around Benchling and manage your account



22 articles in this collection

Written by Lauren Shields, Erica Stanley, Nishant Neel and 10 others



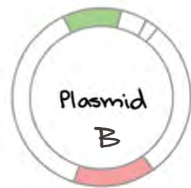
Molecular Biology

Annotate sequences, design primers, perform alignments, and model assemblies using rich design and analysis tools



89 articles in this collection

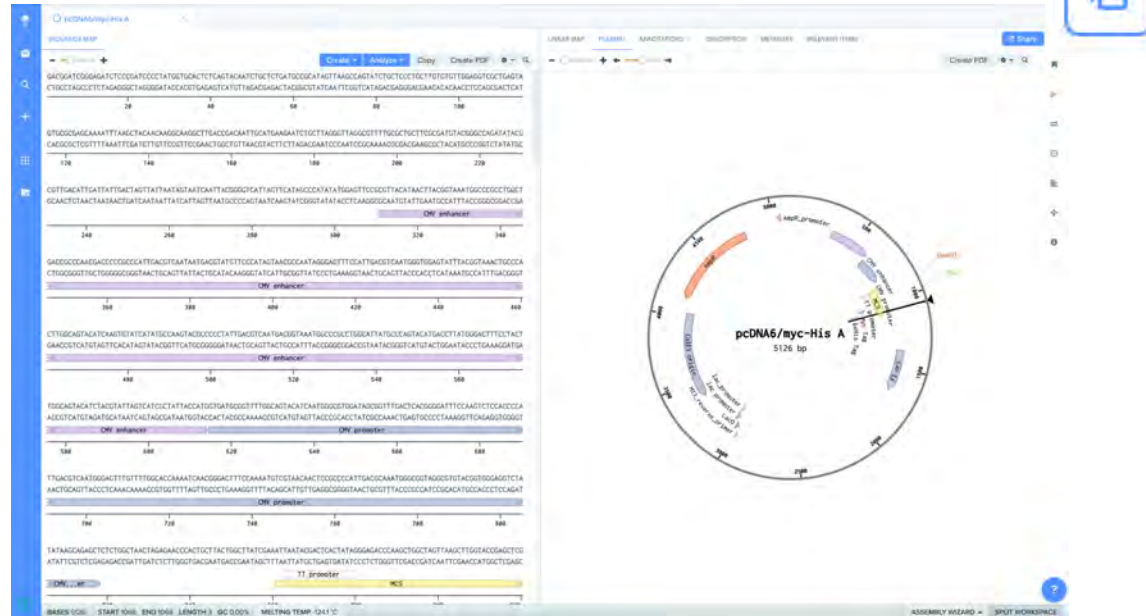
Written by Anshi Saxena, Alex Chao, Damian Costello and 22 others



Save Plasmid Sequences

copy to...

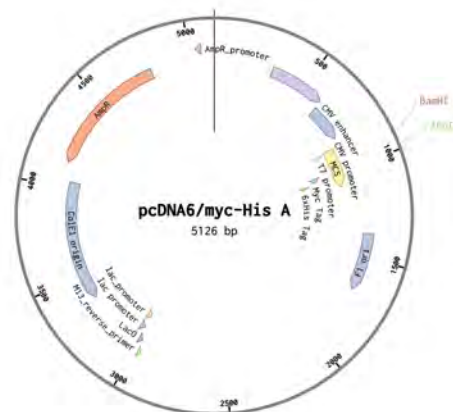
- ✓ open shared files
- ✓ pcDNA6/myc-HIS version A
- ✓ pcDNA6/myc-HIS version B
- ✓ create new project folder
for **each** exercise
- ✓ copy shared sequence file
to the corresponding folder

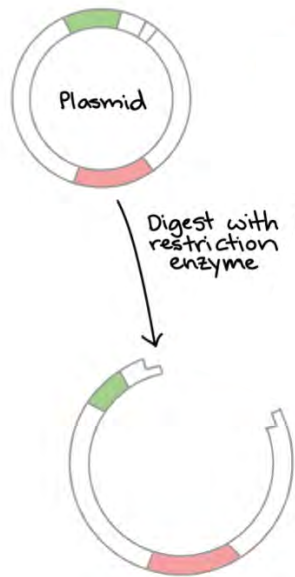


note you can only work on sequences you own, not on shared sequences

Create Project

copy to...





Exercise 1

Create plasmid backbone

in plasmid pcDNA6/myc-His A:

Find multiple cloning site (MCS)

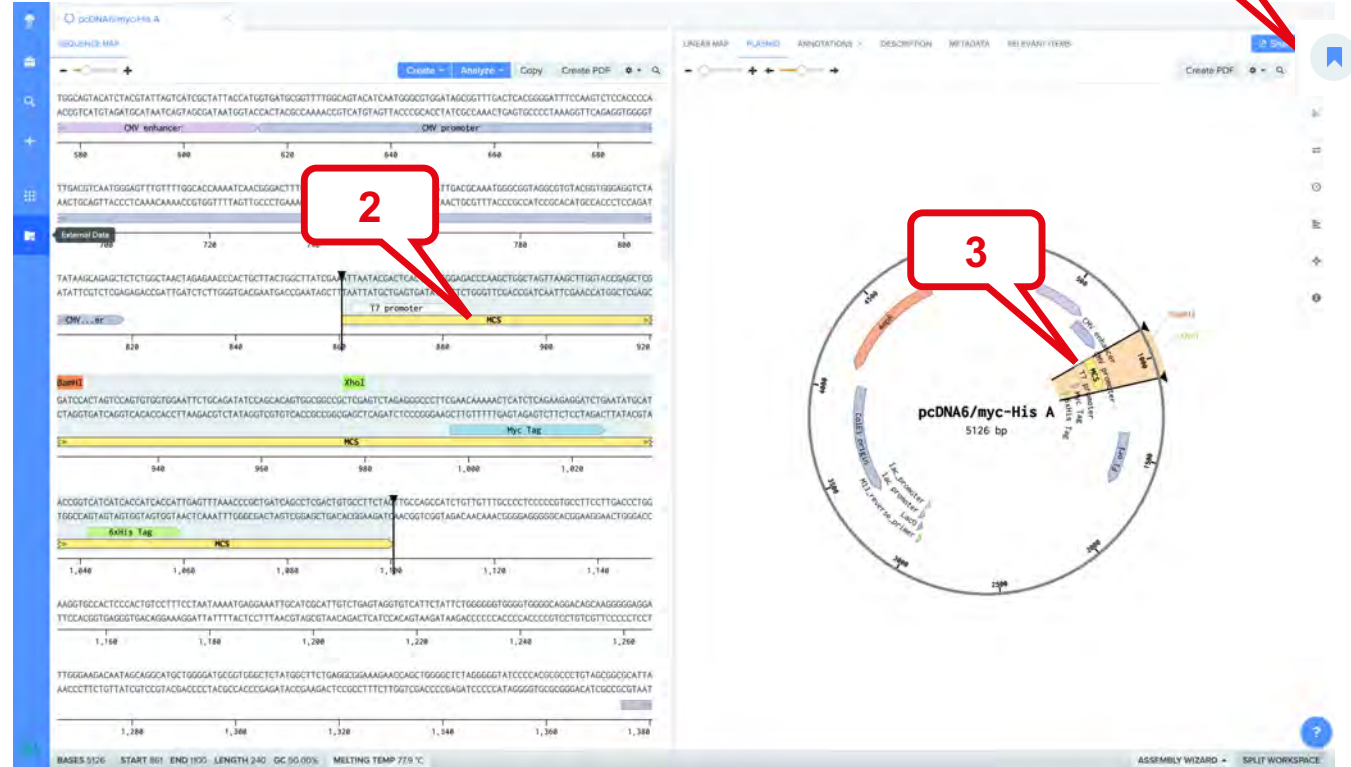
Find restriction sites BamHI and XhoI

Run virtual digest

Create new DNA file with digested plasmid fragment

Options:

- 1) in annotation tool
- 2) in sequence view
- 3) in plasmid view



Help: if too Many Restriction Sites Visible

pcDNA6/myc-His A

SEQUENCE MAP

in sequence

10 20 30 40 50 60 70 80 90

100 110 120 130 140 150 160 170 180 190

BASES 5126 INSERT 2764

pcDNA6/myc-His A

5126 bp

in plasmid

5000 4000 3000 2000 1000

4133-4792

4002-4843

17 origin

2500

ASSEMBLY WIZARD SPLIT WORKSPACE

Help: if too Many Restriction Sites Visible

with this setting you see only the selected restriction sites

1

2 brand 'NEB'

3 'None except...'

pcDNA6/myc-FliA

SEQUENCE MAP

LINEAR MAP PLASMID DESCRIPTION ANNOTATION RELEVANT ITEMS

NEW DIGEST SAVED DIGESTS

Enzyme lists Manage enzyme lists

Cut sites visible on maps

None (except selected and compatible with them)

Find enzyme

Search by name or number of cuts

Name	Cuts	Selected	Color
AatII	5		
AbaSI	307		
Acc65I	1		
AccI	3		
AccI	59		

Show enzymes that cut

anywhere in the sequence

Highlight enzymes with compatible sticky ends

RUN DIGEST

BASES 5126 INSERT 2764

ASSEMBLY WIZARD SPLIT WORKSPACE

- ✓ in digest tool
- ✓ find enzyme
- ✓ search or scroll list
- ✓ select enzymes
- ✓ BamHI
- ✓ XhoI
- ✓ run digest

digest tool

find enzyme

click to select

enzymes selected

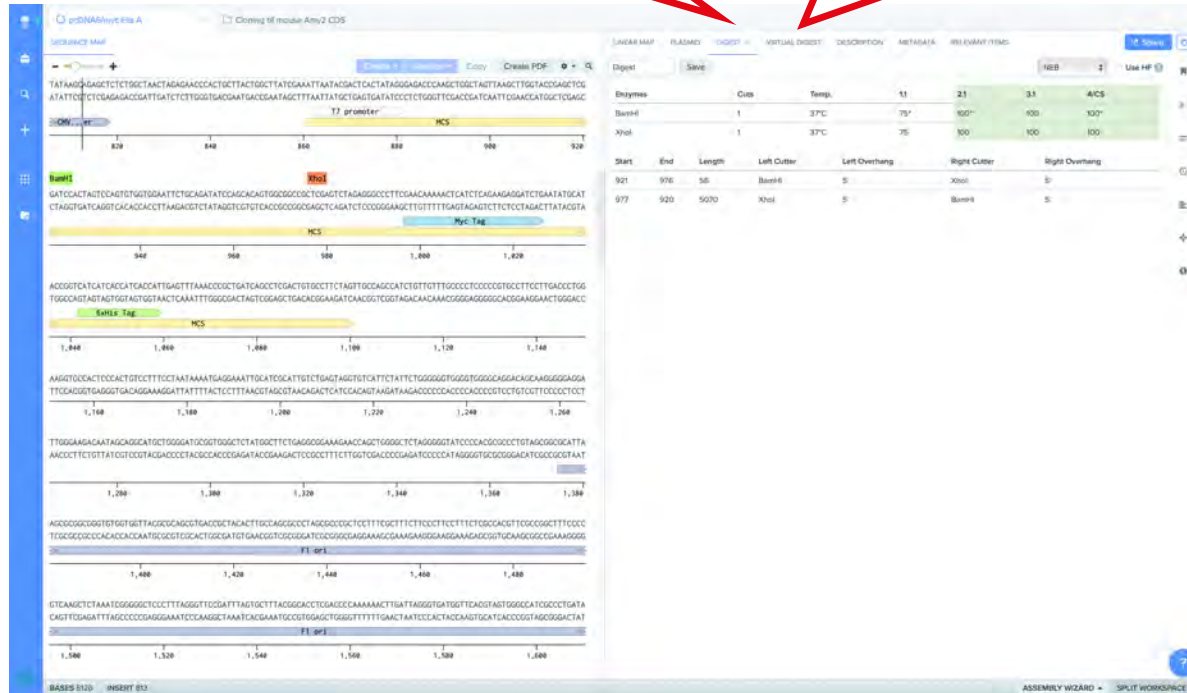
run digest

Name	Cuts	Selected	Color
BamHI	5	☒	Red
AbaSI	307	☒	Green
Acc65I	3	☐	Blue

Digest Results Shown as 2 Alternative Views, Choose What You Like Best

A) Digest

B) Virtual Digest



Select Digested Fragment in Table:

'Digest' Tab

Digest

The screenshot displays the Benchling software interface. On the left, a DNA sequence map is shown with various features labeled, including 'CMV enhancer', 'CMV promoter', 'T7 promoter', 'MCS', and 'Myc Tag'. A red box highlights a specific region of the map. On the right, a table titled 'DIGEST' is visible, showing the results of a digestion. The table has columns for 'Enzymes', 'Cuts', 'Temp.', 'BamHI', 'XbaI', 'Start', 'End', 'Length', 'Left Cutter', 'Left Overhang', 'Right Cutter', and 'Right Overhang'. A red box highlights a row in the table, indicating the selected digested fragment.

Enzymes	Cuts	Temp.	BamHI	XbaI	Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
BamHI	1	37°C	75	100	100	100	100				
XbaI	1	37°C	75	100	100	100	100				
Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang					
921	979	59	BamHI		XbaI						
979	1025	50		XbaI							

click on row to select digested fragment

Select Digested Fragment in Table: 'Virtual Digest' Tab

Virtual Digest

click on band to select digested fragment

The screenshot shows the Benchling software interface. The main window displays a DNA sequence with various features like ORF, enhancer, promoter, and NCS. The 'VIRTUAL DIGEST' tab is selected, showing a table of digested fragments. A band is highlighted at 5.1 kb. A red speech bubble points to the 'VIRTUAL DIGEST' tab, and another red speech bubble points to the highlighted band in the table, with the text 'click on band to select digested fragment'.

Ladder	Size (kb)
1	5.1 kb

From Selected Fragment: Create New DNA

The image shows a screenshot of the Benchling software interface. A red speech bubble labeled "Create" points to the "Create" button in the top navigation bar. Below this, a dropdown menu is open, showing options: Annotation, Primer, Translation, New Protein, **New DNA** (highlighted), New RNA, and New Part. Another red speech bubble labeled "New DNA" points to the "New DNA" option in the menu. To the right, a red speech bubble labeled "window opens: copy with default parameters" points to a dialog box titled "Copy Selection to New DNA". This dialog box contains a section "Customize what gets copied over:" with several checkboxes: "Annotations, translations, and primers" (checked), "Include annotations and translations not fully contained by selection" (unchecked), "Use reverse complement instead" (unchecked), "Preserve sequence indices" (unchecked), "Tags" (checked), and "Description" (unchecked). At the bottom of the dialog are "CANCEL" and "COPY" buttons. The background of the interface shows a DNA sequence viewer with various tracks and a ladder.

Create

New DNA

window opens: copy with default parameters

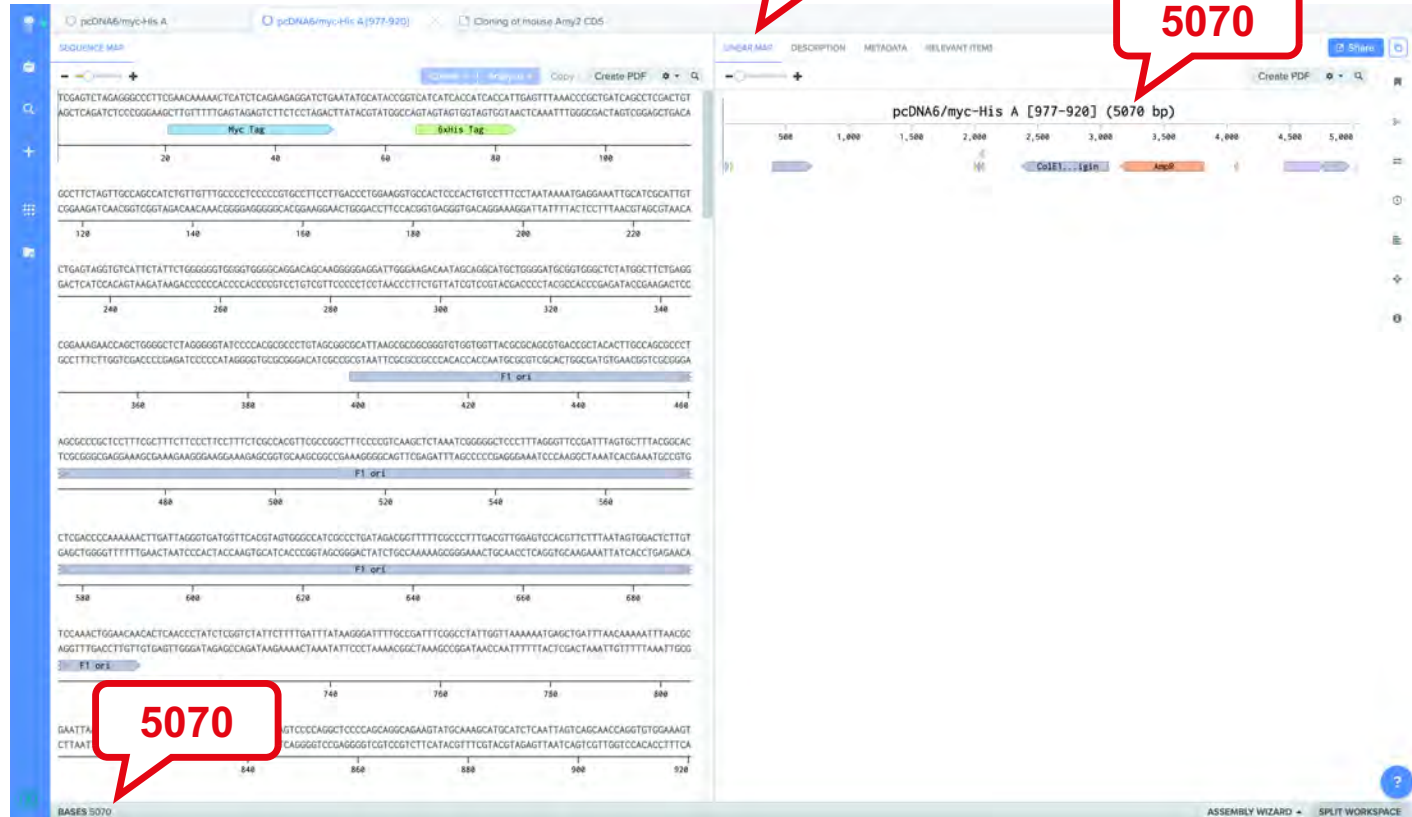
Copy Selection to New DNA

Customize what gets copied over:

- ☒ Annotations, translations, and primers
- ☐ Include annotations and translations not fully contained by selection
- ☐ Use reverse complement instead
- ☐ Preserve sequence indices
- ☒ Tags
- ☐ Description

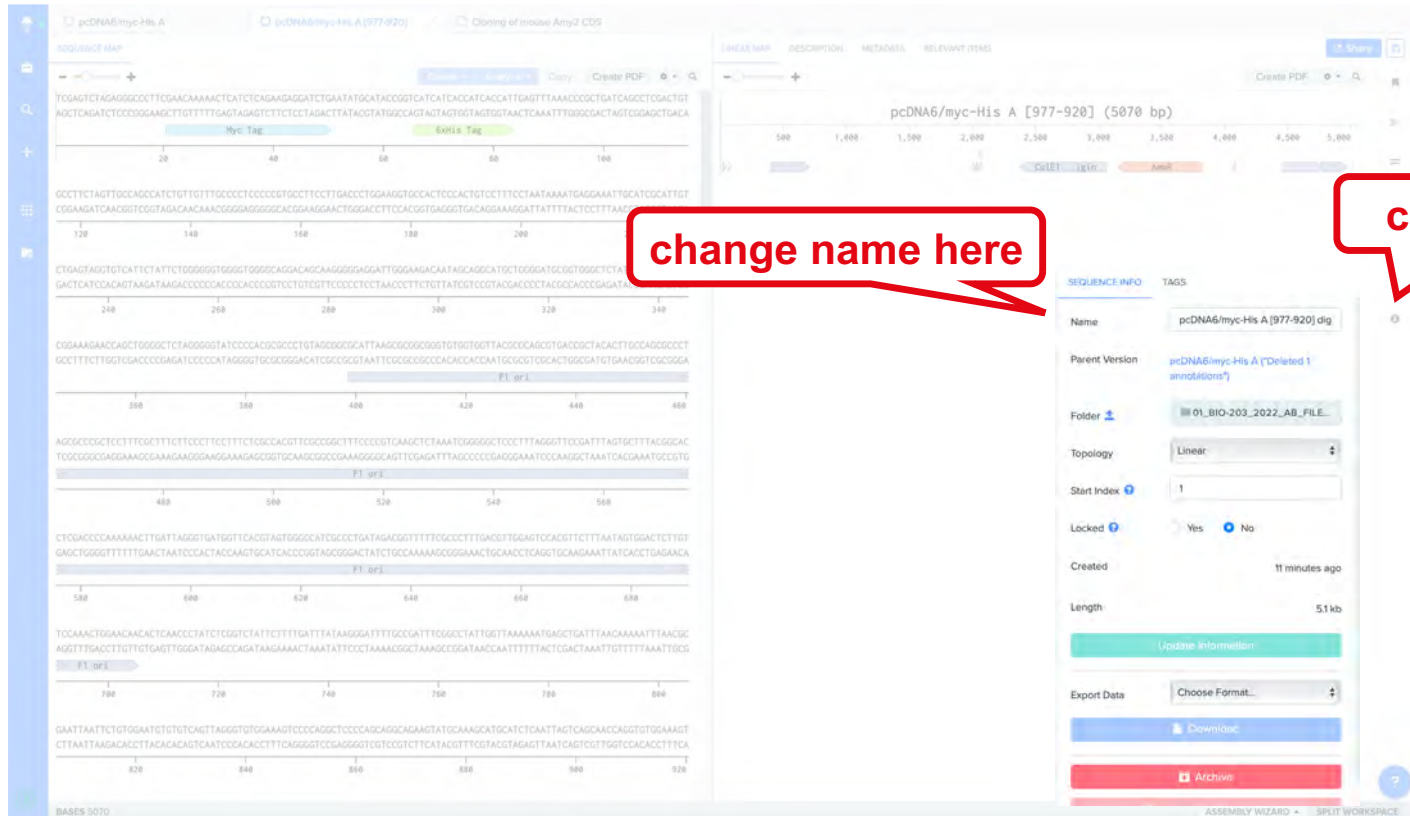
CANCEL COPY

The Digested Fragment is Linear and 5070 bp Long



Rename Digested Fragment:

Linearized pcDNA6/ myc-His A [977-920] digest BamHI and XhoI



The screenshot displays the Bases 5070 software interface, showing a sequence map and a sequence info panel. The sequence map on the left shows a linearized sequence with a 'Myc Tag' and an 'Exon 1 Tag'. The sequence info panel on the right shows the 'Name' field, which is currently 'pcDNA6/myc-His A [977-920] dig'. A red callout points to this field with the text 'change name here'. Another red callout points to the 'Name' field in the 'SEQUENCE MAP' panel, which is 'pcDNA6/myc-His A [977-920] (5070 bp)', with the text 'click on 'i''.

SEQUENCE MAP

pcDNA6/myc-His A [977-920] (5070 bp)

SEQUENCE INFO

Name: pcDNA6/myc-His A [977-920] dig

Parent Version: pcDNA6/myc-His A ("Deleted 1 annotations")

Folder: 01_BIO-2022_AB_FILE...

Topology: Linear

Start Index: 1

Locked: Yes No

Created: 11 minutes ago

Length: 51 kb

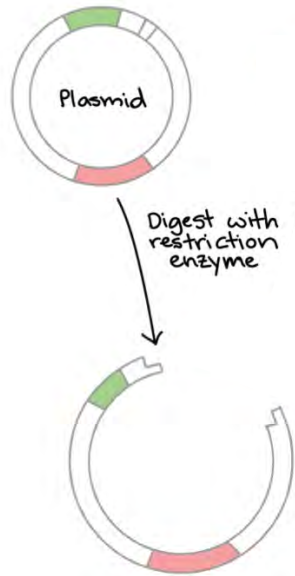
Update Information

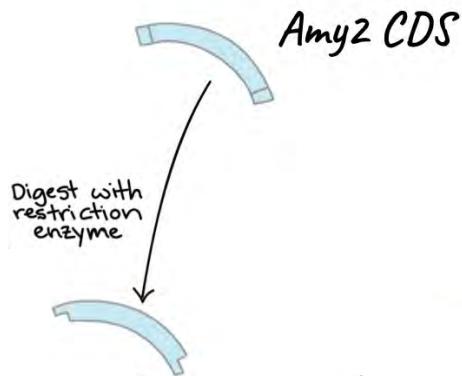
Export Data: Choose Format...

Download

Archive

Plasmid backbone is ready!





Exercise 1

Create insert

Find Amy2 mRNA sequence on NCBI

Create New DNA

Annotate CDS

Attach primer pair

Create PCR product

Digest PCR product

How to Import Sequences

- there are multiple ways how to import
- Option I: copy sequence from NCBI, annotate manually
 - **used for this manual**
 - helps you to learn how to find information on NCBI
- Option II: import annotated sequence from NCBI
 - has many more annotations than we need
 - prevents errors in annotations

Option I

NCBI website : Search Nucleotide Sequence

<https://www.ncbi.nlm.nih.gov>

The screenshot shows the NCBI website homepage. At the top, the NIH logo and "National Library of Medicine" text are on the left, and a "Log in" button is on the right. A red box highlights the "search" button in the top right corner. Below the header, a search bar contains the text "Nucleotide" and "NM_001042711.2", with a red box around it and an arrow pointing to the "Search" button. The main content area is divided into three columns. The left column contains a "NCBI Home" section with a "Resource List (A-Z)" and a list of resources including "All Resources", "Chemicals & Bioassays", "Data & Software", "DNA & RNA", "Domains & Structures", "Genes & Expression", "Genetics & Medicine", "Genomes & Maps", "Homology", "Literature", "Proteins", "Sequence Analysis", "Taxonomy", "Training & Tutorials", and "Variation". The middle column features a "Welcome to NCBI" message, a description of the center's mission, and links to "About the NCBI", "Mission", "Organization", and "NCBI News & Blog". Below this are six interactive tiles: "Submit" (Deposit data or manuscripts into NCBI databases), "Download" (Transfer NCBI data to your computer), "Learn" (Find help documents, attend a class or watch a tutorial), "Develop" (Use NCBI APIs and code libraries to build applications), "Analyze" (Identify an NCBI tool for your data analysis task), and "Research" (Explore NCBI research and collaborative projects). The right column contains a "Popular Resources" section with links to "PubMed", "Bookshelf", "PubMed Central", "BLAST", "Nucleotide", "Genome", "SNP", "Gene", "Protein", and "PubChem". Below this is an "NCBI News & Blog" section with a headline "Announcing GenBank release 252.0" dated "19 Oct 2022", a paragraph about the release, and a link to the "New version of PGAP now available!" dated "15 Feb 2022".

NCBI Home
Resource List (A-Z)
All Resources
Chemicals & Bioassays
Data & Software
DNA & RNA
Domains & Structures
Genes & Expression
Genetics & Medicine
Genomes & Maps
Homology
Literature
Proteins
Sequence Analysis
Taxonomy
Training & Tutorials
Variation

Welcome to NCBI
The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.
[About the NCBI](#) | [Mission](#) | [Organization](#) | [NCBI News & Blog](#)

Submit
Deposit data or manuscripts into NCBI databases

Download
Transfer NCBI data to your computer

Learn
Find help documents, attend a class or watch a tutorial

Develop
Use NCBI APIs and code libraries to build applications

Analyze
Identify an NCBI tool for your data analysis task

Research
Explore NCBI research and collaborative projects

Popular Resources
PubMed
Bookshelf
PubMed Central
BLAST
Nucleotide
Genome
SNP
Gene
Protein
PubChem

NCBI News & Blog
Announcing GenBank release 252.0
19 Oct 2022
Now over 3 billion records! GenBank release 252.0 (10/17/2022) is now available on the NCBI FTP site. This
New version of PGAP now available!
15 Feb 2022

NCBI website : Find Amy2 mRNA- Contains 3'UTR, CDS, 5'UTR

click FASTA
format

GenBank

Send to:

FASTA

Mus musculus amylase 2a5 (Amy2a5), mRNA

NCBI Reference Sequence: NM_001042711.2

[FASTA](#) [Graphics](#)

Go to:

LOCUS NM_001042711 1577 bp mRNA linear ROD 05-MAR-2022
 DEFINITION Mus musculus amylase 2a5 (Amy2a5), mRNA.
 ACCESSION NM_001042711 NM_001042712 NM_009669
 VERSION NM_001042711.2
 KEYWORDS RefSeq; RefSeq Select.
 SOURCE Mus musculus (house mouse)

Name (first line)
+ nucleotide
sequence

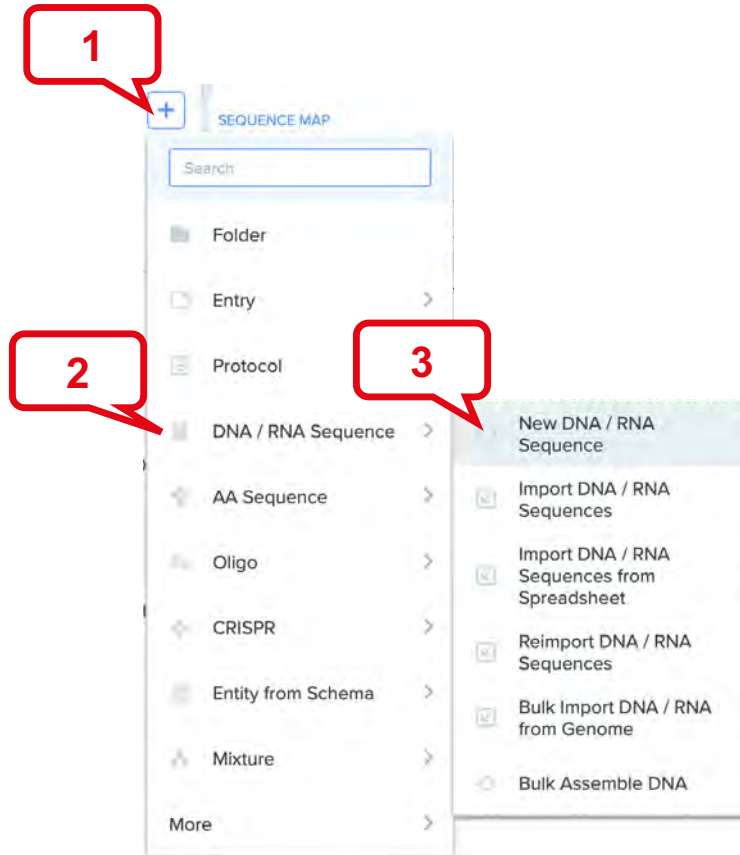
Mus musculus amylase 2a5 (Amy2a5), mRNA

NCBI Reference Sequence: NM_001042711.2

[GenBank](#) [Graphics](#)

>NM_001042711.2 Mus musculus amylase 2a5 (Amy2a5), mRNA
 GACAACCTCAAAGCAAATGAAGTTCGTTCTGCTGCTTCCCTCATTGGGTTCTGCTGGGCTCAATATGA
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 TAGTTCATAACCCATCAAGACCTTGGTGGGAAAGATACCAACCAATCAGCTATATAAAATCTGCACAAGGTC
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 ATCCAAATAACAGGGAATCCCGACAGTCCATACTCTGCTTGGGACCTTAACGATAATAAATGTAATGG
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 CTTGAGAAAGATTATGTTCTGACCAAGGTGGCTGACTATATGAACCATCTCATTGACATTGGAGTAGCAG
 GGTTGACAGCTTGATGCTGCTAAGCACATGTGGCTGGAGACATAAAGGCAGTCTTGGACAACTGCATAA
 TCTCAATACAAATGGTCTCCCAAGGAAGCAGACCTTTCATTTCCTCAAGAGGTCAATGATCTGGGTGGT
 GAGGCAATTAAGGTAGTGAGTACTTTGGAAATGGCGTGTGACAGAATTCAGTATGGTGCAAAATCTG
 GCACAGTTATCCGCAAGTGGAAATGGCGAGAAAGATGTCATTAAAGAACTGGGGAAGAGTTGGGGTTT
 GGTGCTCTCTGACAGAGCCCTTGTGTTTGGGACAACCATGATAATCAGCGAGGACATGGTCTGGAGGA
 TCATCCATCTGACATTCTGGGATGCTAGAATGTATAAAATGGCTGTGGATTATGTTGGCTCATCCTT
 ATGGATTACAAGAGTAATGTCAGATTACCGTTGGAATAGAAATTTCCAGAATGGAAAAGATCAGAATGA
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 AATGACTGGGTCTGTGAACACAGATGGCGTCAAAATCAGGAACATGGTGGCTTCAGGAATGGTCAATG
 GTCAGCCTTTTCAAACCTGGTGGGATAATAACAGCAACCAAGTAGCTTTTAGCAGAGGAACACAGAGATT
 CATTGCTTTAAACAATGATGACTGGGCTTTGTGACGCACCTTACAGACTGGTCTTCTGCTGGCACATAC
 TGTGATGTCATCTGAGAGATAAGGTGATGGCAATTGCACTGGACTTAGAGTGAATGTTGGCAGTGATG
 GCAAAGCTCACTTTTCCATTAGTAACCTGCTGAGGAGCCATTATTGCAATCCATGCTGACTCAAAAT
 GTAAGAATCTATATTAAGAGATTGGATTAAAGCATC

In Benchling: Create New DNA Sequence



Copy + Paste Name and Sequence (from Fasta File in NCBI)

Create DNA / RNA Sequence

[CREATE NEW](#) [UPLOAD FILES](#) [IMPORT FROM DATABASE](#) [SELECT CHROMOSOMAL REGION](#)

1 **Name***

NM_001042711.2 Mus musculus amylase 2a5 (Amy2a5), mRNA

2 **Set nucleotide type***

DNA RNA

Set topology

Linear

3 **Set folder***

01_BIO-203_2022_AB_FILES SHARED WITH STUDENTS

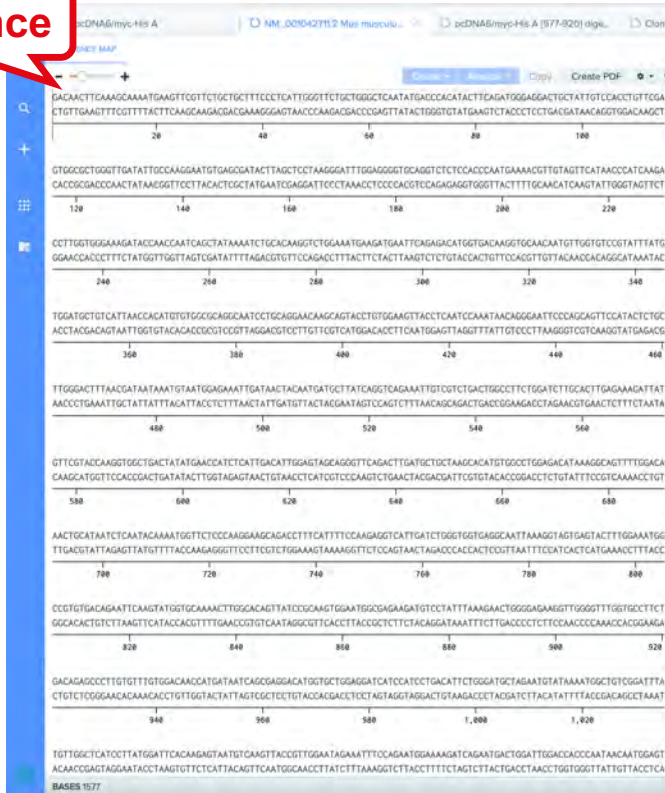
Bases

GACAACTTCAAAGCAAATGAAGTTCGTTCTGCTGCTTCCCTCATTGGGTTCTGCTGGGCTCAATATGACCCACATACTTCAG TGCTAT

4 **Create**

New DNA: Amy2 Nucleotide Sequence

sequence



name



still empty here: let's translate the CDS!

click 'back'
from Fasta
sequence

<https://www.ncbi.nlm.nih.gov>



National Library of Medicine
National Center for Biotechnology Information

Log in

Mus musculus amylase 2a5 (Amy2a5), mRNA

NCBI Reference Sequencer: NM_001042711.2

FASTA Graphics

Go to: [v]

LOCUS NM_001042711 1577 bp mRNA linear ROD 05-MAR-2022
DEFINITION Mus musculus amylase 2a5 (Amy2a5), mRNA.
ACCESSION NM_001042711 NM_001042712 NM_09669
VERSION NM_001042711.2
KEYWORDS RefSeq; RefSeq Select.
SOURCE Mus musculus (house mouse)

18-1544

[CDS](#)

18..1544
/gene="Amy2a5"
/gene_synonym="181008N23Rik; Amy-2; Amy2; mAmy2-2"
/EC_number="3.2.1.1"
/note="amylase 2a5, pancreatic; pancreatic alpha-amylase;
PA; 1,4-alpha-D-glucan glucanohydrolase; amylase 2,
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/db_xref="CCDS:CCDS17775.1"
/db_xref="GeneID:109959"
/db_xref="MGI:MGI:88020"
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DNYNDAYQVRNRLTGLLDLAEKDYVRTKVADYMNHLIDIGVAGFRDLAAKHMWPGD
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MAVGFMALHPYGFTRVMSYRWNRNFQNGKQNDWIGPPNNNGVTKEVTINADTTGCGN
DWVCEHRWRQIRNMVAFRNVVNGQPFNSWDDNSNQVAFSRGNRGFIVFNDDWALSA
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CDS

```

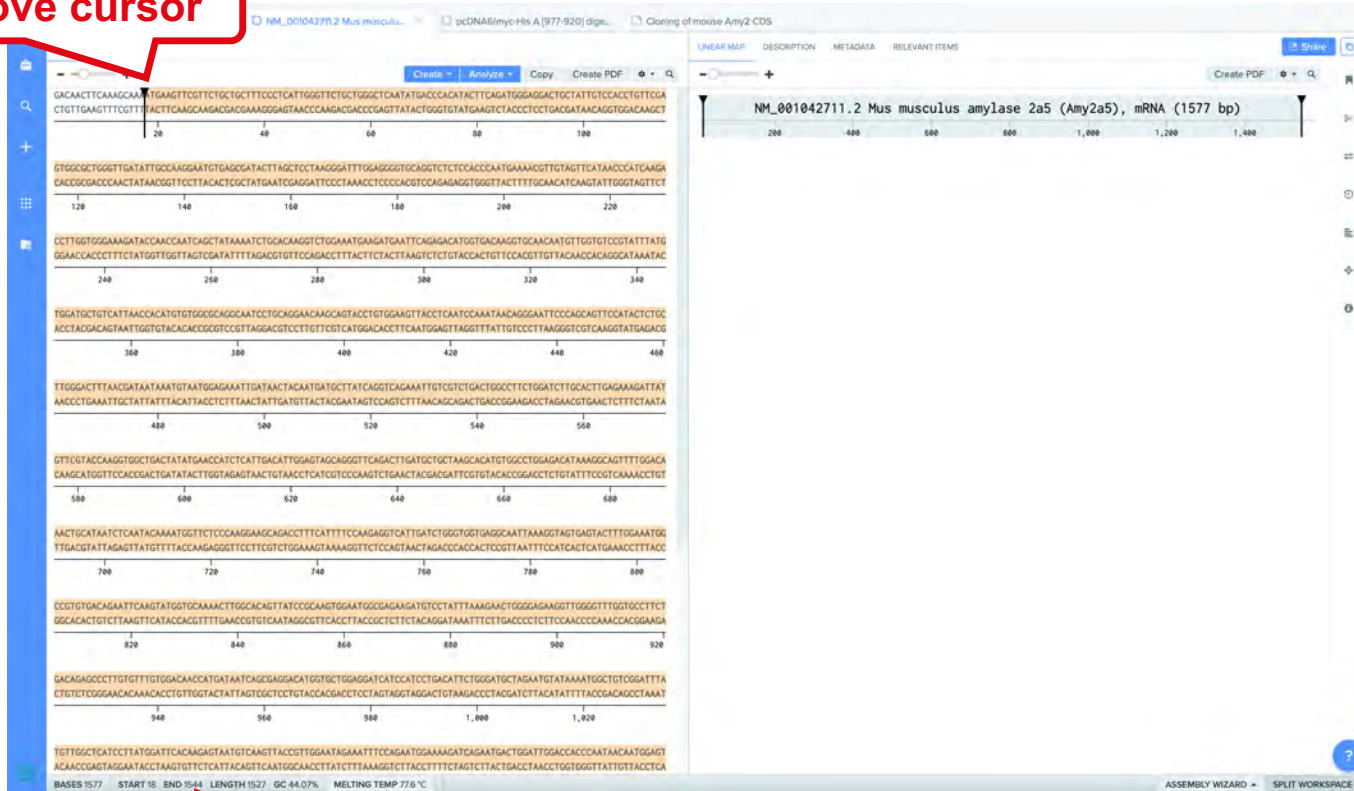
1 gacaacttca aagcaaatg aagttcgttc tgcctgttcc cctcattggg ttcctgtcgg
61 ctcaaatatga cccacatact tcagatggga ggactgctat tgcaccctg ttcagatggc
121 gctgggttga tattgccaag gaattgtgac gatacttagc tcctaaggga ttggaggggg
181 tgcaggtctc tccaccaat gaaaacgttg tagttcataa cccatcaaga cttgtgtggg
241 aaagatacca accaatcagc tataaaatct gcacaaggtc tggaaatgaa gatgaattca
301 gagacatgtg gacaaggtgc aacaatgttg gtgtccgtat ttatgtggat cgtgtcatta
361 accacatgtg tggcgcaggc aatcctgcag gaacaagcag taccgtgga agttaccca
421 atccaaataa cagggaattc ccagcagttc catactctgc ttgggacttt aacgataata
481 aatgtaatgg aaaaattgat aactacaatg atgcttatca ggtcagaaat tgcgtctga
541 ctggccttct ggatcttga cttgagaaga attatgttgc taccaagggtg cgtgactata
601 tgaaccatct cattgacatt ggagtagcag gtttcagact tgatgctgct aagcacatgt
661 ggcctggaga cataaaggca gttttgaca aactgcataa tctcaataca aaatggttct
721 cccaaggaga cagaccttcc attttccaag aggtcattga tctgggtgtg gaaggcaata
781 aaggtagtga gtactttgga aatggcgtg tgacagaatt caagtatgtt gcaaaacttg
841 gcacagttat ccgcaatgg aatggcgaga agatgtccata tttaagaac tggggagaag
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961 gaggacatgg tctggaggga tcattccatc tgacattctg ggaatgtaga atgtataaaa
1021 tgcctgtcgg attatgttg gctatcctt atgagttcac aagatgaatg tcaagtacc
1081 gttggaatg aaatttccag aatggaaaag atcagaatga ctggattgga ccaaccaata
1141 acaatggagt aacaaaagaa gtgaccatta atgcagacac tacttgtgac aatgactggg
1201 tctgtgaaca cagatggcgt caaatcagga acatggttgc cttcaggaat gtggctcaatg
1261 gtcagccttt ttcaaatcgg tgggataata acagcaacca agtagctttt agcagagaaa
1321 acagaggatt cattgtcttt aacaatgatg actgggcttt gtcagccact ttacagactg
1381 gtcttctcgc tggcacatcc tgtgatgtca tctctggaga taaggctgat ggcgaattga
1441 ctggacttag agtgaatgtt gccagtgatg gcaaaagctc cttttccatt agtaactctg
1501 ctgaggaccc attatttga atccatgctg actcaaaatt gtaagaatct atattaagaa
1561 gatttggatt aagcatc

```

click on CDS

Select Amy2 CDS (Nucleotide 18-1544)

to select move cursor



START 18

END 1544

Amy2 Translation (Nucleotide 18-1544)

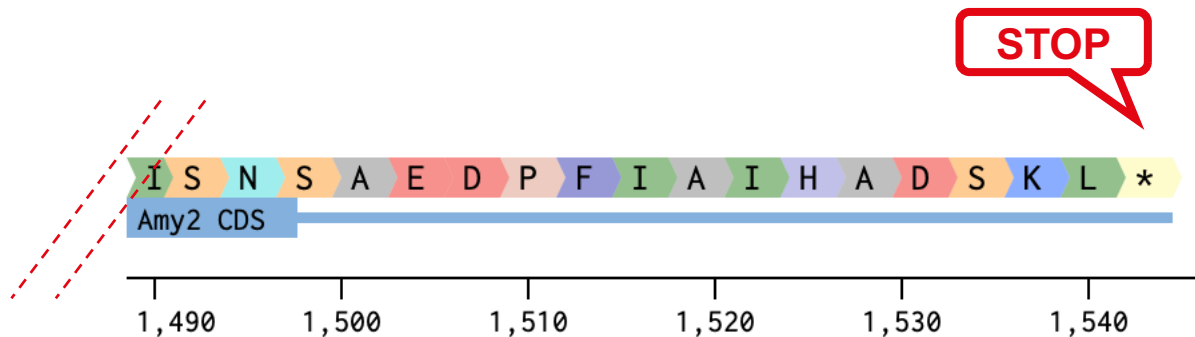
amino acid sequence

The screenshot displays a bioinformatics tool interface for translating a DNA sequence. The main window shows the Amy2 CDS (Coding DNA Sequence) from nucleotide 18 to 1544. The sequence is presented in a multi-line format, with each line showing a segment of the DNA sequence and its corresponding amino acid translation. The amino acid sequence is highlighted in a red box, and a red arrow points to it with the label "amino acid sequence".

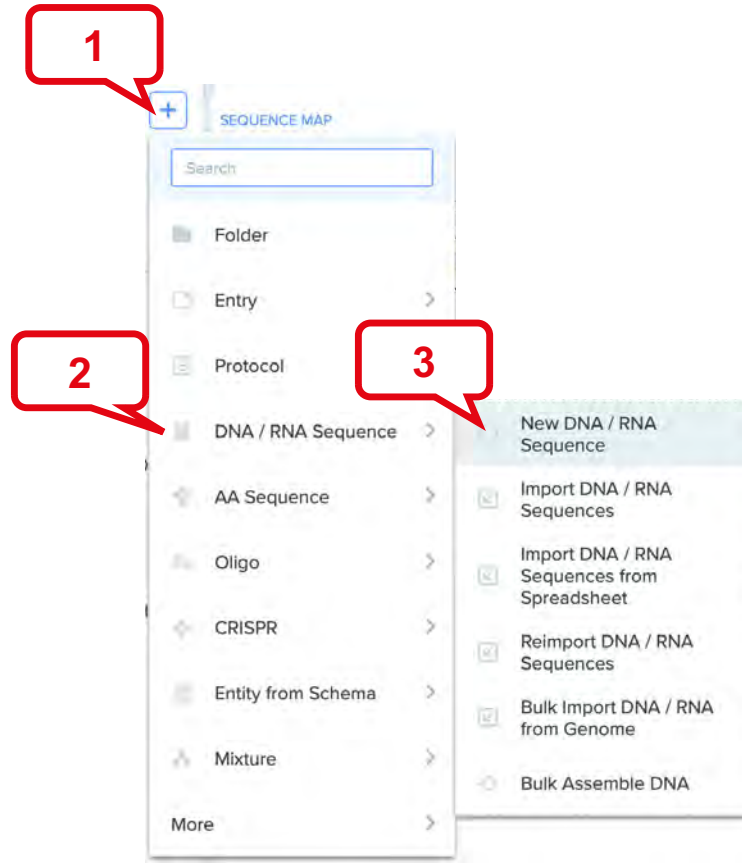
The translation window on the right shows the protein name: **NM_001042711.2 Mus musculus amylase 2a5**. The window also includes a "Create new" button and a "Show expanded view" link.

The bottom status bar provides assembly details: **BASES 1577 START 18 END 1544 LENGTH 1527 GC 44.07% MELTING TEMP 77.6 °C**. It also includes links for **ASSEMBLY WIZARD** and **SPLIT WORKSPACE**.

View Amy2 STOP Codon (1544)



Option II: Import from Database with Annotations



Import from Database with Annotations

1

Create DNA / RNA sequence

2

CREATE NEW UPLOAD FILES IMPORT FROM DATABASE SELECT CHROMOSOMAL REGION

Sequence

NM_001042711.2 Search X Close

NCBI

Entry	NM_001042711	Name	NM_001042711
Database	NCBI Nucleotide (Genbank)		
Length	1577		
Description	Mus musculus amylase 2a5 (Amy2a5), mRNA		

3

Set nucleotide type*

DNA RNA

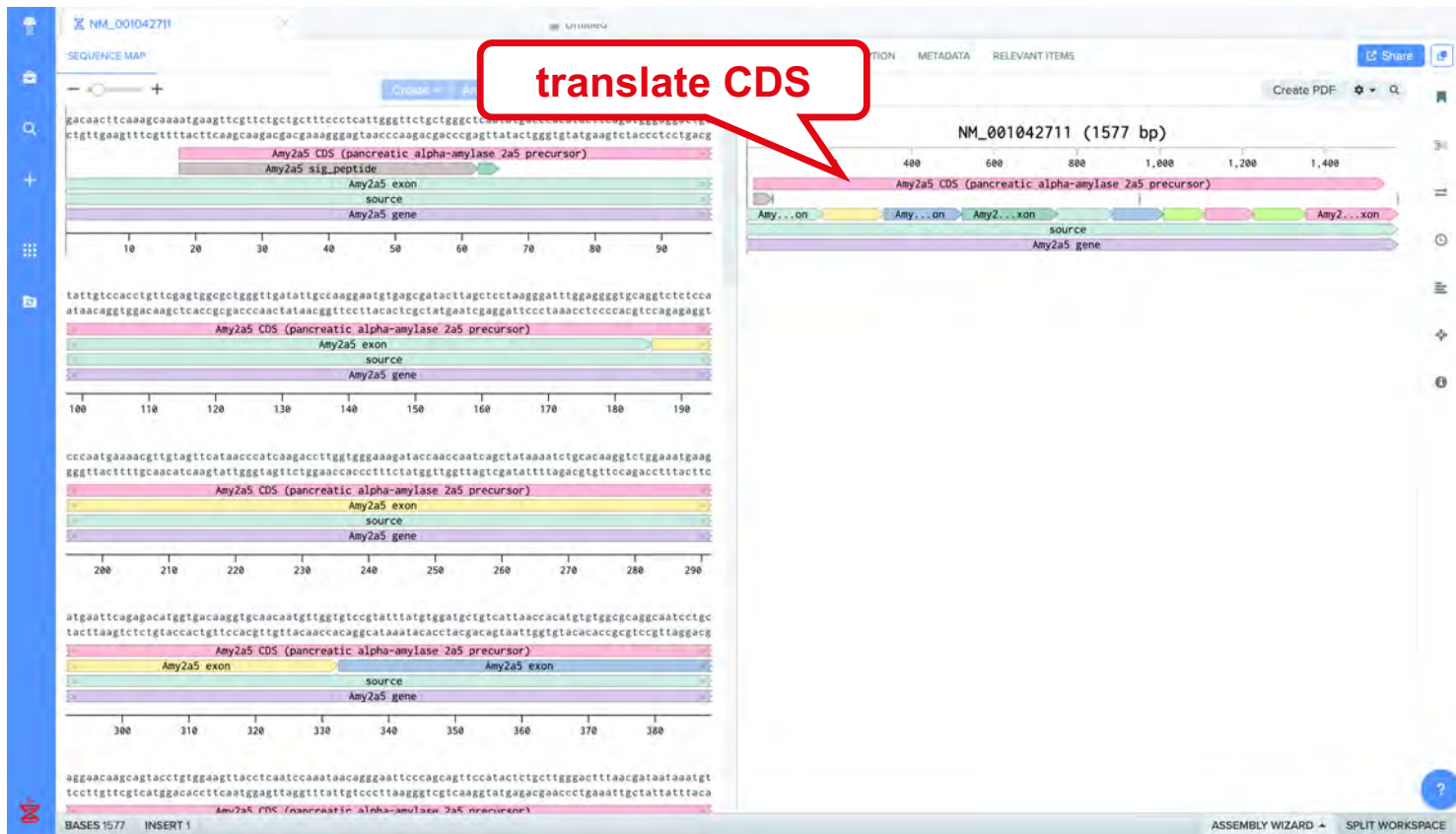
Set folder*

03_Exercises 2021_22

Set schema

Select a schema... 4

Close Import



name	sequence	3'location	overhang
Mm-Amy2_for	CAGGATCCACCATGAAGTTCGTTCTGCTGCTTTC	40	11
Mm-Amy2_rev	GGCTCGAGTTCATACAATTTTGAGTCAGCATGGATTG	1519	8

create primers

manual

CREATE PRIMERS

ATTACH EXISTING

The screenshot displays the 'Create Primer Pair' web application interface. The left panel shows a 'SEQUENCE MAP' for the 'NM_001042711.2 Mus musculus ampless 2a5 (Amy2a5), mRNA' gene. The sequence is displayed in a color-coded format with nucleotide positions (1 to 580) and amino acid positions (1 to 192). The right panel shows the 'DESIGN PRIMER' tab, which includes a 'Primer Pair' dropdown, a 'Jump to Primer' button, and a 'Set from Selection' button. The 'Forward' and 'Reverse' primer sequences are displayed, along with their 3' locations and overhangs. The 'Verify' section shows the GC content, length, and product size. The 'Save To' section includes a 'primers' folder and a 'Choose Folder' button. The 'Save' button is highlighted with a red box.

select primer pair

copy/ paste sequences

3' location

overhang

select restriction site

copy/ paste names

save

Mm-Amy2-for (bezler / Lab3 exercise 1)

You can also [duplicate it](#).

Strand Forward

Bases 5' CAGGATCCACC **ATGAAGTTCGTTCTGC**
TGCTTTC 3'

3' Location 40

Overhang 11

were it binds to the template

non-complementary part

Mm-Amy2-rev (bezler / Lab3 exercise 1)

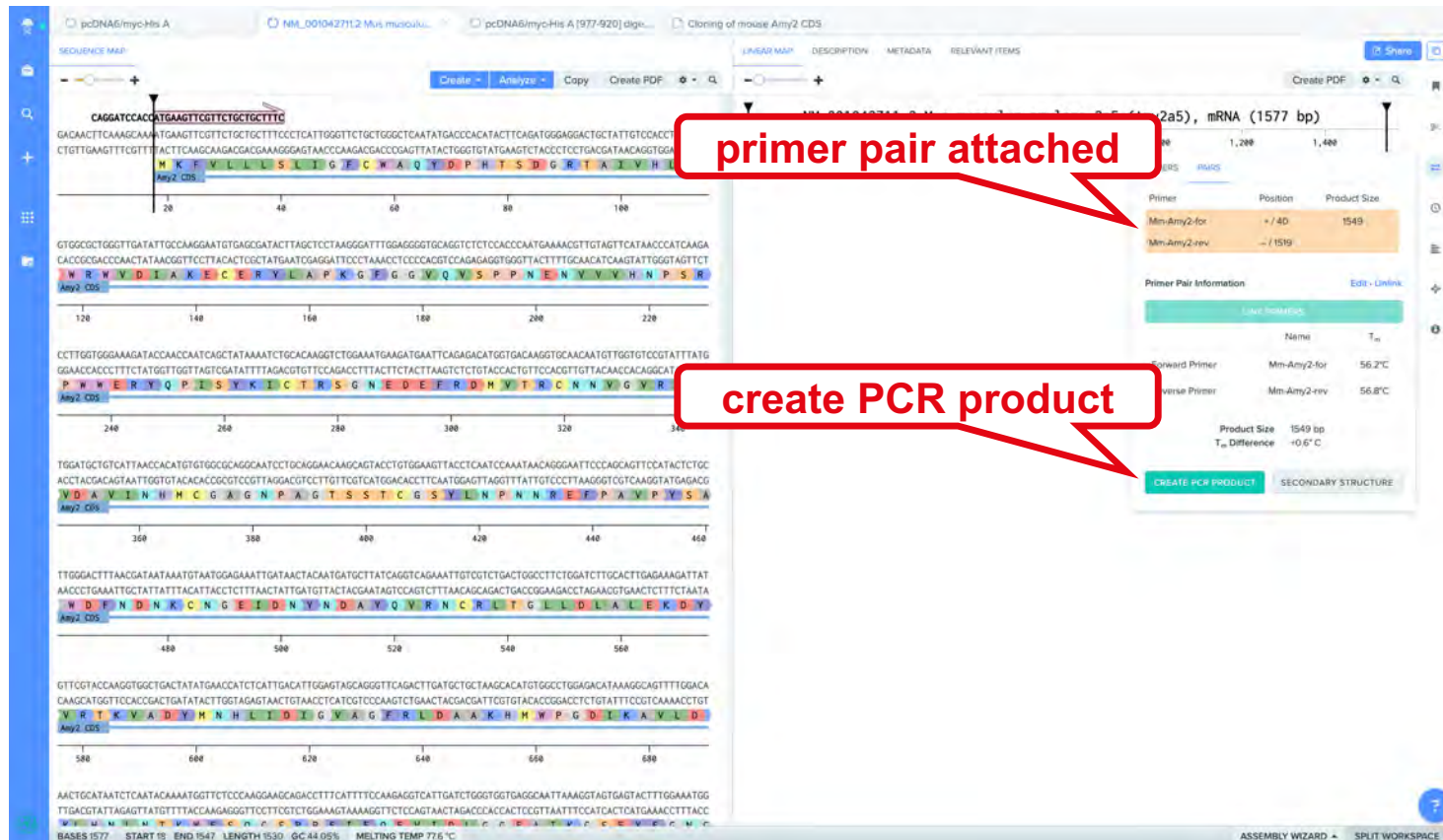
You can also [duplicate it](#).

Reverse

Bases 5' GGctcgag **TTCATACAATTTTGAGTCA**
GCATGGATTG 3'

1519

8



SEQUENCE MAP

pcDNA6/myc-His A

Linear Map

DESCRIPTION

METADATA

RELEVANT ITEMS

Share

primer pair attached

create PCR product

Primer Pair Information

Primer	Position	Product Size
Mm-Amy2-for	+ / 40	1549
Mm-Amy2-rev	- / 1519	

Primer Pair Information

Forward Primer

Mm-Amy2-for

Reverse Primer

Mm-Amy2-rev

Product Size

1549 bp

T_m Difference

+0.6° C

CREATE PCR PRODUCT

SECONDARY STRUCTURE

BASES 1577 START 1547 END 1530 LENGTH 1530 GC 44.05% MELTING TEMP 77.6°C

ASSEMBLY WIZARD SPLIT WORKSPACE

Create PCR Product Including Primers Sequences

what gets copied

Copy Selection to New DNA

Customize what gets copied over:

- ☒ Use primer bases instead of sequence (includes overhang if any)
- ☒ Annotations, translations, and primers
- ☐ Include annotations and translations not fully contained by selection
- ☐ Use reverse complement instead
- ☐ Preserve sequence indices
- ☒ Tags
- ☐ Description

copy

amy1ase 2a5 (Amy2a5), mRNA (1577 bp)

Primer	Position	Product Size
Mini-Amy2-for	+ / 40	1549
Mini-Amy2-rev	- / 1519	

Primer Pair Information

Name	T _m
Mini-Amy2-for	56.2°C
Mini-Amy2-rev	56.6°C

Product Size: 1549 bp
T_m Difference: +0.6°C

CREATE PCR PRODUCT SECONDARY STRUCTURE

ASSEMBLY WIZARD SPLIT WORKSPACE

1549 bp

change name here
PCR...

click on 'i'

g

1549 bp

change name here PCR...

pcDNA3/myc-His A [397-920] dige... Cloning of mouse Amy2 CDS

SEQUENCE INFO TAGS

Name PCR_NM_001042711.2 Mus musc

Parent Version NM_001042711.2 Mus musculus amylase 2a5 (Amy2a5), mRNA ("Linked primers Mm-Amy2-for and Mm-Amy2-rev")

Folder 01_BIO-203_2022_AB_FILE...

Topology Linear

Start index 1

Locked Yes No

Created 7 minutes ago

Length 1.5 kb

Export Data Choose Format...

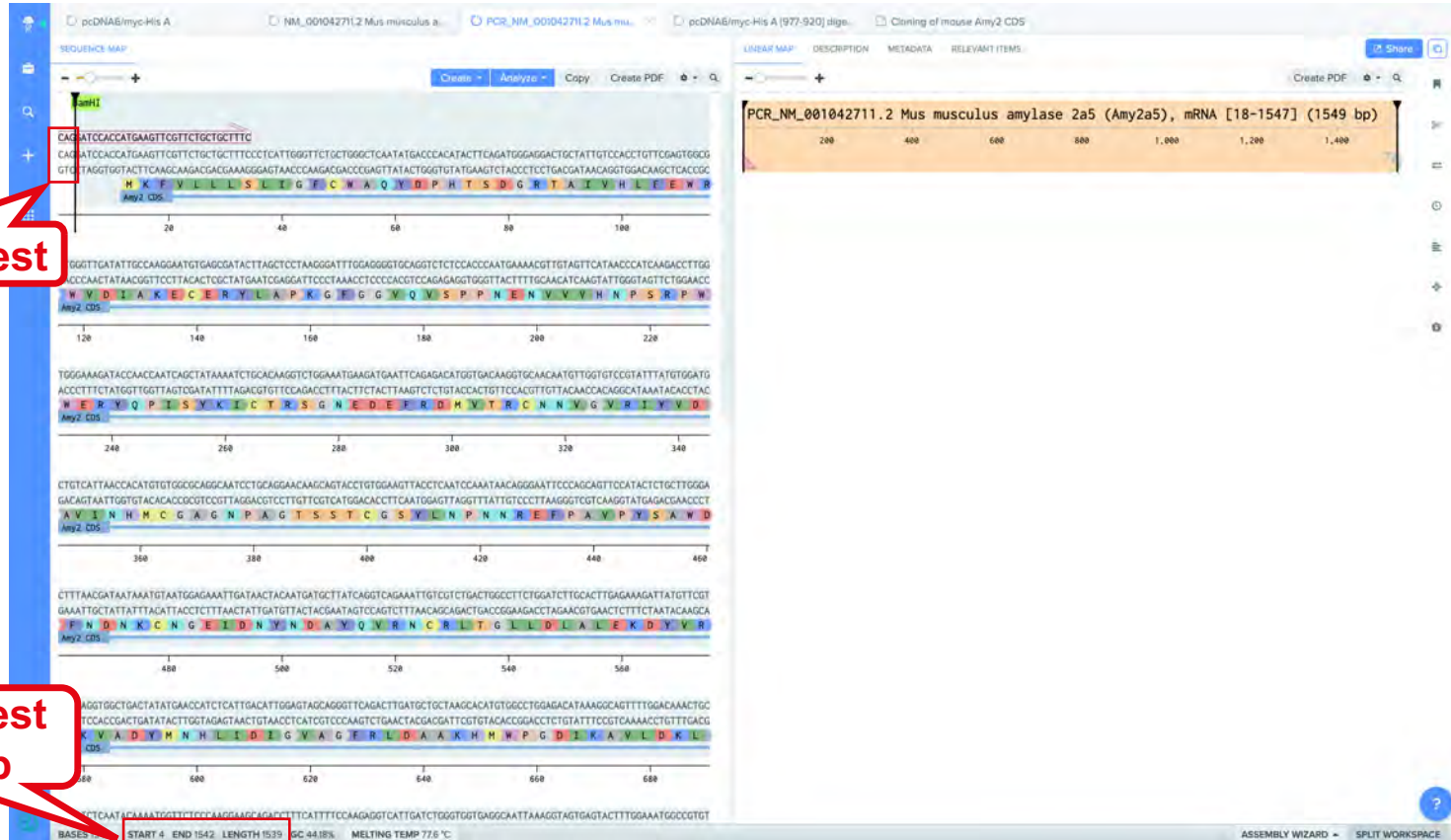
Download

Archive

ATATCTCAATAAAATGGTCTCCCAAGGAAGACAGCTTATTTTCCAAGAGGTCAATGATCTGGGTGGTGAAGCAATAAAGGTAGTGAGTACTTTGAAATGGCGTGT

BASES 1549

In PCR: Manually select sequence between BamHI + XhoI (Alternative: Run digest, create new DNA)



Copy selected sequence between BamHI + XhoI

copy

DNA

include annotations

Copy Special

The following bases were copied from the sequence.
Click the sequence type you would like to copy.

DNA Sequence

```
GATCCACCATGAAGTTCGTTCTGCTGCTT
TCCCTCATGGGTCTGCTGGGCTCAATA
TGACCCACATACTTCAGATGGGAGGACTG
CTATTGTCACCTGTTGAGTGCGCTGG
GTTGATATTGCCAAGGAATGTGAGCGATA
```

DNA Reverse Complement

```
gTTCATACAATTTTGAAGTCAGCATGGATT
GCAATAAATGGGTCTCAGCAGAGTTACTT
AATGGAAGAGTGAAGTTGCCATCACTGC
CAACATTCACCTCAAGTCCAGTGAATTG
CCATGACCTTATCTCCAGAGATGACATC
```

AA Translation

```
DPP*SSFCCFSLGAGLNMTHLLQMGLL
LLSTCSSGAGLILPRNVSOT*LLRDLGCG
RSLHPMKT*FIHQDLGGKDTNQAIS
AQGLEMKMNS*QATMLVSFMMML
LTTCAQAILQEAPVEVTSIQITGNSQ
```

AA Reverse Translation

```
VHTILSQHGLQ*MGPOQSY*WKSELCHIC
QHSL*VQCNCHRPYLQR*HHSMCQEDQS
VKWLTKPSHC*HQ*ILCFLC*KLLGCCY
YPTSLKKADH*PHS*RPQCS*FDAICVHR
PSHCHK*CLH*NSLLLLLHCYVWYQSSHS
```

RNA Sequence

```
GAUCCACCAUGAAGUUGUUGUCUGCUU
UCCUCUUAUGGGUUGUCUGGCUCAUA
UGACCCACAUUCUAGUUGGAGGACUG
CUAUUGUCCACCUUGUCAGUGGCGCUGG
GUUGAUUGCCAAGGAUGUGAGCGAUA
```

RNA Reverse Complement

```
gUUAUUAUUAUUUGAGUCAGCAUGGAUU
GCAUUAUUGGUUCCAGCAGAUUAU
AAUGGAAUAGAGUUGGCAUACUGC
CAACAUUACUCUAAGUCCAGUGCAUUG
CCAUCGACUUAUUCAGAGAUAGCAUC
```

Additional options when copying the sequence or its reverse complement inside Benchling:

- ☒ Include annotations/translations
- ☐ Include annotations/translations not fully contained by selection

SEQUENCE MAP

PCR_NM_001042711.2 Mus musculus a...

Cloning of mouse Amy2 CDS

SEQUENCE MAP

DESCRIPTION

METADATA

RELEVANT ITEMS

Create PDF

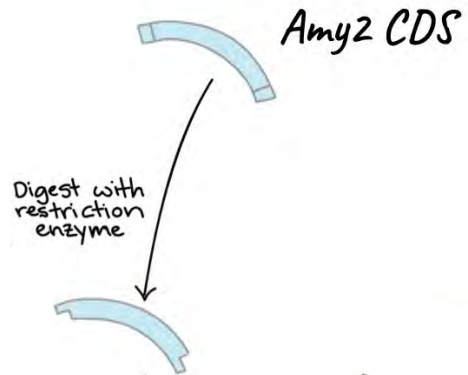
01042711.2 Mus musculus amylase 2a5 (Amy2a5), mRNA [18-1547] (1549 bp)

200 400 600 800 1,000 1,200 1,400

ATCATCTCAATACAAATGGTCTCCCAAGGAGCAGACCTTTCATTTTCCAGAGGCTCATGATCTGGGTGTGAGGCAATTAAGGTAGTACTTGGAAATGCCGTGT

BASES 1549 START 8 END 1542 LENGTH 1539 GC 44.8% MELTING TEMP 77.6 °C

ASSEMBLY WIZARD SPLIT WORKSPACE



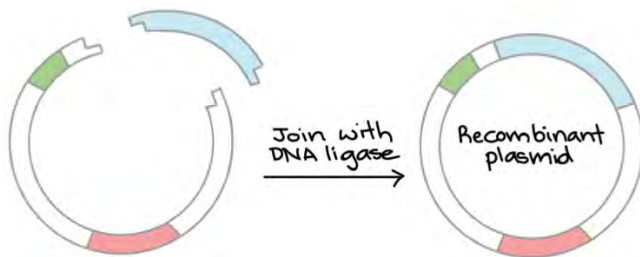
The insert is ready!

Exercise 1

Combine insert and plasmid

ligate digested insert Amy2 PCR product and digested plasmid pcDNA6/myc-His A using BamHI and XhoI with copy/ paste

annotate recombinant fusion protein



Paste into Linearized Plasmid Backbone File at Position 1 (BamHI cut site)

2 put cursor at position '1'

1 open file

3 CTR+V

SEQUENCE MAP

Linearized pcDNA6/myc-His A [977-920] BamHI XhoI (5070 bp)

GATCCACCATGAAGTTCGTTCTGCTGCTTCCCTC

You're about to paste a sequence that includes 0 annotations, 1 translation, 0

4 confirm

Press ENTER to insert 1539 bases at position 1. ESC to cancel.

BASES 5070

Make Circular + Change Name

The image shows a screenshot of the Benchling software interface, specifically the 'ASSEMBLY WIZARD' and 'SPLIT WORKSPACE' sections. The main window displays a linear map of a plasmid named 'Amy2 CDS pcDNA6/myc-His A' with a length of 6609 bp. The map shows the sequence of the plasmid, including the Amy2 CDS, pcDNA6, and myc-His A regions. The sequence is displayed in a color-coded format (A, T, C, G) and includes a scale bar from 0 to 600 bp.

Annotations on the image include:

- A red callout bubble pointing to the '6609 bp' label: **6609 bp**
- A red callout bubble pointing to the 'Name' field: **click on 'i'**
- A red callout bubble pointing to the 'Name' field: **change name here**
- A red callout bubble pointing to the 'Update Information' button: **update**

The 'Name' field is currently set to 'Amy2 CDS pcDNA6/myc-His A'. The 'Parent Version' is 'pcDNA6/myc-His A ("Deleted 1 annotation")'. The 'Folder' is '01_BIO-202_2022_AB_FILE...'. The 'Topology' is set to 'Circular'. The 'Start Index' is '1'. The 'Locked' status is 'No'. The 'Created' date is 'a day ago'. The 'Length' is '6.6 kb'. The 'Update Information' button is highlighted in green.

At the bottom of the interface, the following information is displayed:

BASES 6609 START 9 END 1535 LENGTH 1527 GC 44.07% MELTING TEMP 77.6 °C



Translate Recombinant Protein

Amy2-CDS-myc-His including STOP codon

The image displays a bioinformatics tool interface for translating a recombinant protein. The main window shows a DNA sequence (pcDNA6/myc-His A) and its corresponding amino acid translation. The sequence is divided into segments, with the Amy2 CDS (Catalytic Domain) highlighted in blue. The translation shows the protein sequence, including a STOP codon (TGA) at position 9-1625. The interface includes a 'SEQUENCE MAP' on the left, a 'TRANSLATIONS' tab on the right, and a circular map of the plasmid construct. Red callouts 1, 2, and 3 highlight specific features: 1 points to the 'Create new' button, 2 points to the 'Amy2_CDS_myc_HIS 9-1625' entry, and 3 points to the 'Show expanded view' button.

SEQUENCE MAP

pcDNA6/myc-His A

SEQUENCE MAP

1 2 3

TRANSLATIONS

Amy2_CDS_myc_HIS 9-1625

4696-5355 4696-5355

Show expanded view

Amy2 CDS pcDNA6/myc-His A 6609 bp

1ac promoter
lac promoter
His reverse primer
amy2
myc tag
his tag

BASES 6609 INSERT 2405

ASSEMBLY WIZARD SPLIT WORKSPACE

Annotate Recombinant Protein

Amy2-CDS-myc-His including STOP codon

The image shows the Benchling software interface for annotating a recombinant protein construct. The left panel displays the DNA sequence with the Amy2 CDS highlighted in blue. The right panel shows the 'ANNOTATIONS' tab with a list of features: Amy2_CDS-myc-His (9-1625), Myc Tag (1560-1589), 6xHis Tag (1605-1622), F1 ori (1938-2244), and M13_reverse_primer (3528-3546). A circular map of the plasmid is shown below the list. Red callouts 1, 2, and 3 point to the 'Create new' button, the 'Amy2_CDS-myc-His' entry, and the 'Auto annotate' button respectively.

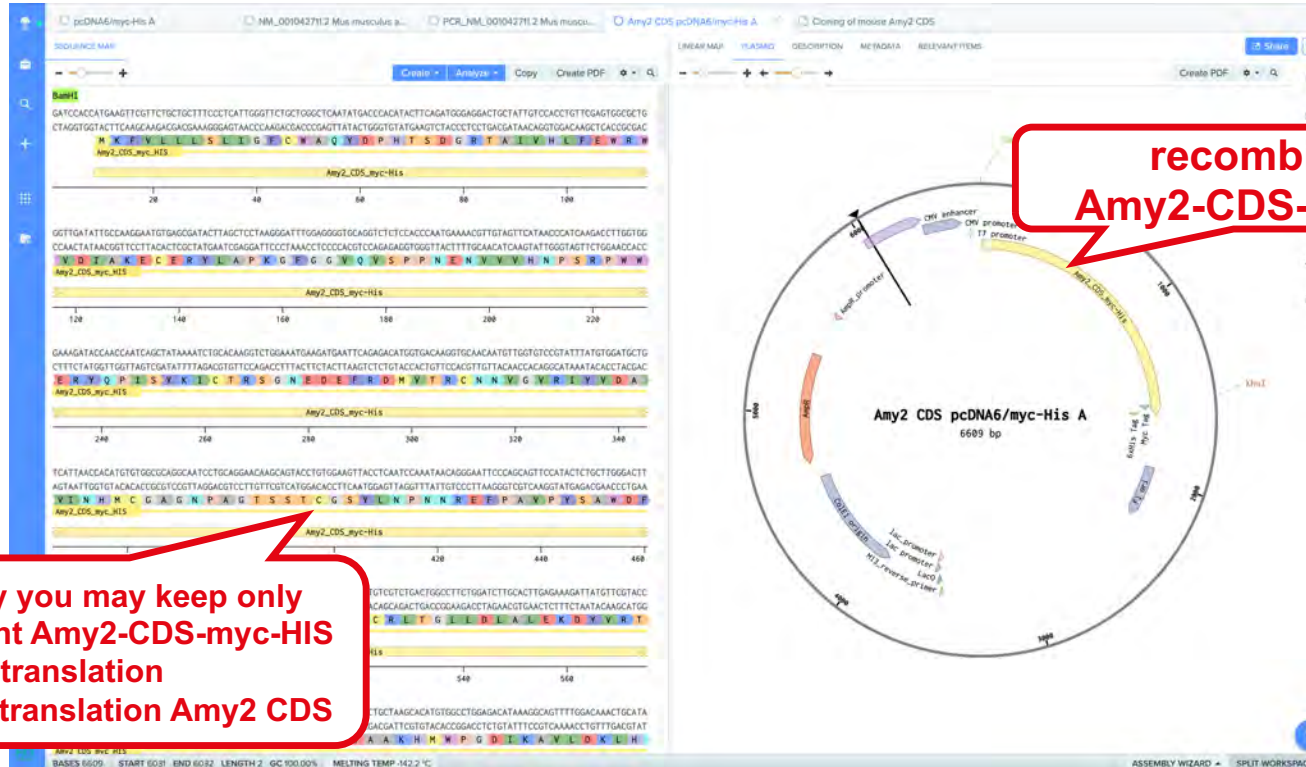
1. Create new

2. Amy2_CDS-myc-His 9-1625

3. Auto annotate

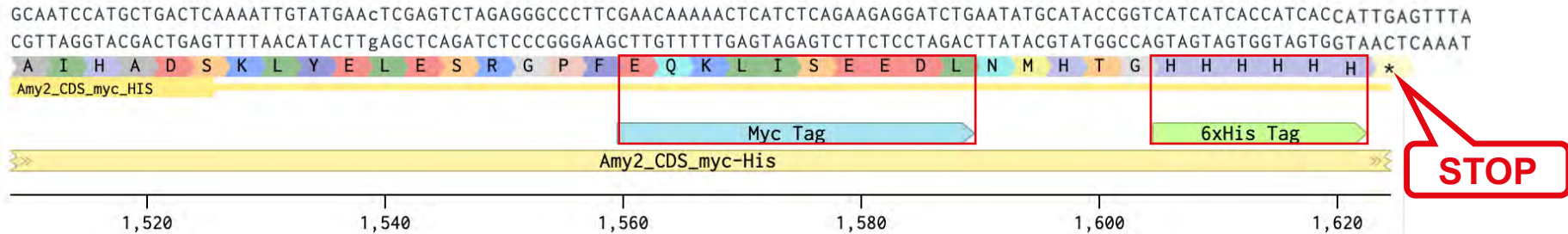
Annotate Recombinant Protein

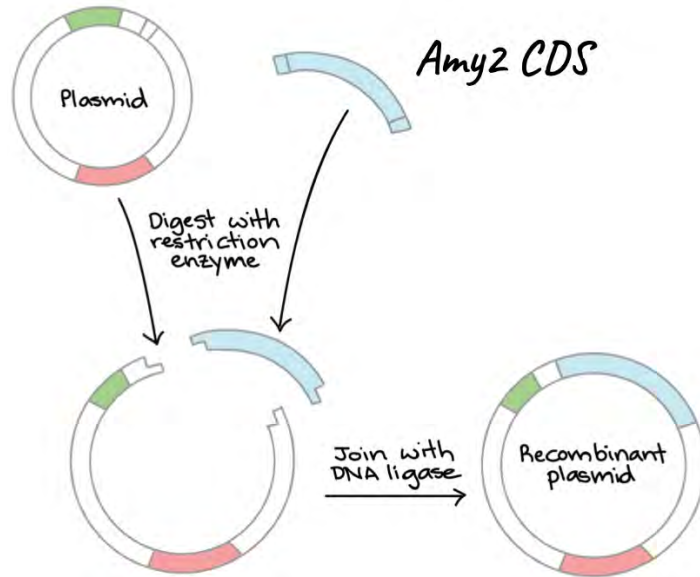
Amy2-CDS-myc-His including STOP codon



Recombinant Protein Amy2-CDS-myc-His

Check Tag Translation and STOP codon



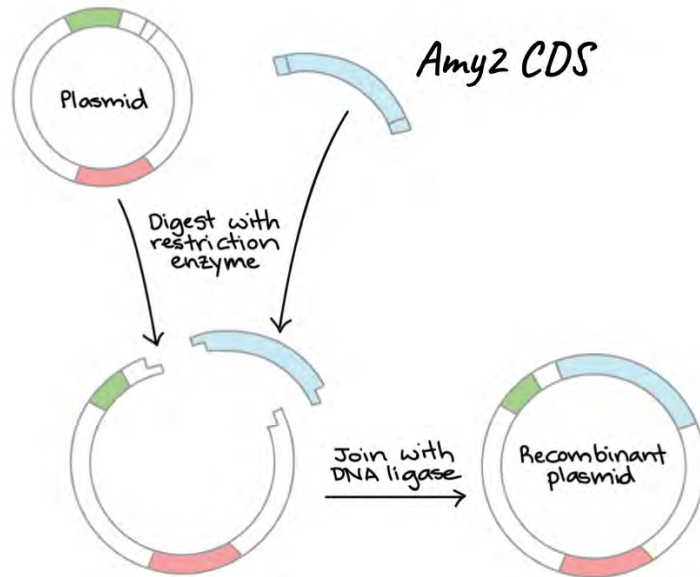


**The recombinant plasmid is ready:
virtual cloning
completed!**

Files You Created

(as orientation only- you might have more or less files, names could be different)

Do a clean-up/ rename files - next semester you will need them again



backbone

- ☐ pcDNA6/myc-His A
- ☐ Linearized pcDNA6/myc-His A [977-920] BamHI XhoI

insert

- ☐ Template_NM_001042711.2 Mus musculus amylase 2a5 (Amy2a5), mRNA
- ☐ PCR_NM_001042711.2 Mus musculus amylase 2a5 (Amy2a5), mRNA [18-1547]

primers

- ☐ Mm-Amy2-for
- ☐ Mm-Amy2-rev

recombinant plasmid

- ☐ Recombinant Amy2 CDS pcDNA6/myc-His A

Exercise 2

Follow steps of Exercise 1

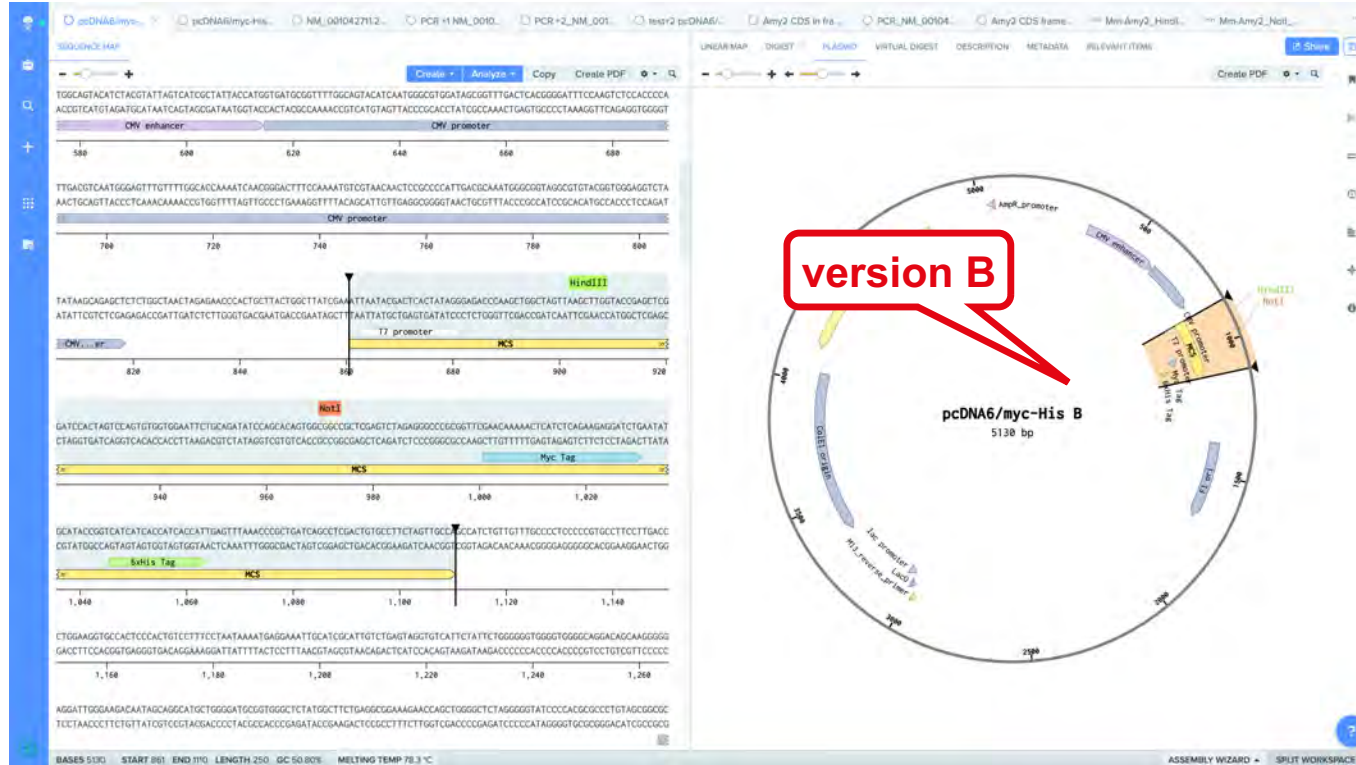
plasmid: pcDNA6/myc-His **version B**

insert: copy Amy2 mRNA file (no not
download again from NCBI)

primers exercise 2

Copy Sequence File in Your Project Folder

careful: do not mix up A and B version of the plasmid- different reading frames!



Steps for Plasmid Backbone (same as for exercise 1)

- ✓ find restriction enzymes HindIII and NotI
- ✓ virtual digest
- ✓ create new DNA with digested linearized fragment (plasmid backbone)

name	sequence	3'location	overhang
Mm_Amy2_HindIII_F	CGAAGCTTACCATGAAGTTCGTTCTGCTGCTTTC	40	11
Mm_Amy2_NotI_R	ATGCGGCCCGCCCATACAATTTTGAGTCAGCATGGATTGC	1518	11

Attach New Primer Pairs to Amy2 Nucleotide Sequence (use mRNA sequence you imported in exercise 1)

The screenshot displays a bioinformatics tool interface with two main panels. The left panel shows the 'SEQUENCE MAP' for 'pcDNA6/myc-His A' and 'NM_001042711.2 Mus musculus...'. It features a sequence map with a scale from 0 to 600 bp. The right panel shows the 'LINKAGE MAP' for 'NM_001042711.2 Mus musculus amylase 2a5 (Amy2a5), mRNA (1577 bp)'. It includes a table of primer pairs and a section for primer pair information.

Primer Pairs Table:

Primer	Position	Product Size
Min-Amy2-for	+ / 40	1549
Min-Amy2-rev	- / 1519	

Primer Pair Information:

Link PrimePairs

use primers exercise 2
this image is from exercise 1!

You can also duplicate it.

Strand: Forward

Bases: 5' CGAAGCTTACCGATGAAGTTCGTTCTGC TGCTTTC

3' Location: 40 1518

Overhang: 11 9

Cut Site: AatI

Use the dropdown above to look up restriction sites.

Steps for Insert and Ligation (same as for exercise 1)

- ✓ attach primer pair
- ✓ create PCR product
- ✓ copy digested PCR product into digested plasmid backbone
- ✓ annotate + translate recombinant protein (from START to STOP codon)
- ✓ check sequence of tags and location of stop codon

End



Calvin & Hobbes
by Bill Waterson