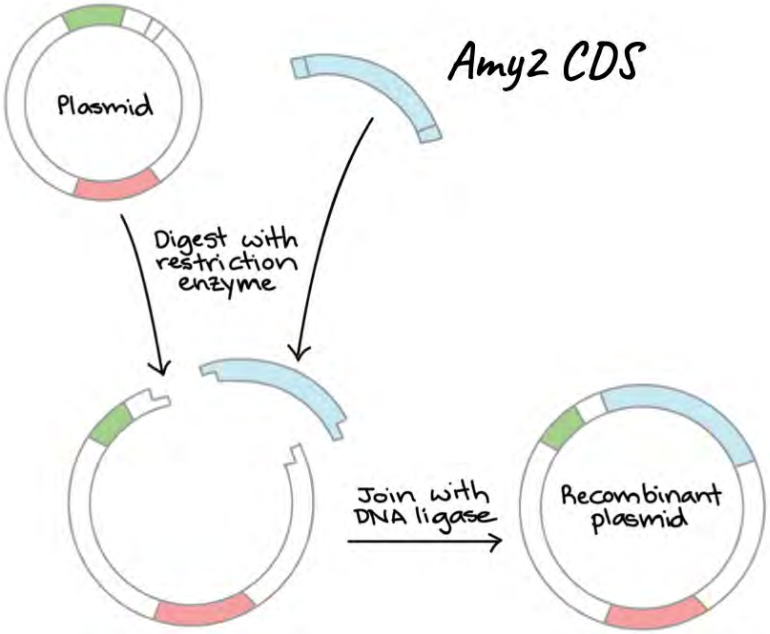
Benchling Exercises lab3 Virtual Cloning



Dr. Alexandra Bezler

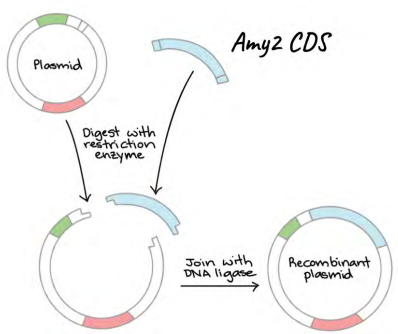
BIO-203

image source
Benchling.com

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 andvanced users!

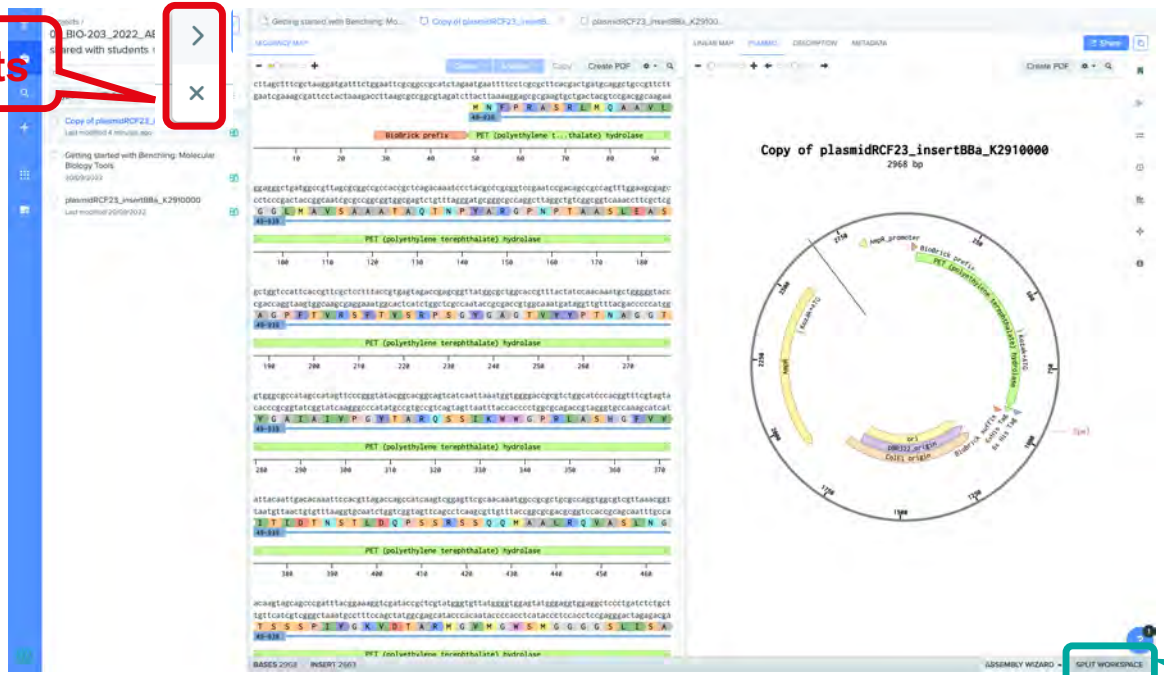
Basic Benchling Functions

Tools Used in Lab3

explore all buttons to see what they are used for

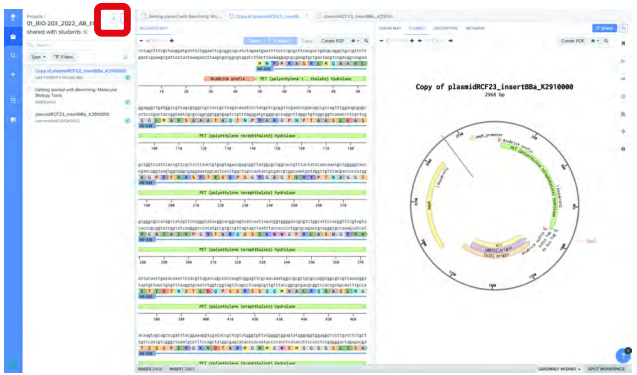
To Change the Window Layout

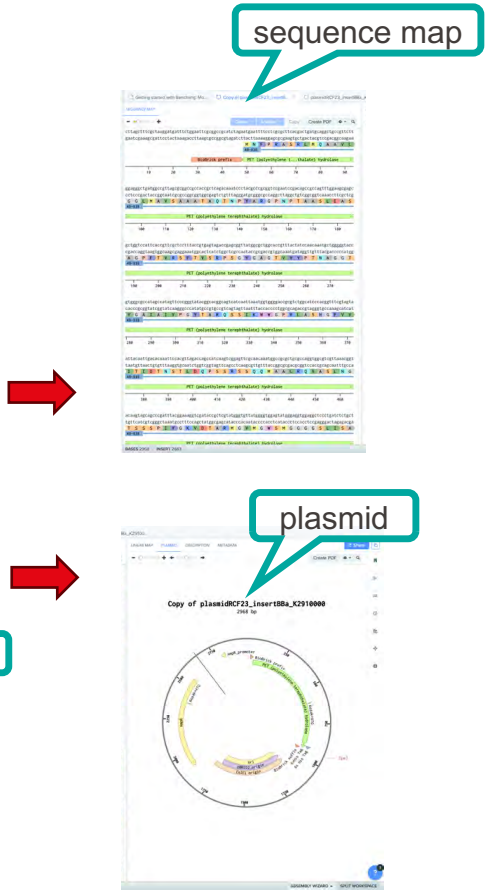
close projects



split workplace

To Change the Window Layout

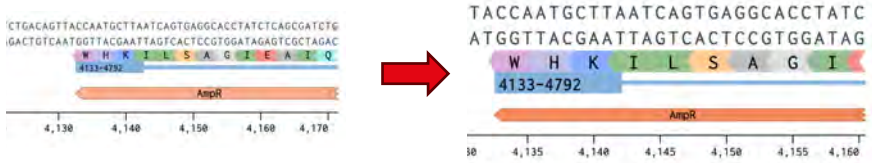


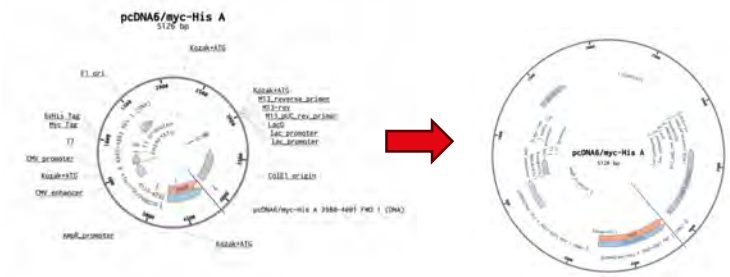


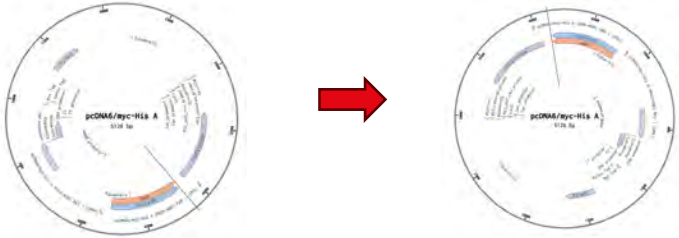
Explore Useful Display Functions

use two fingers to zoom/ rotate

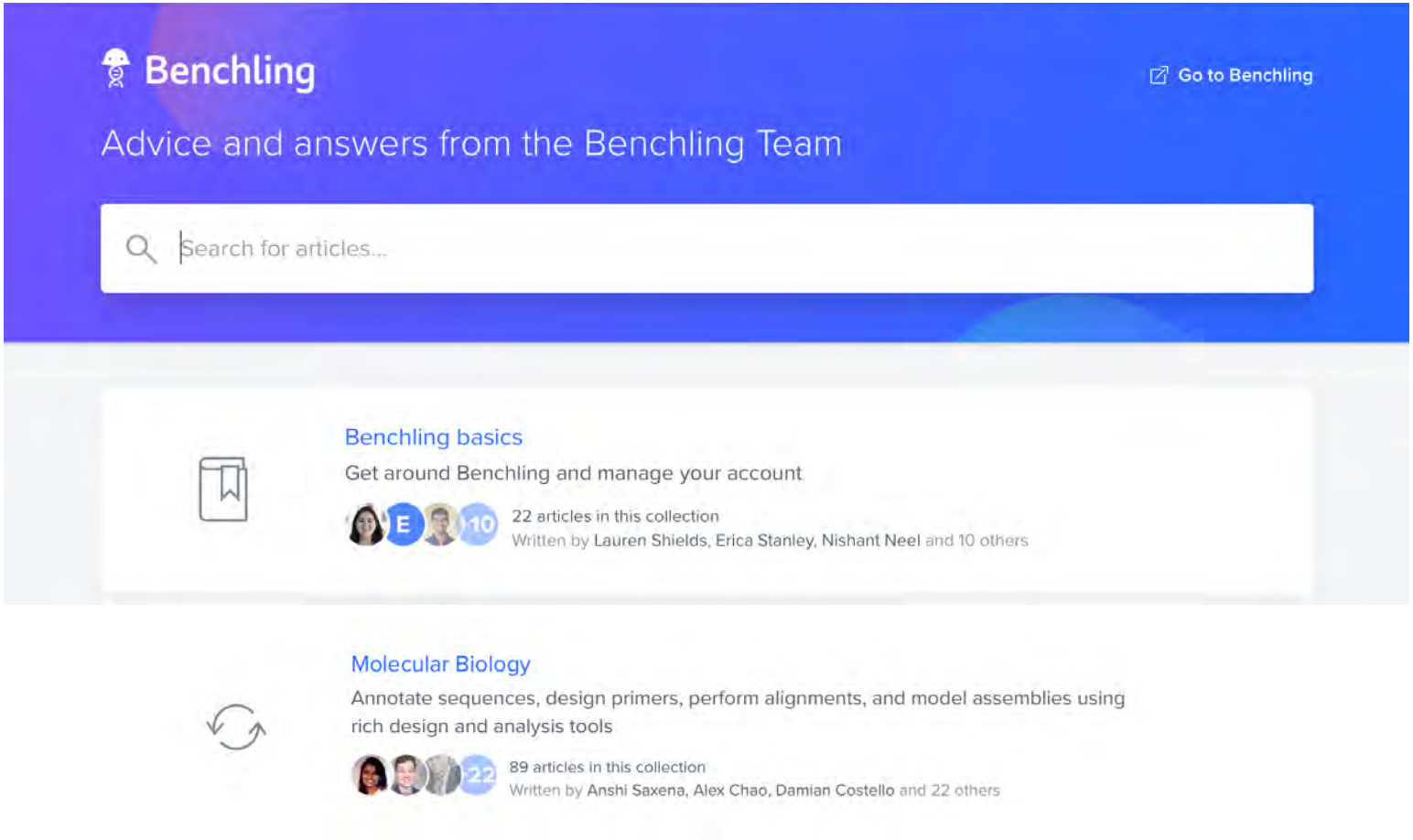
- zoom in/ out sequence
- zoom in/ out plasmid
- rotate map







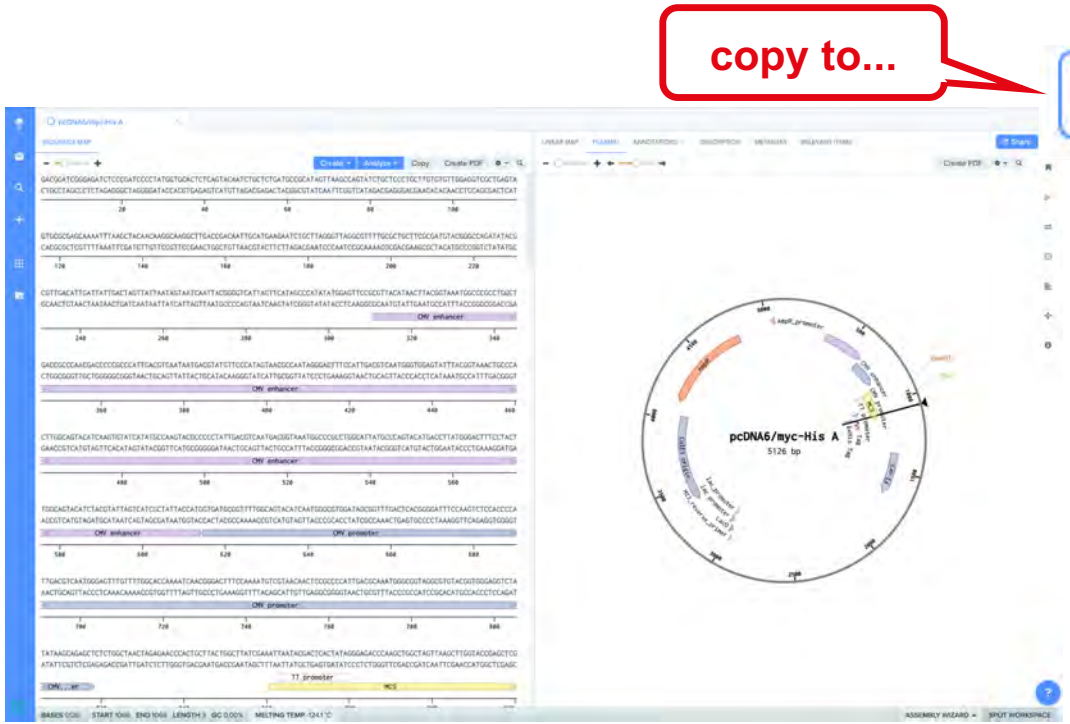
Benchling Help : How to + Videos



Save Plasmid Sequences

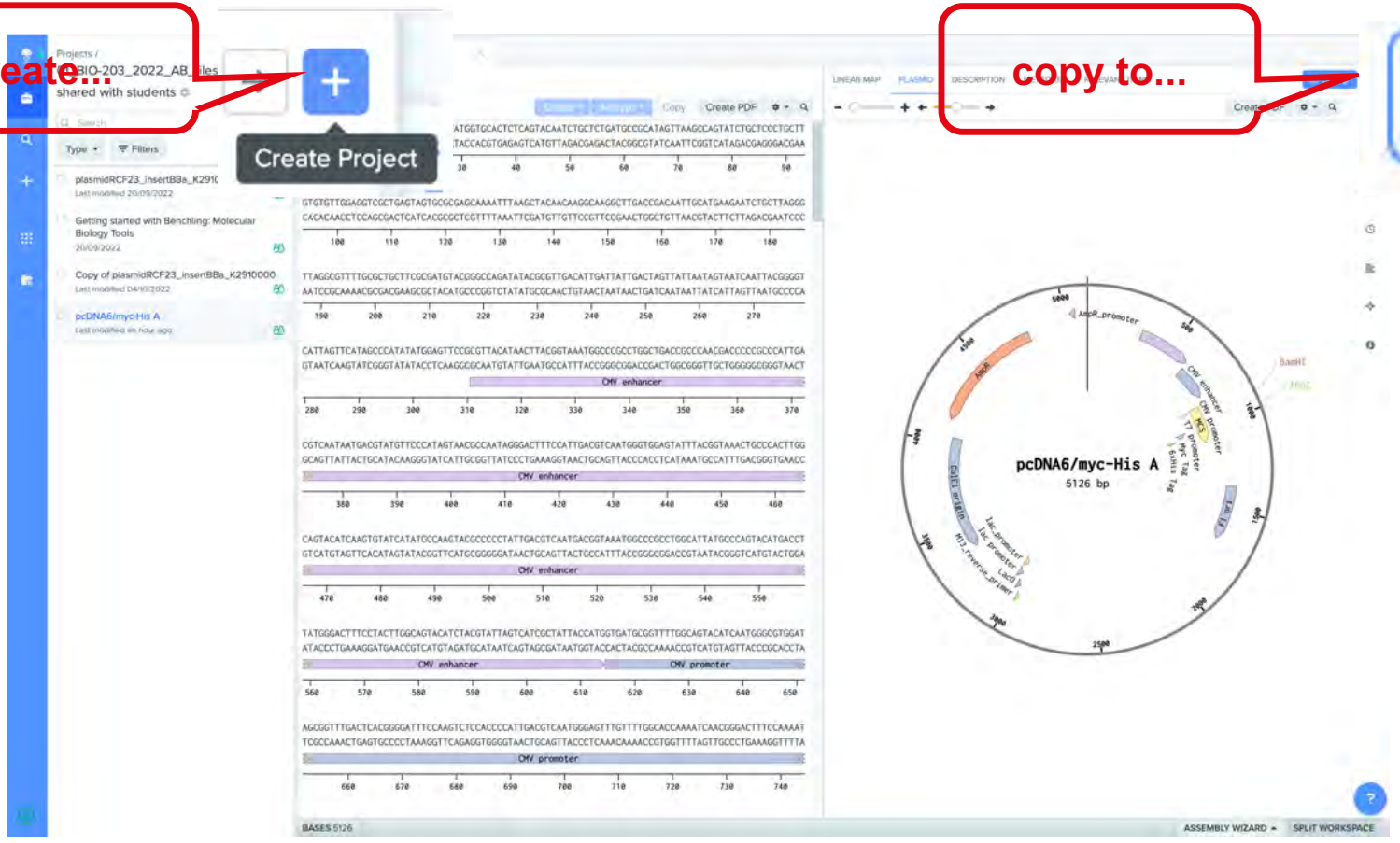
Virtual Cloning Using Benchling Lab 3

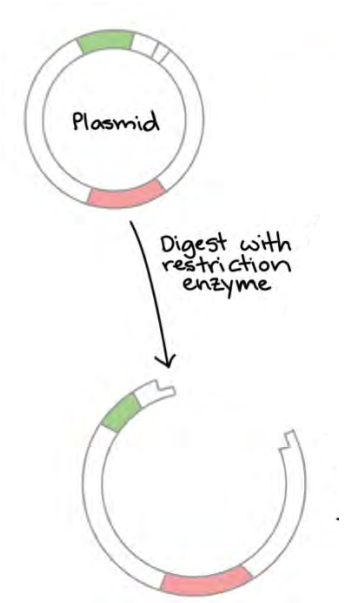
- ✓open shared files
  - ✓[pcDNA6/myc-HIS version A]
  - ✓[pcDNA6/myc-HIS version B]
- ✓create new project folder for **each** exercise
- ✓copy shared sequence file to the corresponding folder



Copy to New Project Folder

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





Exercise 1
Create plasmid backbone

in plasmid pcDNA6/myc-His A:

Find multiple cloning site (MCS)

Find restiction sites BamHI and XhoI

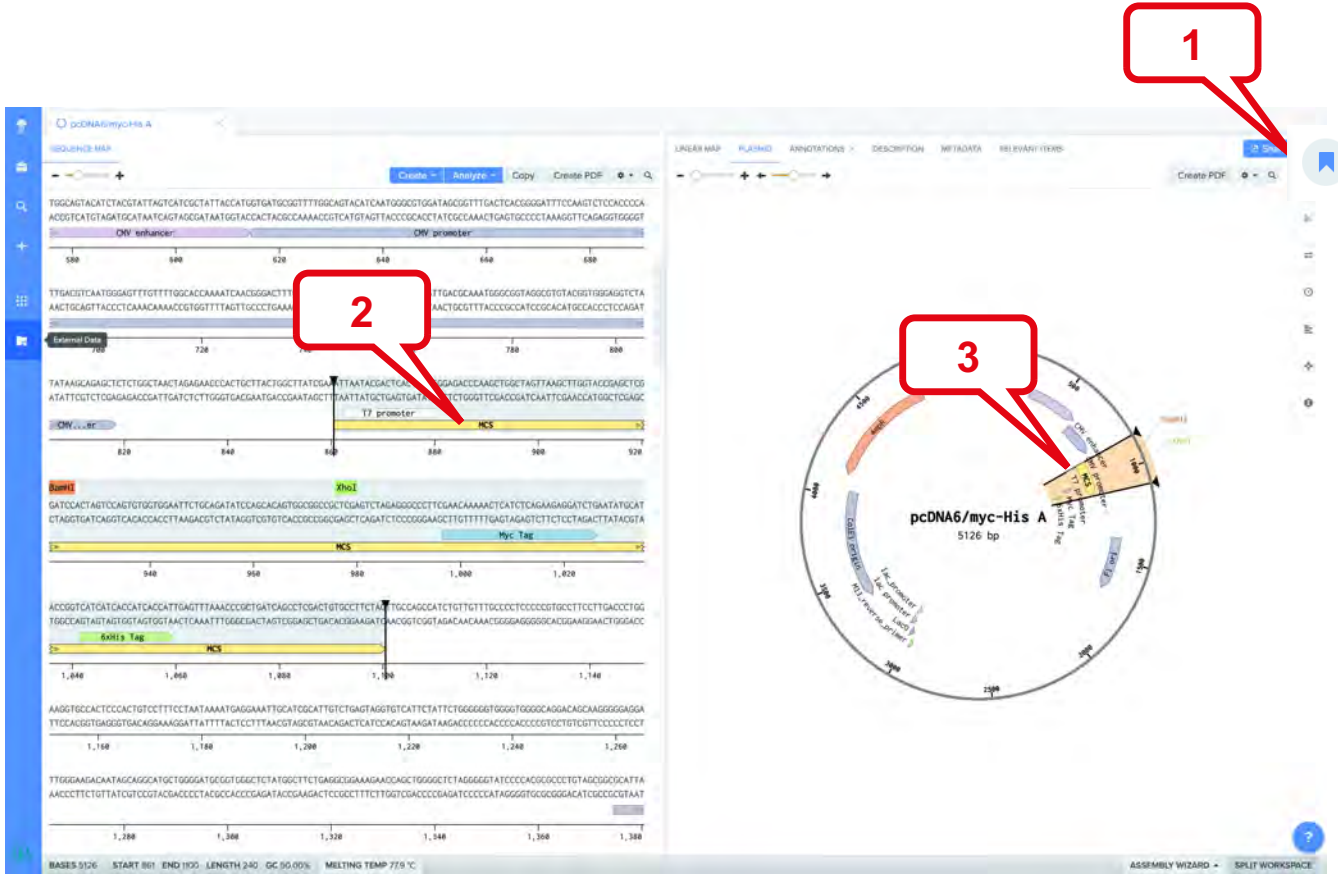
Run virtual digest

Create new DNA file with digested plasmid fragment

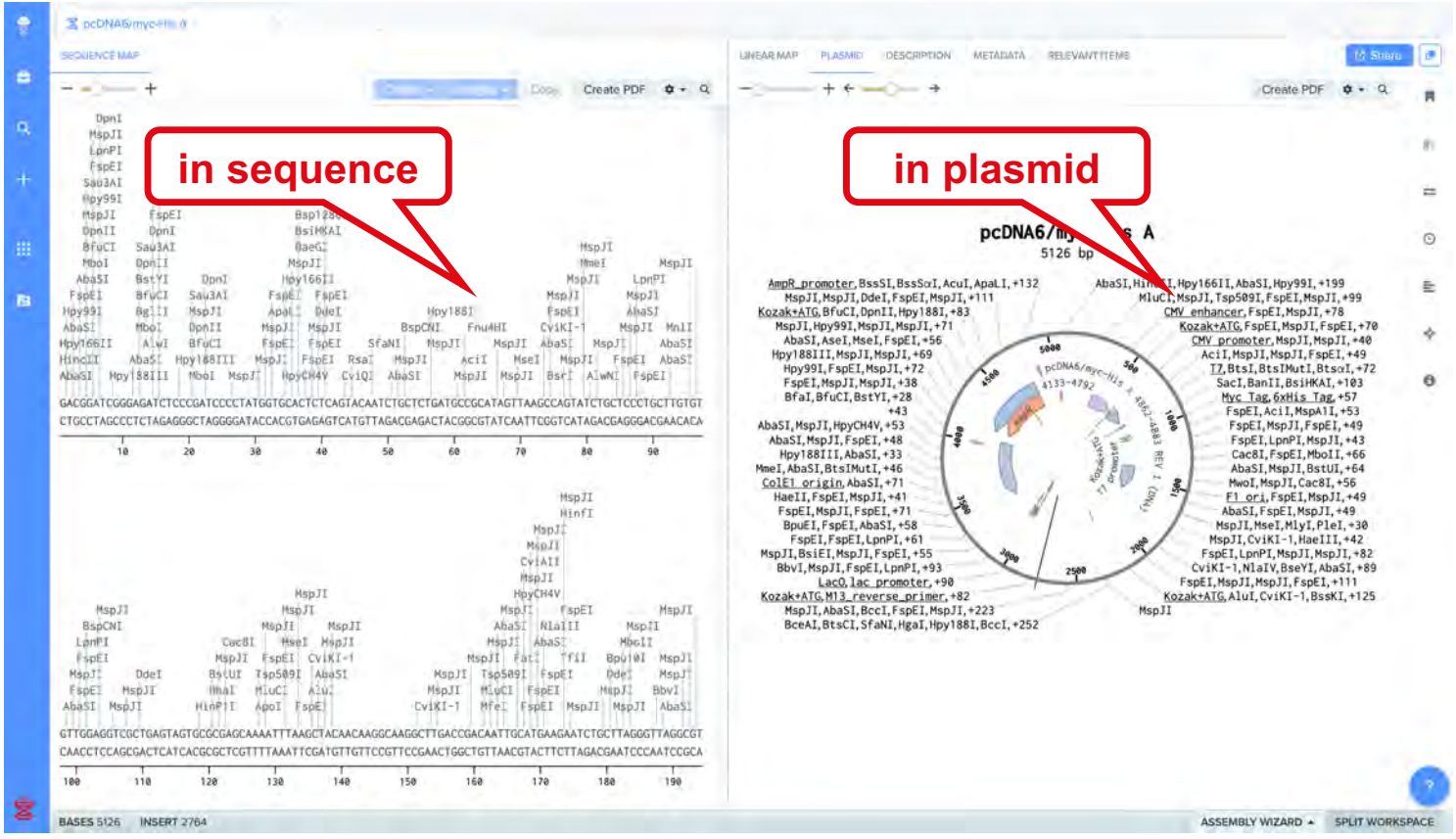
Find Annotation for Multiple Cloning Site

Options:

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

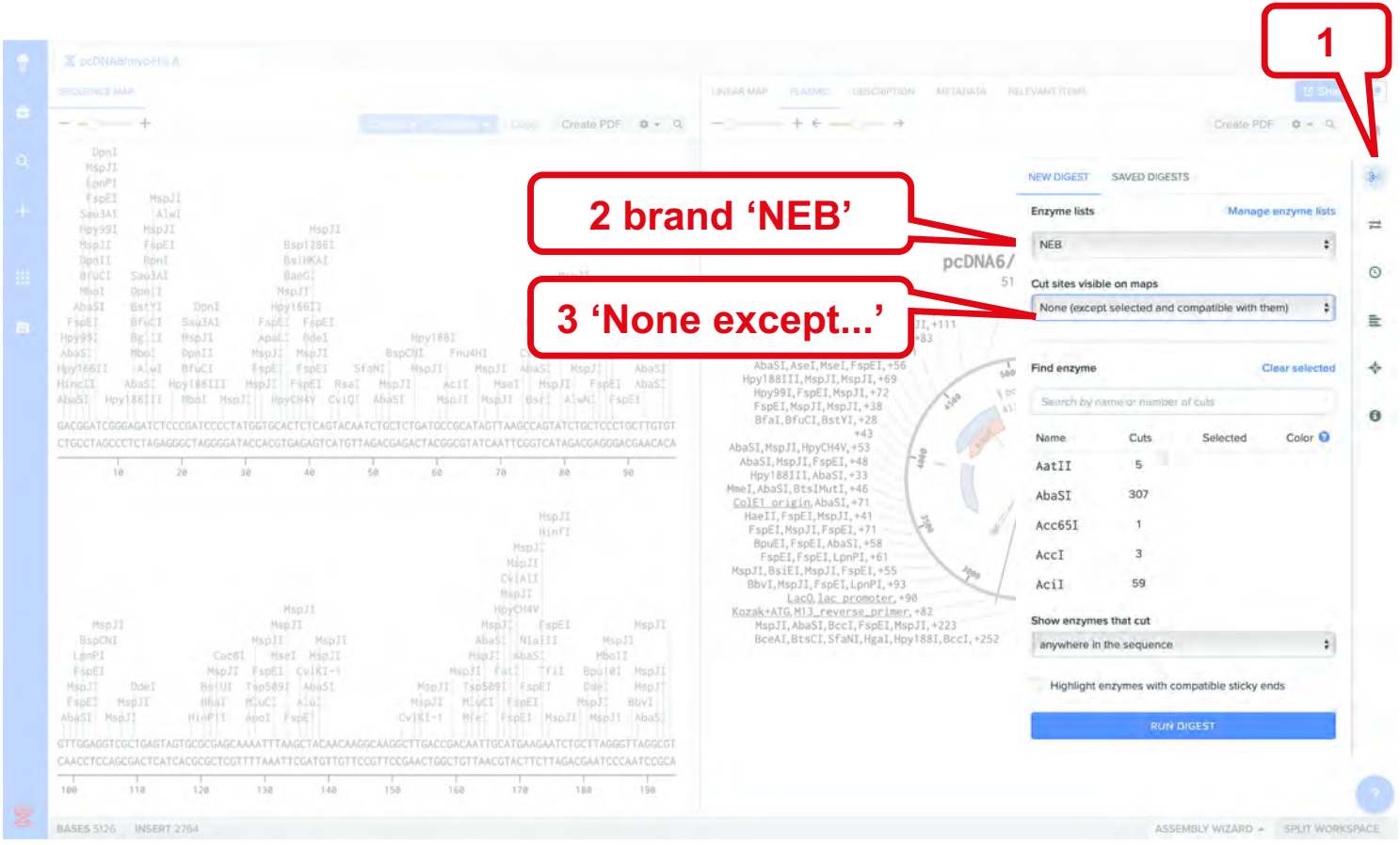


Help: if too Many Restriction Sites Visible



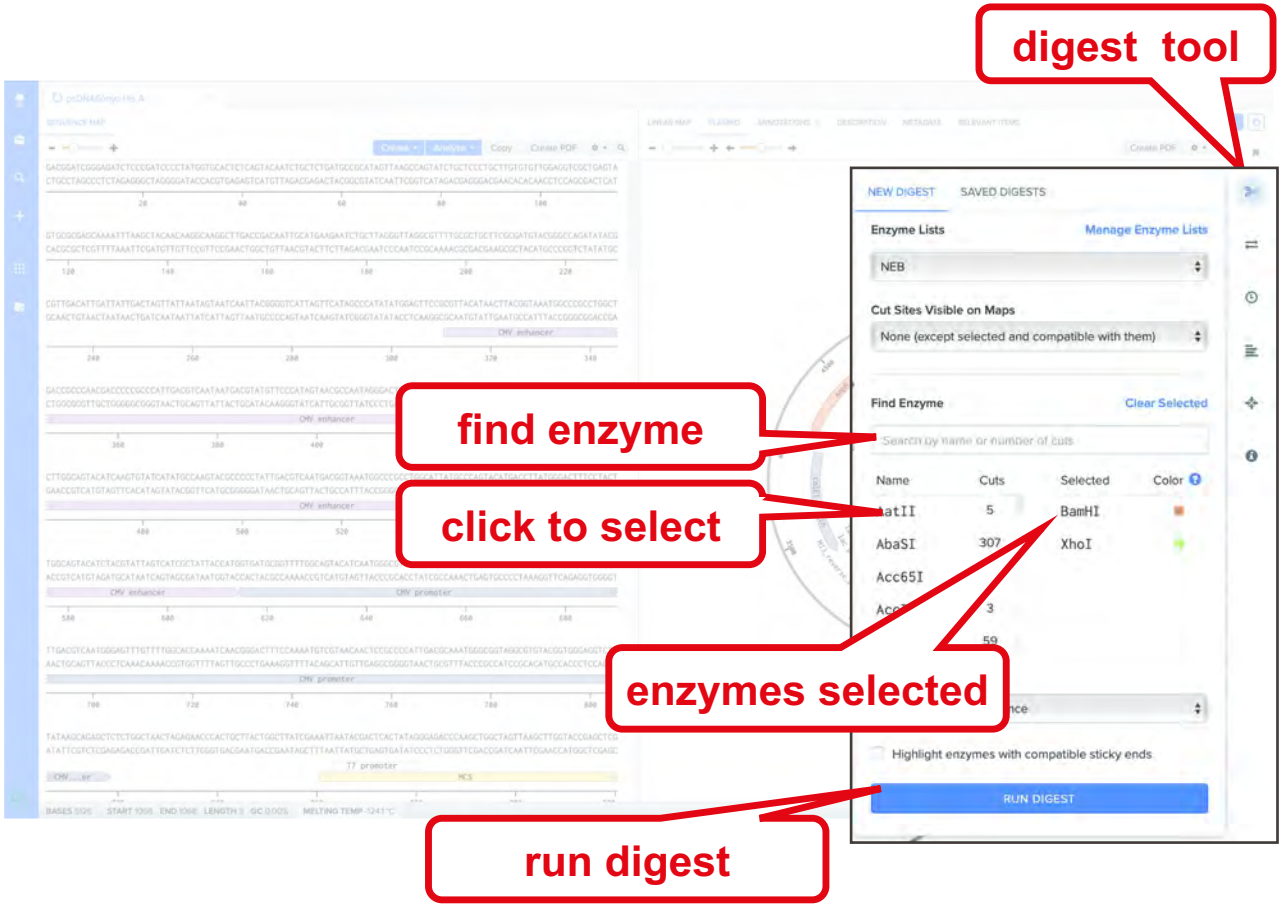
Help: if too Many Restriction Sites Visible

with this setting you see only the selected restriction sites

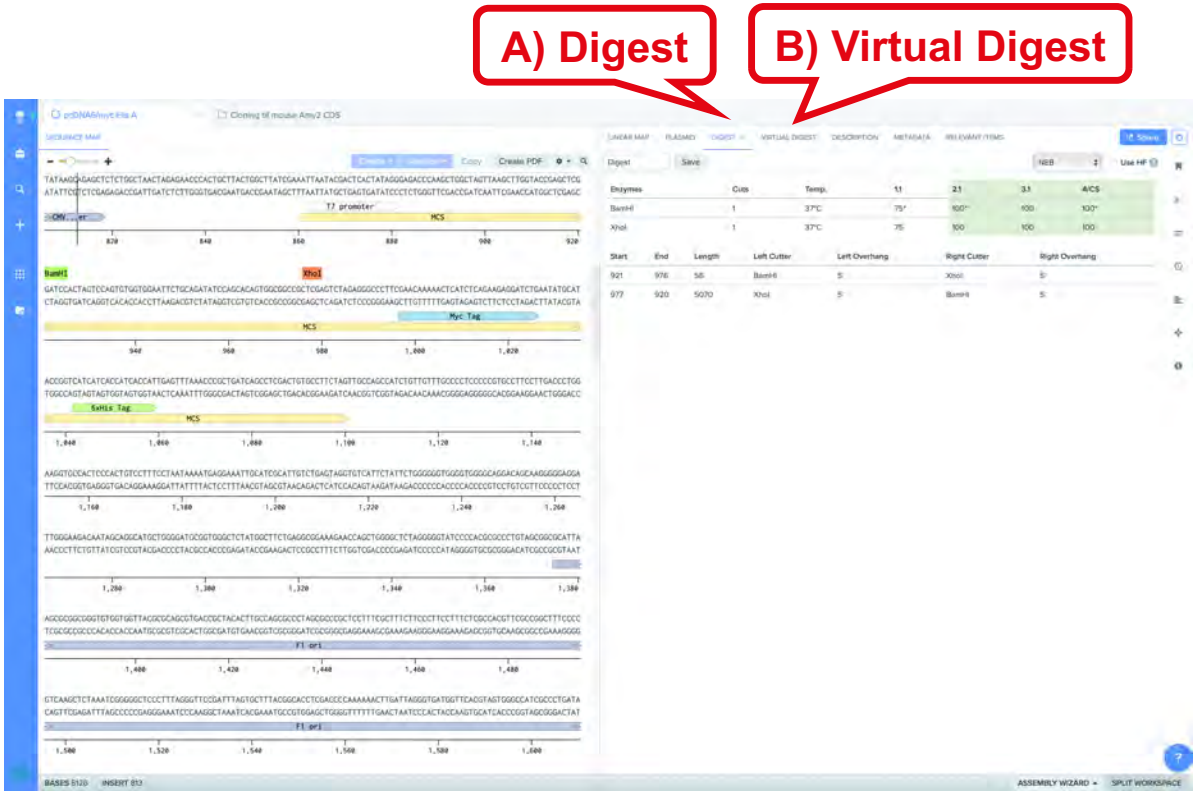


Find Restriction Sites

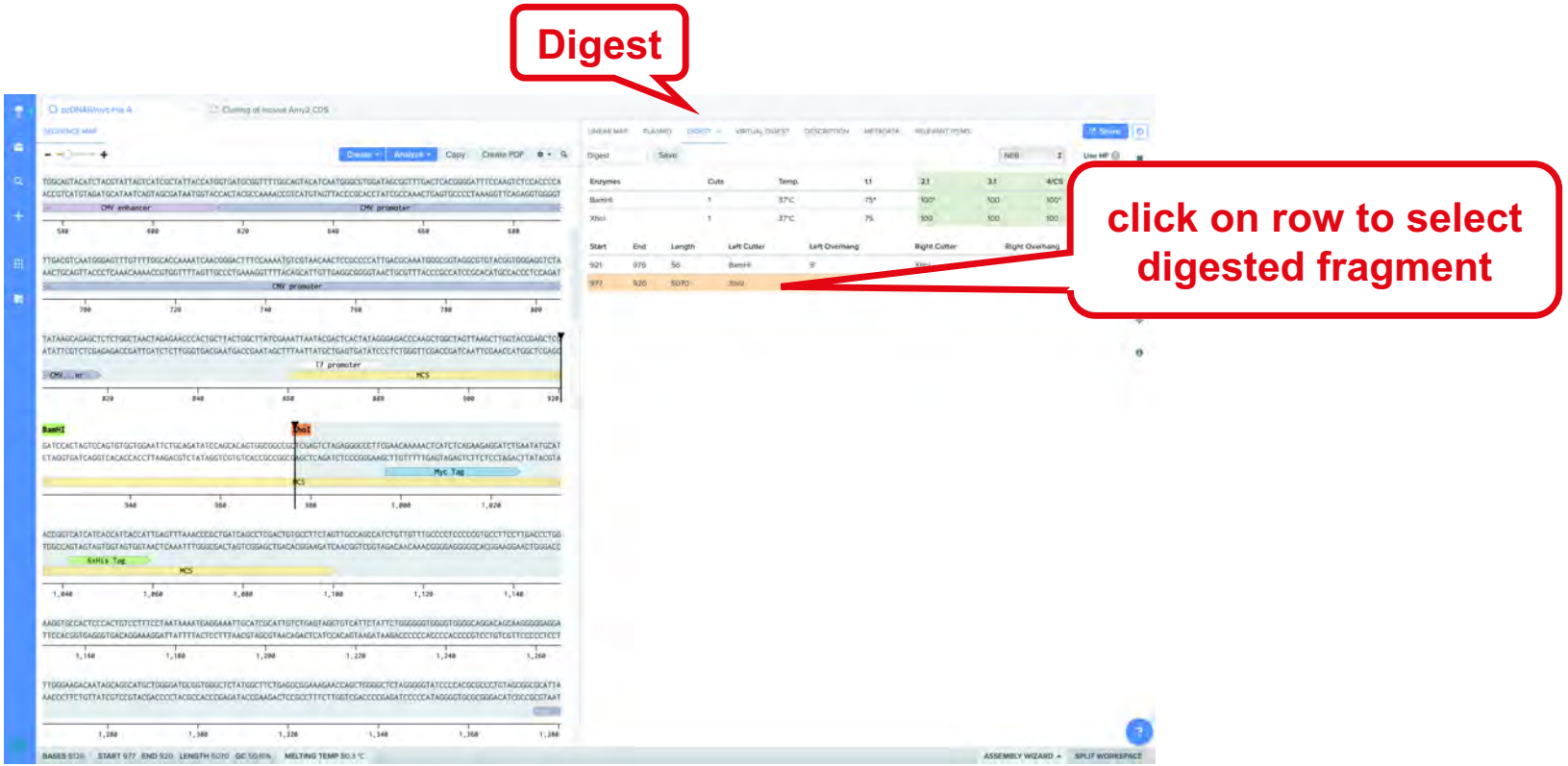
- ✓in digest tool
- ✓find enzyme
  - ✓search or scroll list
- ✓select enzymes
  - ✓BamHI
  - ✓XhoI
- ✓run digest
-



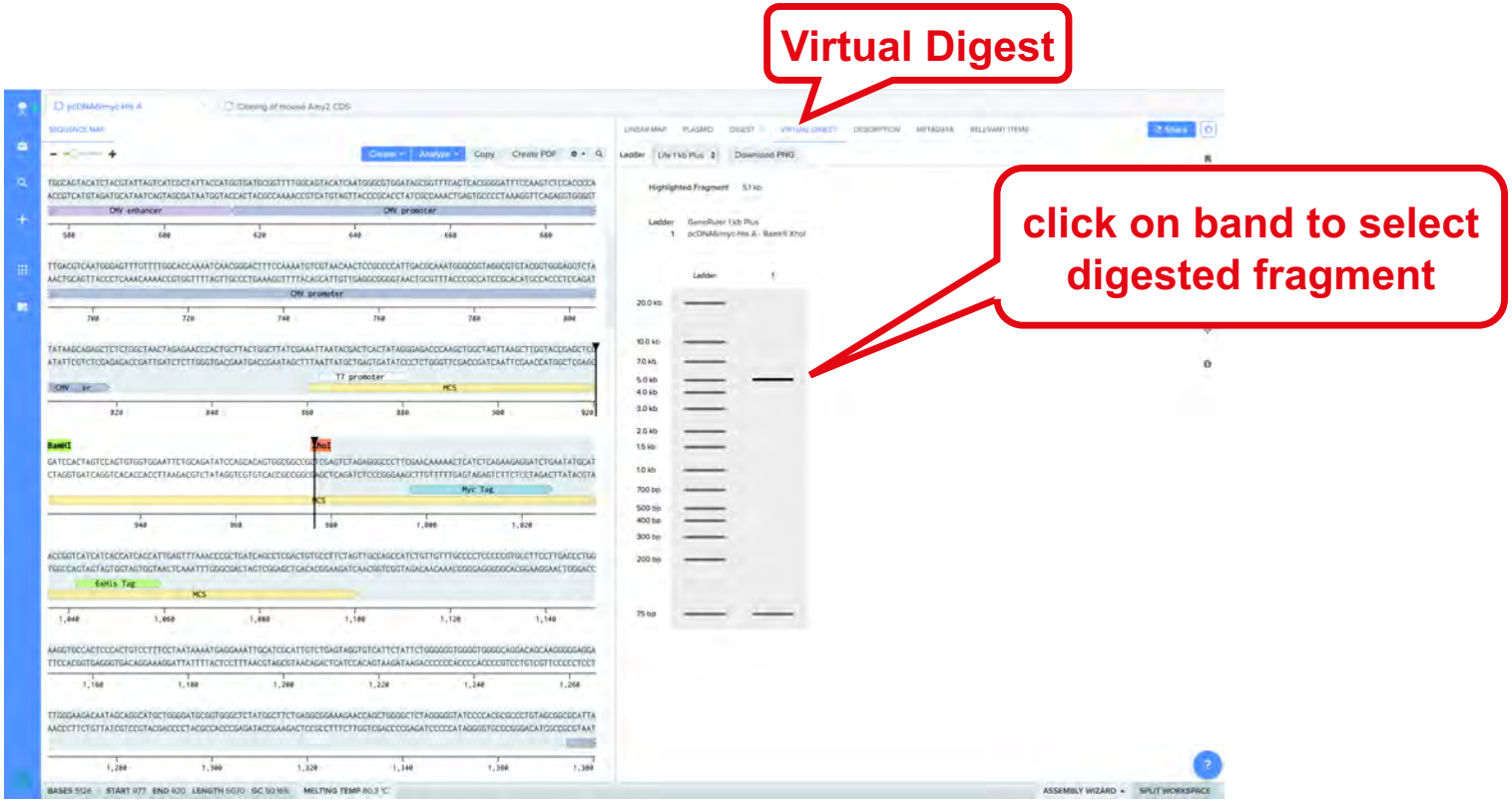
Digest Results Shown as 2 Aternative Views, Choose What You Like Best



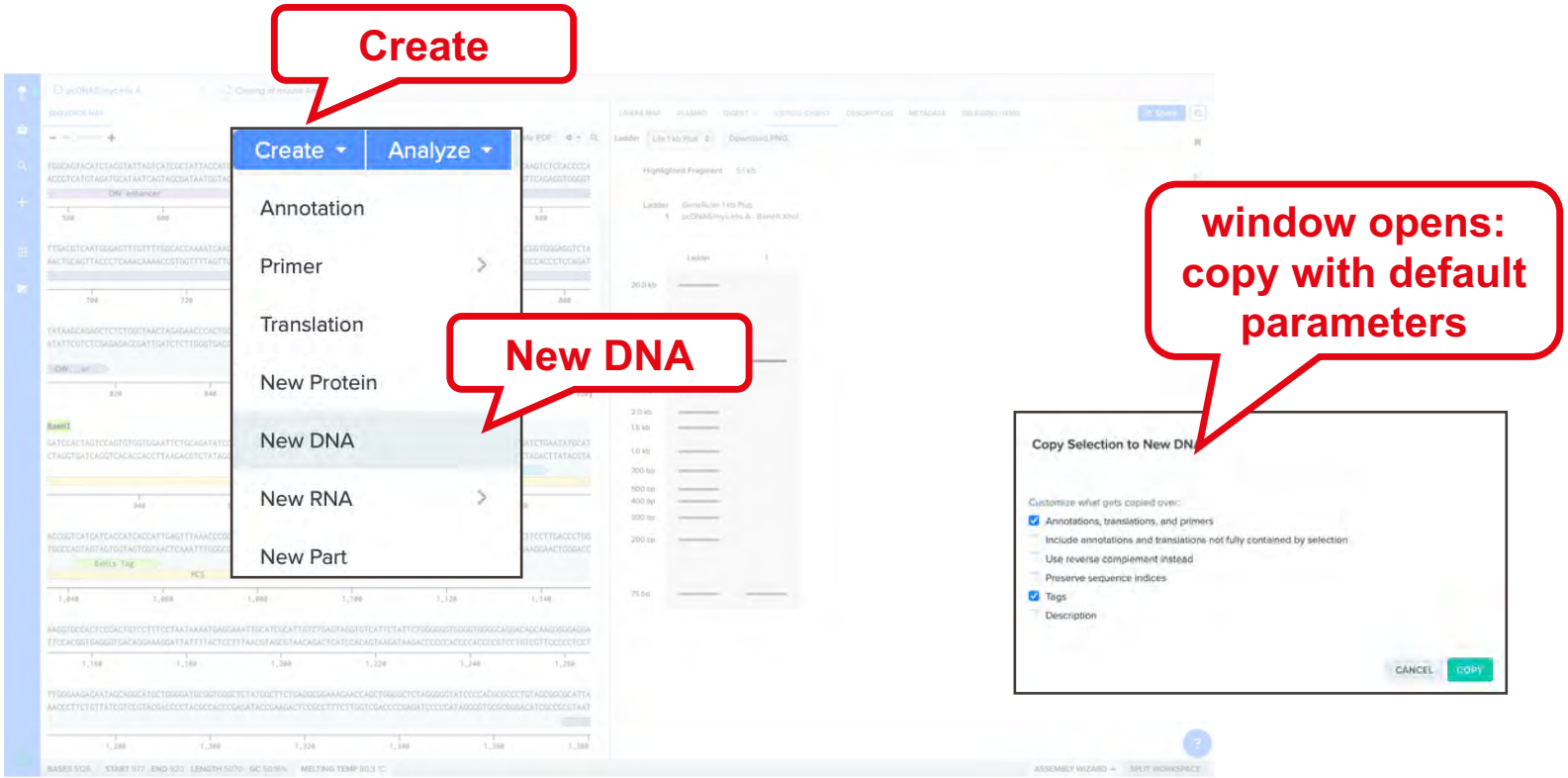
Select Digested Fragment in Table: ‘Digest’ Tab



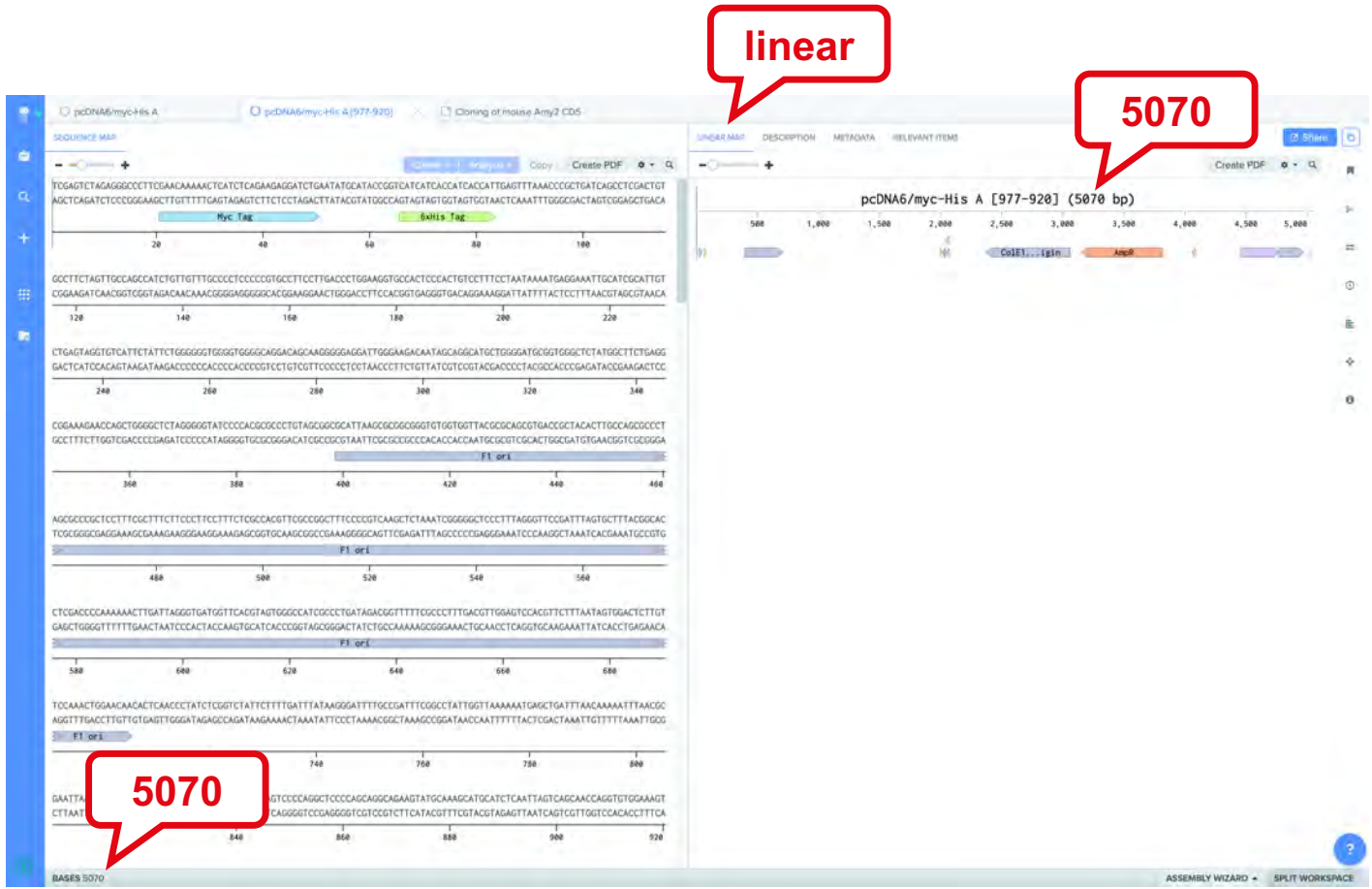
Select Digested Fragment in Table: ‘Virtual Digest’ Tab



From Selected Fragment: Create New DNA

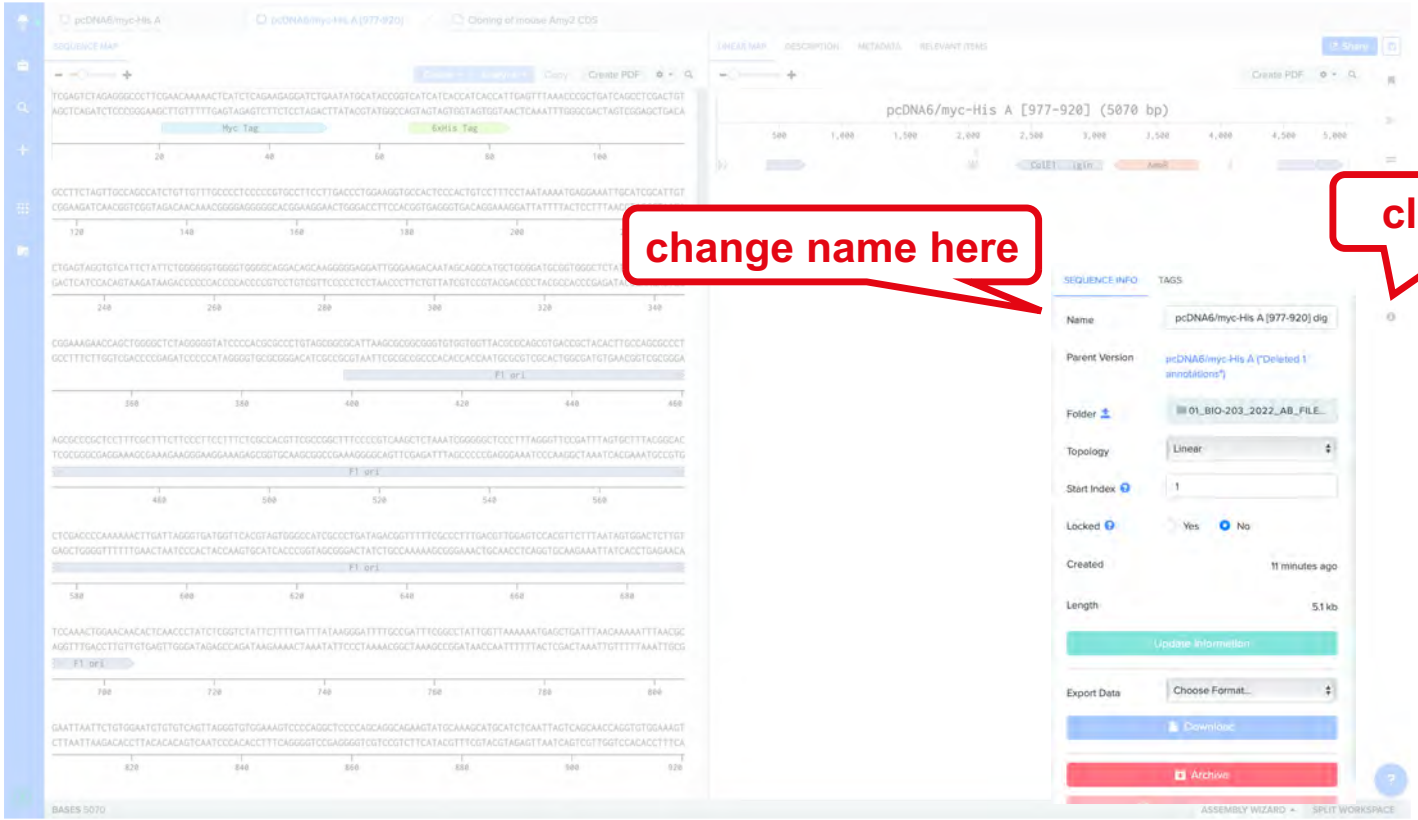


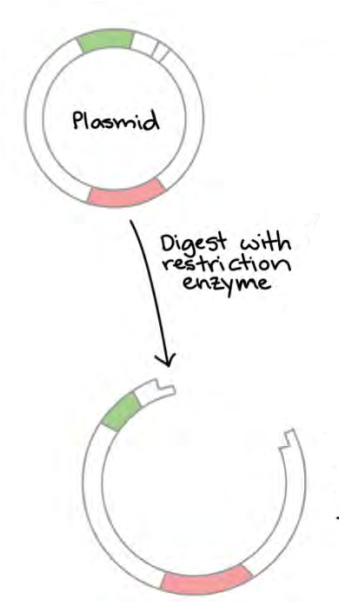
The Digested Fragment is Linear and 5070 bp Long



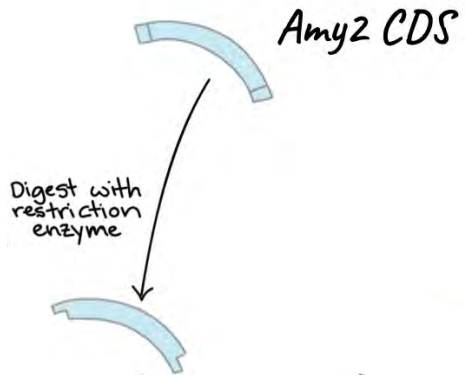
Rename Digested Fragment:

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





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 muliple ways how to import

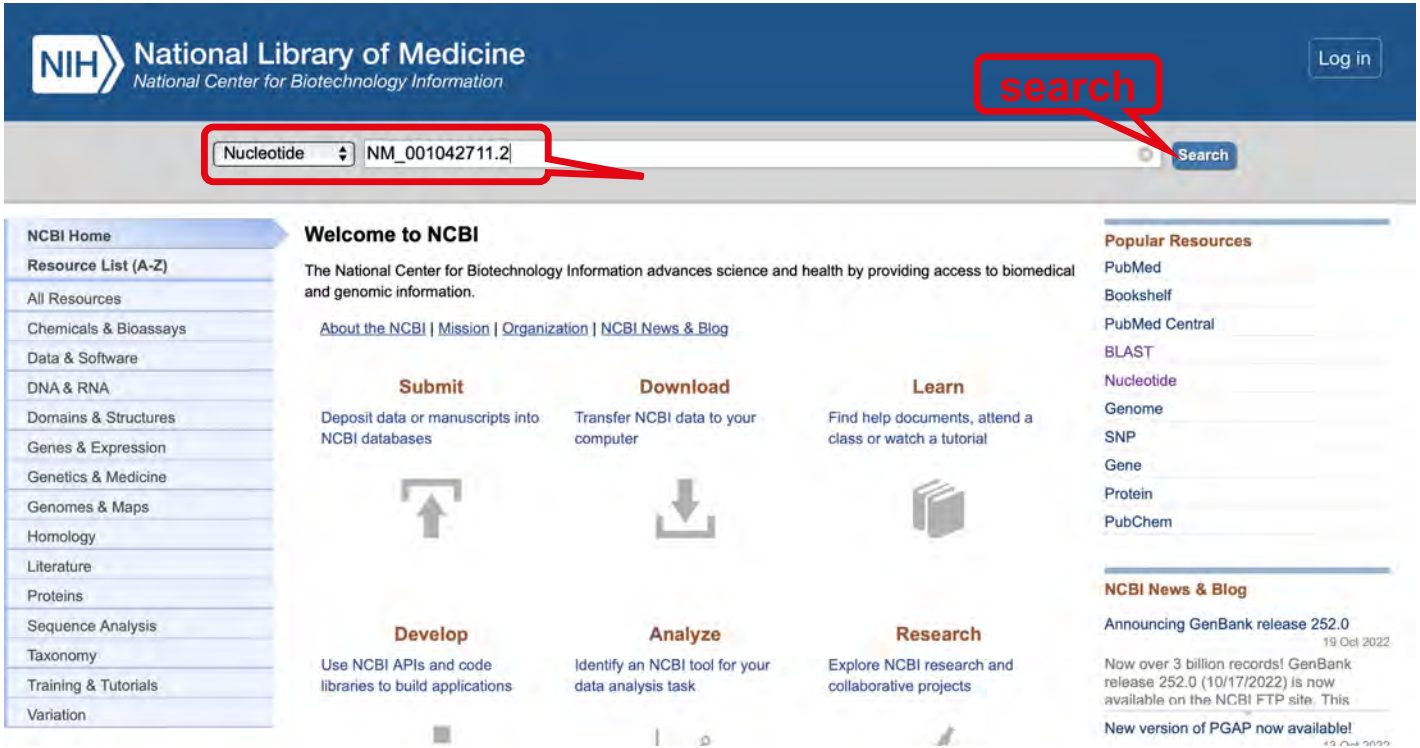
- Option I: copy sequence from NCBI, annotate manually
  - **used for this manual**
  - helps you to learn how to find information on NCBI

- Option II: import annotated sequence from NCBI
  - has many more annotations than we need
  - prevents errors in annotations

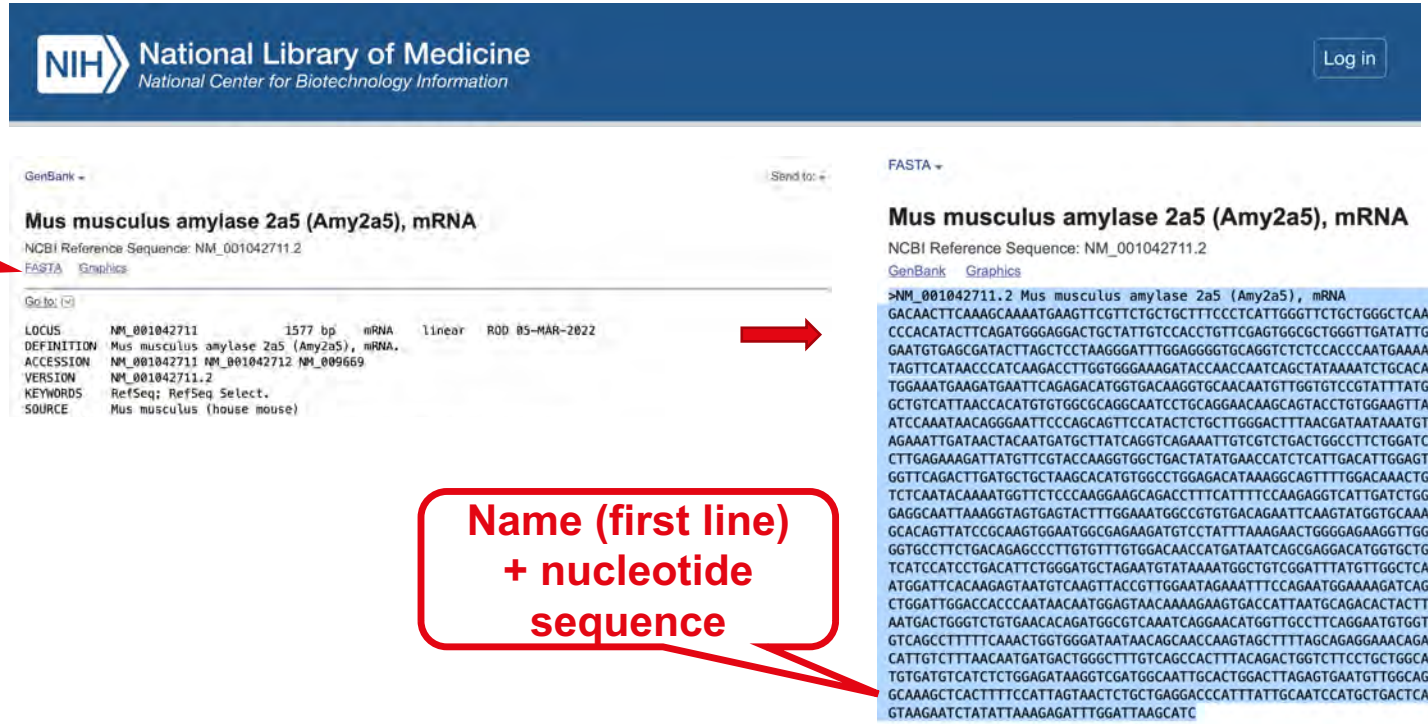
Option I

NCBI website : Search Nucleotide Sequence

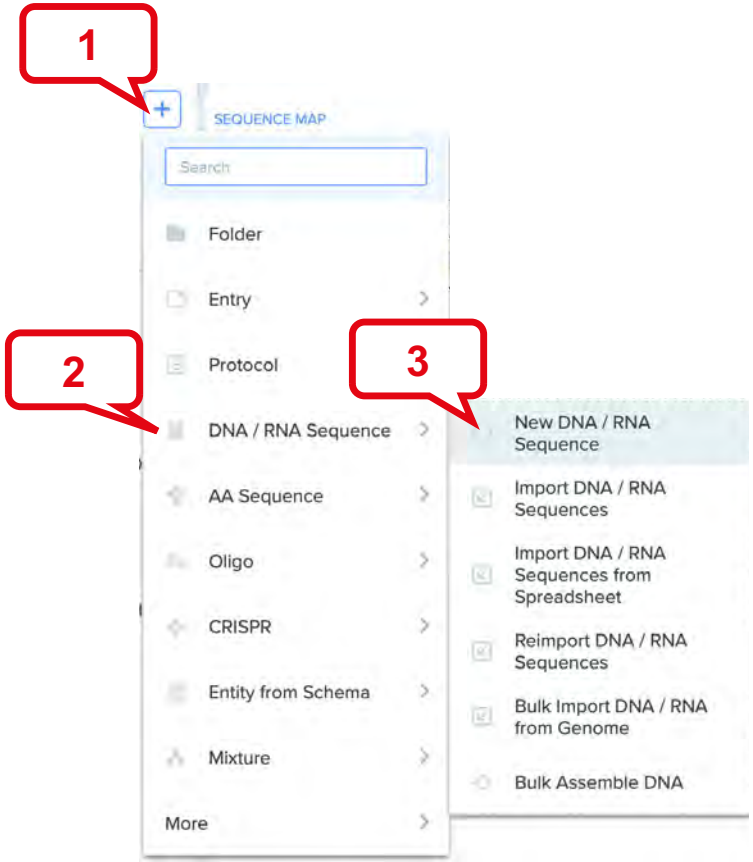
https://www.ncbi.nlm.nih.gov



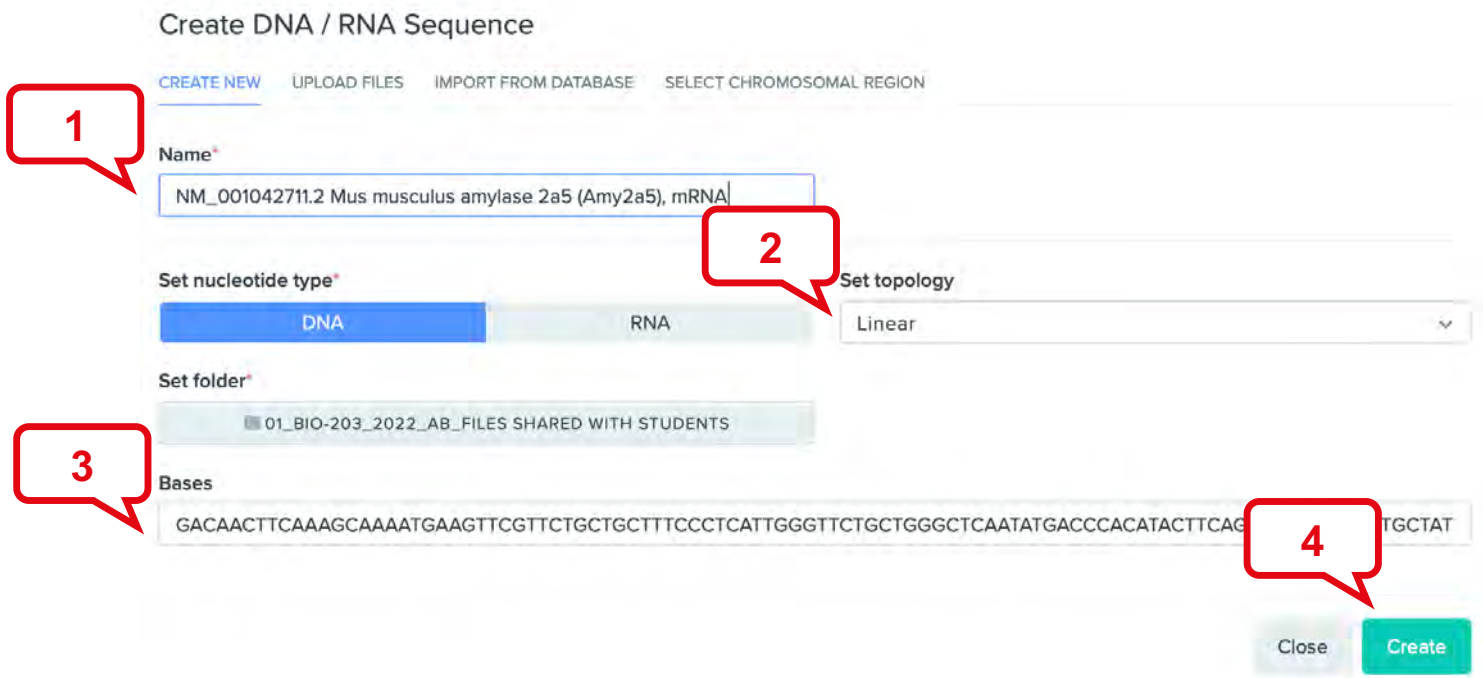
NCBI website : Find Amy2 mRNA- Contains 3'UTR, CDS, 5'UTR



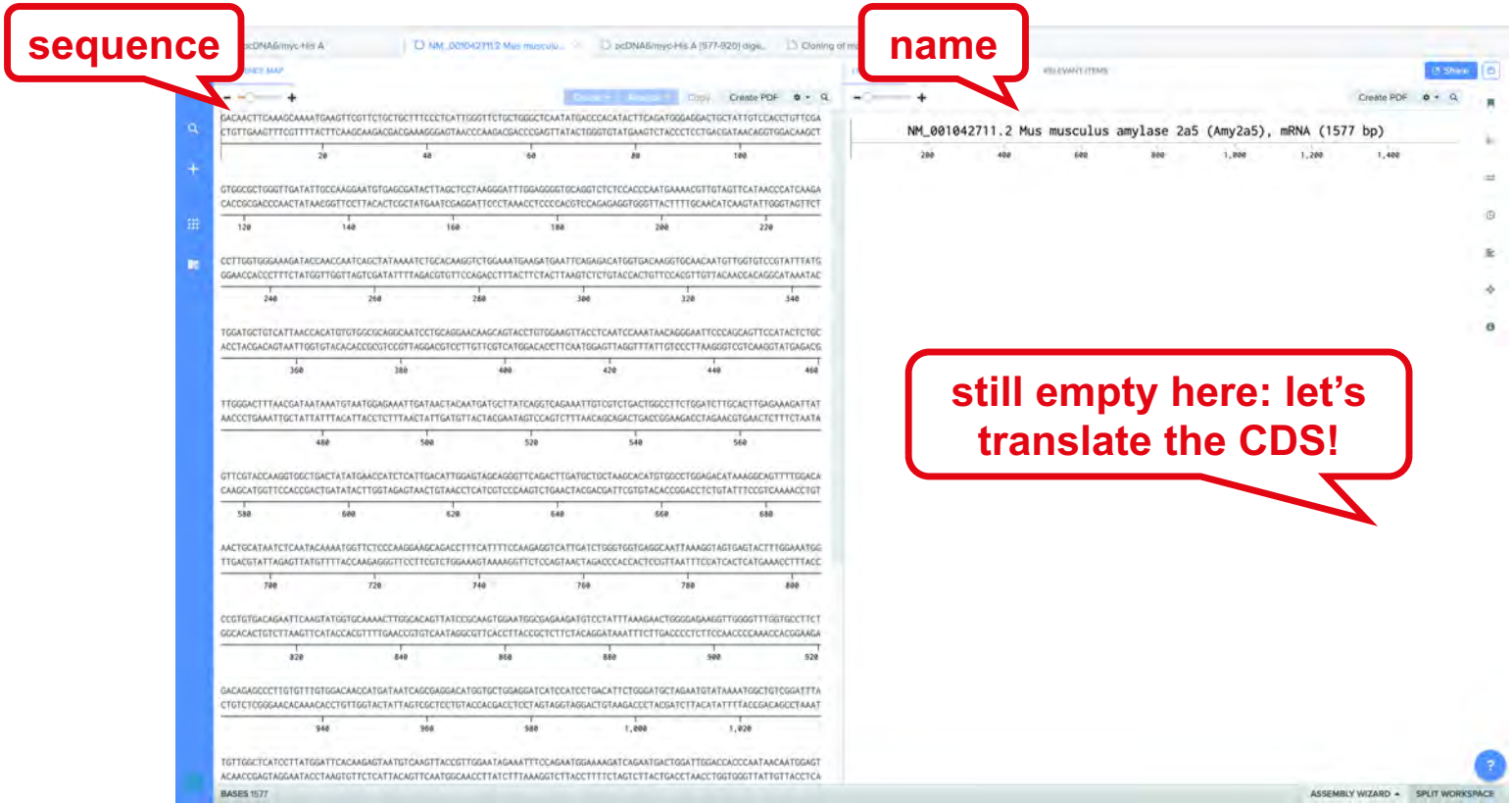
In Benchling: Create New DNA Sequence



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



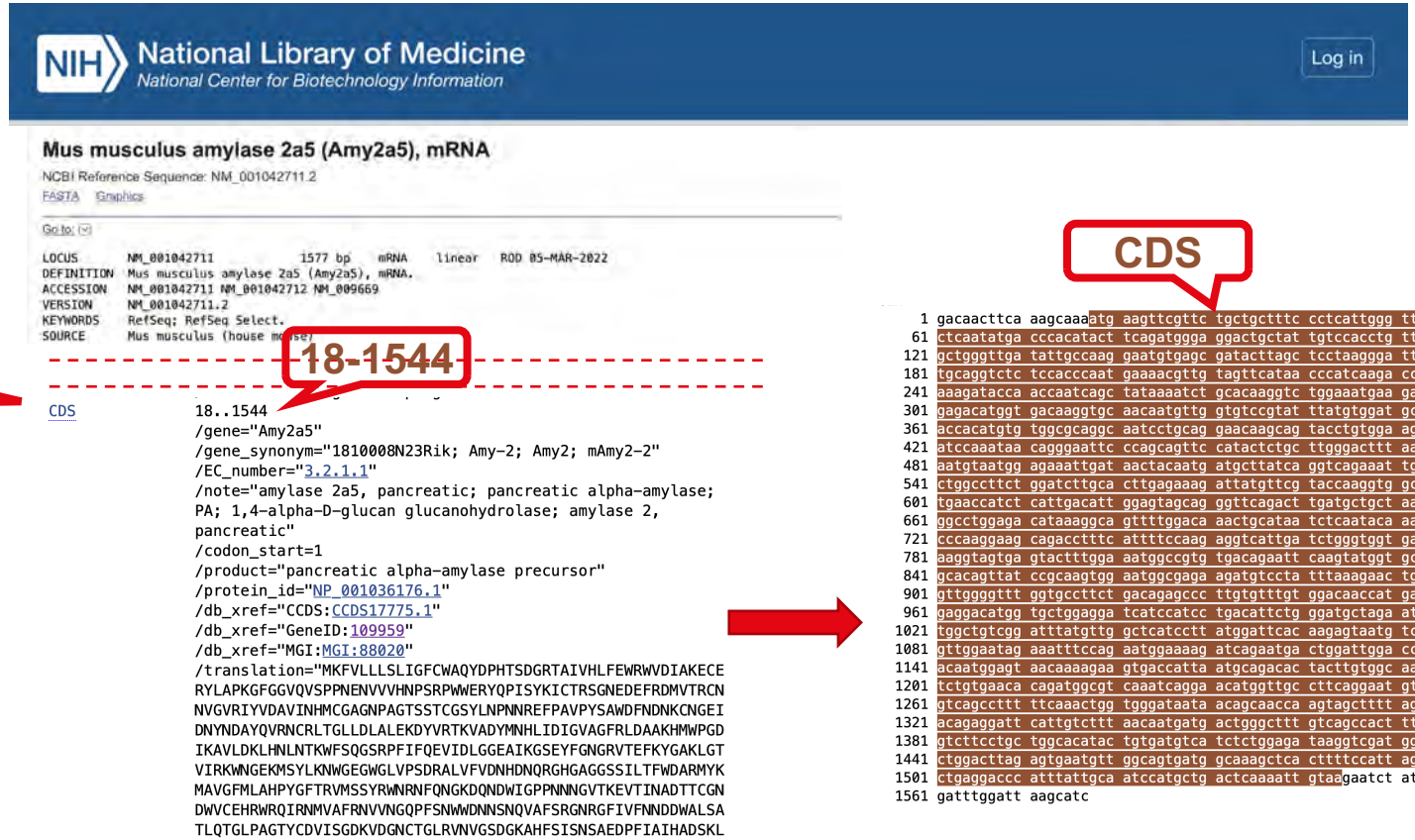
New DNA: Amy2 Nucleotide Sequence



NCBI website : Find Amy2 Coding Sequence (CDS)

click 'back' from Fasta sequence

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

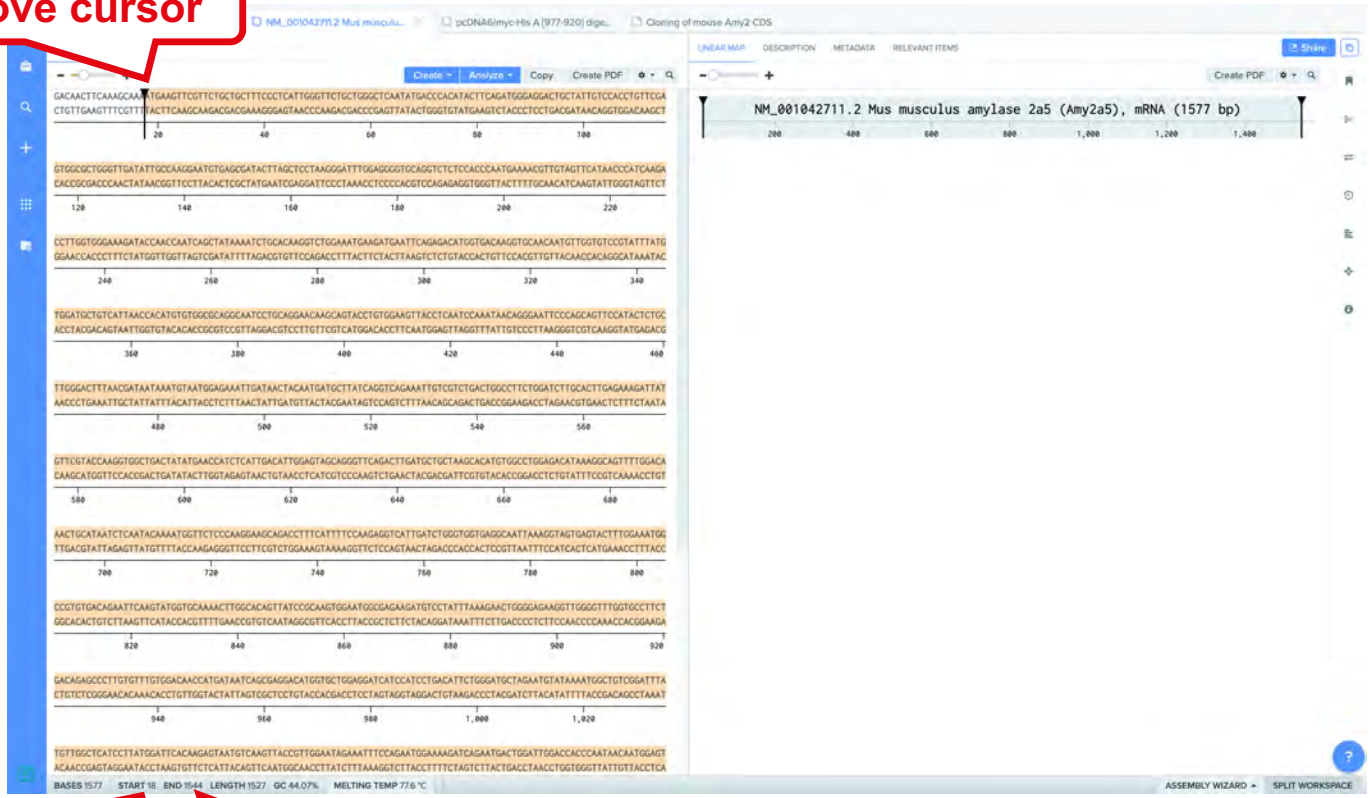


click on CDS

CDS

Select Amy2 CDS (Nucleotide 18-1544)

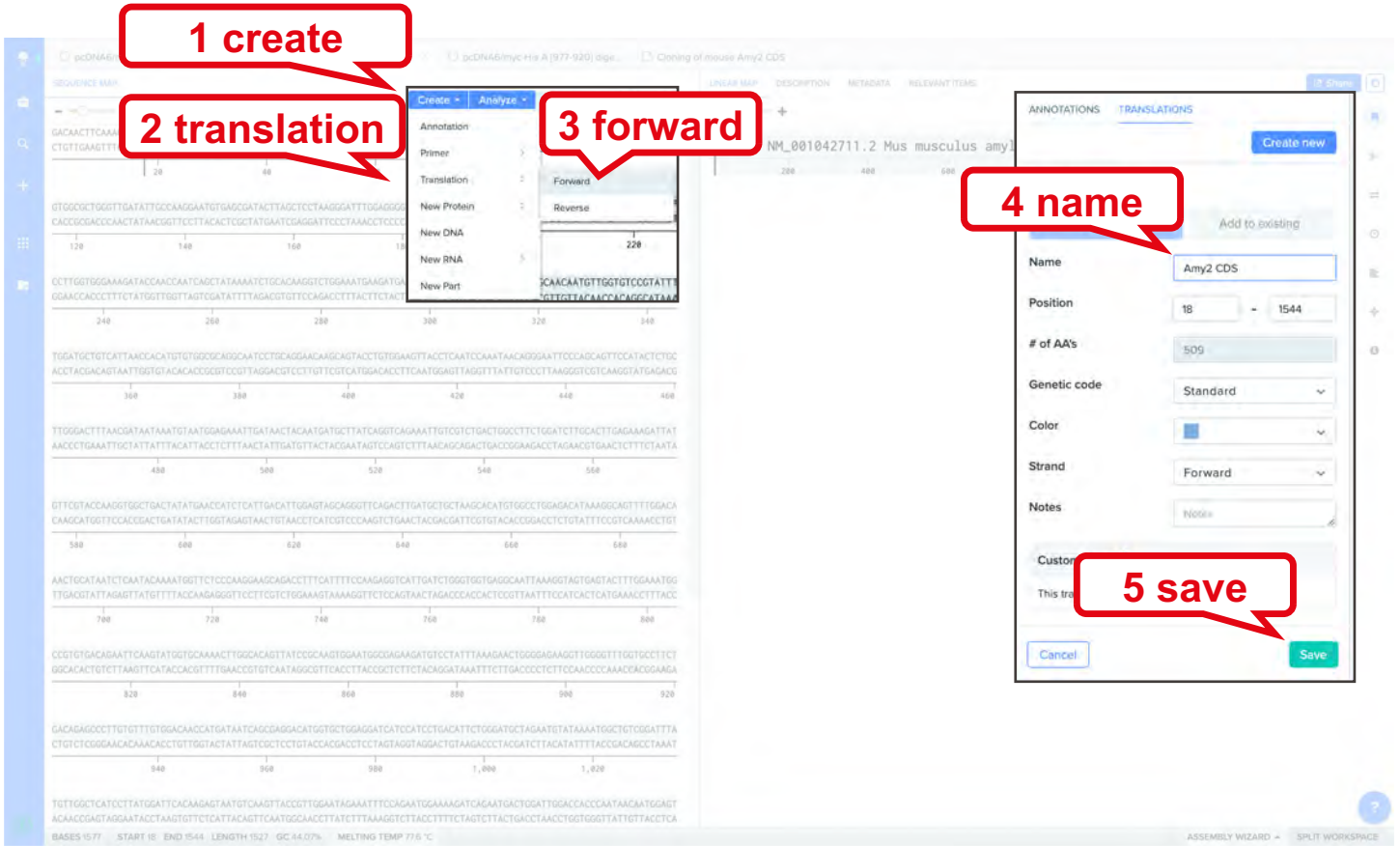
to select move cursor



START 18

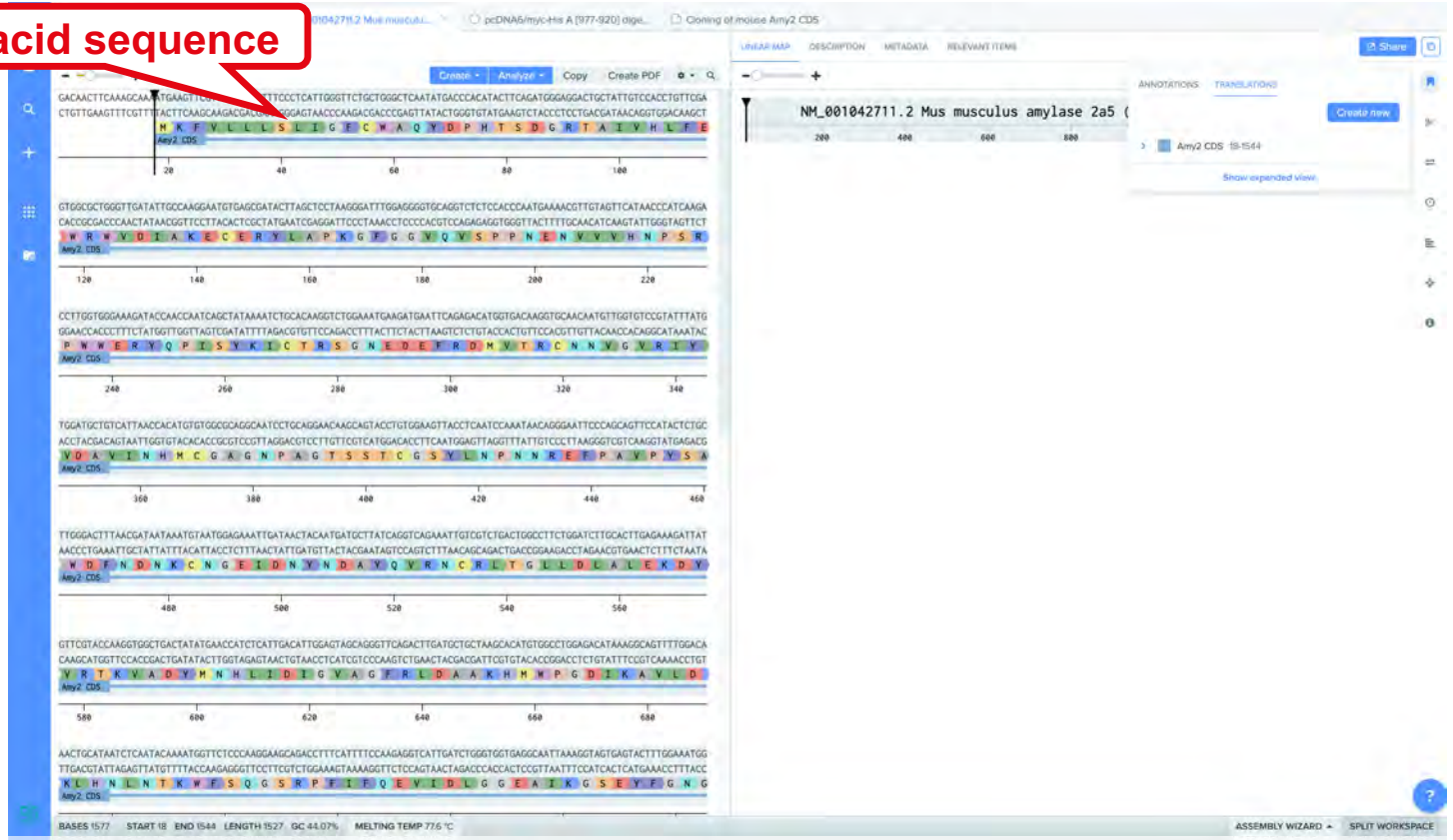
END 1544

Create Amy2 Translation (Nucleotide 18-1544)

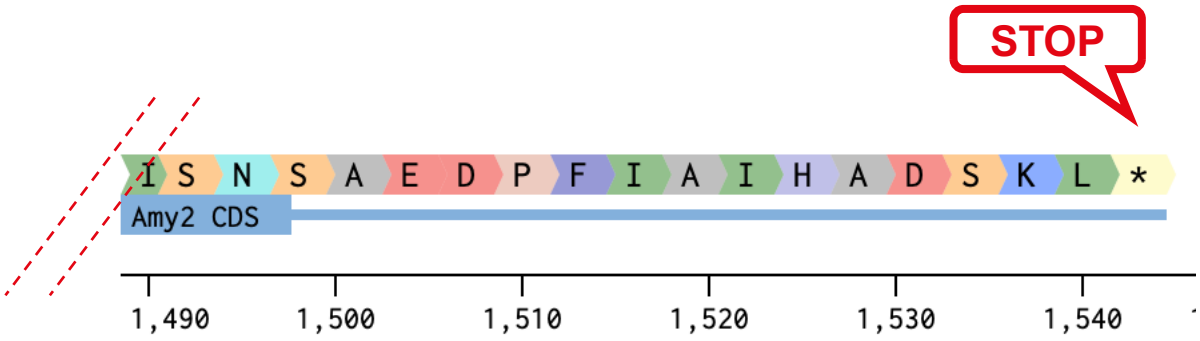


Amy2 Translation (Nucleotide 18-1544)

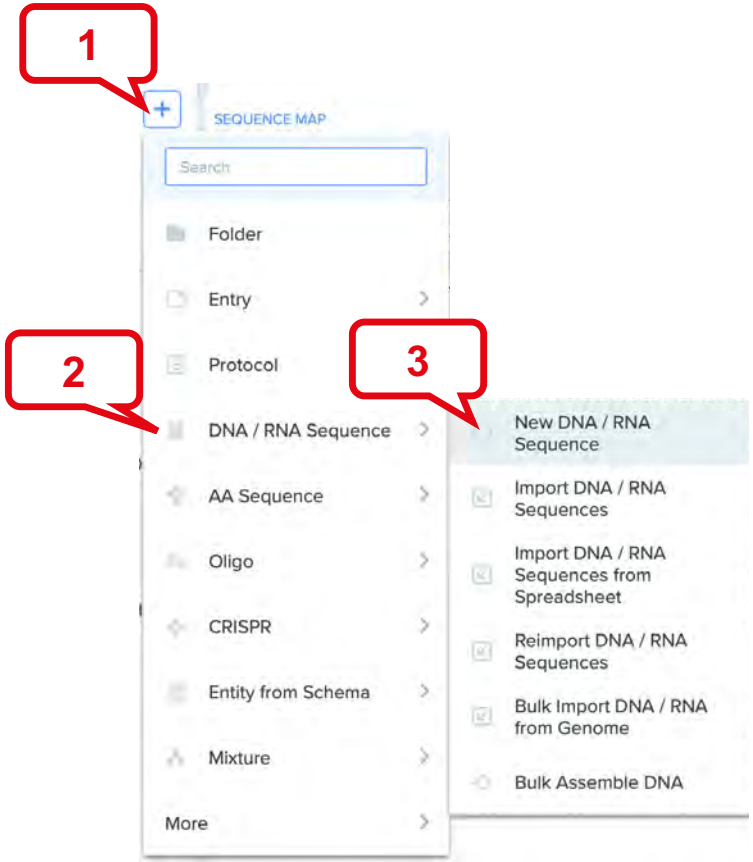
amino acid sequence



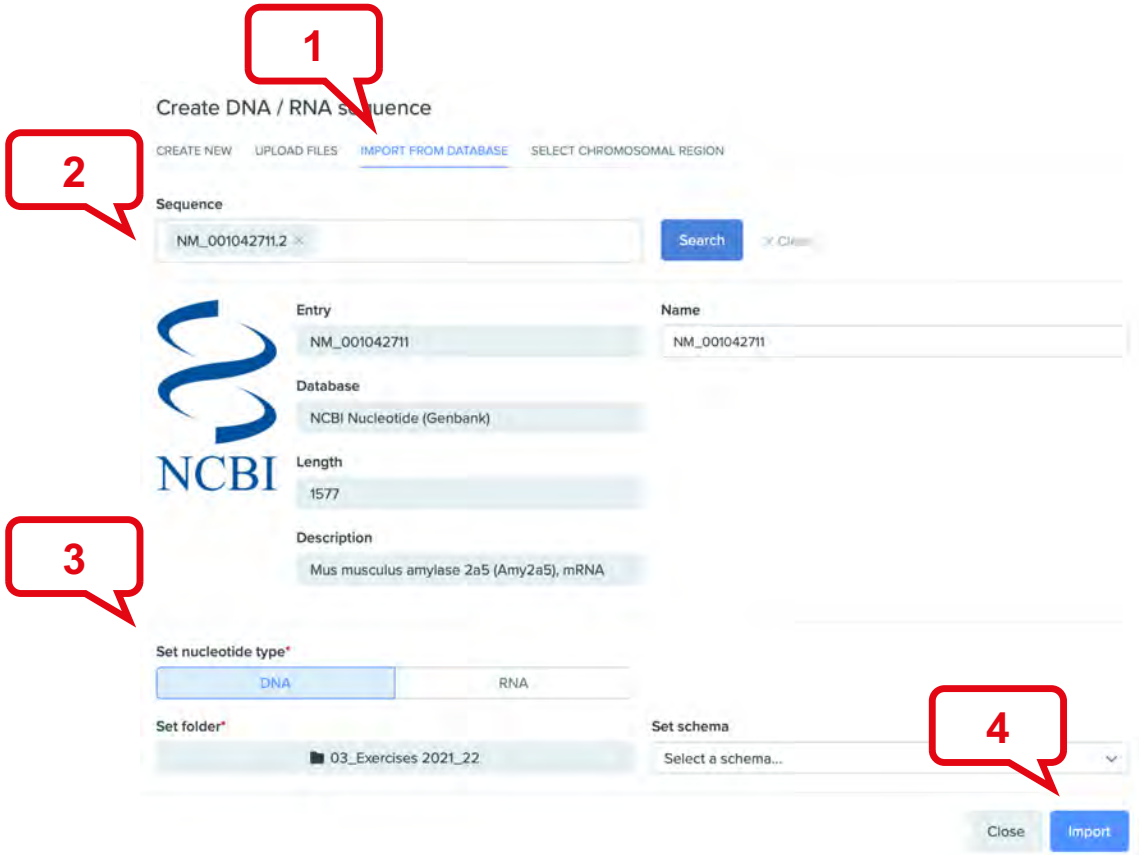
View Amy2 STOP Codon (1544)



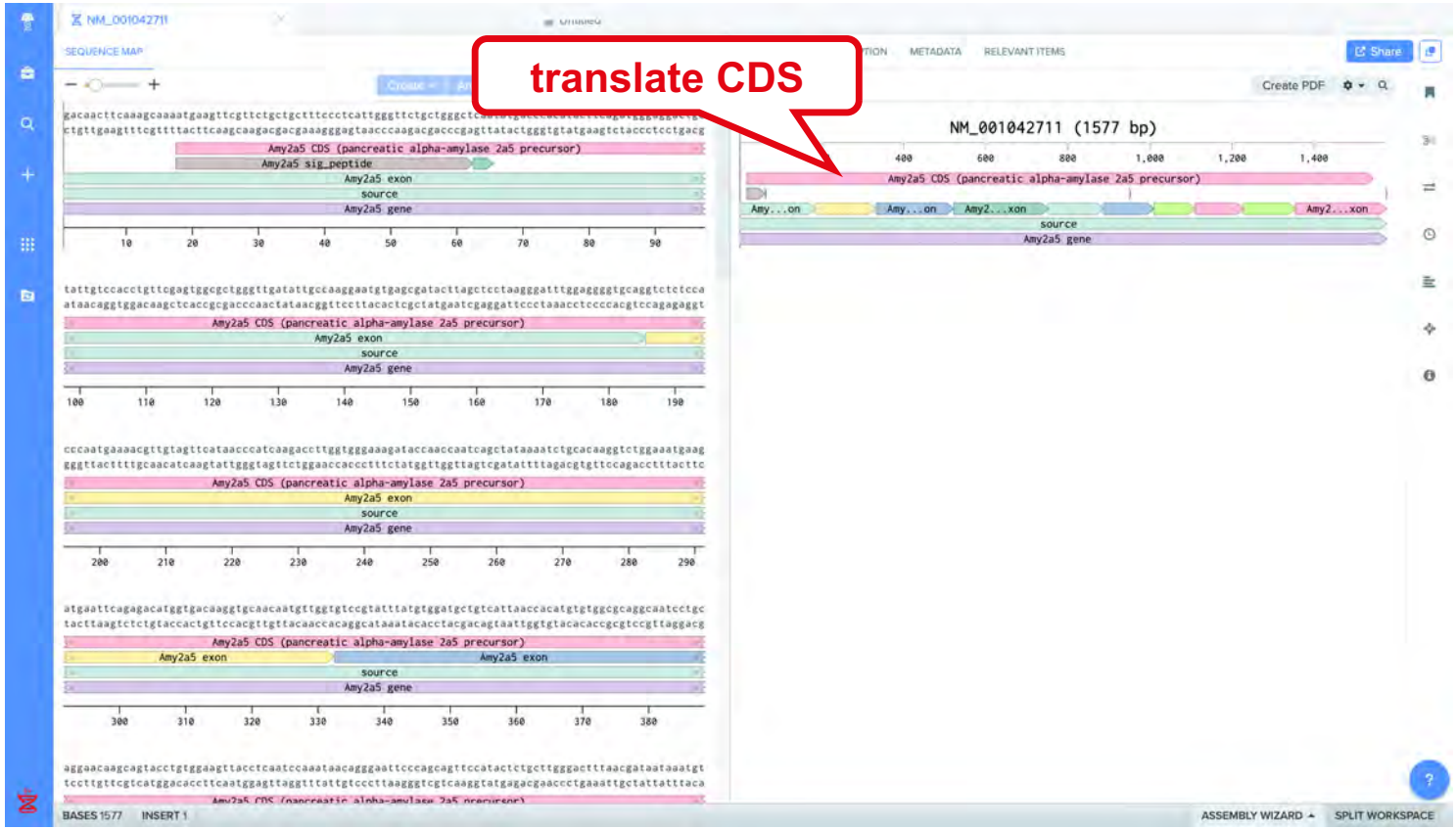
Option II:
Import from Database with Annotations



Import from Database with Annotations



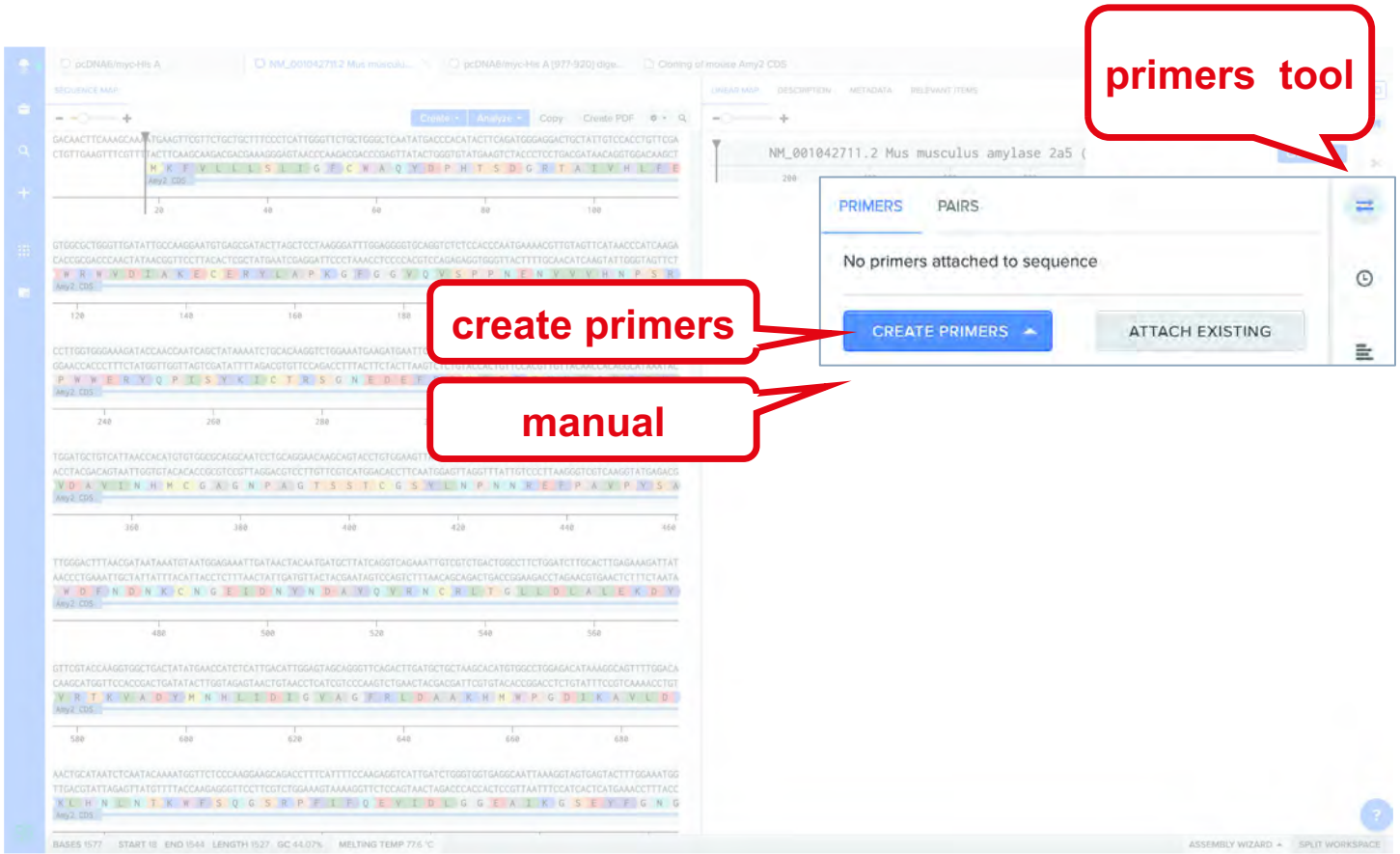
Default Annotations Amy2:



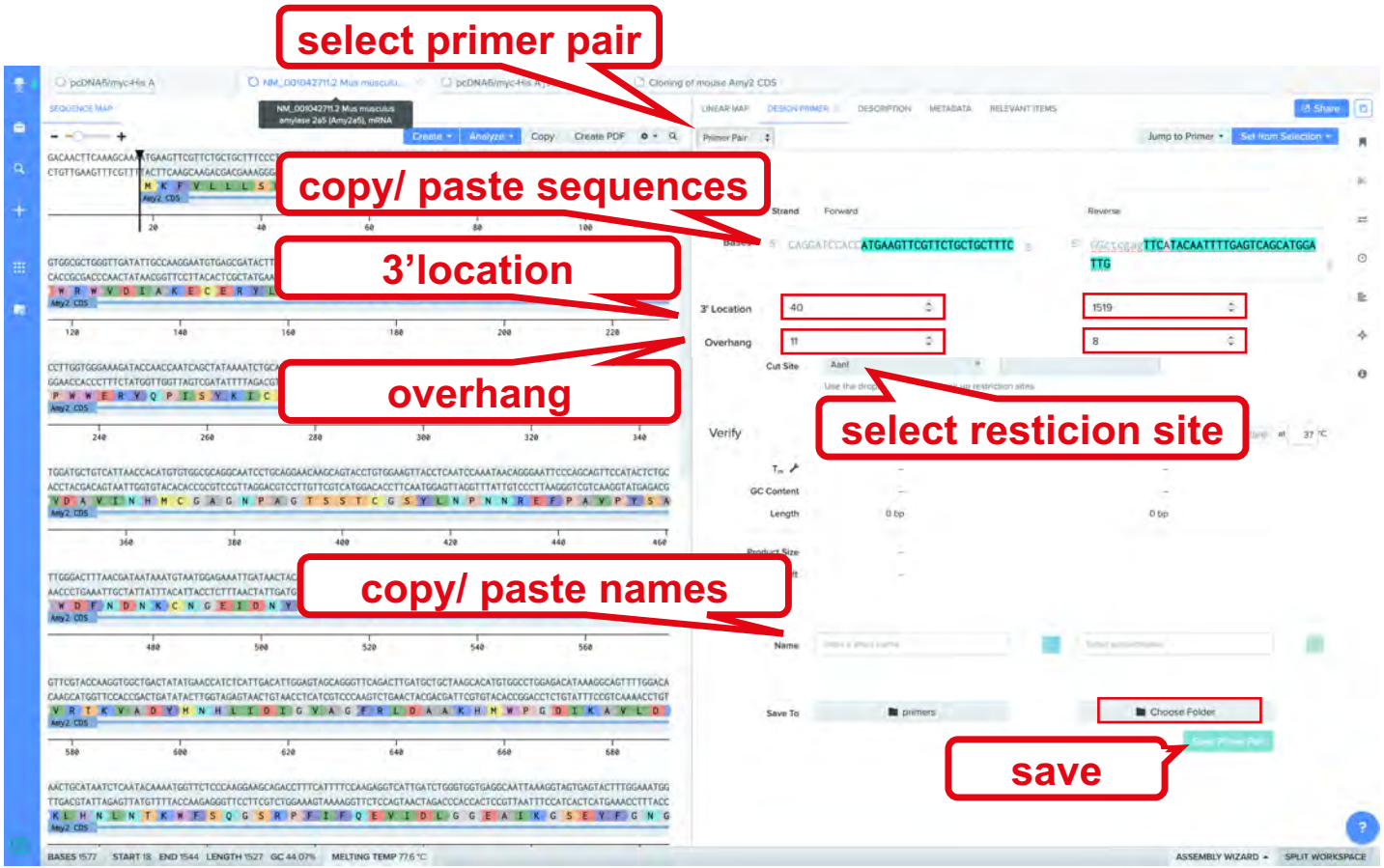
Primers exercise 1

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

Create Primers

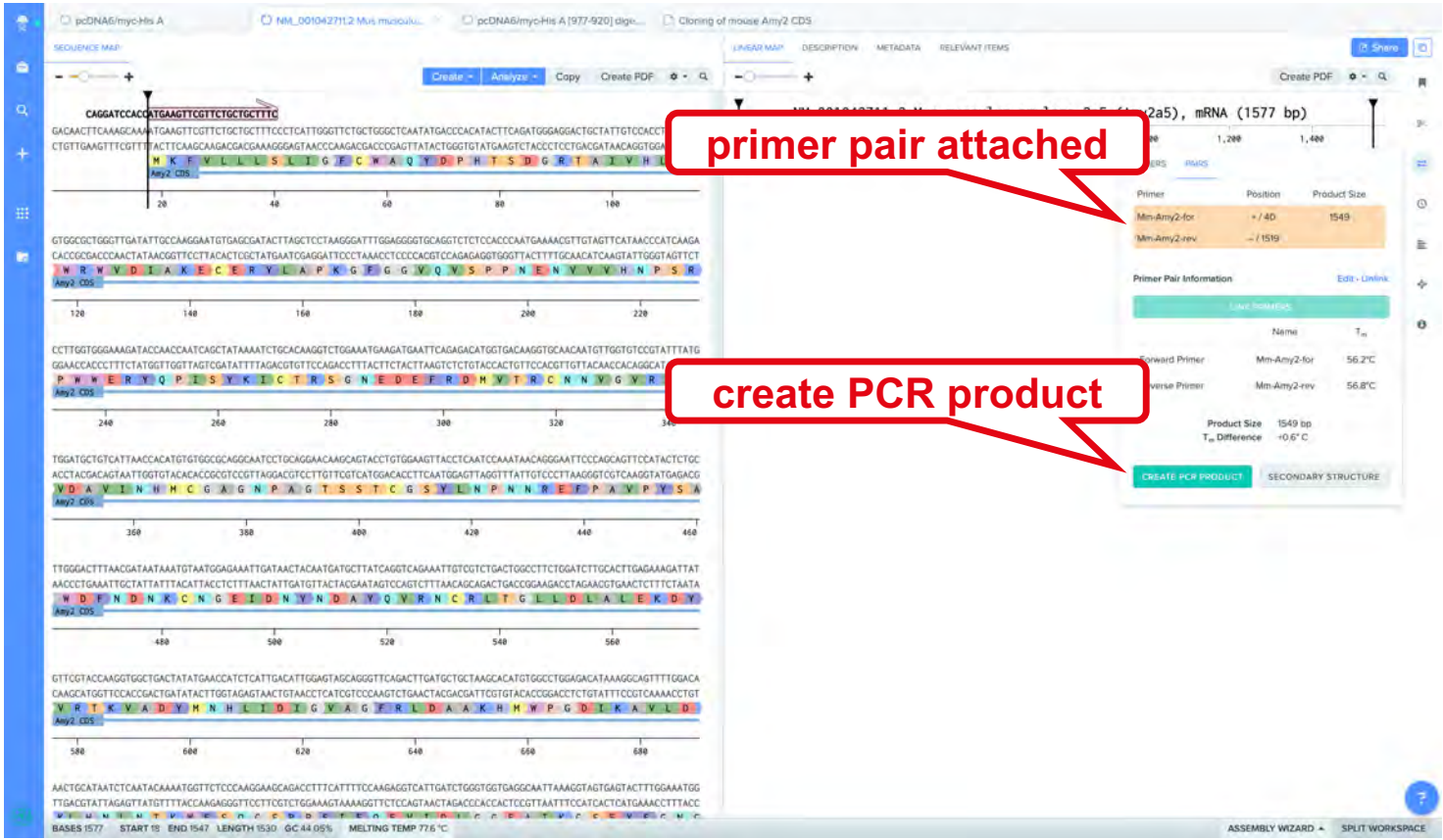


Create Primer Pair

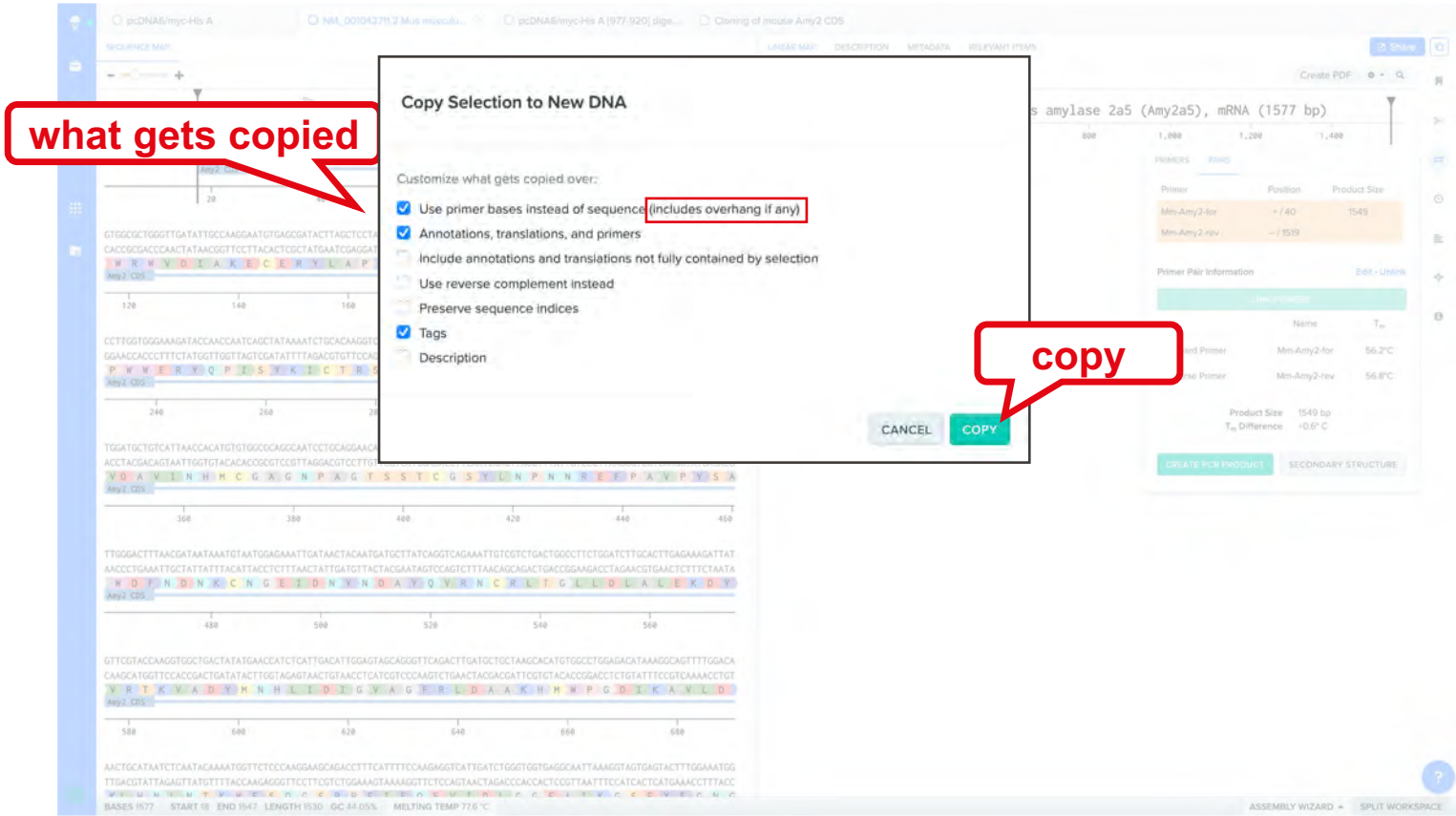


Primer Pair: 3'Location and Overhang

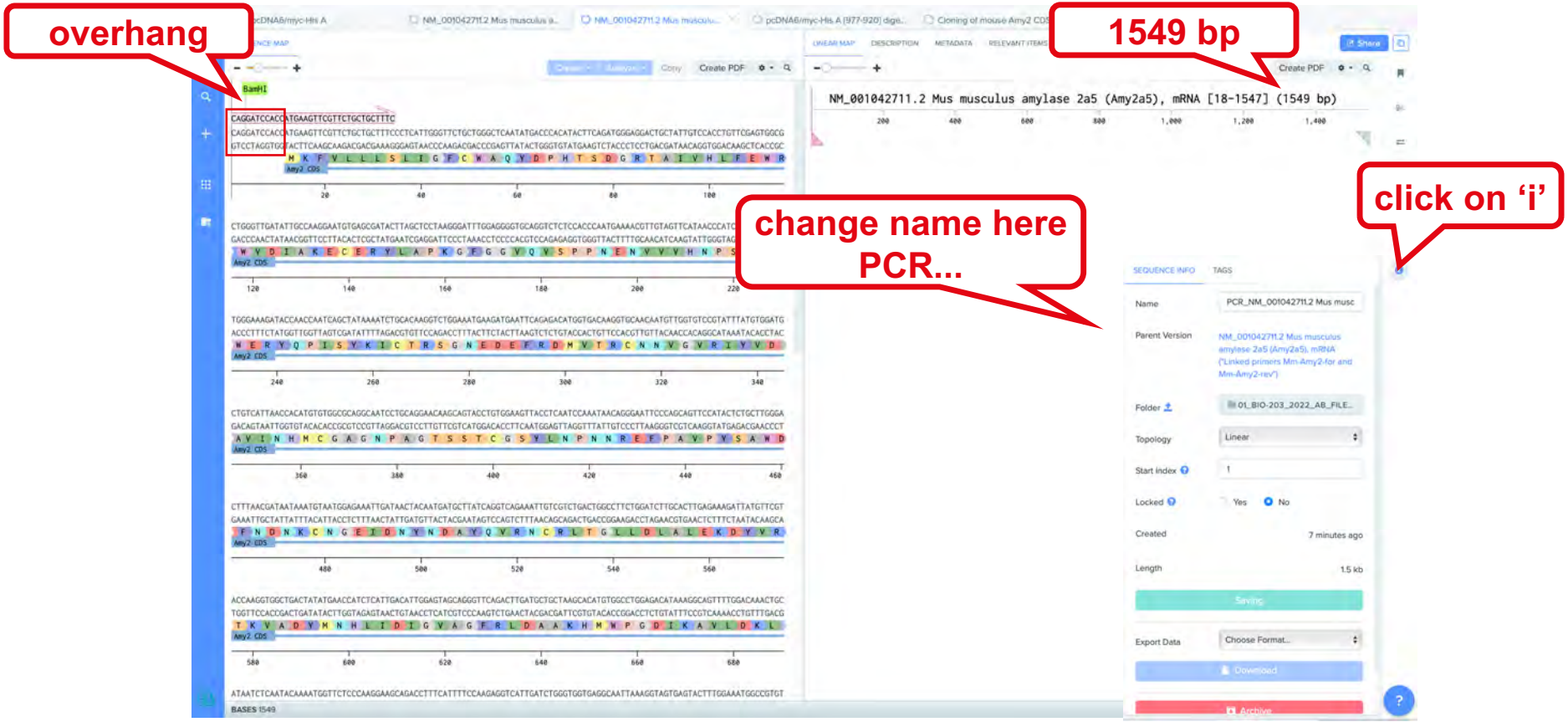
Create PCR Product



Create PCR Product Including Primers Sequences

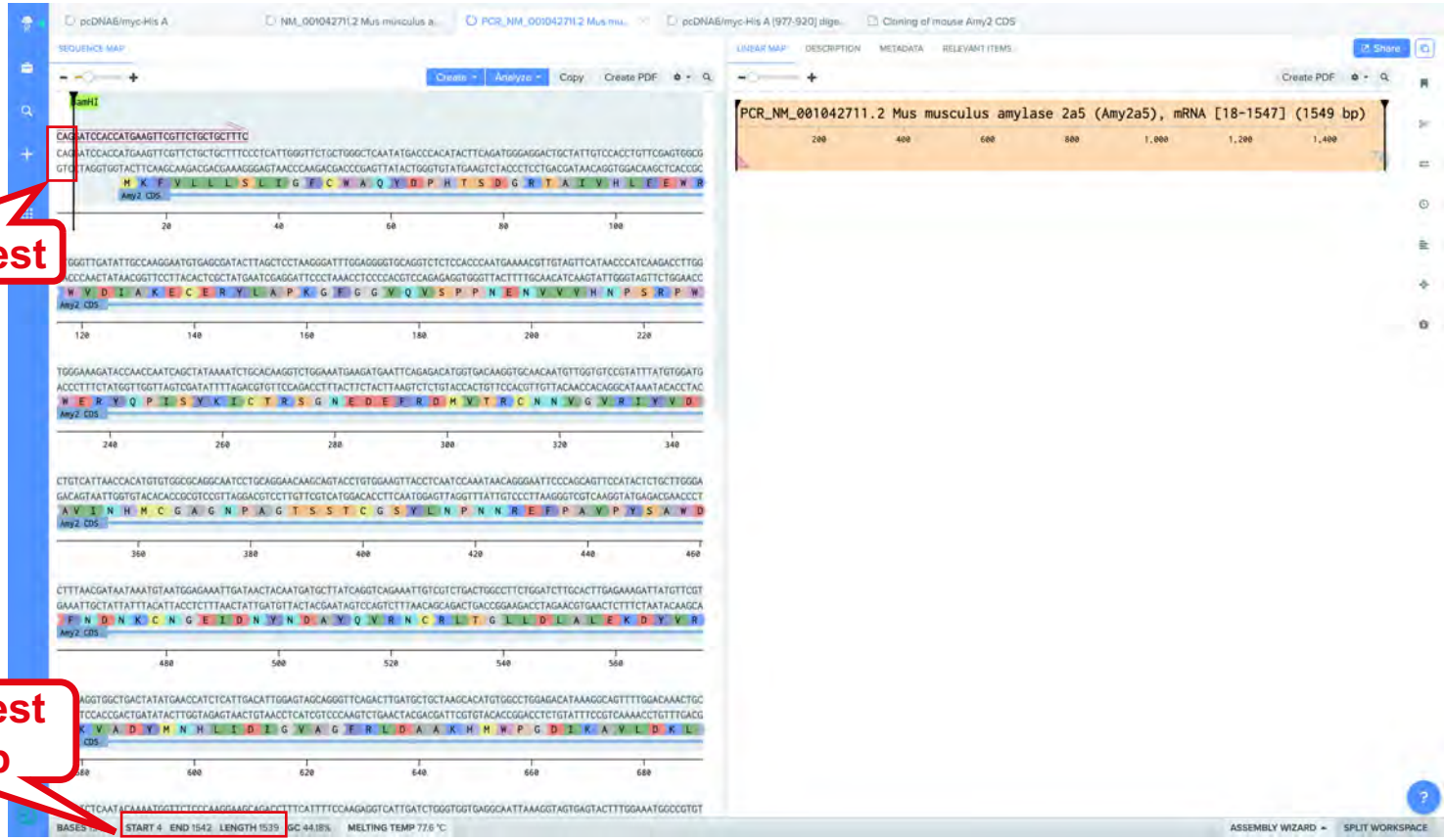


PCR Product with Overhangs

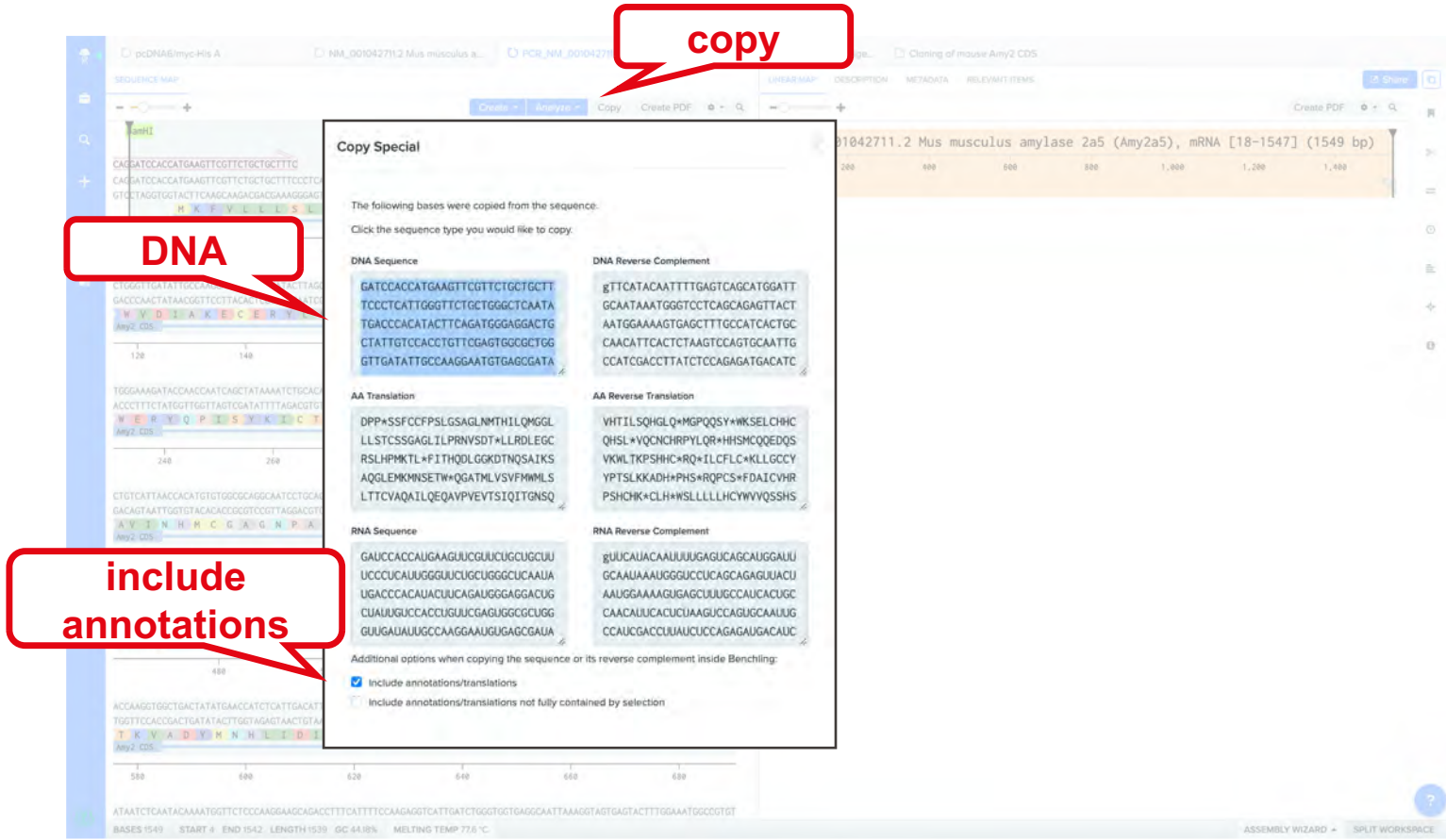


In PCR: Manually select sequence between BamHI + XhoI

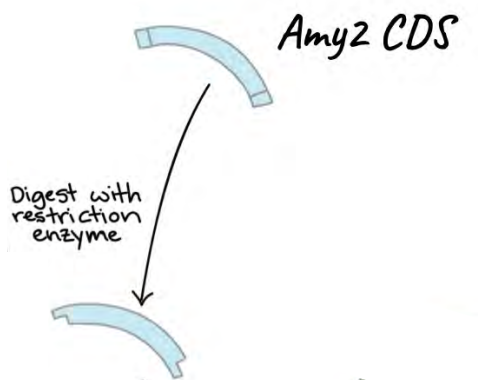
(Alternative: Run digest, create new DNA)



Copy selected sequence between BamHI + XhoI



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

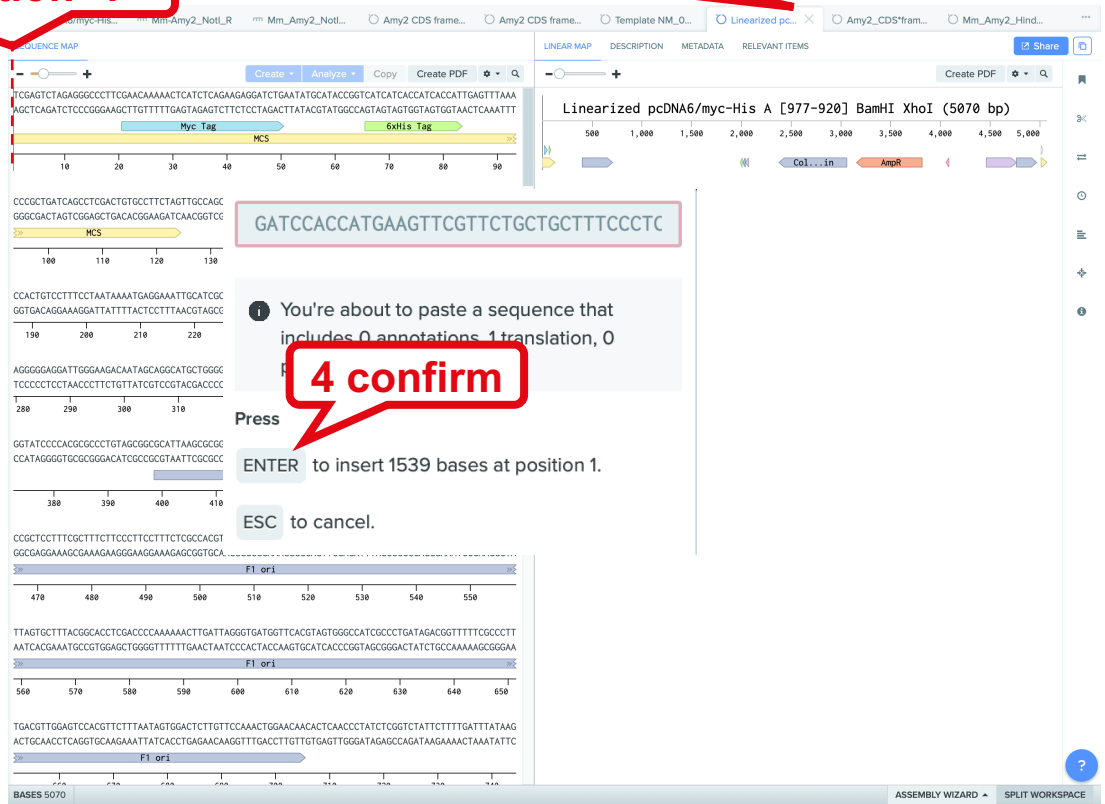
annotate recombinant fusion protein

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

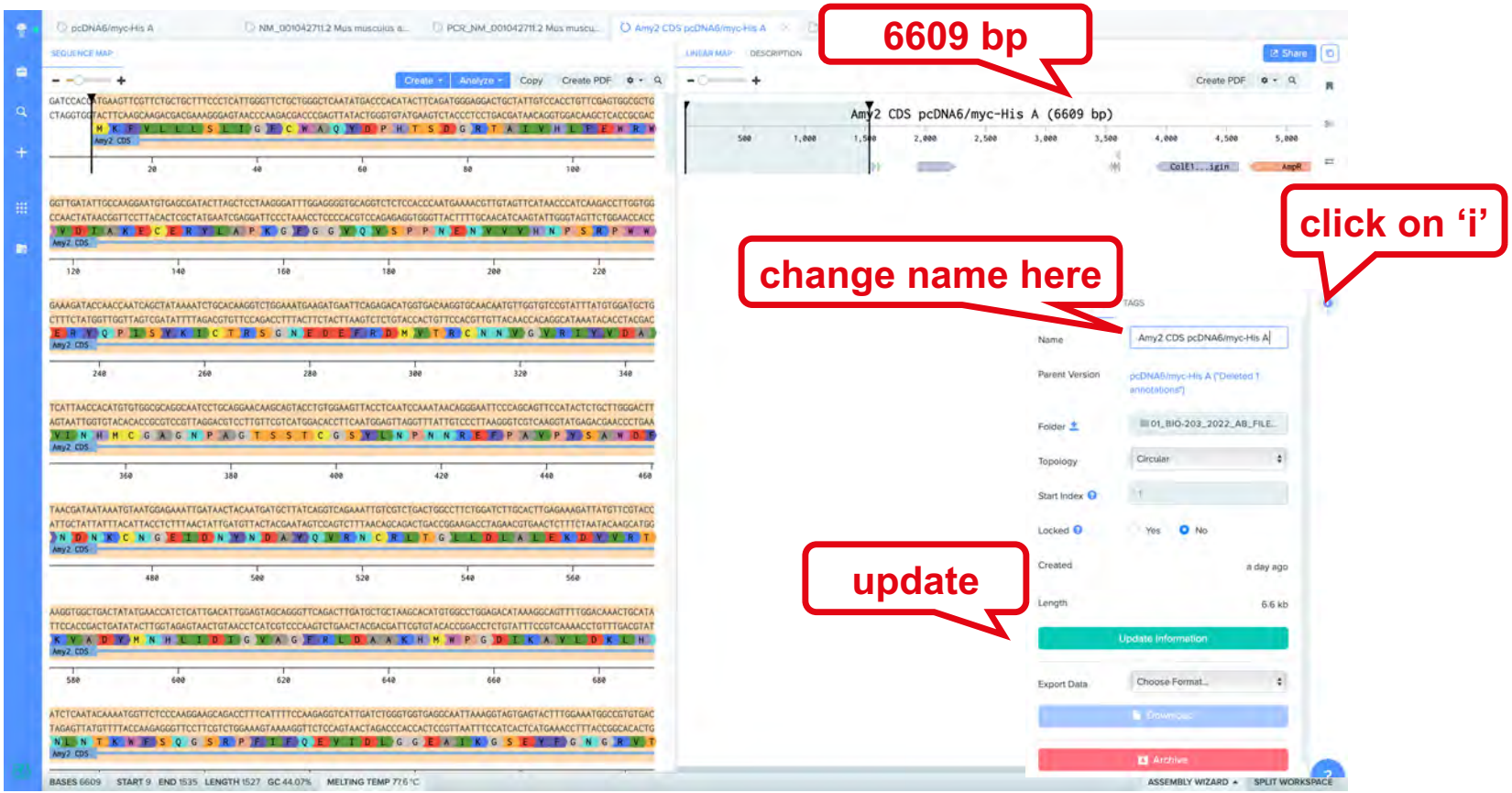
2 put cursor at position ‘1’

1 open file

3 CTR+V



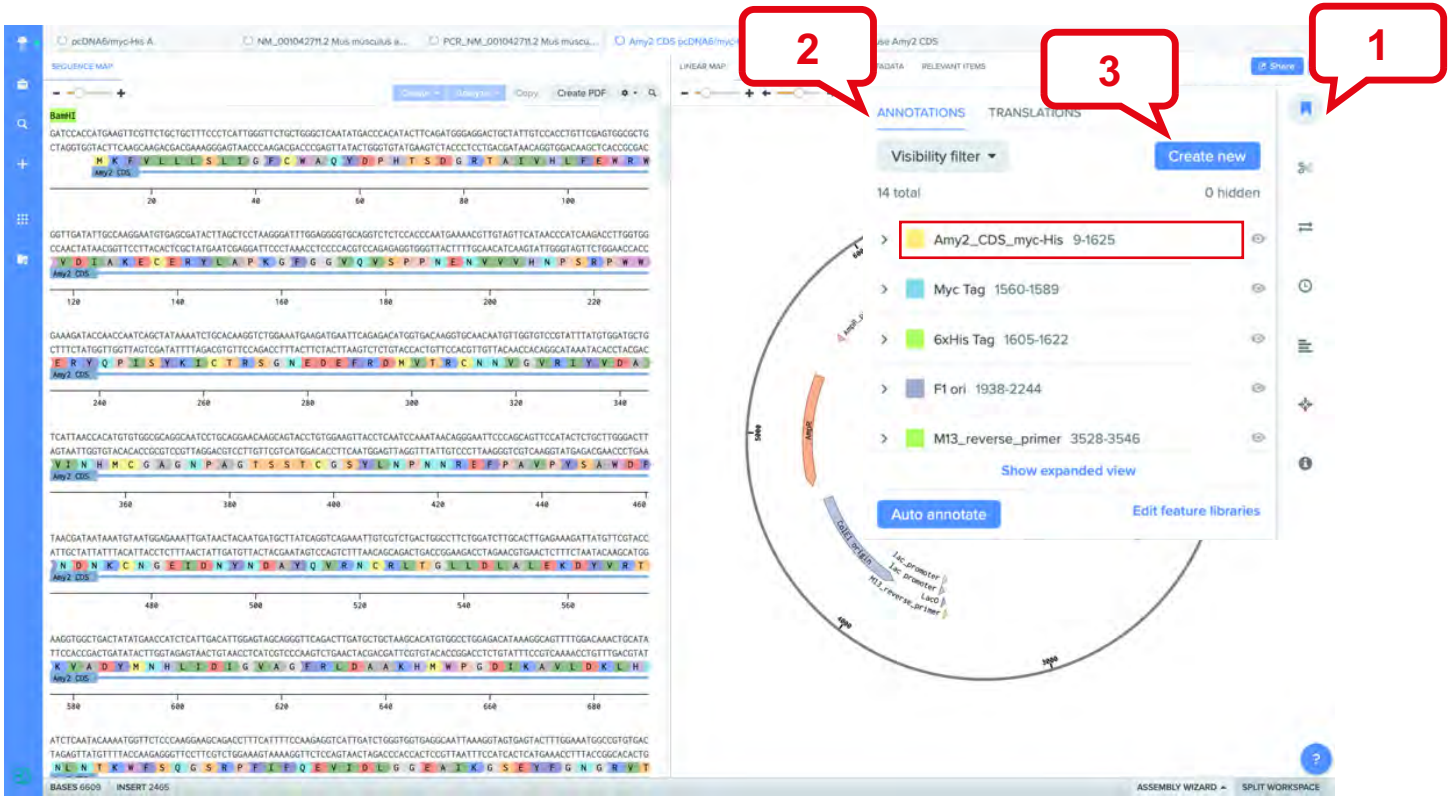
Make Circular + Change Name



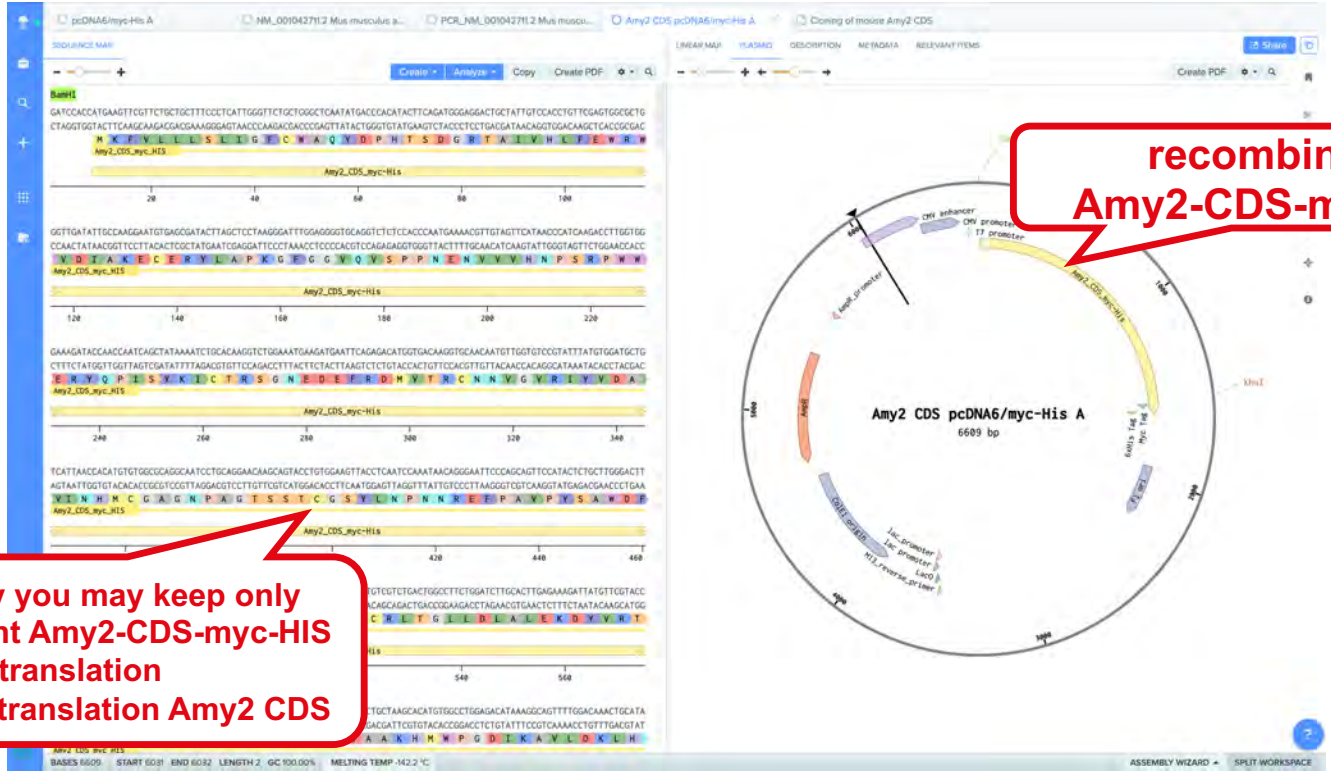
Virtual Cloning Completed

Translate Recombinant Protein Amy2-CDS-myc-His including STOP codon

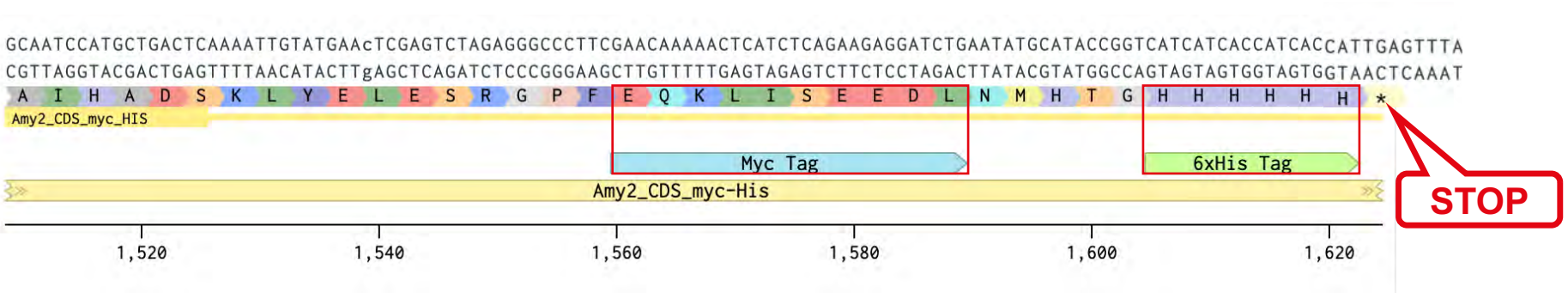
Annotate Recombinant Protein Amy2-CDS-myc-His including STOP codon

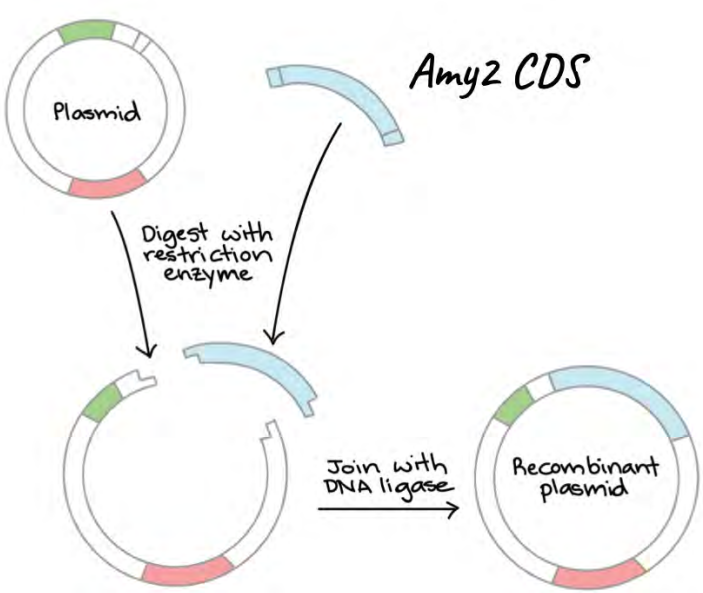


Annotate Recombinant Protein Amy2-CDS-myc-His including STOP codon



Recombinant Protein Amy2-CDS-myc-His Check Tag Translation and STOP codon

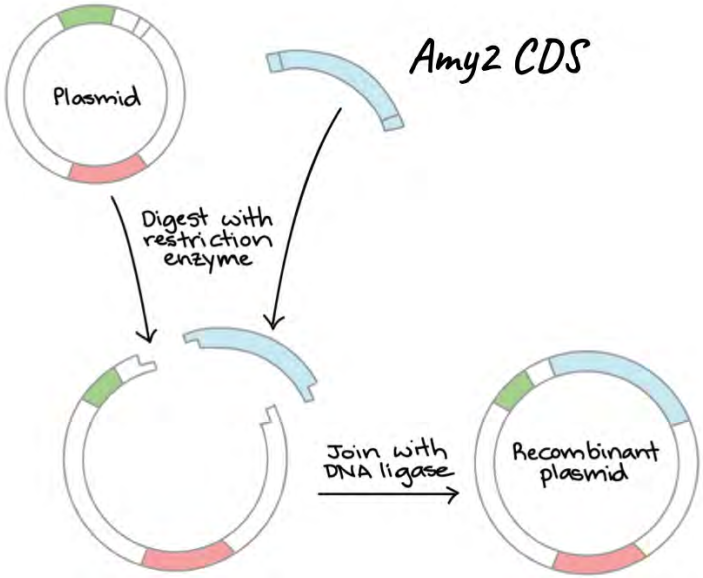




The recombinant plasmid is ready: virtual cloning completed!

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

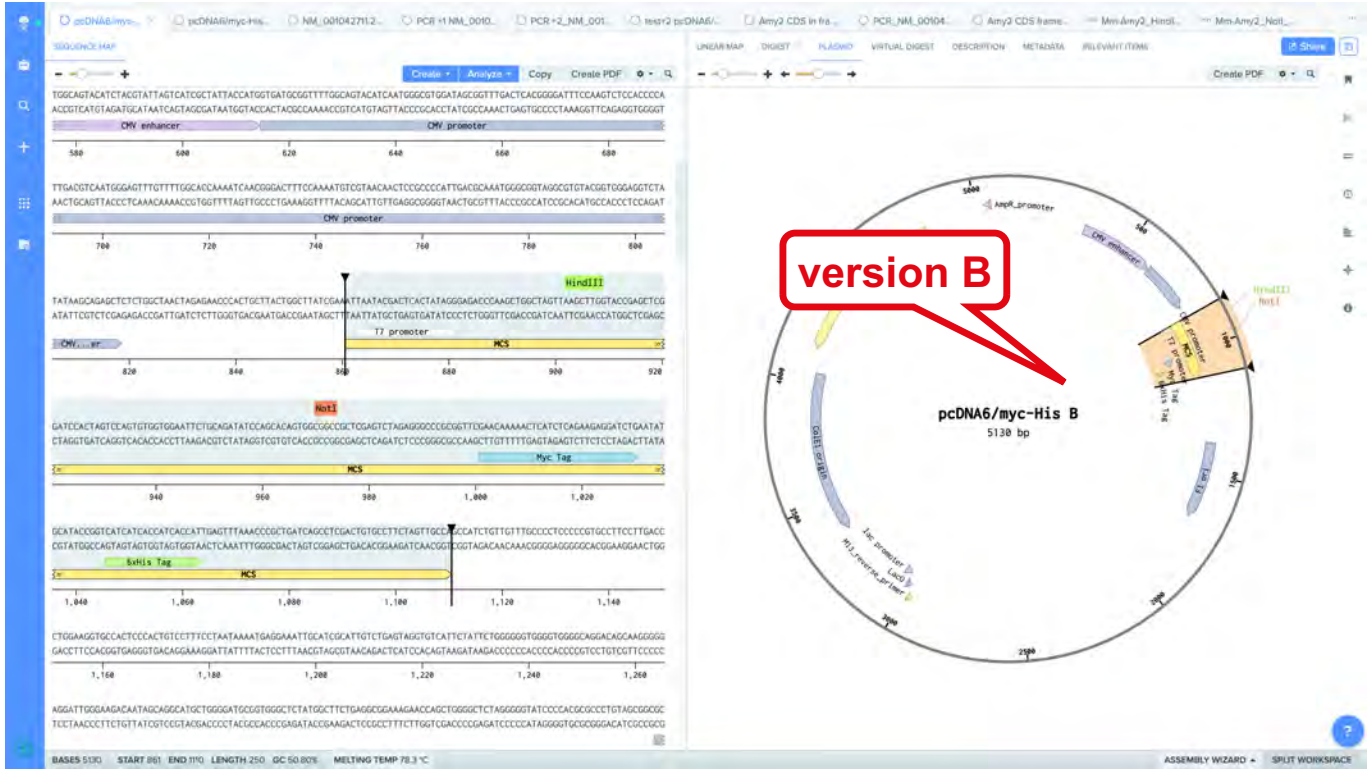
plasmid: pcDNA6/myc-His **version B**

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 plamid- different reading frames!



Steps for Plasmid Backbone (same as for exercise 1)

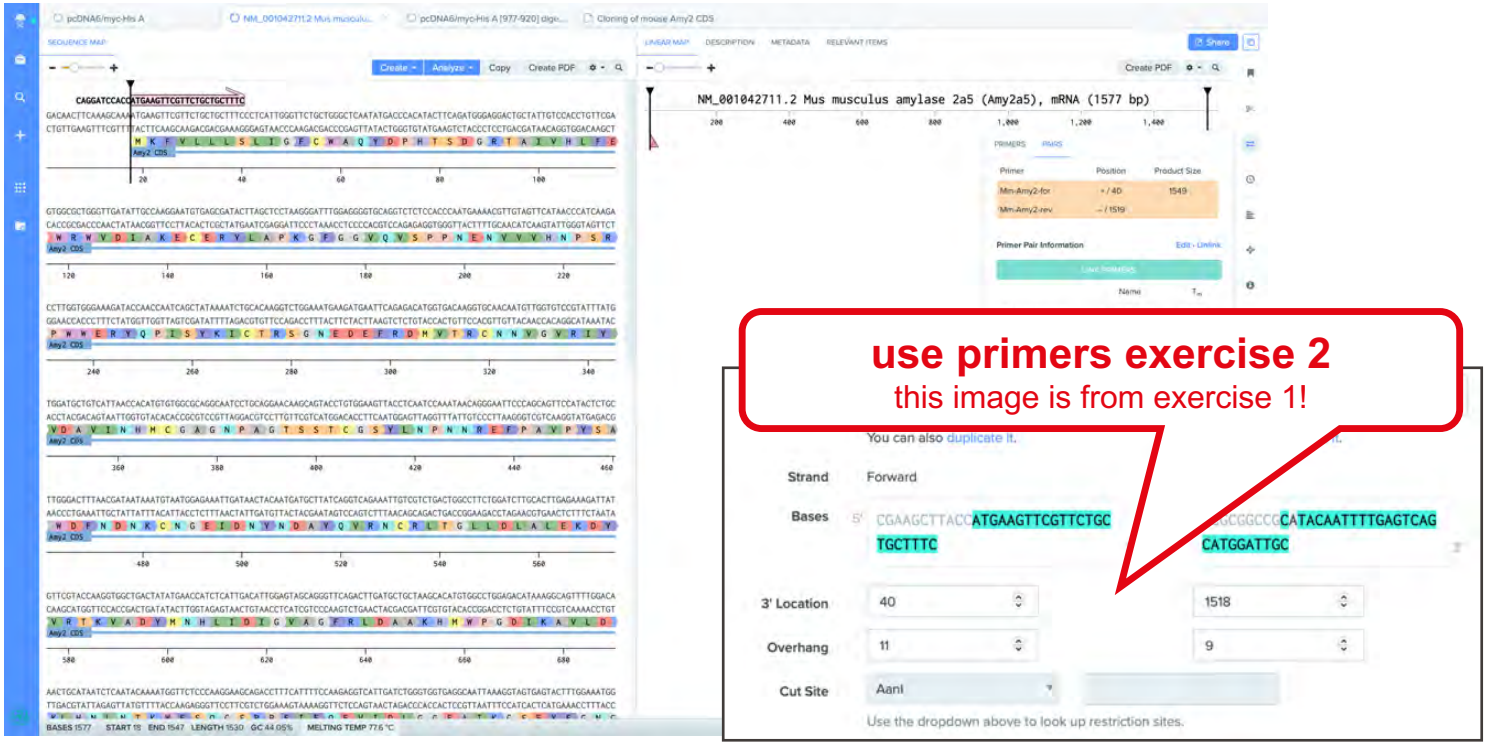
- ✓find restriction enzymes HindIII and NotI
- ✓virtual digest
- ✓create new DNA with digested linearized fragment (plasmid backbone)

Primers exercise 2

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

Attach New Primer Pairs to Amy2 Nucleotide Sequence

(use mRNA sequence you imported in exercise 1)



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