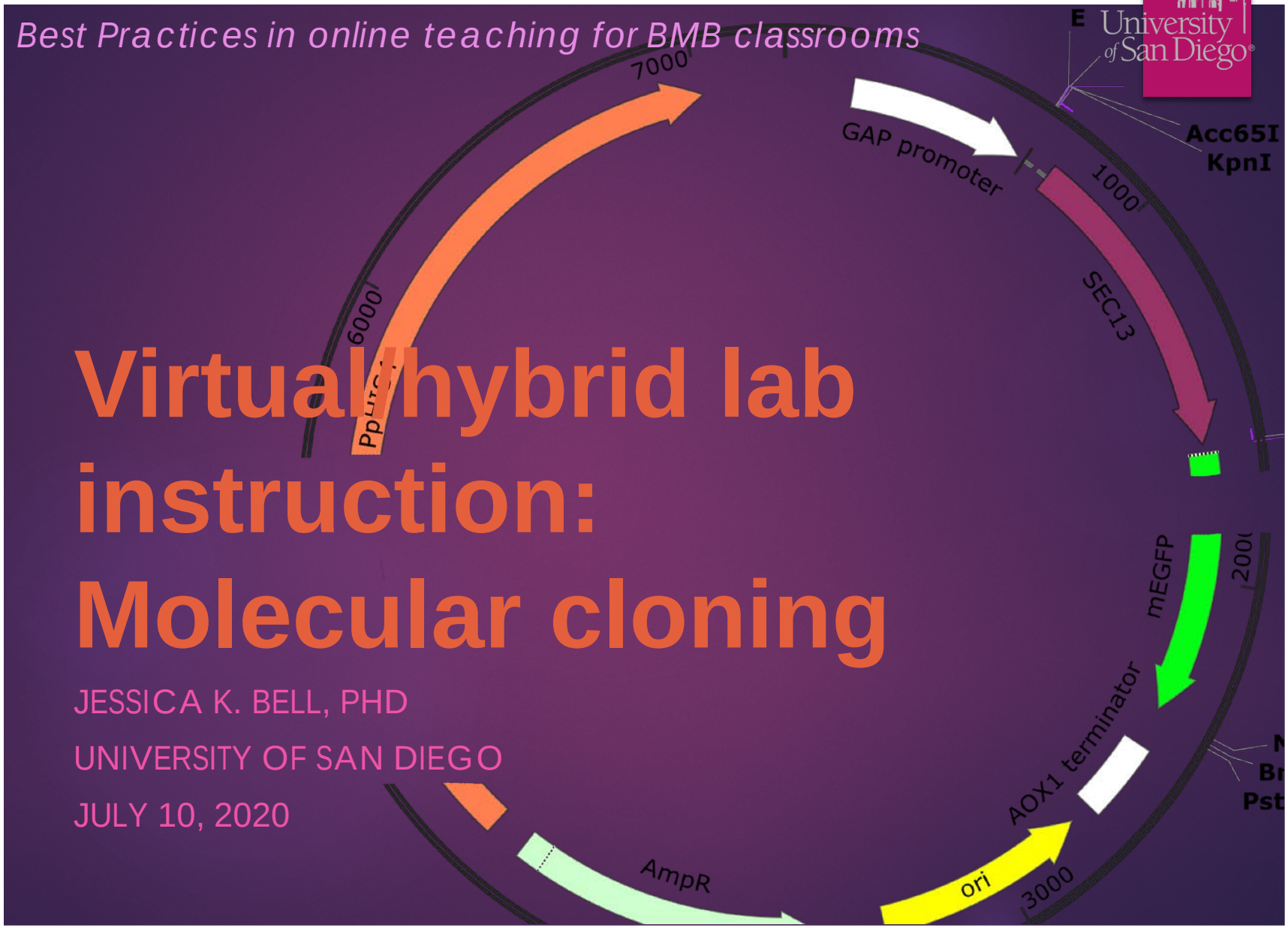

Virtual/hybrid lab instruction: Molecular cloning

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UNIVERSITY OF SAN DIEGO

JULY 10, 2020

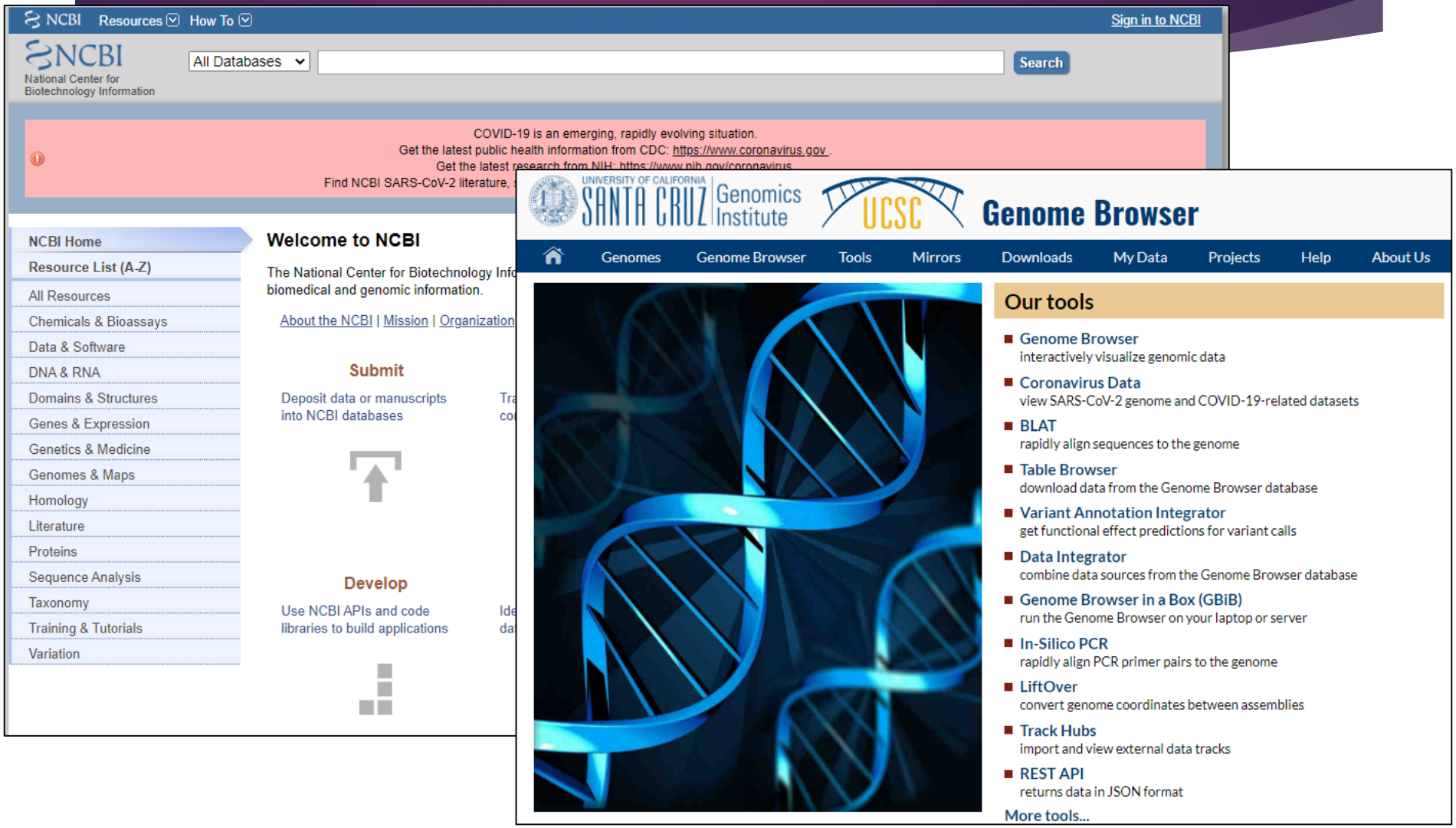
Techniques in Molecular Biology: Virtual Edition

Several labs have been or are being developed as virtual experiences…

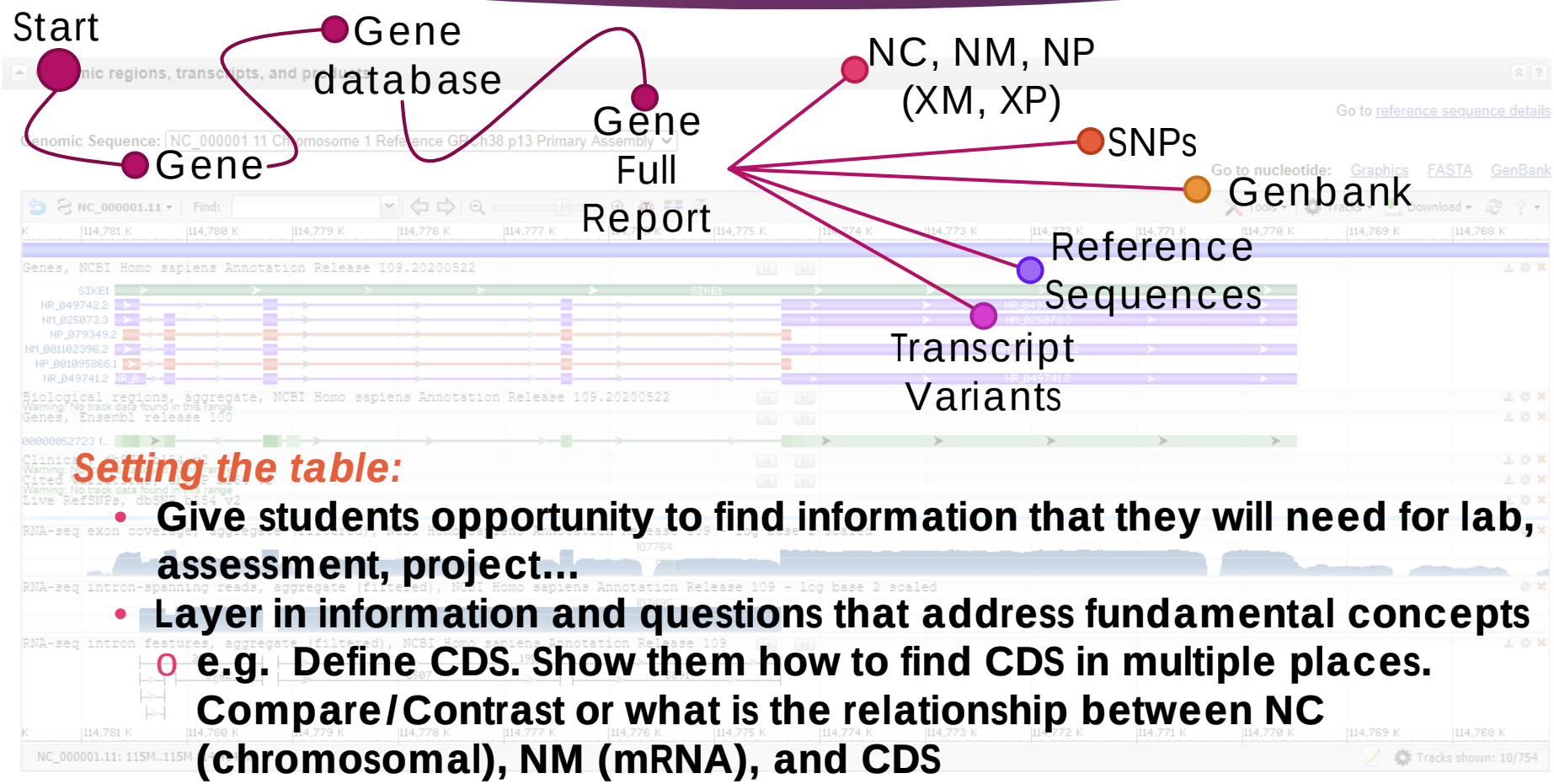
In a normal year:

- ***Dry lab for bioinformatics:***
  - Exploring resources of NCBI, UC-Santa Cruz Genome Browser
  - e.g. NC, NM, NP designators; SNPs database, identify CDS
- **Components of molecular cloning completed in silico (SnapGene):**
  - Primer design
  - Restriction Enzyme based
  - Gibson Assembly based
- ***Used bioinformatics and SnapGene in assessment:***
  - Cloning question where students locate gene given chromosomal identifier, find corresponding CDS, design primers with compatible restriction enzyme sites for target vector, demonstrate in silico cloning is successful.

Bioinformatics



Bioinformatics: Examples



Setting the table:

- **Give students opportunity to find information that they will need for lab, assessment, project…**
- **Layer in information and questions that address fundamental concepts**
  - **e.g. Define CDS. Show them how to find CDS in multiple places. Compare/Contrast or what is the relationship between NC (chromosomal), NM (mRNA), and CDS**

https://mdh-cures-community.squarespace.com/virtual-cures-and-ures

Bioinformatics Ex.

Lactase Persistence lab

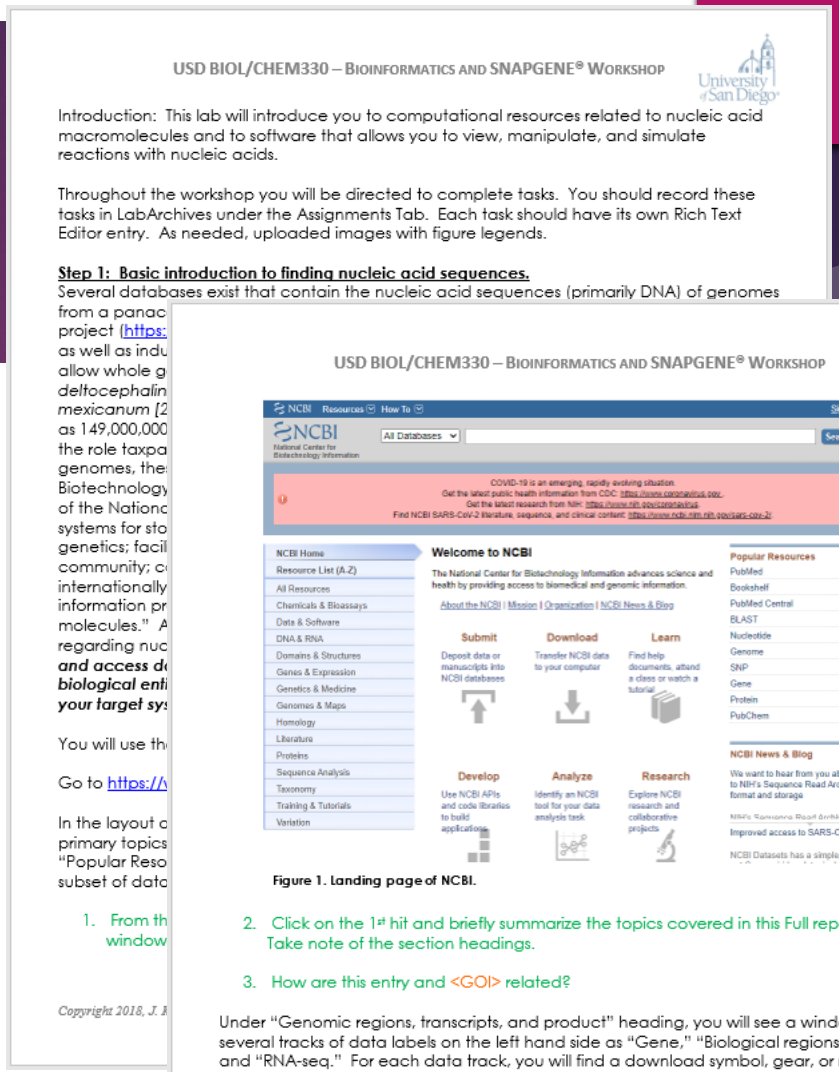
- Given 3 SNPs of LCT gene (lactase) design a primer set to amplify a 500 bp region encompassing these 3 SNPs
- Based upon Schultheis & Bowling BMBEd 39:133-140

qPCR lab

- Evaluate TaqMan primer sets for targets

Takehome exams

- Given either gene name or identifier, use information to complete in silico cloning



USD BIOL/CHEM330 – BIOINFORMATICS AND SNAPGENE® WORKSHOP

Introduction: This lab will introduce you to computational resources related to nucleic acid macromolecules and to software that allows you to view, manipulate, and simulate reactions with nucleic acids.

Throughout the workshop you will be directed to complete tasks. You should record these tasks in LabArchives under the Assignments Tab. Each task should have its own Rich Text Editor entry. As needed, uploaded images with figure legends.

Step 1: Basic introduction to finding nucleic acid sequences.

Several databases exist that contain the nucleic acid sequences (primarily DNA) of genomes

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Figure 1. Landing page of NCBI.

2. Click on the 1st hit and briefly summarize the topics covered in this Full report. Hint: Take note of the section headings.

3. How are this entry and <GOI> related?

Under "Genomic regions, transcripts, and product" heading, you will see a window with several tracks of data labels on the left hand side as "Gene," "Biological regions," "dbSNP," and "RNA-seq." For each data track, you will find a download symbol, gear, or red X that allows you to export the data, alter its presentation, or remove it from view, respectively. Click on the gear associated with the "Gene" track data. Select "Show All" from the drop-down menu and click apply. (If you want to learn more about the colors and symbols used in this section, the "Track Legend" hyperlink in the Gear menu provides information.) In the "Genes" track data, note the letter and numerical delineators that begin with NC, NM, or NP. These refer to complete genomic, mRNA, or protein sequences, respectively. If "N" is replaced by an "X" this refers to a predicted sequence, lacking experimental validation.

4. How many sequences are available for <GOI>? What are their NM reference numbers? Why does <GOI> have more than one entry? Click on the 1st NM number.

https://mdh-cures-community.squarespace.com/virtual-cures-and-ures

SnapGene®
Molecular Cloning *in silico*

Advantages:

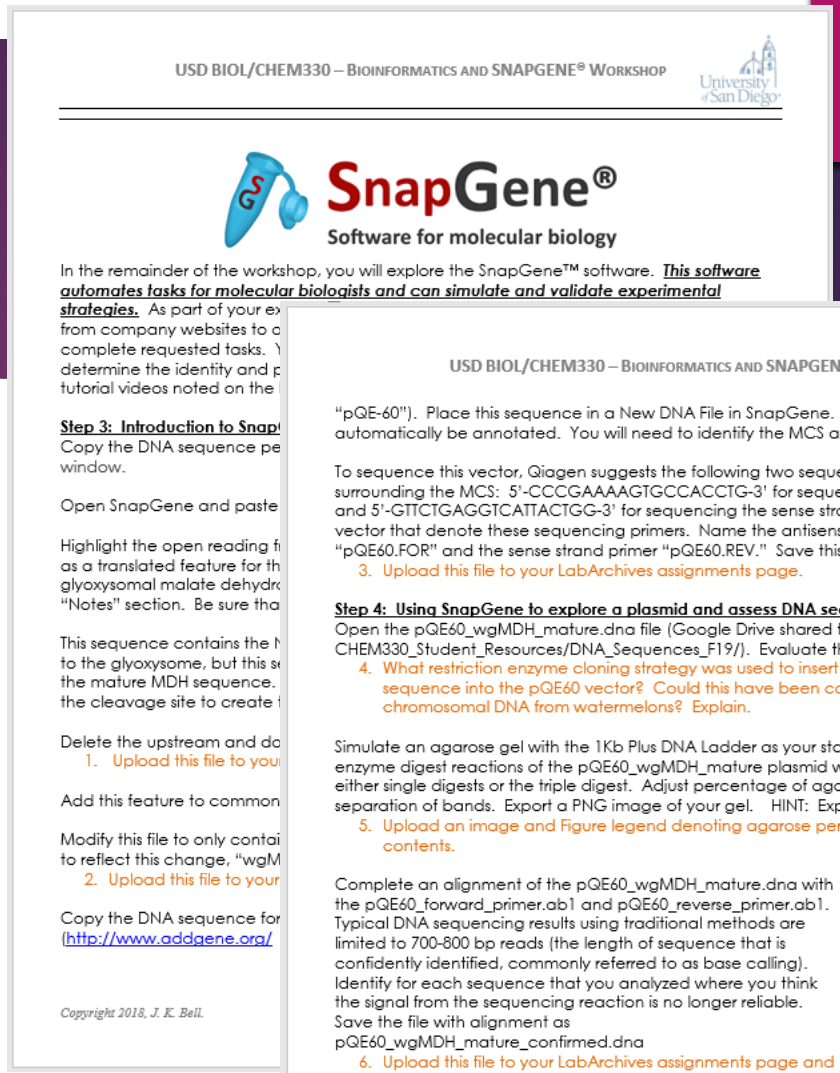
- LOTS of video tutorial support
- Ease of use

Disadvantages:

- Free Viewer software has limited functionality
- Licenses cost $$$

Some freeware alternatives:

- Serial Cloner (http://serialbasics.free.fr/Serial_Cloner.html)
- ApE (https://jorgensen.biology.utah.edu/wayned/ape/)



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SnapGene®
Software for molecular biology

In the remainder of the workshop, you will explore the SnapGene™ software. ***This software automates tasks for molecular biologists and can simulate and validate experimental strategies.*** As part of your ex
from company websites to c
complete requested tasks. Y
determine the identity and p
tutorial videos noted on the

Step 3: Introduction to Snap
Copy the DNA sequence pe
window.

Open SnapGene and paste

Highlight the open reading f
as a translated feature for th
glyoxysomal malate dehydr
"Notes" section. Be sure tha

This sequence contains the N
to the glyoxysome, but this s
the mature MDH sequence.
the cleavage site to create

Delete the upstream and do
1. Upload this file to you

Add this feature to common

Modify this file to only contai
to reflect this change, "wgM
2. Upload this file to your

Copy the DNA sequence for
(http://www.addgene.org/

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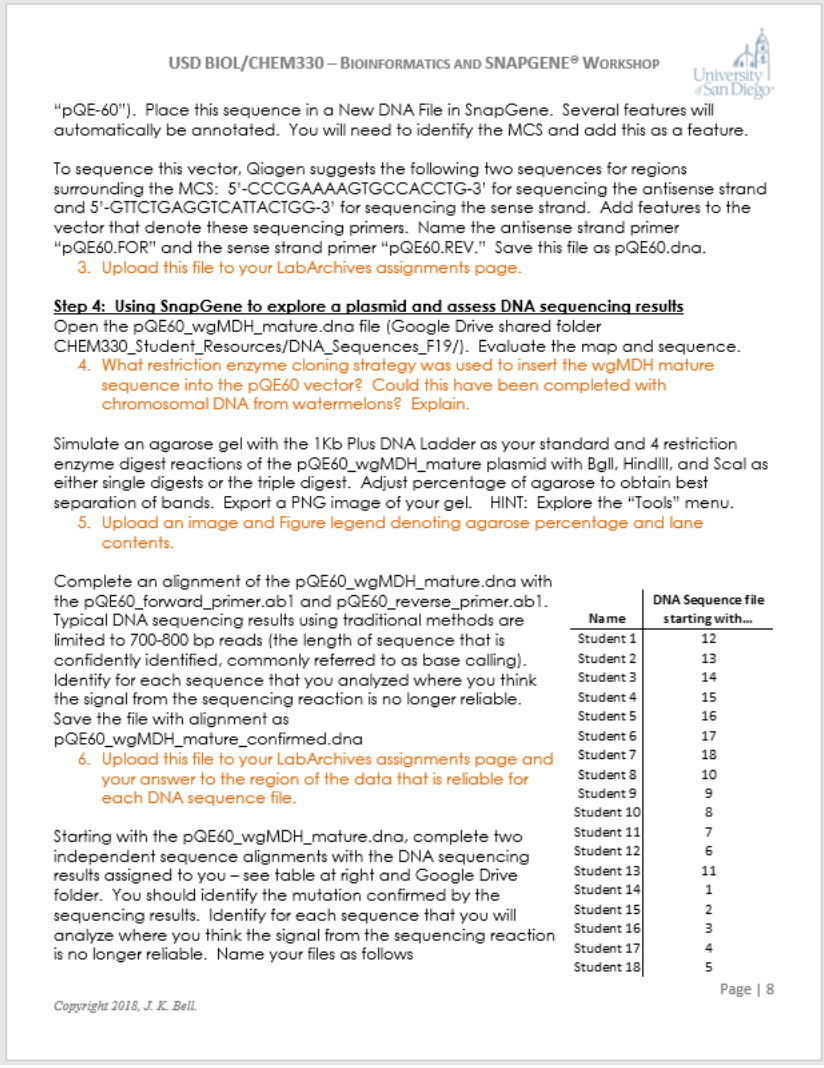

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"pQE-60"). Place this sequence in a New DNA File in SnapGene. Several features will automatically be annotated. You will need to identify the MCS and add this as a feature.

To sequence this vector, Qiagen suggests the following two sequences for regions surrounding the MCS: 5'-CCCGAAAAGTGCCACCTG-3' for sequencing the antisense strand and 5'-GTTCTGAGGTCATTACTGG-3' for sequencing the sense strand. Add features to the vector that denote these sequencing primers. Name the antisense strand primer "pQE60.FOR" and the sense strand primer "pQE60.REV." Save this file as pQE60.dna.

3. Upload this file to your LabArchives assignments page.

Step 4: Using SnapGene to explore a plasmid and assess DNA sequencing results
Open the pQE60_wgMDH_mature.dna file (Google Drive shared folder CHEM330_Student_Resources/DNA_Sequences_F19/). Evaluate the map and sequence.

4. What restriction enzyme cloning strategy was used to insert the wgMDH mature sequence into the pQE60 vector? Could this have been completed with chromosomal DNA from watermelons? Explain.

Simulate an agarose gel with the 1Kb Plus DNA Ladder as your standard and 4 restriction enzyme digest reactions of the pQE60_wgMDH_mature plasmid with BglI, HindIII, and ScaI as either single digests or the triple digest. Adjust percentage of agarose to obtain best separation of bands. Export a PNG image of your gel. HINT: Explore the "Tools" menu.

5. Upload an image and Figure legend denoting agarose percentage and lane contents.

Complete an alignment of the pQE60_wgMDH_mature.dna with the pQE60_forward_primer.ab1 and pQE60_reverse_primer.ab1. Typical DNA sequencing results using traditional methods are limited to 700-800 bp reads (the length of sequence that is confidently identified, commonly referred to as base calling). Identify for each sequence that you analyzed where you think the signal from the sequencing reaction is no longer reliable. Save the file with alignment as pQE60_wgMDH_mature_confirmed.dna

6. Upload this file to your LabArchives assignments page and your answer to the region of the data that is reliable for each DNA sequence file.

Starting with the pQE60_wgMDH_mature.dna, complete two independent sequence alignments with the DNA sequencing results assigned to you – see table at right and Google Drive folder. You should identify the mutation confirmed by the sequencing results. Identify for each sequence that you will analyze where you think the signal from the sequencing reaction is no longer reliable. Name your files as follows

Name	DNA Sequence file starting with...
Student 1	12
Student 2	13
Student 3	14
Student 4	15
Student 5	16
Student 6	17
Student 7	18
Student 8	10
Student 9	9
Student 10	8
Student 11	7
Student 12	6
Student 13	11
Student 14	1
Student 15	2
Student 16	3
Student 17	4
Student 18	5

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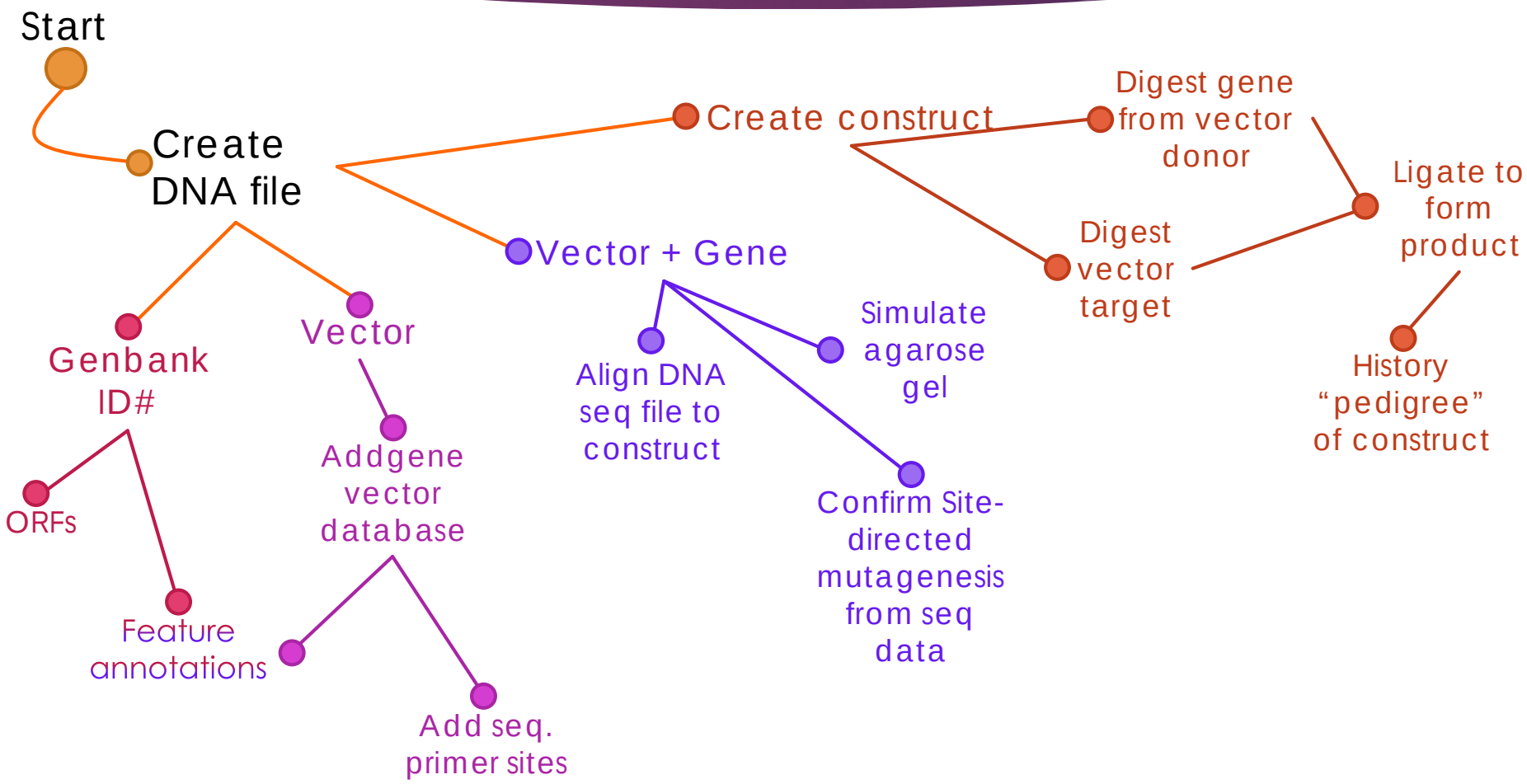
Molecular Cloning: Examples



Molecular Cloning: Examples

Start

Cr
DN

Genbank ID#

ORFs

Feature annotations

Addgene vector database

Add seq. primer sites

Align DNA seq file to construct

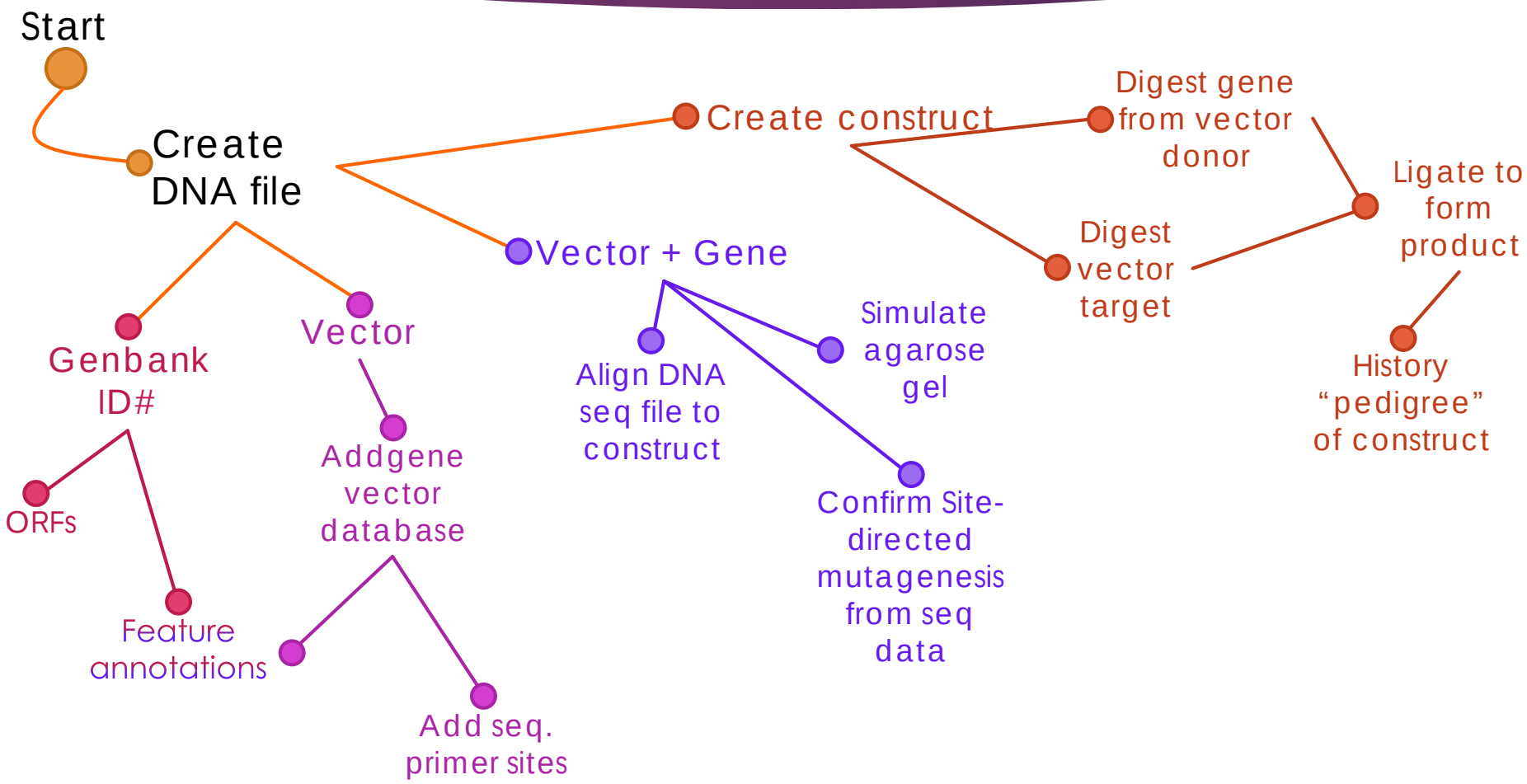
agarose gel

Confirm Site-directed mutagenesis from seq data

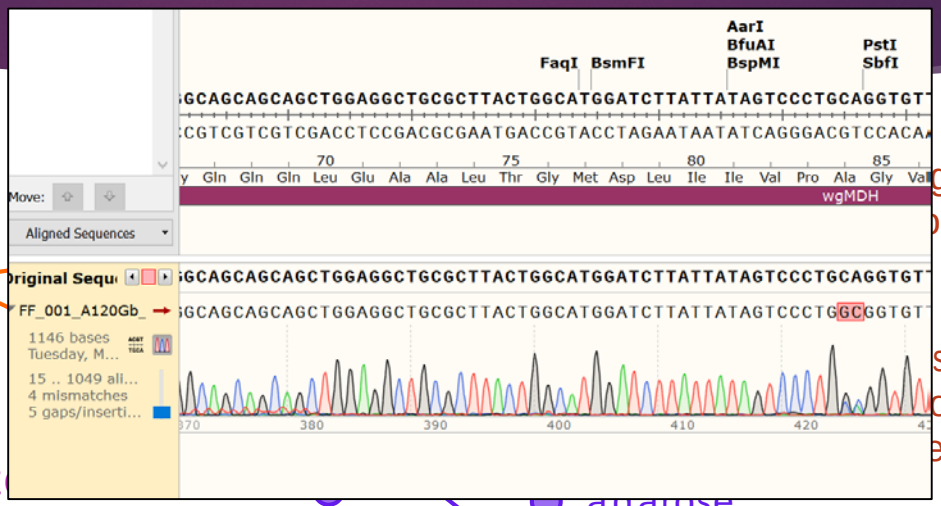
Ligate to form product

History "pedigree" of construct

Molecular Cloning: Examples



Molecular Cloning: Examples



Start

Create DNA file

Genbank ID#

ORFs

Feature annotations

Vect

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agarose gel

Confirm Site-directed mutagenesis from seq data

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Ligate to form product

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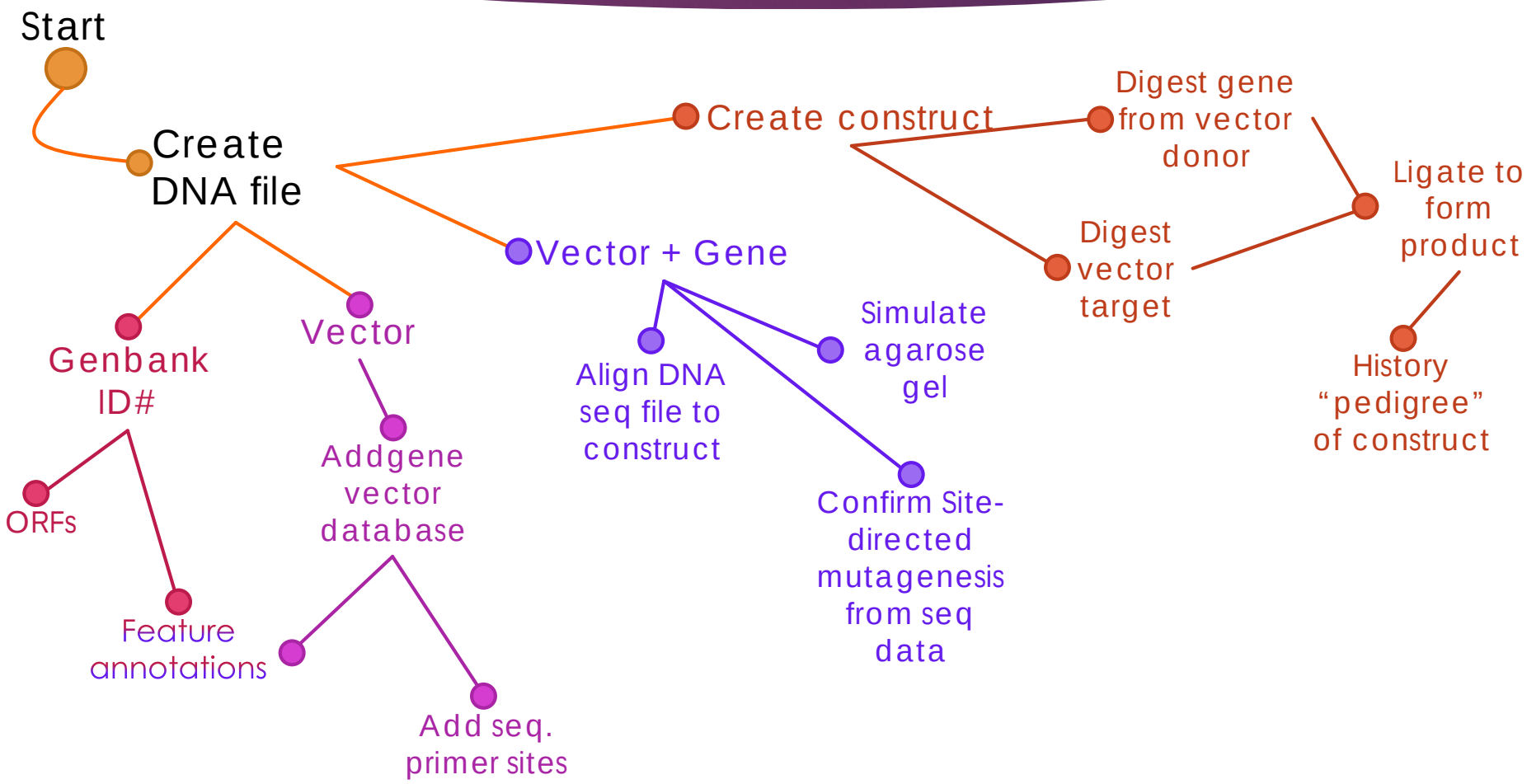
Molecular Cloning: Examples



Molecular Cloning: Examples

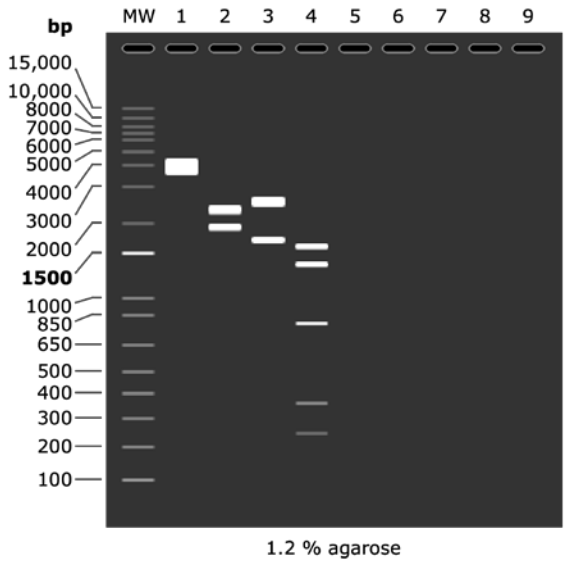
Start

Create DNA file

G

ORFs

Vector



1.2 % agarose

Create construct

Vector + Gene

Align DNA seq file to construct

Simulate agarose gel

Confirm Site-directed mutagenesis from seq data

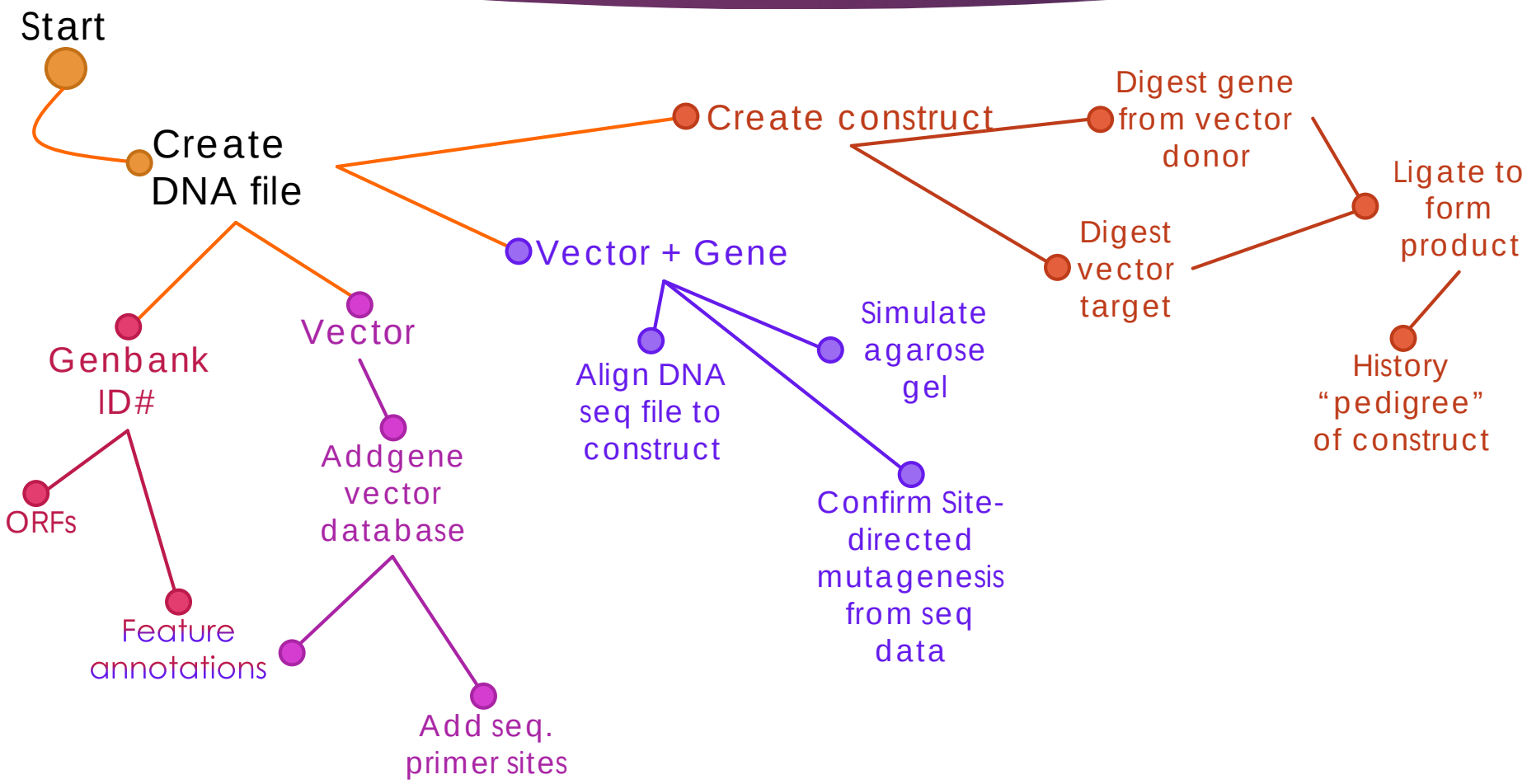
Digest gene from vector donor

Digest vector target

Ligate to form product

History "pedigree" of construct

Molecular Cloning: Examples



Molecular Cloning: Examples

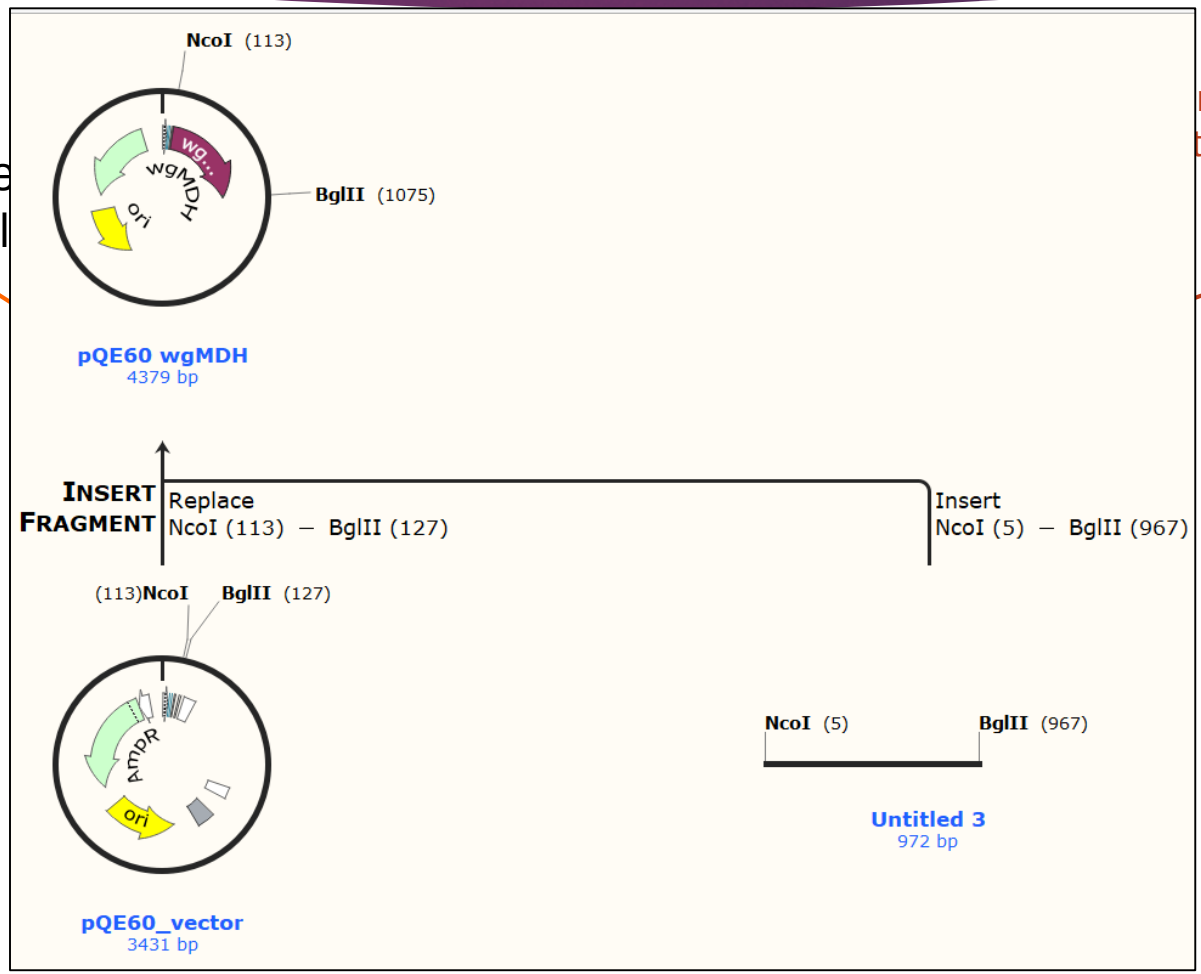
Start

Create
DNA fil

Genbank
ID#

ORFs

Feature
annotations



ne
tor

Ligate to
form
product

History
"pedigree"
of construct

USD Schedule for Hybrid Techniques in Molecular Biology

Tentative Schedule Section 01 – Tuesday Lab:
The schedule is tentative and may change based on the pace of the class. ***Be prepared to adjust depe… …rojects proceed!***

Basic Skills

Adv PCR

Adv Nucleic Acid Manipulation

Independent Projects

Basic Nucleic Acid Manipulation

Basic PCR

Lecture – Monday 8 – 9:30 am

Day	Block	Activity Description	…te	Assign.	NB	Project	Exam
		Introduction, Safety, ELNs, Solns, Lab Math, Sterile & Bacteriological techniques	8/17				
		Bacterial Transformation, Plasmid & components	8/24				
		Restriction enzymes, Intro to cloning and subcloning	8/31				
		Restriction enzymes, Intro to cloning and subcloning					
		Basic PCR	9/14				
		Basic PCR	9/21				
		Sequencing	9/28				
		Adv PCR	10/5				
		Adv PCR	10/12				
		CRISPR systems	10/…				
		CRISPR systems	10/26				
		Wrap-up, Review	11/2				
13		Project Presentations	11/9				

Laboratory – Tuesdays 8 am – 11:50 am

Group 1

In Person			*Virtual*		
1	Pipetting, Sterile/streak/culture technique, Transformation	8/18	1	Standard Operating Procedure	8/25
2	Plasmid DNA Purification	9/1	2	Bioinformatics/SnapGene Intro	9/8
3	Restriction Digest/Agarose gel	9/15	3	Primer design – Lactase Expt.	9/22
4	Lactase expt.	9/29	4	Adv. Primer design; RNASeq and/or CHIPSeq	10/6
5	qPCR	10/13	5	Project Design	10/20
6	Project	10/27	6	COVID-19 Exploration of Mol. Tech Applications	11/3

Group 2

In-Person			*Virtual*		
1	Pipetting, Sterile/streak/culture technique, Transformation	8/25	1	Standard Operating Procedure	8/18
2	Plasmid DNA Purification	9/8	2	Bioinformatics/SnapGene Intro	9/1
3	Restriction Digest/Agarose gel	9/22	3	Primer design – Lactase Expt.	9/15
4	Lactase expt.	10/6	4	Adv. Primer design; RNASeq and/or CHIPSeq	9/29
5	qPCR	10/20	5	Project Design	10/13
6	Project	11/3	6	COVID-19 Exploration of Mol. Tech Applications	10/27
7	Data Analyses; Wrap-up projects				11/10

Final oral exam: 15 min interview, 11/16 8 am – 10 am; 11/17 8 am – 1 pm

Acknowledgements

USD

Chemistry & Biochemistry

Anthony Bell
J. Ellis Bell
Margaret Daley
Joseph Provost

Biology

Curtis Loer
Martha Stauderman

My students for being fabulous beta testers!