9/14/20

Project: VA iGEM 2020 Shared Project
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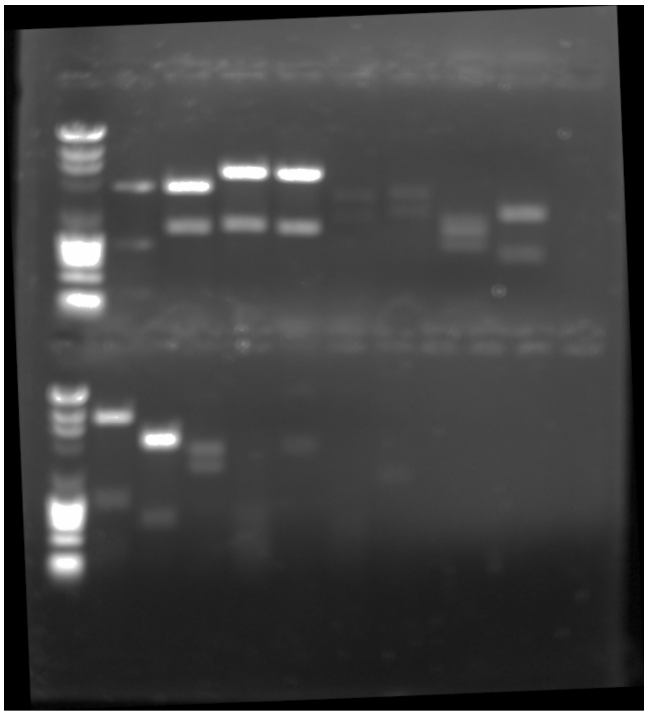
SUNDAY, 9/13/2020

Lab Log

	A	B	C
1	**Procedure**	**Researcher**	**Comments**
2	Plasmid Digestions	CM	See Below for more details Digestion Start Time: 10:17 pm Digestion End Time: 10:48 pm
3	0.8% Agarose Gel Creation	JB SL	Edits were made to the procedure and documented on the procedure on the notebook page. The actual procedure was edited to reflect the recommendations of Prof K and constraints of our gel tray.
4	Agarose Gel Electrophoresis	JB SL CM	Gel was run at 80V
5			
6			

All_Plasmid_Digest_9.14.20.png



Digestions:

Buffer 2.1 = 10x
Buffer 3.1 = 10x
Cutsmart Buffer = 10x

Make Master Mixes:

Enzyme Master Mixes

	A	B	C	D	E	F	G	H
1	Enzyme	Buffer	Enzyme Amount (uL)	Buffer Amount (uL)	Water (uL)	Total Volume (uL)	Volume Per Rxn	# Rxns
2	EcoRI	2.1	2	4	30	36	18	2
3	PstI	3.1	3	6	45	54	18	3
4	SpeI	2.1	2	4	30	36	18	2
5	HindIII-HF	2.1	3	6	45	54	18	3
6	XhoI	2.1	2	4	30	36	18	2
7	AluI	2.1	2	4	30	36	18	2

Use Master Mixes To assemble Following Digestions:

Digestion Reactions

	A	B	C	D	E	F	G	H	I	J	K	L
1		Plasmid	Link	Enzyme	Buffer	DNA Volume	Enzyme Amount (uL)	Buffer Amount (uL)	Water (uL)	Total Volume (uL)		
2	1	GE114	(Addgene #120731) GGE114 (pSB1A3-GB3)	EcoRI	2.1	2	1	2	15	20		
3	2	GE114	(Addgene #120731) GGE114 (pSB1A3-GB3)	PstI	3.1	2	1	2	15	20		
4	3	pHIV_pTp66p51	pHIV_pTp66p51	PstI	3.1	2	1	2	15	20		
5	4	pHIV_pTp66p51	pHIV_pTp66p51	SpeI	2.1	2	1	2	15	20		
6	5	pMLRT	pMLRT	PstI	3.1	2	1	2	15	20		
7	6	pMLRT	pMLRT	SpeI	2.1	2	1	2	15	20		
8	7	pSB1K3 containing mRFP1	pSB1K3 containing mRFP1	HindIII-HF	2.1	2	1	2	15	20		
9	8	pSB1K3 containing mRFP1	pSB1K3 containing mRFP1	XhoI	2.1	2	1	2	15	20		
10	9	pSB1C3-Lux	(Addgene #109383) pSB1C3-Lux	HindIII-HF	2.1	2	1	2	15	20		
11	10	pSB1C3-Lux	(Addgene #109383) pSB1C3-Lux	XhoI	2.1	2	1	2	15	20		
12	11	pAC-4CL1	pAC-4CL1	EcoRI	2.1	2	1	2	15	20		
13	12	pAC-4CL1	pAC-4CL1	AluI	2.1	2	1	2	15	20		
14	13	pUC-STS	pUC-STS	HindIII-HF	2.1	2	1	2	15	20		
15	14	pUC-STS	pUC-STS	AluI	2.1	2	1	2	15	20		

*NEB Time-Saver qualified Restriction Enzyme Digestion

Introduction

Restriction Enzyme Digestion

Materials

- 10X NEBuffer
- DNA
- Restriction Enzyme
- Nuclease-free Water
- Time:
  - 5-15 min or overnight if needed

Procedure

Steps

1. Find Buffer Needed For Enzyme Here: https://www.neb.com/tools-and-resources/usage-guidelines/nebuffer-performance-chart-with-restriction-enzymes
2. Set up reaction as follows:

Table1

	A	B
1	COMPONENT	50 µl REACTION
2	DNA	1 µg
3	10X NEBuffer	5 µl (1X)
4	Restriction Enzyme	1.0 µl (20 units)†
5	Nuclease-free Water	to 50 µl

3. Incubate at 37°C for 5–15 minutes.

*Agarose Gel Electrophoresis

Introduction

https://www.addgene.org/protocols/gel-electrophoresis/

Materials

- TAE (one liter 50X stock)
  - Tris-base: 242 g
  - Acetate (100% acetic acid): 57.1 ml
  - EDTA: 100 ml 0.5M sodium EDTA
  - Add MilliQ H_2O up to one litre.
  - To make 1x TAE from 50X TAE stock, dilute 20ml of stock into 980 ml of DI water
- Agarose
- Ethium bromide (stock concentration of 10 mg/mL)

Procedure

Pouring a Standard 0.8% Agarose Gel:

- [x] 1. **See *0.8% Agarose Gel Creation and Pour Procedure**

Loading Samples and Running an Agarose Gel:

- [x] 2. Add loading buffer to each of your DNA samples. (*4 microliters of 6x loading buffer*)
- [x] 3. Once solidified, place the agarose gel into the gel box (electrophoresis unit).

  The gel was made in the gel box.
- [x] 4. Fill gel box with 1xTAE (or TBE) until the gel is covered.

  Remove combs by pulling straight up.
- [x] 5. Carefully load a molecular weight ladder into the first lane of the gel. *10 microliters Quick-Load DNA Marker Broad Range*

  Pro-Tip Remember, if you added EtBr to your gel, add some to the buffer as well. EtBr is positively charged and will run the opposite direction from the DNA. So if you run the gel without EtBr in the buffer you will reach a point where the DNA will be in the bottom portion of the gel, but all of the EtBr will be in the top portion and your bands will be differentially intense. If this happens, you can just soak the gel in EtBr solution and rinse with water to even out the staining after the gel has been run, just as you would if you had not added EtBr to the gel in the first place.

Note: When loading the sample in the well, maintain positive pressure on the sample to prevent bubbles or buffer from entering the tip. Place the very top of the tip of the pipette into the buffer just above the well. Very slowly and steadily, push the sample out and watch as the sample fills the well. After all of the sample is unloaded, push the pipettor to the second stop and carefully raise the pipette straight out of the buffer.

- [x] 6. Carefully load your samples into the additional wells of the gel.

   We had 16 samples.
   **note* well 2 the tip was moved slightly and some sample was sloshed*

- [ ] 7. Run the gel at *80-100 V* until the dye line is approximately 75-80% of the way down the gel. Typical run time is about 1-1.5 hours, depending on the gel concentration and voltage. *We used 80 V and ran it for 48 minutes and 30 seconds.*

   Note: Black is negative, red is positive. The DNA is negatively charged and will run towards the positive electrode. **Always Run to Red.**

- [ ] 8. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.

- [x] 9. ~~*(Optional)* If you did not add EtBr to the gel and buffer, place the gel into a container filled with 100 mL of TAE running buffer and 5 µL of EtBr, place on a rocker for 20-30 mins, replace EtBr solution with water and destain for 5 mins.~~

- [ ] 10. ~~Using any device that has UV light, visualize your DNA fragments.~~ *Use the **Gel Doc EZ Image.** Go to "Application" select "Nucleic Acid Gels" then "Ethidium Bromide". For "Image Exposure" use "Automatically optimize for faint bands". For "Display Options" use "EtBr" for "Color Option".*

   The fragments of DNA are usually referred to as ‘bands’ due to their appearance on the gel.
   Pro-Tip If you will be purifying the DNA for later use, use long-wavelength UV and expose for as little time as possible to minimize damage to the DNA.