10/05/20

Project: VA iGEM 2020 Shared Project
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Created at: 2020-10-05T23:42:48.119524+00:00

MONDAY, 10/5/2020

Lab Log

	A	B	C
1	**Procedure**	**Researcher**	**Comments**
2	PCR Purification	VG	Purify PCR 3 from 10/3 and 2 DNA Team PCR from 10/2 Nanodrop Concentration: PCR 3 from 10/3: 1 ng/uL
3	Enzyme Digest	CH	Digested pNUT and pNU with pstI for further comparison (first gel needed clarity)
4	Agarose Gel	CH VG	Look at protocol for more info on what was run
5			
6			

*Agarose Gel Electrophoresis

Introduction

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

see the procedure on 9/14/20 to see the updates to this procedure based on practice and advice from Prof. K

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 1% Agarose Gel:~~

1. **Don't do this!!!!! Instead see *0.8% Agarose Gel Creation and Pour Procedure**
2. ~~Measure 1 g of agarose.~~
3. ~~Mix agarose powder with 100 mL 1xTAE in a microwavable flask.~~

   ~~Agarose gels are commonly used in concentrations of 0.7% to 2% depending on the size of bands needed to be separated – see FAQs below. Simply adjust the mass of agarose in a given volume to make gels of other agarose concentrations (e.g., 2 g of agarose in 100 mL of TAE will make a 2% gel).~~
4. ~~Microwave for 1-3 min until the agarose is completely dissolved (but do not overboil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel. Many people prefer to microwave in pulses, swirling the flask occasionally as the solution heats up).~~
5. ~~Let agarose solution cool down to about 50 °C (about when you can comfortably keep your hand on the flask), about 5 mins.~~

   ~~It is a good idea to microwave for 30-45 sec, stop and swirl, and then continue towards a boil. Keep an eye on it the solution has a tendancy to boil over. Placing saran wrap over the top of the flask can help with this, but is not necessary if you pay close attention.~~
6. ~~*(Optional)* Add ethidium bromide (EtBr) to a final concentration of approximately 0.2-0.5 µg/mL (usually about 2-3 µl of lab stock solution per 100 mL gel). EtBr binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light.~~

~~***CAUTION:** EtBr is a known mutagen. Wear a lab coat, eye protection and gloves when working with this chemical.*~~
~~***Note:** If you add EtBr to your gel, you will also want to add it to the running buffer when you run the gel. If you do not add EtBr to the gel and running buffer, you will need to soak the gel in EtBr solution and then rinse it in water before you can image the gel.*~~

7. ~~Pour the agarose into a gel tray with the well comb in place.~~

8. ~~Place newly poured gel at 4 °C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.~~

Loading Samples and Running an Agarose Gel:

9. Add loading buffer to each of your DNA samples.

10. Fill gel box with 1xTAE (or TBE) until the gel is covered.

11. Remove combs by pulling straight up.

12. Carefully load a molecular weight ladder into the first lane of the gel.

    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.*

13. Carefully load your samples into the additional wells of the gel.

14. Run the gel at 80-150 V until the dye line is approximately 75-80% of the way down the gel. A typical run time is about 1-1.5 hours, depending on the gel concentration and voltage.

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

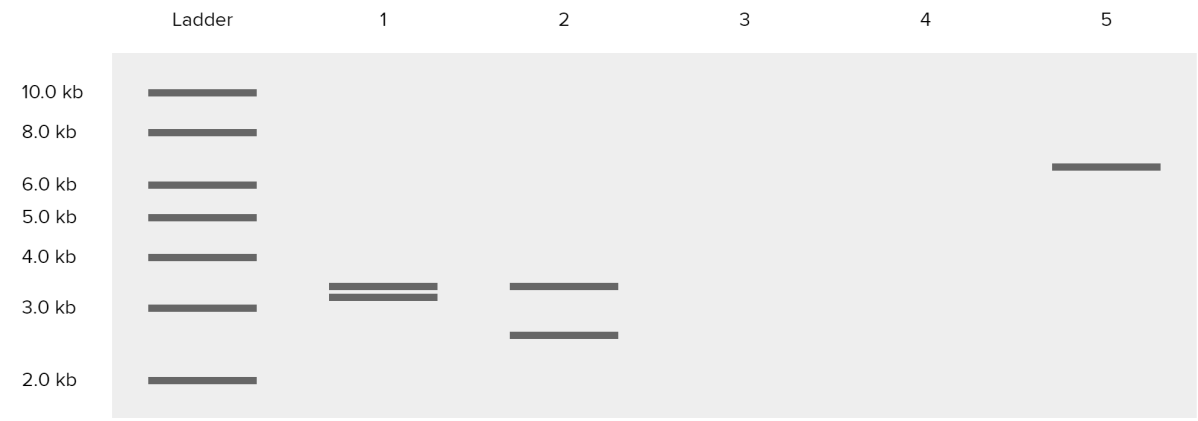
15. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.

16. *(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.

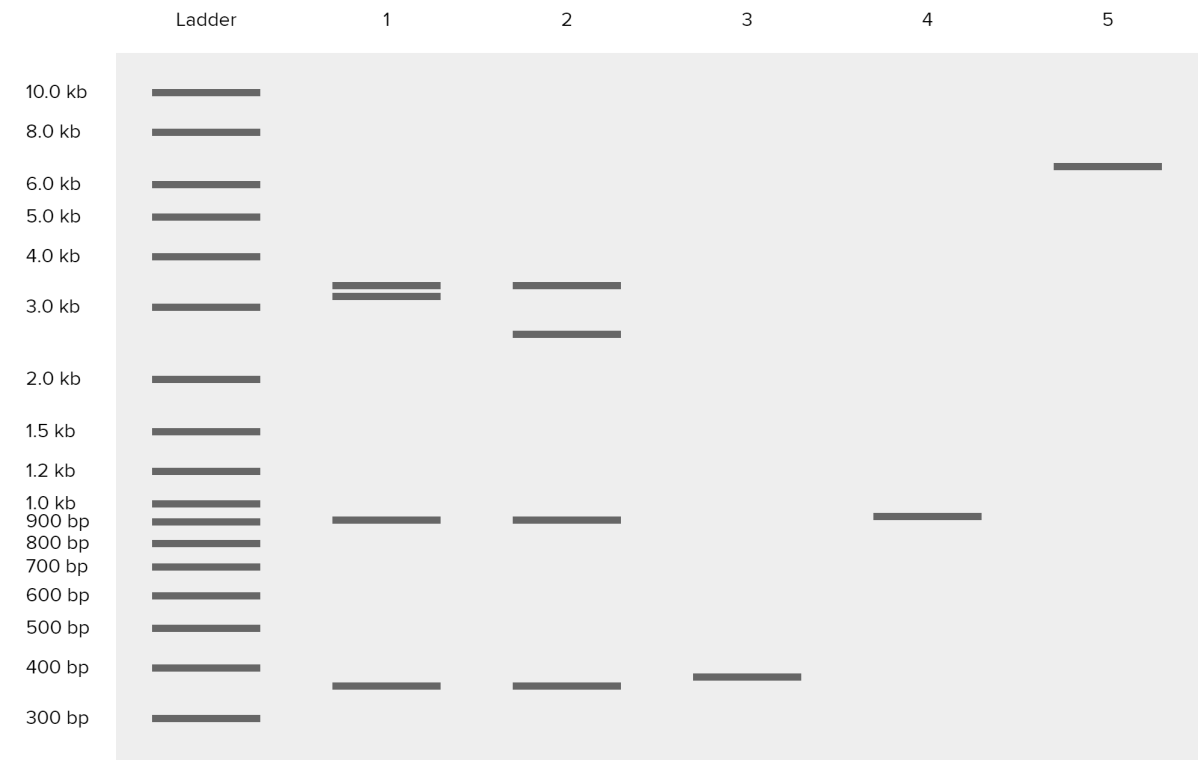
17. Using any device that has UV light, visualize your DNA fragments. The fragments of DNA are usually referred to as ‘bands’ due to their appearance on the gel.

    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.

Ladder	NEB 1kb PLus
1	pET-pduABJKNUT - PstI
2	pET-pduABJKNU (Steffi's) - PstI
3	Frag 1 PCR 1 - PsuI
4	Frag 2 PCR 2 - PsuI
5	Frag 3 PCR 3 - RsrII



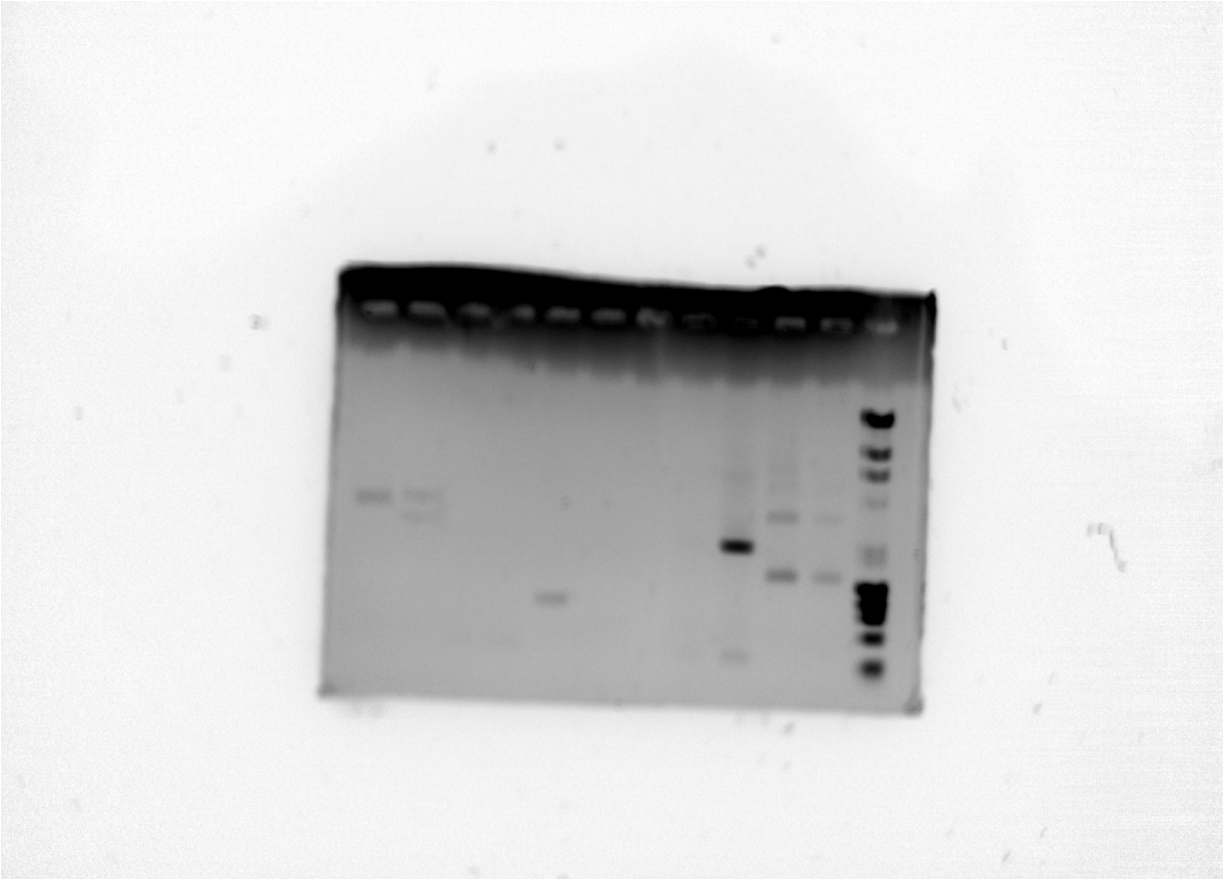
✔ 18.



✔ 19.

Table1

	A	B	C	D	E	F	G	H	I	J	K	L
1	Well 1	2	3	4	5	6	7	8	9	10	11	12
2	pduABJKNUT	pduABJKNU	BMC PCR Frag 1 Run 1	BMC PCR Frag 1 Run 2	BMC PCR Frag 2	BMC PCR Frag 3	Empty	Collin PCR Run 1 (*rxn name rubbed off)	Collin PCR Run 2	pHIV BB PCR 1	pHIV BB PCR 2	Ladder



20.

PCR Purification Kit (GenScript)

Introduction

Source: https://www.genscript.com/site2/document/13324_20100901221333.PDF

Done to 3A

Materials

- Binding Buffer
- Wash Buffer
- Elution Buffer
- Spin Columns
- 1.5 mL tubes

Procedure

Using Materials from the Kit:

1. Transfer PCR reaction product to 1.5 mL micro-centrifuge tubes
2. Add 2 volumes of Binding Buffer to 1 volume of PCR or enzymatic reaction product (ie. if your PCR product is 50 uL, add 100 uL of Binding Buffer)

   Note: Do not exceed 200 uL of Binding Buffer
3. Apply mixture to Spin Column by pipetting, centrifuge for 1 min at 6,000 x g
4. Discard all flow-through and place the column back in the same tube
5. Wash the Spin column by 650 uL Wash Buffer in centrifuge for 30-60 sec at 12,000 x g. Discard flow-through liquid and **repeat Step 5 again.**
6. Centrifuge for an additoinal 1 minute at 12,000 x g and transfer the Spin column to a sterile 1.5 mL micro-centrifuge tube.
7. Add 50 uL Elution Buffer to the center of the Spin column and let stand for 1 min at room tempterature, then centrifuge for 1 min at 12,000 x g.
8. Store the micro-centrifuge tube containing purified plasmid DNA at -20*C if not using immediately.

*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. Used PstI and 3.1 Buffer
3. Set up reaction as follows:

Table1

	A	B	C	D
1	COMPONENT	30 µl REACTION	Actual	
2	DNA	1 µg	1 ul	
3	10X NEBuffer	3 µl (1X)	3 ul	
4	Restriction Enzyme	1.0 µl (20 units)†	1 ul	
5	Nuclease-free Water	to 30 µl	25 ul	

4. Incubate at 37°C for 5–15 minutes.