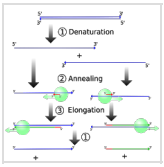

Oct 13, 2020

Protocol 2: PCR Wet Lab

Forked from Protocol 2: PCR Wet Lab

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[1]UCSC

1 *Works for me* This protocol is published without a DOI.

UCSC BME 22L

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PROTOCOL CITATION

2020. Protocol 2: PCR Wet Lab . **protocols.io**
https://protocols.io/view/protocol-2-pcr-wet-lab-bnddma26

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Forked from Protocol 2: PCR Wet Lab , Alyssa Ayala

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CREATED

Oct 13, 2020

LAST MODIFIED

Oct 13, 2020

PROTOCOL INTEGER ID

43141

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BEFORE STARTING

We will be using protocols from the Bento lab website.

Hazards:

Careful when handling hot agarose gel. If there is no protective gear, do not attempt to pick up the hot container. Wait some time for it to cool down where the container is bearable to touch, around 55°C.

Careful when loading the centrifuge. Counter weight must always be used to balance the microcentrifuge. Correct loading is necessary for microcentrifuge function. Incorrect loading can lead to injuries and permanent damage to the rotor.

BentoLab set-up

PCR description and machinery usage

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PCR: Components and Physical layout
Centrifuge: Components and Physical layout
Gel electrophoresis and Transilluminator: Components and Physical layout

Timeline

Day 1 (2.75 hours)

DNA extraction (30 min)

- Roughly ~17 minutes hands on
- 10 minutes thermocycler heating
- 6 minutes centrifugation

PCR (2.25 hours)

- Roughly 15 minutes hands on
- 2 hours of thermocycler

Day 2 (2-2.5 hours)

Gel electrophoresis (1.5 hours)

- 20 minutes hands on
- 30 minutes gel cooling

40 running the gel

1 DNA Extraction

DNA Extraction: Saliva (Bento lab)

This protocol extracts DNA from Saliva using a microcentrifuge and thermocycler. The microcentrifuge separates the cells in the sample into a pellet at the bottom of the microcentrifuge tube. Extra liquid is drawn out of the microcentrifuge tube. Cells are then heated in the thermocycler and placed in the microcentrifuge to separate DNA from other cellular material.

2 PCR

Lactose intolerance: PCR (Bento lab)
Bitterness tasting: PCR (Bento lab)
Athlete gene: PCR (Bento lab)

This protocol uses a thermocycler in order to amplify sections of DNA genes. Students will amplify genes specific to lactose intolerance, bitterness tasting, and an athletic gene. DNA template is extracted from the previous protocol and added to a PCR master mix and designated primer. PCR master mix is composed of Taq polymerase, dNTPs, MgCl2, and pH buffer. After adding all reagents, students will set parameters for the thermocyclers. Each PCR experiment will have different parameters due to the nature of its respective primers.

3 Gel Electrophoresis

Gel electrophoresis (Bento lab)

This protocol uses a gel electrophoresis to identify the amplified sequences from the previous protocol, PCR. Students will set up their gel electrophoresis by creating an agarose gel with necessary lanes, adding 0.5X TBE buffer, and attaching electrodes. After set up, DNA ladders and amplified DNA will be added to lanes in the gel. The gel will run at 50V for 40 minutes. Then students will analyze the results and determine their allele type and phenotype.

3.1 **Take a picture** of your gel electrophoresis results and upload it to your Lab Notebook.

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