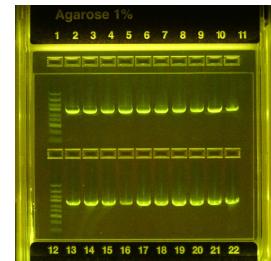


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E-Gel Protocol EFGL

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Protocol status: Working

We use this protocol and it's working

Created: July 12, 2024

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Protocol Integer ID: 103325

Abstract

The purpose of this protocol is to determine successful polymerase chain reaction (PCR) amplification. This is achieved by preparing PCR sample and ladder droplets, preparing the E-Gel™ Power Snap, depositing all samples into appropriate lanes on an E-gel of the E-gel, and viewing the results on the E-Gel™ Power Snap Camera. This will result in an image of a gel, that has a ladder that can be compared to the desired PCR samples.

Materials

- PCR Sample(s)
- Single channel pipette (2-20 µL)
- Pipette tips
- E-Gel 1 Kb Plus DNA Ladder
- Lab grade nucleus free water
- E-Gel Agarose cassette
- PCR Product
- Parafilm wax paper
- Tape
- Kim wipe
- Flash drive
- E-Gel™ Power Snap and Camera
- Gloves

Safety warnings

 *This machine uses UV that will damage your eyes if you don't use the protective orange screen as intended.*

WARNING

- 1 *This machine uses UV that will damage your eyes if you don't use the protective orange screen as intended.*

Required Materials:

- 2
 - PCR Sample(s)
 - Single channel pipette (2-20 µL)
 - Pipette tips
 - E-Gel 1 Kb Plus DNA Ladder
 - Lab grade nucleus free water
 - E-Gel Agarose cassette
 - PCR Product
 - Parafilm wax paper
 - Tape
 - Kim wipe
 - Flash drive
 - E-Gel™ Power Snap and Camera
 - Gloves

Protocol:

- 3 Prepare the working stock of E-Gel 1 Kb Plus DNA Ladder, it is stored in the EFGL primers fridge
 - a. Vortex and spin
- 4



E-Gel Ladder

- 5 Prepare the PCR Sample(s)

- a. Vortex and spin PCR sample(s)
- b. At EFGL, E-Gels are used to confirm successful PCR amplification

6 Prepare working space

- a. Vortex and spin PCR sample(s)
- b. At EFGL, E-Gels are used to confirm successful PCR amplification

7 Prepare working space

- a. Secure Parafilm wax paper to bench top with tape

8 Choose an appropriate E-Gel Agarose cassette with SYBR stain, and the appropriate agarose percentage.

- a. DO NOT OPEN CASSETTE PACKAGE (at this point)
- b. At EFGL there are at least two options, a single comb (11 lanes) or a double comb (22 lanes).

The single comb is NOT obviously marked. If you are uncertain, with the package closed, you

should be able to feel one lump (single comb) or two lumps (double comb) through the front

side of the package.

- c. Choose which one is less wasteful based on how many samples you have, and the type of band

resolution you need. Remember, you will have to fill empty wells with water

- b. These are in the cabinet below the E-Gel station

9



10 Prepare the E-Gel™ Power Snap

WARNING – *This machine uses UV that will damage your eyes if you don't use the protective orange screen as intended.*

- a. There are two parts to the E-Gel™ Power Snap
- i. The E-Gel™ Power Snap, which is where you will insert the cassette and has the orange UV protective screen.
 - ii. E-Gel™ Power Snap Camera, which detaches from the machine. It is used to view the gel progress and take images.

11



12



13



14 Prepare the E-Gel™ Power Snap (continued)

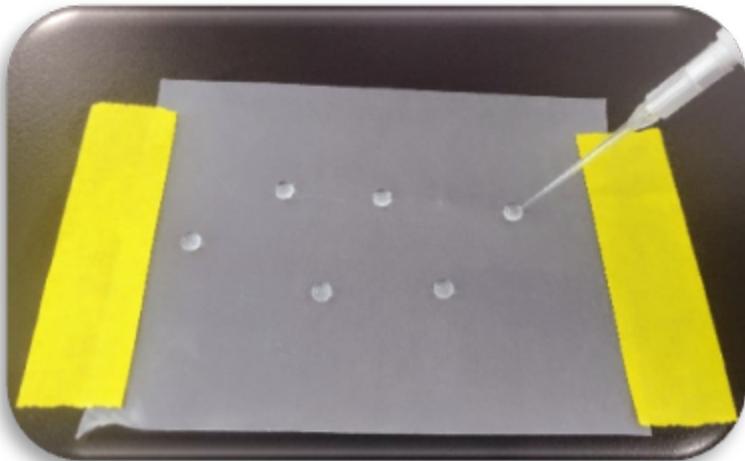
- a. With the machine closed and the camera sitting on top, flip the switch at the back of the machine to make sure it is plugged in, and it turns on.
- b. Insert your flash drive to make sure it syncs with the machine.
- c. **TURN OFF THE MACHINE**, by flipping the switch at the back again.

15 *Note: These small samples evaporate quickly. Fully prepare your supplies first so you can move through the next steps efficiently.*

16 Prepare Sample(s) for E-gels

- a. On the wax paper, aliquot out 18 µL of water for the ladder and each sample.
- b. Dispense 2 µL of the ladder into the first 18 µL water droplet
- c. Dispense 2 µL PCR product into the corresponding 18 µL water droplets
 - i. Use a new tip every time
 - ii. Each droplet should only have a total volume of 20µL.

17



18 Unwrap the E-Gel

19 Remove the comb protector by popping it off from the edges of the cassette

20 Check the cassette for irregularities.

- a. Clean the cassette face with a Kim wipe if you splashed any liquid when removing the comb protector

- b. The gel should be clear and transparent
- 21 Load the cassette onto the machine
- a. MACHINE OFF
 - b. Lift off the camera
 - c. Open the orange protective screen, by pressing the white latch release button under the warning symbol
 - d. With the comb openings facing up, lower the right-hand side of the cassette into the machine first. Then press the left-hand side down until it clicks into place.
- 22 Moving quickly, use the single channel pipette set to 20 µL to transfer samples to their respective wells
- a. Careful not to overfill the wells
 - b. Careful not to pierce the gel walls
 - c. Use a new tip every time
- 23 In empty wells, add 20 µL of water.
- a. Use a new tip every time
- 24 When the gel is loaded, snap the lid close and turn ON the machine.
- 25 Select the appropriate E-Gel program.
- a. Based on the agarose%, single/double comb, and stain
- 26 Begin the run.
- 27 Place the camera back onto the machine
- 28 Capture and export images to a flash drive throughout the gel run.
- 29

Gel Type	Recommended Program	EFGL's Suggested Run Time	Maximum Run Time
E-Gel™ EX Agarose Gel, 1% and 2%	E-Gel EX 1-2%	1-4 min	20 min
E-Gel™ EX Double Comb Agarose Gels, 1% and 2%	E-Gel EX 1-2%	1%: 4 min 2%: 6-7 min	8 min
E-Gel™ Double Comb SYBR™ Safe Agarose Gels, 1% and 2%	E-Gel Double Comb	13 min	18 min

