Ciências ULisboa | Biologia Vegetal

Fisiologia Molecular do Stress

Laboratory classes
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Analysis of the results of RNA extraction using agarose gel electrophoresis

You will need an electrophoresis equipment, power supply, agarose, TAE buffer, GreenSafe, MW marker, UV transilluminator

1- Assemble the comb in the tray.

2- Prepare a 1% agarose solution in electrophoresis buffer (TAE 1X), in a final volume of 100 mL in an Erlenmeyer.

3- Heat the agarose solution in the microwave until the agarose is completely fused. Wear protective gloves because of the heat. Let the solution cool until it doesn't burn your hands and add the GreenSafe (5 uL). Mix gently.

4- Pour the agarose solution into the assembled tray and wait for the gel to cool and polymerize.

5- Carefully take off the comb and put the gel in the electrophoresis tank. Add electrophoresis buffer until the surface of the gel is covered with buffer.

6- Prepare the RNA samples: mix RNA samples (1000 ng) with 6X loading buffer

7- Transfer the samples to the wells of the gel using a micropipette. Load the MW marker (4 µL) on the first well.

8- Put the lid on the electrophoresis tank and turn on the power supply. Adjust the voltage to 80V (constant voltage) and start the run.

9- Check the run until the stain from the loading buffer reaches the bottom of the gel. Turn off the power supply. Take the gel off the tank and transfer into the transilluminator. Turn on the transilluminator (UV light) and photograph the gel.