### Today’s Tasks

#### First task: Design PCR reactions.

See page 163 of your lab manual. Make sure you understand which primers to use.

#### Secnd Task: Program the thermocycler.

The manufacturer recommends:

|  |  |  |  |
| --- | --- | --- | --- |
| **Cycle step** | **Temp** | **Time** | **Cycles** |
| Initial denaturation | 98°C | 5 min | 1 |
| DenaturationAnnealingExtension | 98°C\_\_°C72°C | 5 sec5 sec20 sec/kb | 40 |
| Final extension | 72°C | 1 min | 1 |

#### Third Task: Choose Salk plants to genotype

You will need DNA from 11 Salk plants. Pick those whose genotypes you ***most*** wish to know. This may be any combination of seedlings grown on soil or on plates. Your 12th sample will be from a potted wild type plant. If reagents remain, you may set up one reaction with your previously prepared gDNA.

#### Fourth Task: Prepare your primers.

Your instructor will tell you how far the left border primer is from the left border, its Tm, and its concentration.

If you ordered new primers, multiply the number of nmol of in the vial by 10 to give you the volume of sterile water to add to produce a 100 µM stock.

Dilute this stock[[1]](#footnote-1) to make a working solution of 10 or 12.5 µM (match the concentration of the left border primer).

#### Fifth Task: Prepare template DNA from plants

Young leaves are recommended.

Take one small leaf or a piece of leaf (no more than 1mg, about 2mm square) and place it in 20 µL of Dilution Buffer in a clean 1.5 mL tube.

Crush the leaf sample with a sterile 100 µL pipette tip by pressing it briefly against the tube wall.

After the leaf is crushed, the solution should be greenish in color. Spin the plant material down briefly (10 sec). You will use 0.5 µL of the supernatant as a template for a 20 µL PCR reaction. Keep samples on ice.

***Before going on to the next leaf sample, clean your fingers or change your gloves to prevent cross-contamination.***

#### Sixth Task: Set up PCR reactions

Make 2 master mixes: one for your two gene-specific primers and one for the Salk primer. As usual, make enough for one more reaction than you actually intend to perform.

|  |  |  |  |
| --- | --- | --- | --- |
| **Component** | **per 20 µL rxn (µL)** | **Master mix for \_\_\_ rxns (µL)** | **Final conc** |
| 2× Phire Plant PCR Buf | 10 |  | 1× |
| L primer[[2]](#footnote-2) | [[3]](#footnote-3) |  | 0.5 µM |
| R primer |  |  | 0.5 µM |
| LBP |  |  | 0.5 µM |
| Phire hot start polymerase[[4]](#footnote-4) | 0.4 |  |  |
| Plant squish supernate | 0.5 | don’t add this to master mix! |  |
| sterile H2O to final vol | to make 20 µL | To make 20×#rxns |  |

Set up the individual reactions

Aliquot 19.5 µL master mix to each of your pcr tubes, then add 0.5 µL of each crushed plant supernate to the appropriate tube. ***Be careful not to cross contaminate—be sure to change tips between every tube.***

You are now ready to run your reactions. Carry your reactions ON ICE over to the pre-programmed thermocycler. Start the cycle running. When the temperature of the block reaches 98°C, place your reactions in the block, and close the lid.

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1. $v\_{i}=\frac{v\_{f}×c\_{f}}{c\_{i}}$ [↑](#footnote-ref-1)
2. One master mix contains GSP1 + GSP2; the other one contains LBP + the appropriate GSP. Be sure you know which primers go in which master mix. [↑](#footnote-ref-2)
3. Figure out how much you need of each primer, based on the concentration of the working stock, the final volume you are trying to make, and the 0.5 µM final concentration. $v\_{i}=\frac{v\_{f}×c\_{f}}{c\_{i}}$ [↑](#footnote-ref-3)
4. ***Add last! Keep on ice! Keep the master mixes cold at all times.*** [↑](#footnote-ref-4)