# PCR protocol

## (plant DNA detection from leafhoppers)

1. Turn the thermocycler on (the black one on the left)
2. Take out from the freezer: PCR PreMix, primers (working solution; 4 µM)
3. Prepare on the bench: $ddH_2O$ (in the right drawer), one 1.5 ml microcentrifuge tube.
4. Prepare PCR cocktail (#reactions = #samples+1; to cover pipetting error):

| **PCR reagent** | **1 reaction** | **5 reactions** | **10 reactions** |
|---|---|---|---|
| PCR PreMix | 10 µl | 50 µl | 100 µl |
| Primer 1 (2 µM) | 2 µl | 10 µl | 20 µl |
| Primer 2 (2 µM) | 2 µl | 10 µl | 20 µl |
| $ddH_2O$ | 5.2 µl | 26 µl | 52 µl |

5. Prepare 0.2 ml PCR strip tubes (in the drawer, next to the window)
6. Take DNA samples out from the fridge.
7. Place **19.2 µl** of PCR cocktail in each tube.
8. Add **0.8 µl** of a DNA sample in each tube. Mixed by pipetting.
9. Place strip tubes in the thermocycler (in any block: right or left)
10. Click on "RUN" -> "PROCEED". "Under "MAIN" find protocol ALINA1LH. Run it.

    PCR settings:

    - $94^0$C for 4 min
    - ×35 cycles:
      $94^0$C for 30 sec
      $57^0$C for 30 sec
      $72^0$C for 30 sec
    - $72^0$C for 2 min
    - Hold at $4^0$C for 9 hours

11. To stop the protocol, press on "CANCEL". Turn the thermocycler off. Take the tubes out and place them in the freezer.

* Annealing temperature and amount of DNA in a tube can be adjusted if needed.

** After step 11 you can proceed immediately to gel electrophoresis if needed.