PCR protocol

(DNA barcoding of isopods)

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 (4 µM)	2 µl	10 µl	20 µl
Primer 2 (4 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 ISOTEST1. Run it.

    PCR settings:

    - 94^0C for 3 min
    - ×34 cycles:
        94^0C for 45 sec
        48^0C for 45 sec
        72^0C for 1 min
    - 72^0C for 3 min
    - Hold at 4^0C 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.

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