


Protocol

1. Take the  to transfer 1 mL milk into the green tube.
2. Take out the thermos flask and fill with water.
3. Place the immersion heater inside the thermos flask and connect to electricity.




4. Wait until the water boils and place the green tube inside the thermos flask - let it boil for 10 minutes.

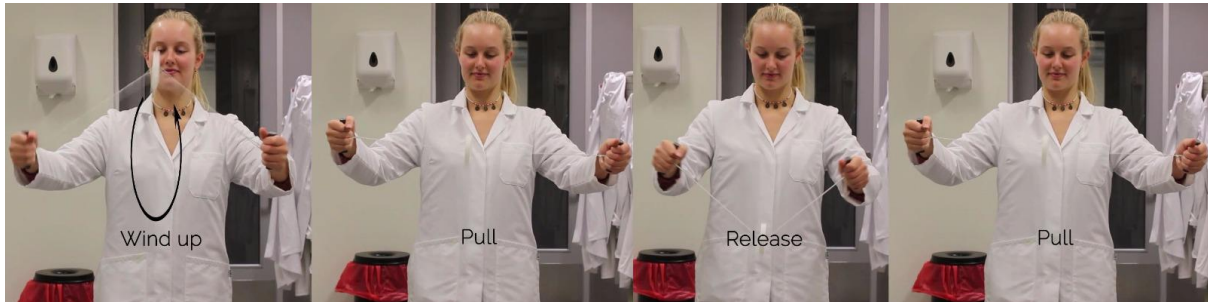


5. Disconnect the immersion heater and wait 15 seconds before lifting; scoop out the tube while lifting.



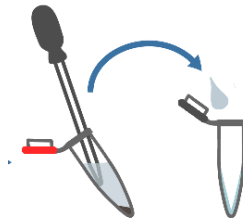
6. Transfer with  0.5 mL from the green tube into the red tube.

7. Centrifuge for 5 minutes.



8. Pipette with  32 μ L of the mixture A into the white tube with dried content

9. Pipette with  6.9 μ L of the red tube as shown in the picture into the white tube



10. Take out the thermometer and bring the water in the thermos flask to 45C.



11. Place the white tube in the thermos flask and close the lid

12. Call up *External support* and wait for 3 hours

-----External support arrives-----

13. Add DNase I (quantity depends on volume of RPA mix, 0.05 wt% final concentration).

14. Purify the RNA using the RNeasy Minelute (Qiagen) columns and the centrifuge. Measure the RNA concentration. Ideally, it should be around ~30 ng/μL but it will probably be around 300 ng/μL.

15. Add your purified RNA to a final concentration of 0.3 ng/μL to a dried powder that mixture of 0.05 wt% (or 50 nM) Cas13a, 0.3 ng/μL crRNA, 0.1 wt% polyU and buffer (40 mM Tris-HCl, 60 mM NaCl, 6 mM MgCl₂, pH 7.3).

16. Adjust the final volume of this reaction mixture to 45 μL using nuclease free water.

-----External support leaves -----

17. Put the immersion heater in the thermos flask and bring to 37 °C

18. Place the tube received from the external support in the thermos flask and close the lid.

19. Wait for 1 hour.

20. Add 5 μL of mixture to the tube, if the solution in the tube becomes turbid, no antibiotic resistance is present, if the solution stays clear, antibiotic resistance is detected!

antibiotic resistance revealed



no antibiotic resistance

