RNA extraction from punches

Punch homogenizing and prep for RNA extraction

Material:

- TRIzol Reagent (4°C, foil because light sensitive)
- Chloroform - yellow cabinet
- Ethanol 70% (4°C)
- QIAGEN RNeasy mini kit – RNA room
- Timer

Make sure that the centrifuge is at 4°C.

1. Add 200uL of TRIzol Reagent to the eppendorf with the punches.
2. Homogenize tissues with homogenizer (RNA room).
3. Incubate 5 min at RT.
4. Add 40uL of chloroform in each Eppendorf.
5. Shake VIGOROUSLY by hands for 15 seconds (DO NOT VORTEX).
6. Incubate at RT for 3 min.
7. Centrifuge 12000G for 15min at 4°C.
   After this step, you will see 3 phases in the tube: Lower red phase, central opaque phase and upper clear phase that contains RNA.
8. During that time, prepare new Eppendorfs with 500uL of 70% ethanol.
9. Transfer the clear upper phase in a new Eppendorf.

RNA extraction

Prepare collection tube + filter, collection tube alone and Eppendorf (1 for each condition). Spray everything with RNAse solution, prepare the sterile field and add the blue pad.

1. Transfer RNA solution to Qiagen RNeasy minikit RNA binding columns - 700 uL at a time. If you have more, repeat steps 1 and 2.
2. Centrifuge 12000g for 15s
3. Discard flow through (inside the tube and add 700 uL wash buffer 1 to column.
4. Centrifuge 12000g for 15s
5. Discard flow through (inside the tube and add 700 uL wash buffer 2 to column (make sure ethanol has been added in the solution!)
6. Centrifuge 12000g for 15s
7. Repeat steps 5 and 6.
8. Discard flow through and centrifuge again at 12000g for 1 min to dry column.
9. Transfer column to labelled RNA collection tube.
10. Add 30uL RNAse-free water to column and let incubate for 3 - 5 min.
11. Centrifuge 12000g for 2 min.
12. Analyze RNA yield (ng/mL) and quality (260/280) with nanodrop.
13. Store RNA samples at -80°C indefinitely.

Protocol for the Nanodrop (7th floor)

Preparation

1. Important to wear new gloves every time we use it.
2. Clean everything with ethanol before and after use.
3. Clean with ethanol both sides of the 2 ul wells on the sample plate
4. Bring your own pipette and tips.

Computer

5. Account access POM (password: POM)
6. Run *Gene 5 application*.
7. Go to *Nucleic Acid Quantification*
8. Select the number of wells you want to analyse (by clicking on it)
9. Open the black case next to the computer screen to take out the sample plate.
10. Measure a blank for each well you will put a sample on after.

10a. Clean both sides of plate with ethanol

10b. Pipette 2 ul of NFW into each well (close the plate after)

11. Open the machine with the black round button (•)
12. Put the plate on machine drawer, gray part facing upward and towards the machine.
13. In the program, press READ, and then OK for the temperature.

14a. If samples on the computer screen are green, press APPROVE.

14b. If samples are red, press RESET and go back to 8

15. Wipe the plate with Kimwipe (DO NOT use ethanol, only the wipe)
16. Pipette 2 ul of each sample into separate wells
17. Close the plate and put it on the machine drawer (gray part facing upwards)
18. Press READ, and then OK for temperature.
19. Save the pop-up window with results on the desktop.
20. Close everything on the computer.
21. Clean the sample plate with ethanol, put the white protective foam in between and close it
22. Clean the computer and the machine with ethanol.

cDNA synthesis with Maxima™ H Minus cDNA Synthesis Master Mix, with dsDNase kit

Material:

- Kit components: (all stored at -20°C)
  10X DNAse buffer
  dsDNAse
  Nuclease-free water
  Maxima cDNA h minus synthesis Master
- RNAse-free tubes
- 1.5 mL Eppendorfs
- dd water

1. Thaw kit components on ice. Expect this will take about an hour.
2. Centrifuge briefly.
3. Add the following reagents in RNAse-free tubes on ice in this order:

10X dsDNAse buffer	1 uL
dsDNAse	1 uL
RNA	Depends on your concentration calculated with Nanodrop
Nuclease-free water	Idem
	10 uL total

4. Mix gently and centrifuge briefly.
5. Incubate at 37°C for 2 min (in water or dry bath)
6. Add the following reagents:

Maxima cDNA H minus synthesis Master	4 uL
Mix	6 uL
Nuclease-free water	20 uL total

7. Mix gently and centrifuge.
8. Make a solution of all reagents except Maxima H minus and another solution of all reagents except RNA to verify the cDNA synthesis.
   No RT control: Add nuclease-free water to have the same volume.
   NTC: 4uL of Maxima + 16uL of nuclease-free water.
9. Put your samples in the thermocycler.
10. Click on the Menard program on the thermocycler. The cycle takes around 30min.
    If you use the yellow thermocycler, you have to take it out right when it's done because it doesn't go back to 4°C when it's done.
11. Identify 1.5 mL Eppendorfs with your samples.
12. Add the amount of dd water required to have the final volume (calculated with the excel sheet).
13. Add all your cDNA in the Eppendorfs.
    I suggest you rince the small Eppendorf that went in the thermocycler with a bit of dd water to get as much cDNA as possible.
14. Store the cDNA in -20°C. It can be kept there for about a month.

qPCR avec le QuantStudio

Material:

- qPCR plaque
- 1.5mL Eppendorf
- Primers (at -20°C)
- SYBR green (at 4°C)
- dd water
- The funky pipette with its tips (tips are on the bench)
- Sticker
- USB key with your experiment on it

1. Thaw the primers on ice. This step takes about an hour.
2. Identify 1.5mL Eppendorf tubes according to the number of genes you want to study.
3. In those Eppendorf, make the mixed primers solutions with SYBR green, the primers and the dd water. The quantity of each must be calculated with the plaque excel sheet.
   DON'T FORGET TO CONSIDER THE NTC WELLS.
   Don't forget to covert the tubes from light with aluminium (SYBR green photobleaches!)
4. Add 3 uL of your diluted cDNA samples in the right wells of the plaque.
   Vortex briefly the diluted cDNA before pipetting it to mix it properly.
5. Add 7 uL of your mixed primer solution in the right wells of the plaque with the funky pipette.
   Be careful not to touch the plaque with the tip.
6. Add the sticker to close the wells.
   Make sure to get rid of all the air by sealing the sticker on the plaque with some tool that looks like a credit card.
7. Centrifuge your plaque 3 X 10 seconds with the centrifuge beside the qPCR machine.
8. Turn on the qPCR. Code: 7224
9. Position your plaque with the A1 well at the top-left in the qPCR.
10. Plug in your USB key directly on the machine and load your experiment by selecting the right file.
11. Start the run. This is going to take 2 hours.
12. When it is done, click on transfer and select your USB key.
13. The plaque can go in the trash.