ARIA

RNA Isolation Protocol

iGEM upf. prbb arquicalcul TASALIA SOCIEDAD DE TASACIÓN

RNA isolation

Introduction

In this protocol we expose how to isolate the IVT-RNA. We always used the meassures for a 24W-plate. In this case, instead of TriZol we use Quiazol.

Materials

- Quiazol
  - Aspect: pink
  - Localization: Under the air flow cabin, right drawer, inside a carton box
  - Use in air flow cabin
  - Maintain at RT
- Chloroform
  - **Always** use in an air flow cabin!
- Isopropanol
  - Maintain at RT
  - We have a 50mL falcon (iGEM bench)
- Ethanol 85%
  - Dilution is in our 50mL falcon (iGEM bench)
  - If not diluted--> Ethanol 100% is under the air flow cabin, right drawer, the biggest bottle
- Glicoblue
  - For visualization of the RNA pellet!!!

Procedure

Obtaining of two-phases

1. **Lyse** cells by resuspending directly in **Quiazol**: pipet up and down until homogeneized
2. **Lyse** cells by resuspending directly in **Trizol**, pipet up and down until homogenized

   Well of a 6W plate – 1ml Trizol
   Well of a 24W plate – 0.5ml Trizol
3. For a well of a **24W plate**:

   Add 0.1 ml chloroform (or chloroform-isoamyl alcohol) per 0.5 ml TRIzol Reagent initially used. Cap tubes and shake vigorously for 15 seconds. Do not vortex.
4. **Centrifuge** samples at no more than 12,000 x g for 10 minutes at 2–8°C.

5. **Examine phasing**. Clear, the aqueous phase should be the entire atop Phase Lock Gel. The phenol-chloroform phase and cloudy interphase should be below the Phase Lock Gel layer. If this is not the case, add another 0.1 ml chloroform (or chloroform-isoamyl alcohol) per 0.5 ml TRIzol Reagent used initially and shake vigorously. Repeat centrifugation and re-examine phasing.

6. **Transfer** aqueous phase containing RNA to a fresh tube

7. **Precipitate** RNA with isopropanol: use 400 µl isopropanol per ml Trizol initially used (1ul of Glicoblue will help to visualize the pellet)

8. **Incubate** > 20 min at –20 °C

9. **Centrifuge** > 20 min full speed at 4°C

10. **Discard** supernatant

11. **Wash** pellet by gently pipetting with 0.5 ml 85% ethanol for every 0.5 ml of TRIzol used. Mix by flicking and inverting tube or vortexing (flying pellet)

12. **Centrifuge** 5 min full speed at 4°C

13. Carefully **aspirant** supernatant.

14. **Repeat** ethanol 85% wash 2x

15. Air-dry RNA **pellet** for 5-10 minutes (be careful not to overdry)

16. **Dissolve** pellet in 40 ul of H2O at 30°C for 5 min with shaking (65°C if overdried)

17. **Pipette** up and down and quantify in nanodrop.

** Note: the protocol expose in this pdf is not exactly the same as those set out in the bibliography part. We did it followinf a lab protocol. **

Bibliography

1. (N.d.-f). Retrieved October 19, 2021, from Thermofisher.com website: https://tools.thermofisher.com/content/sfs/manuals/1330M_G.pdf
2. Addgene: Kit Free RNA Extraction. (n.d.). Retrieved October 19, 2021, from Addgene.org website: https://www.addgene.org/protocols/kit-free-rna-extraction/