

DEX/CHX treatment and RNA extraction

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1. Materials:

1.1 DEX/CHX treatment

1. Dexamethasone (DEX) stock solution: 3 mM DEX in 100% ethanol.
2. Cyclohexamide (CHX) stock solution: 10 mM CHX in 100% ethanol.
3. MS medium containing DEX or CHX is made by adding DEX or CHX stock solution to autoclaved MS medium + 5.5% plant agar. When cooled down to 60°C add DEX to a final concentration of 30µM and CHX to 100µM. $\frac{1}{50}$ volume 100% ethanol is added to make MS-Mock.

1.2 RNA extraction:

1. liquid nitrogen
2. Trizol reagent (Invitrogen)
3. Chloroform
4. Isopropanol
5. Ethanol
6. Nuclease free water
7. RNesay[®] mini columns (Qiagen)

2. Methods:

2.1 Tissue collection:

1. Grow *Arabidopsis thaliana* seedlings on MS media at 22°C, under a photoperiod of 16 hours light and 8 hours dark.
2. 15 to 16 days after germination, transfer seedlings to MS media containing 30 µM DEX, 100 µM CHX, 30 µM DEX + 100 µM CHX or $\frac{1}{50}$ volume 100% ethanol.
3. Collect seedling tissues after 4 hours of treatment.
4. Freeze tissue immediately in liquid nitrogen.

2.2 RNA extraction:

1. Grind approximately 30 to 40 seedlings in liquid nitrogen.
2. Immediately add ground seedlings to 7.5 ml Trizol[®] reagent and vortex.
3. Incubate at room temperature for 5 min.
4. Remove the insoluble material from the homogenate by centrifugation at 12,000 xg for 10 min at 4°C.
5. Add 1.5 ml chloroform to the supernatant from the previous step and mix by vortexing for 30 sec.
6. Incubate samples at room temperature for 3 min and then centrifuge at 10,000 xg for 15 min at 4°C.
7. Precipitate RNA from the aqueous phase by mixing the aqueous phase with 3.75 ml isopropanol.
8. Incubate at room temperature for 20 min.
9. Centrifuge the samples at 10,000 xg for 10 min at 4°C.
10. Wash the RNA precipitates with 10 ml 70% ethanol and re-centrifuge.
11. Dry RNA pellets for 10 min at room temperature.
12. Dissolve RNA pellets in 150 µl nuclease free water by incubating at 60°C for 10 min.
13. Further concentrate the RNA samples through Qiagen RNeasy[®] mini columns following “RNeasy mini protocol for RNA cleanup”.