http://www.flychip.org.uk/protocols/gene_expression/large_extraction.php

Large scale extraction of total RNA from *Drosophila melanogaster*

Overview

The RNA that is to be labelled must be of high quality. It must be undegraded and contain no genomic DNA contamination. Several extraction methods have been tested for use with Drosophila samples. However, extraction using TRIzol gives consistent, reliable results and is considerably cheaper than kit−based products and is therefore our method of choice.

Poly A+ mRNA constitutes approximately 2% of total RNA from a Drosophila embryo. Labelling of 50 µg total RNA using an oligo(dT) primer gives similar results to approximately 1 µg poly A+ RNA and it is therefore unnecessary to purify poly A+ RNA from the total RNA prep.

This protocol is based on a method from Kevin White's web site (http://quantgen.med.yale.edu/). This particular protocol has been optimised to extract total RNA from very large samples, e.g. 0.4 g adult flies to make 2.5 mg of total RNA.

We only require 30 µg total RNA per labelling reaction. Please ensure that you have a sufficient amount of tissue before sending us your samples.

Equipment and reagents

- TRIzol (Gibco/BRL; Cat. No. 15596−018)
- DEPC − Diethyl pyrocarbonate (BDH; Cat. No. 44170 3D)
- Chloroform (BDH; Cat. No. 100775A)
- Isopropanol (BDH; Cat. No. 102246L)
- Phenol:Chloroform:Isoamyl alcohol (25:24:1 mixture, pH 4.3) (Fisher BioReagents; Cat. No. UN2821)
- DEPC−treated MilliQ water
- 70% ethanol/DEPC MilliQ water
- RNAlater (Ambion; Cat. No. 7020)
- Ultra−Turrax T8 homogeniser, Labortechnik
- RC−55 refreigerated superspeed centrifuge with SS−34 rotor, Du Pont instruments
- Jouan GR422 Centrifuge
- 50 ml Falcon tube (Falcon; Cat. No. 352070)
- Clear glass corex tube (Du Pont Instruments; Cat. No. 00152)

Removal of RNase

All materials should be autoclaved and only handled using gloves. Glassware should be baked at 180 °C overnight. Water and solutions should be treated with DEPC. The work area can be cleaned using RNase Zap to further limit the risk of RNase contamination. If at all possible, it is also a good precaution to use a separate set of pipettes for RNA work.

Procedure

1. For adult flies, imaginal discs and other tissues, transfer tissue to a 50 ml Falcon tube and weigh on microbalance. For embryos, dechorionate first, rinse thoroughly with water and blot off excess before weighing (do not fix!). If samples are ready to be homogenised immediately, skip to step 2. If samples

are not yet ready for processing, then either:

- flash freeze tube in liquid nitrogen then store in −80 °C freezer until ready to homogenise. Thaw on ice before continuing with step 2, or;
- add 5 volumes of RNAlater. The tissue can be stored safely at 25 °C for a couple of days, at 4 °C for up to a week, and at −20 °C or −80 °C for at least a month. When ready to continue, remove RNAlater before continuing with step 2.

2. Place sample on ice and add 1 ml of TRIzol per 50 to 100 mg of tissue.
3. Homogenise the sample for using a Ultra−Turrax T8 homogeniser set to full speed. Avoid making sample hot.
4. Centrifuge at 4000 rpm in a Jouan GR422 centrifuge for 10 minutes at 4 °C to pellet debris such as the chorion, vitelline membrane, cuticle etc. Transfer the supernatant to an autoclaved corex tube.
5. Add an equal volume of Phenol:Chloroform:Isoamyl alcohol to the supernatant and mix by vortexing.
6. Centrifuge for 15 minutes at 13,000 rpm at 4 °C using an SS−34 rotor within the RC−55 refrigerated superspeed centrifuge.
7. Transfer the upper phase to a fresh autoclaved corex tube without touching the interphase or the side of the tube.
8. Add 0.2 volumes chloroform and vortex
9. Centrifuge at 13,000 rpm for 15 minutes at 4 °C using an SS−34 rotor within the RC−55 refrigerated superspeed centrifuge.
10. Transfer the upper phase to a fresh autoclaved corex tube without touching the interphase or the side of the tube.
11. Add 0.8 volumes of isopropanol to precipitate the RNA. Incubate at −20 °C for at least 1 hour.
12. Leave the sample at room temperature for a few minutes and then centrifuge at 13,000 rpm for 15 minutes at 4 °C using an SS−34 rotor within the RC−55 refrigerated superspeed centrifuge.
13. Discard the supernatant and wash the RNA pellet with 1 ml 70% ethanol/DEPC MilliQ water per 1 ml of the original TRIzol volume and centrifuge at 13,000 rpm for 10 minute at 4 °C using an SS−34 rotor within the RC−55 refrigerated superspeed centrifuge.
14. Air dry the pellet for a few minutes (leave on work bench). Resuspend in an appropriate volume of DEPC MilliQ water that has been pre−heated to 55 °C for 3 minutes
15. Transfer to a 1.5 or 2 ml microfuge tube
16. Verify quality of RNA according to the RNA quality control / assessment protocol.