Array Tips

Useful tips and quick updates to help you get the most from your microarray experiments

RNA Concentration and RNA Clean-up Methods

When RNA is too dilute:

UHNMAC labelling protocols recommend that RNA samples have a minimum concentration of 1 µg/µL in order to use with labelling master mixes in order to ensure optimal concentrations and volumes of the other labelling reagents. When RNA is too dilute, we recommend concentrating it prior to labelling.

The typical method we use for concentrating RNA is isopropanol precipitation. It has been our experience that 75-90% of the initial sample can be recovered. Other column-based methods are available [RNeasy (Qiagen), Absolutely RNA (Stratagene/Agilent), Versagene (Gentra)] but often less than 75% of the initial sample is recovered using these methods.

Isopropanol Preciptation:

- Add 1/10 volume 3M sodium acetate
- Add 1 volume isopropanol
- Incubate at –20°C for at least 1 hour
- Centrifuge at maximum speed (20,000 x g) for 15 minutes
- Remove supernatant and briefly rinse pellet with 70% ethanol
- Centrifuge at maximum speed for 2 minutes
- Remove supernatant and allow pellet to dry
- Resuspend in a small volume of RNase-free water (10 –15 µL)
- OD sample to quantify/run on Agilent 2100 Bioanalyzer to check integrity

When RNA is contaminated:

Occasionally we receive RNA samples with a brown-tinged appearance (often from samples isolated from tissue), indicating that the RNA sample may be contaminated. While the Agilent 2100 Bioanalyzer results may suggest that the RNA quality is good, contaminants may affect the labelling reaction. Depending on the amount of sample we have, and other factors, we may try to label a portion of the sample and carry out a test microarray hybridisation to see how the sample labels.

If the RNA sample requires clean-up, we try using the RNeasy Purification kit, following the manufacturer's recommendations. Depending on the type of contaminant present in the sample, this clean-up method may or may not work. Using the RNeasy (Qiagen) purification kit for this purpose, we have found that less than 75% of the initial sample is often recovered. If the RNeasy column does not remove the contaminant, the Trizol Reagent (Invitrogen), following the manufacturer's protocol, is tried. Be aware that some of the initial sample will likely be lost.
