RNA Extraction Project - Methods

Extraction

Standard extractions are performed using TRIzol® Reagent (Invitrogen) according to manufacturer recommended protocols. This method maintains the profile of total RNA of all sizes, but is not the most efficient for small RNA extraction. For projects with a primary focus on small RNA profiles, we recommend use of the miRNEasy® (Qiagen) extraction method as being the method with highest recovery efficiency of small RNA molecules.

RNA Purification

On column DNase I digestion can be performed during RNA extraction or on previously extracted RNA to ensure removal of residual genomic DNA. Please be advised that column methods vary in the retention of small RNA molecules and investigators must specify at the time of requesting DNAse treatment whether retention of small RNA molecules is desired. Should RNA purity be below requirements of downstream applications, RNeasy or MiRNeasy purification (Qiagen) can be performed on previously extracted RNA. RNA purity is assessed via ratio of OD260/OD280. In good quality RNA this ratio is >1.8. As above, small RNA molecule retention is effected by the column method chosen.

Quality Control

Upon completion of the final purification, RNA quantity and quality is measured by obtaining an A260/A280 nm wavelength ratio. High quality RNA preparations return a A260/A280 nm wavelength ratio of 1.8 - 2.1. RNA integrity is verified via Agilent 2100 bioanalyzer.

Sample Storage

Following extraction and purification, the RNA is stored at -80°C in nuclease-free water. The sample may be banked with the Core for a period of up to 3 months following all downstream GRC processing. There will be additional fees to maintain the samples for a longer time period.

Reporting

The Investigator is provided with a spreadsheet containing the A260/A280 nm wavelength ratio, RNA concentration, volume, total yield and a pdf showing bioanalyzer results. Your laboratory must be a registered user of our facility in order to access your data.