Protocol for Preparation of mRNA from *E. coli* for Use in Microarray Hybridizations

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This protocol has been used successfully on 1-25 mL of *E. coli* culture. The amount of starting material should adjusted to achieve the desired RNA yield depending on strain and culture conditions. For example 1 mL of log phase MG1655 will yield approximately 30 ug of RNA. 20 mL of a stationary phase culture is required for similar yield. Also, an RNeasy column with appropriate capacity (Mini, Midi or Mega) should be selected depending on the scale of the prep.

Reagents:

5% Buffer Equilibrated Phenol (pH 7.4) in Ethanol "Stop Solution" (1/10th of sample volume)
Ice
RNeasy Kit

Cells are grown to desired density under conditions of interest. Before harvest centrifuge tube are labeled and filled with 1/10th the sample volume of "Stop Solution." For example if 4 mL of culture were being prepped, one would use 0.4 mL of the stop solution. The stop solution was initially used as an inhibitor of ribonucleases in RNA decay experiments. It also inhibits transcription to some extent.

When the cultures are ready, samples are transferred rapidly to the Stop solution containing centrifuge tubes. The tubes are capped and the sample mixed with the stop solution by inversion. The sample is the placed on ice. The samples can sit on ice for up to 20' without complication.

When all samples are collected the cells are spun down at 4 degrees C. The supernatant is then poured/pipetted off. Try to leave as little media as possible.

At this point the pellets can be snap frozen in liquid nitrogen for storage for up to a week and possibly longer.

If the cells have been frozen they are allowed to thaw at room temperature. As soon as they thaw begin the RNeasy protocol for gram-negative bacteria. If the cells were not frozen proceed directly to the RNeasy protocol.

At the end of the RNeasy protocol DNase digestion may be performed on the RNeasy column as described in the product manual. Alternatively, DNase digestion may be performed at the completion of the RNeasy protocol, but this necessitates either phenol extraction or the use of a second RNeasy column to remove the DNase from the RNA sample.

Yield determination is made by measurement of A_{260}/A_{280} at appropriate dilution. If necessary, samples can be concentrated in an RNase free speed-vac or by precipitation. If precipitation is chosen it is very important to wash the pellets thoroughly to remove excess salts.