This protocol is derived from QIAGEN's RNeasy Protect Bacteria Mini Kit [1].

Experiment

Materials

- *QIAGEN RNeasy Mini Kit Protect Bacteria - containing:*
  - *RLT buffer*
  - *TW1 buffer*
  - *RPE buffer*
  - *Purification columns*
  - *RNase-free collection tubes.*
  - *RNAprotect Bacteria Reagent*

- *Two cultures of E.coli, approx. 6ml each, grown in M9-media to an average OD_{600} of 0,75. One culture is grown alongside with live strongyles while the other one isn't.*
- *TE-buffer (1M Tris-HCl, 0,1M EDTA)*
- *Lysozyme (s)*
- *Proteinase K (20mg/ml)*
- *2-mercaptoethanol (99%)*
- *Ethanol (99%)*
- *RNase-free water*
- *DNase enzyme (1500U)*
- *Centrifuge*
- *Pipettes, tips and appropriate tubes*

- All relevant buffers and reagents were prepared to working solutions:
- TE-buffer - 2ml, mixed with 15mg/ml lysozyme, 20µl/ml Proteinase K
- RLT-buffer - 2,5ml, mixed with 10µl/ml 2-mercaptoethanol
- RPE-buffer - 11ml, four volumes of ethanol (99%) are added to a total of 55ml.
- DNase (diluted with RNase-free water to 3U/µl)

Method

1. Three samples are taken from each *E.coli* culture - one aliquot of 1,6ml, 1,7ml and 1,8ml. This is to measure any difference in RNA yield or quality when performing the experiment with different cell amounts.

2. RNAprotect Bacteria Reagent is added to each aliquot to a volume totaling 3x the original volume.

3. The aliquots are centrifuged for 10min at 13.000rpm. Supernatant discarded, only pellet remains.

4. 200µl of TE-buffer were added to the samples.

5. Samples were vortexed briefly and incubated for 5 minutes with shaking at 25 degrees celsius.

6. 700µl of RLT-buffer were added to each sample. The samples were centrifuged at 13.000rpm for 2min to remove particulate matter - the supernatants were moved to new tubes.

7. 500µl of ethanol were added to each sample.

8. 700µl of the samples were moved over to RNeasy spin columns (six columns in total).

9. 350µl of RW1 buffer were added to each sample. The columns were centrifuged for 15sec at 13.000rpm. Flowthrough discarded.

10. 10µl of DNase solution were mixed with 70µl of RDD-buffer. 80µl of the DNase/RDD buffer was added to each sample. The samples were incubated for 15min at 25c.

11. 700µl of RW1 buffer were added to each sample. Incubated for 5min at 25c. The samples were then centrifuged for 15sec at 13.000rpm. Flowthrough and collection tubes discarded; new collection tubes attached to the spin columns.

12. 500µl of RPE-buffer were added to each sample. The samples were centrifuged for 15sec at 13.000rpm. Flowthrough discarded.

13. 500µl of RPE-buffer were added to each sample. The samples were centrifuged for 2min at 13.000rpm. Flowthrough and collection tubes discarded. New collection tubes were attached to the spin columns.

14. 30µl of RNase-free water were added to each column. The columns were centrifuged for 1min at 13.000rpm. The flowthrough was collected and put on ice - it contains purified RNA.

15. 15µl of RNase-free water were added to each column to collect any remaining RNA. The columns were centrifuged for 1min at 13.000rpm. The flowthrough was collected and put on ice.

16. The RNA suspensions (twelve in total) were measured with Thermo Fishers NanoDrop to check the nucleic acid concentration and sample purity. A gel electrophoresis was also performed using the samples collected in step 15.

References

[1] QIAGEN, 2018. RNAprotect Bacteria Reagent Handbook. https://www.qiagen.com/us/resources/resourcedetail?id=95346297-ae79-41a8-9259-e48f702d4f36&lang=en Date of Visit 2018-10-15