Denaturing gel (formaldehyde)

To quantify RNA samples on a gel w/o effects of secondary structure, we've got to run it denaturing. This protocol is taken from Benjy’s RNAseq spike-in protocol.

(also consider http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3699176/ -Darach)

Denaturing Gel

1. Add 0.6g agarose to 50.8ml DEPC water.
2. Microwave till dissolved, cool to ~60-65C.
3. Add 6ml 10x MOPS.
   In hood:
4. Add 3.24ml formaldehyde.
5. Pour gel and let sit 1hr.
6. Remove comb and wrap in saran wrap.

Prepping RNA samples for gel

1. Make master mix:

<table>
<thead>
<tr>
<th></th>
<th>1x</th>
<th>10x</th>
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<tbody>
<tr>
<td>10x MOPS</td>
<td>2ul</td>
<td>20ul</td>
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<tr>
<td>Formaldehyde</td>
<td>4ul</td>
<td>40ul</td>
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<tr>
<td>Formamide</td>
<td>10ul</td>
<td>100ul</td>
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<tr>
<td>EtBr (dilute 1:10?)</td>
<td>1ul</td>
<td>10ul</td>
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2. Distribute 17ul of master mix to eppendorfs, add 2-4ul of sample. Do not forget the appropriate RNA ladder!
3. Incubate 60min @ 55C.
4. Chill 10min on ice water.
5. Centrifuge, add 2ul of 10x formaldehyde gel loading buffer, mix.
6. Put on ice until ready to load.

Running gel

1. Once gel has sat for 1hr, set it up with ~400ml or more of 1xMOPS buffer.
2. Run gel blank for 5min at 100V.
3. Load samples, run ~30-40 minutes. Dye runs about ~500 bases fast, so adjust time as appropriate.
4. Take picture for EtBr staining.

References: