1) DNA Mix
   32P labeled probe
   10 ul 10% polyvinyl alcohol
   1 ul 10 A 260 unit/ml poly d(I-C) (from Sigma P9514)
   H2O to 25 ul

2) Add Z' or TM buffer to a final volume of 50 ul allowing room for the amount of protein extract used.

3) Add protein extract last.

4) incubate on ice 15 min.

5) DNase digestion. (amount of DNase to use must be titrated for best results)
   a) 1 ul of 2.5 mg/ml DNase I is added to 1 ml ice cold water, mix thoroughly by inversion.
   b) Add 100 ul from above to 200 ul ice cold water.
   c) Add 50 ul 10 mM Mg Cl2, 5mM Ca Cl2 to the DNA protein mix leave at room temp for 1 min.
   d) Add 2.0 ul diluted DNase, mix quickly , incubate at room temp for 1 min. (if done at 1 min intervals, one can do three reactions at 15-20 second intervals.)
   e) Add 90 ul STOP Mix, vortex, and store on ice.
   f) After all reactions are completed to all;
      Phenol extract.
      Chloroform extract. This may require 1 min microfugation for good seperation.
      Ethanol precipitate.
      70% ethanol wash and vacuum dry.
   g) Add 4-10 ul loading buffer, boil for three minutes and load on sequencing gel.

Footprinting solutions.

<table>
<thead>
<tr>
<th></th>
<th>Stock</th>
<th>1 ml</th>
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<tbody>
<tr>
<td>TM buffer</td>
<td></td>
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</tr>
<tr>
<td>50mM Tris-HCl pH 7.9</td>
<td>2M</td>
<td>25 ul</td>
</tr>
<tr>
<td>12.5mM Mg Cl2</td>
<td>1M</td>
<td>12.5 ul</td>
</tr>
<tr>
<td>1mM EDTA</td>
<td>0.5M</td>
<td>2 ul</td>
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<tr>
<td>Component</td>
<td>Concentration</td>
<td>Volume</td>
</tr>
<tr>
<td>--------------------</td>
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<td>--------</td>
</tr>
<tr>
<td>1mM DTT</td>
<td>1M</td>
<td>1 ul</td>
</tr>
<tr>
<td>20% glycerol</td>
<td>100%</td>
<td>200 ul</td>
</tr>
<tr>
<td>0.1M K Cl</td>
<td>1M</td>
<td>100 ul</td>
</tr>
<tr>
<td>H₂O</td>
<td>660 ul</td>
<td></td>
</tr>
<tr>
<td>STOP Mix</td>
<td></td>
<td>5 ml</td>
</tr>
<tr>
<td>200mM Na Cl</td>
<td>1M</td>
<td>1 ml</td>
</tr>
<tr>
<td>20mM EDTA</td>
<td>0.5 M</td>
<td>250 ul</td>
</tr>
<tr>
<td>1% SDS</td>
<td>10%</td>
<td>500 ul</td>
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<tr>
<td>250 ug/ml tRNA</td>
<td>5 mg/ml</td>
<td>250 ul</td>
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