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Reverse transcription of complementary DNA

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MATERIALS

STEP MATERIALS

AMV Reverse Transcriptase - 1,000 units **New England Biolabs Catalog**
#M0277L

OPEN ACCESS

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Reverse Transcription using AMV RT

- 1 Obtain an RNase -free microcentrifuge tube 400 μL

Add the following components in the described order:

 - Water 15 μL
 - 10 X Buffer
 - 2 μL 50 mM Tris-acetate, 75 mM Potassium Acetate, 8 mM Magnesium Acetate, 10 mM DTT (pH 8.3)
 - 5'-P32 Radiolabeled DNA 1 μL < 1 pmol
 - RNA 2 μL app. 2 pmol
- 2 Place the tube in a heat block at 90 °C and turn off the heat block to allow for slow cooling.

02:00:00
- 3 Remove the tube upon reaching room temperature and centrifuge for 00:00:05 using a microcentrifuge
- 4 The solution is then transferred to a separate microcentrifuge tube 3 μL

- 5 Add the desired dNTP solution 1 μL 10 millimolar (mM)
- 6 Add the reverse transcriptase solution 2 μL . The reverse transcriptase solution consists of RT 2 μL dissolved in water 58 μL . This enzyme was purchased from NEB.
AMV Reverse Transcriptase - 1,000 units **New England Biolabs Catalog #M0277L**
- 7 Place the solution in an incubator at 37 °C for 00:45:00
- 8 Remove from water bath and add urea 7 μL 6 Molarity (M)
- 9 Centrifuge for 00:00:05 using a microcentrifuge
- 10 Place the tube in a heat block at 90 °C for 00:10:00
- 11 Centrifuge for 00:00:05 using a microcentrifuge while allowing to cool to rt
- 12 Withdraw the solution 8 μL This typically accounts for app 4000 cpm and load onto a denaturing 20 % PAGE

- 13 On a separate well, add a marker composed of xylene cyanol and bromophenol blue in 90% formamide buffer
- 14 Turn on the power supply and allow to run until the bromophenol blue marker is 3/4 down the gel. Settings will vary depending on the gel size and thickness.
- 15 Remove the gel from the gel stand and remove one glass slide.
- 16 Develop using an autoradiography cassette Overnight
- 17 Visualize and quantify the gel using a Molecular Dynamics Phosphorimager 840

Reverse Transcription using MMLV RT

- 18 Repeat all steps above, except with the amount of reverse transcriptase added. Add the reverse transcriptase solution 2 μL . The reverse transcriptase solution consists of RT 1 μL dissolved in water 10 μL . This enzyme was purchased from NEB M0253L at 200,000 U/mL

Reverse Transcription using HIV RT

- 19 Repeat all steps above, except with the amount of reverse transcriptase added. Add the reverse transcriptase solution 2 μL . The reverse transcriptase solution consists of RT 0.75 μL dissolved in water 1.5 mL; followed by a second 1:1 dilution in water. This enzyme was purchased from Worthington LS05003 at 27.5 units/ μL