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Reverse transcription of complementary DNA

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.birskd6e

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STEPS MATERIALS

NAME	CATALOG #	VENDOR
AMV Reverse Transcriptase - 1,000 units	M0277L	New England Biolabs

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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 Milimolar (mM)**

6 Add the reverse transcriptase solution **2 µl** . The reverse transcriptase solution consits of RT **2 µl** dissolved in water **58 µl** . This enzyme was purchased from NEB.

AMV Reverse Transcriptase - 1,000 units
by 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)**

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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 thicknes.

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 consits 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 consits 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 Worthtington LS05003 at 27.5 units/uL

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