

Primer Extension Analysis Using AMV Reverse Transcriptase

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[Abstract] Primer extension analysis is a useful method to determine the transcription start point or a processing site on an RNA molecule. It can also allow a quantitative measurement of an RNA species.

Materials and Reagents

1. AMV reverse transcriptase from the avian myeloblastosis virus (Finnzyme, catalog number: F570) (other reverse transcriptases can also be used, by adapting the reaction buffer)
2. T4 polynucleotide kinase + 10x reaction buffer (Biolabs, catalog number: M0201)
3. RNasin Plus RNase inhibitor (Promega Corporation, catalog number: N2611)
4. Glycogen (Acros Organics, catalog number: 422950010)
5. NaCl
6. Tris HCl (pH 7.5)
7. EDTA
8. MgCl₂
9. EtOH
10. dATP, dCTP, dGTP, dTTP (Promega Corporation, catalog number: U120D, U121D, U122D, U123D)
11. γ -³²P ATP (3,000 Ci/mmol, 10 μ Ci/ μ l) (PerkinElmer, catalog number: BLU502A)
12. DTT (Promega Corporation, catalog number: P117B)
13. 5x ss-Hybridization buffer (see Recipes)
14. 1.25x RT buffer (see Recipes)

Equipment

1. Incubator

Procedure

A. Primer labelling

1. Labelling mix (total volume 10 μ l):
 - a. 1 μ l oligonucleotide (20-25 mer, 10 pmoles/ μ l)
 - b. 3.5 μ l γ^{32} P ATP (10 μ Ci/ μ l)
 - c. 1 μ l T4 DNA polynucleotide kinase
 - d. 1 μ l 10x polynucleotide kinase reaction buffer
 - e. 3.5 μ l H₂O
2. Incubate 30 min at 37 °C, stop reaction on ice.

B. Precipitation

1. Add 1/10 vol 10 M LiCl.
2. Add 3 vol EtOH (96 %).
3. Incubate 30 min at -80 °C.
4. Centrifuge 20 min at >10,000 $\times g$ at room temperature (RT), take off supernatant, don't wash.
5. Air-dry pellet and take up in 10 μ l (consider concentration to be 0.5 pmoles/ μ l).

C. Annealing step

1. Annealing mix (total volume 10 μ l):
 - a. 10 - 20 μ g total RNA
 - b. 10 U RNasin RNase inhibitor
 - c. 0.5 pmole of 5' labelled primer (1 μ l)
 - d. 2 μ l 5x ss-hybridization buffer
 - e. Add water to 10 μ l

D. Extension step

1. Add directly to the annealing mix:
 - a. 40 μ l 1.25x RT buffer (pre-warmed at 50 °C)
 - b. 10 U AMV transcriptase
 - c. 10 U RNasin inhibitor
2. Incubate 30 min at 50 °C (extension).

E. Extension termination

1. Add to the reaction mix:
 - 1 μ l EDTA (0.5 M)

- 6 μ l NaOH (1 M)
 2. Incubate 10 min at 55 °C
 3. Add 6 μ l HCl (1 M) (neutralization).
- F. Precipitation
1. Add 1/10 vol 10 M LiCl
 2. Add 25 μ g glycogen
 3. 2.5 vol EtOH
 4. Incubate at -20 °C for > 1 h.
 5. Centrifuge 10 min at >10,000 $\times g$ at room temperature, wash pellet 1x with 70% EtOH.
 6. Air-dry pellet and take up in 15 μ l of classic DNA Loading dye buffer.
- G. Separation of reaction products on denaturing polyacrylamide gel.

Recipes

1. 5x ss-Hybridization buffer
 - 1.5 M NaCl
 - 50 mM Tris HCl (pH 7.5)
 - 10 mM EDTA
2. 1.25x RT buffer
 - 1.25 mM dATP
 - 1.25 mM dCTP
 - 1.25 mM dGTP
 - 1.25 mM dTTP
 - 12.5 mM DTT
 - 12.5 mM Tris HCl (pH 8)
 - 7.5 mM MgCl₂

Acknowledgments

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References

1. Taverniti, V., Forti, F., Ghisotti, D. and Putzer, H. (2011). [*Mycobacterium smegmatis* RNase J is a 5'-3' exo-/endoribonuclease and both RNase J and RNase E are involved in ribosomal RNA maturation.](#) *Mol Microbiol* 82(5): 1260-1276.