

## **Primer extension**

(submitted by Joanna Kufel)

Based on Beltrame and Tollervey, 1992, EMBO J. 11: 1531-42, modified in David Tollervey's and Joanna Kufel's groups.

### **1. Labelling the oligo using T4 PNK**

10 pmole DNA oligo (60 ng for 20 mer)  
1.5µl kinase buffer (commercial, New England Biolabs)  
2-3 µl [<sup>32</sup>γ ATP, 20-30 µCi, specific activity 370 Bq/ml, 3000 Ci/mmol, Amersham]  
5-10 u T4 polynucleotide kinase (New England Biolabs)  
Water to 15 µl final volume

Incubate 30 min 37°C

**(Optional: extract once with 1x vol phenol/chloroform/isoamyl alcohol (25:24:1))**

Add 7.5 µl 7.5 M NH<sub>4</sub>Ac (1/2 vol)  
2 µl glycogen (2 mg/ml)  
90 µl 96% EtOH

Incubate 30 min @ -20°C

Centrifuge 10 min @ +4°C

Wash with 70% cold EtOH

Air dry briefly

Resuspend in 25 µl

## **1 Annealing**

Mix 4-8 µg RNA  
2 µl SS buffer  
2 µl kinased oligo  
water to 10 µl

Incubate 5 min @ 80°C

Cool down slowly to 42-46°C (RT reaction temperature)

**(Alternative: Heat 2 min @ 95°C**

**Snap freeze in liquid N<sub>2</sub>**

**Thaw on ice, while preparing RT mixture)**

## **2 RT reaction**

Prepare reaction buffer: **1.25x RT buffer** (commercial, Promega) + **1mM dNTP** (final each dNTP, use 10mM or 20mM stock), 39 µl per reaction, make enough for n+1 reactions

Pre-heat reaction buffer @ reaction temperature (42-46°C, depending on the oligo)

Add 0.5µl RNasin (Promega) and 0.5 µl Reverse Transcriptase (Promega) per reaction to RT buffer (remember to add for n+1 reactions)

Add 40 µl of RT buffer + enzymes to each sample @ 42-46°C

Incubate 40 min @ 42-46°C

SS hyb buffer            1.5M NaCl  
                                  50 mM Tris-Hcl pH 7.5  
                                  10 mM EDTA pH 8.0

**(Optional:**

**Add 6 µl 1M NaOH and 1 µl 0.5 M EDTA pH 8.0**

**Incubate 30-60 min @ 55°C**

**Add:            2 µl 2mg/ml glycogen**

**30 µl 7.5 M NH<sub>4</sub>Ac**

**6 µl 1 M HCl**

**250 µl 96% EtOH)**

**Denaturation of RNA can be OMITTED**

Precipitate:

Add            2 µl 2mg/ml glycogen

                  25 µl 7.5 M NH<sub>4</sub>Ac (1/2 vol)

                  190 µl 96% EtOH

Incubate 30 min @ -20°C

Centrifuge 10 min @ 4°C

Air dry briefly

Resuspend in 8-10 µl of water

Take 2-3 µl to run on the 6-8% polyacrylamide gel (19:1)

Add 2x vol of formamide+XC+BPB loading dye

Heat @ 80°C before loading on the gel