Poisoned Primer Extension Protocol

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1. Label your oligo

10pmol/µl oligo	2 µl
10x PNK buffer	1.5 µl
10 U/µl T4 PNK	1.0 µl
Gamma 32P ATP	1.0 µl
Water	9.5 µl

- ➔ 37°C – 30 min
- ➔ 65°C – 20 min
- ➔ On ice add :
  - + 35 µl water
  - + 13 µl 7.5 M NH4Oac
  - + 125 µl **ice cold** EtOH 100%
- ➔ Several hours –20°C
- ➔ 15000 rpm - 20min
  - 200 µl EtOH 70 %
- ➔ 15000 rpm - 10 min
- ➔ Speed-vac a few minutes to dry
  - Resuspend in 50 µl Tris pH 8.3 (20 pmol/50 µl)
  - Determine the number of counts in your probe by DE81 filter binding
  - Dilute probe to ~100,000 counts/ul

2. Hybridization of oligo to RNA

5x annealing buffer	500 ul
50mM Tris 8.5	25ul of 1M
500mM KCl	63ul of 4M
Filtered Water	412 ul

- ➔ Add 1µl of labelled oligo (~100,000 counts) to 3µl of RNA (~4ug)
- ➔ Heat small water beaker to 95°.
- ➔ Place samples in water and remove beaker from heat.
- ➔ Add 1 ul of annealing buffer
- ➔ Let water and samples cool to ~50° (this takes ~10 minutes)
- ➔ Place samples in temperature block at 45°.
- ➔ Set up mastermix

3. **Reverse transcription reaction**

Make a mastermix for (n+1) reactions.

MasterMix (5ul/rxn)

Water	1.75 ul
5x RT buffer	2.0 µl
dNTP/ddNTP mix	0.5µl (500µM FC)
RNAsin	0.25 µl
AMV-RT	0.5µl (u / rxn)
Total vol. + RNA/oligo	10 µl

5x RT Buffer 500 ul

250mM Tris 8.5	125 ul of 1M stock
500mM MgCl	25 ul of 1M stock
50mM DTT	25 ul of 1M stock
Filtered Water	325 ul

dNTP/ddNTP mix:

For unknown reasons, reactions with ddATP and ddCTP give better results than ddGTP. Avoid ddGTP if possible. No info on ddTTP available.

20 mM ddNTP	10.0 µl (10mM FC)
Other three dNTPs at 100 mM	2.0 µl each (10mM FC)
water	12 µl

➔ Return to annealing temperature 45°– 30 min

➔ Stop by adding 12µl of a 80% formamide/0.25 x TBE / BPB /XC buffer

4. **Run products on gel**

- Make short (30 cm) 12 %, 7M urea PAGE gel 1 x TBE ; kitchen TBE is fine
- Boil samples for 2 min
- Load 10 µl of each sample
- Run until BPB is 4 cm from bottom at 900 V (several hours)
- Dissemble gel apparatus and transfer gel to old piece of film *(film can be found in box in lab kitchen area)* . You may be able to transfer to Whatman and dry the gel for sharper bands and strongest signal, but this may not be possible with such high percentage gels.
- You will have to remove ~5cm from the top of gel to fit the film.
- Wrap in saran wrap and expose to phosphor screen for 1 hour