Recombinant Ppr2 (TFIIS)

For *in vitro* Transcription (IVT) assays with RNApII

NOTE 1: For general notes on recombinant protein expression see the *E.coli* lab protocols section.

NOTE 2: The eukaryotic RNApII transcription elongation factor TFIIS is encoded by non-essential **PPR2** in *Saccharomyces cerevisiae*. *ppr2*Δ strains have normal growth on YPD but are hypersensitive to 6AU (>10μg/ml) and MPA (15μg/ml) (see: ***Media Additives*** protocol). In RNApII IVTs *ppr2*Δ extracts are defective but can be easily repaired to WT levels by addition of recombinant protein: thus a great positive control for these assays. TFIIS stimulates the intrinsic 3'-5' nuclease activity of RNApII, allowing the removal of mis-incorporated nucleotides during chain extension (Thomas *et al* (1998) *Cell* **93:**627).

See: Mkeogh notebook pH8.30 – recombinant production
Mkeogh notebook pH8.44 – Use in RNApII IVTs

1. Transform expression plasmid pET15b-Ppr2 (BE87 / b745; **Amp^R**) into *E.coli* strain BL21/DE3.

2. Grow 100ml culture overnight at 37^{o}C (LB^{Amp}; LBA). Sub-innoculate next morning 1/100 into 2L LBA and grow to $OD_{600} \approx 0.6$ at 37^{o}C. Chill the cultures 15 minutes on ice and induce with 1mM IPTG at RT^o for three hours.

NOTE 3: All steps from this point are performed at 4^{o}C, and all buffers contain protease inhibitors as standard.

3. Harvest cells by centrifugation, wash pellet with ice-cold ddH_2O and transfer to a 50ml Falcon tubes in (<u>can freeze pellets -80^{o}C at this point</u>). Resuspend in 30mls ***buffer A*** (4^{o}C) and sonicate on ice (3 x 20s pulses 50% output, 40 duty cycle). Add Triton X-100 to 1% and mix by inversion.

4. Clarify by centrifugation (10K, 15m, 4^{o}C) and transfer the <u>supernatant</u> to a 50ml falcon tube on ice. Add 2ml Ni-NTA resin (Qiagen) (pre-equilibrate in ***buffer A***) and incubate at 4^{o}C overnight with gentle rolling.

5. Collect beads by centrifugation (1.5K, 5m, 4^{o}C) and wash in batch with 20mls ***buffer A*** and 20mls ***buffer E / 20mM Imidazole***. Collect after each resuspension by centrifugation and discard the supernatants (keep some to test by Western analysis). Transfer the beads to a disposable column and elute with ***buffer E / 200mM Imidazole***. Collect 500μl fractions.

6. Analyze fractions by Biorad Dc and SDS-PAGE (**expected size ≈35kD**). Estimate the cleanliness of the prep (how many bands visible on commassie staining SDS-PAGE) and yield per L (**NB**. Original prep (mkeogh notebook pH8.30) >90% pure).

7.1 (Option 1) Pool positive fractions and dialyze against 2 x 1L ***Transcription buffer B*** (**Tx-b^0 B**; 2 hrs per, 4^{o}C). Compare concentration (by Biorad Dc) before and after dialysis (to ensure no holes in the membrane) and store in small aliquots at –80^{o}C (recommend 500μg/ml).

7.2 (Option 2) Positive fractions were pooled and the buffer changed to ***Transcription buffer B*** (**Tx-b^0 B**) by three successive 10ml concentrations in a Centriprep YM-10. The final concentrate was made to 500μg/ml in **Tx b^{0}B** and stored in aliquots at -80^{o}C.

Buffers:

Buffer A -

10mM Tris.HCl (pH 8.0)
10% Glycerol
500mM NaCL
0.1% Tween 20
10mM Imidazole
10mM β-ME
Protease inhibitors as standard (incl. 1mm PMSF)

Buffer E -

20mM HEPES.KOH (pH 7.6)
20% Glycerol
100mM NaCL
1mM EDTA
1mM DTT
Protease inhibitors as standard (incl. 1mM PMSF)
Imidazole as indicated

Transcription Buffer B -

20mM HEPES.KOH (pH 7.5)
20% Glycerol
150mM KAc
10mM MgAc
10mM EGTA
5mM DTT
Protease inhibitors as standard (incl. 1mM PMSF)

For a manuscript methods section:

Recombinant Ppr2 (TFIIS): E. coli strain BL21/DE3 was transformed with pET15b-Ppr2. A 1L culture was grown at 37^{o}C to $OD_{600} \approx 0.5$ and induced by the addition of 1mM IPTG. Cells were further cultured for three hours at room temperature. All steps from this point

on were performed at 4^{o}C. Lysates were prepared and Ni-NTA purified as above. Positive fractions were pooled and the buffer changed to Transcription buffer B (Tx b^{o}B) (see *In vitro transcription* protocol) by three successive 10ml concentrations in a Centriprep YM-10. The final concentrate was made to 500μg/ml in Tx b^{o}B and stored in aliquots at -80^{o}C.