Making Hot RNA Probe

Mix in 1.7ml eppendorf tube:

5λ	Txn. Buffer
4λ	H_2O
3λ	0.3mM CTP
2.5λ	3.3mM ATP, UTP, GTP
4λ	^{32}P CTP
2.5λ	0.1M DTT
1λ	rRNasin
1.5λ	DNA (linearized w/)
1.5λ	T7 RNA Polymerase
25λ	→ Incubate reaction @37°C for 1 hr

Add 2λ RQ1 DNase → Incubate reaction @37°C for 15 min
Add H_2O to make 200λ (+175λ)
Add 200λ phenyl/chloroform (PCA) → vortex, spin max 3 min
Transfer top phase to new tube:

Add:	500λ	EtOH
	22λ	3M NaOAc
	1λ	glycogen

Vortex, spin 10 min. Pipette off ethanol and discard
Wash pellet in 500λ 70% ethanol
Spin, max 2 min. Pipette off ethanol and discard. Allow pellet to air dry 2-5 min
Resuspend pellet in 8λ formamide buffer. Boil 5 min. Run on 10% acrylamide gel for ~30 min (depending on fragment size)