

Materials List for:

RNA Secondary Structure Prediction Using High-throughput SHAPE

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Materials

Name	Company	Catalog Number	Comments
REAGENTS			
N-methylisatoic anhydride (NMIA)	Life technologies	M25	Dissolve in anhydrous DMSO
1-methyl-t-nitroisatoic anhydride (1M7)	see ref. 22		
Superscript III Reverse Transcriptase	Life technologies	18080044	10,000 units
Thermo sequenase cycle sequencing kit	Affymetrix	78500	
Materials provided by the user			
RNA of interest			6 pmol per reaction (the limit of detection will be determined by the instrument)
Sets of four 5' labeled primers (Cy5, Cy5.5, WellRed D2 and WellRed D1/Licor IR800)			Primers are complementary to the RNA and are used in reverse transcription and sequencing reactions. The listed fluorophores are optimal for the Beckman Coulter 8000 CEQ. Primers may be purchased or synthesized in house.
DNA template			DNA is used for sequencing reactions, and must contain the sequence of the RNA being studied - including any 3'terminal extension, if present. Where applicable, it is often convenient to use the RNA transcription template.
Buffers			
10x RNA renaturation buffer			100 mM Tris-HCl pH 8.0, 1 M KCl, 1 mM EDTA
5X RNA folding buffer			200 mM Tris-HCl pH 8.0, 25 mM MgCl ₂ , 2.5 mM EDTA, 650 mM KCl. (This buffer might be changed depending on the case (e.g. pH, EDTA, Mg, RNase inhibitor)
2.5X RT mix			4 µl 5X buffer, 1 µl 100 mM DTT, 1.5 µl water, 1 µl 10 mM dNTPs, 0.5 µl SuperScript III. Note that the 5X buffer and 100 mM DTT are provided with purchase of SuperScript III (Invitrogen).
GenomeLab Sample Loading Solution (Beckman Coulter)			Attention: Avoid multiple freeze-thaw cycles

EQUIPMENT			
Capillary electrophoresis	Beckman	CEQ8000	
Thermocycler	varies		