

1.3 Sample Protocol

1. i) Experiment title and date.
2. ii) Specific purpose and questions addressed.
3. iii) Columns across the page: tube #; DNA to be transcribed; volume of DNA; volume of master mix; volume of H₂O; total volume. Down the page, list numbered tubes and volumes for each component.
4. Master T1 mix for 10 uL transcripts:

WG		RRL	
CB5XSP6T1 (WG)	2.0 uL	CB5XSP6T1 (RRL)	2.0 uL
4 NTP (5X) capped	2.0 uL	4 NTP (5X) uncapped	2.0 uL
10X Gcap	1.0 uL		
0.1 M DTT	1.0 uL	0.1 M DTT	1.0 uL
tRNA 10 mg/mL	0.2 uL	tRNA 10 mg/mL	0.2 uL
SP6 pol 10 U/uL	0.4 uL	SP6 pol 10 U/uL	0.4 uL
RNA guard 20 U/uL	0.4 uL	RNA guard 20 U/uL	0.4 uL
add DNA and H ₂ O --> 10 ul		add DNA and H ₂ O --> 10ul	

Sample Protocol - Transcription for RRL

MMT1

10 uL reaction for 350
uL reaction

CB5X	2	70
4 NTP	2	70
0.1 M DTT	1	35
tRNA	0.2	7
SP6P	0.4	14

Rnasin	0.4	14
TOTAL= 6 uL		

Tube	Plasmid	DNA	MMT'	H2O	Total Volume
1	A@0.5 mg/mL	2	6.0	2.0	10
2	B@ 1.0 mg/mL	1	6.0	3.0	10
3	C@ 1.0 mg/mL	2	12.0	6.0	20

etc.