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SOP for cDNA synthesis by RT (Reverse Transcription) Promega kit

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ABSTRACT

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Protocol status: Working
We use this protocol and it's working

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Before Begining:

1 Take from the kit: OligodT, RT buffer (5x), MgCl2, dNTP mix, and H_2O to thaw- you can keep them on ice or at RT for a little while.

Protocol:

2 Prepare per sample:

	A	B
	total RNA (1 pg-1ug)	max vol 4ul
	Water	Fill upti 4ul
	OligodT	1ul
	Final Volume	5ul

3 Heat samples at 70°C for 5 min (prepare mix for step 4).

4 Snap freeze samples at +4°C or on ice for a minimum of 5 min.

5 Prepare RT mix (vol per samples, calculate for all your samples + 1):

	A	B
	RT buffer (5X)	4ul
	MgCl2 (25 mM)	3ul
	dNTP Mix (10 mM)	1 ul
	Rnasine*	0.5 ul
	Rtase*	1 ul
	Water	5.5ul
	final volume	15 ul

* add them in the last step before adding the mix to each sample. Meanwhile keep them at 20°C.

6 Brief centrifugation.

7 RT program

	A	B
	hybridization	5 min at 25°C
	elongation	60 min at 42°C
	inactivation of the enzyme	15 min at 70°C

8 Add H2O to bring final concentration to a desired amount – this will likely depend on the abundance of your targets.

9 Once it is done, cDNA can be stored at -20°C