PROKARYOTIC TARGET LABELING PROTOCOL/WORKSHEET

(Target prep takes ~ 6.5 hours)

RNA prep (one of multiple options):

- Hot phenol extractions should be followed by column purification (MinElute, for example).
- Alternatively, RNA can be purified using the RNeasy Mini Purification Kit.
  - No more than 10^9 cells should be applied to a single column
  - Lysozyme should be increased to 1 mg/mL (not 400 ug/mL)
  - DNaseI treatment may be necessary if cultures grown at high density
  - 16S and 23S rRNA bands should be clearly visible without smears on agarose gel using a 1 ug load volume (or use Agilent Bioanalyzer to analyze)

CONCENTRATION AND PURITY (1A=40 ug/mL RNA; Dilution: _____)

Sample	A260	A260/A280 (1.8-2.1 OK)	Yield (ug)
_1	_____	_____	_____
_2	_____	_____	_____
_3	_____	_____	_____
_4	_____	_____	_____
_5	_____	_____	_____
_6	_____	_____	_____
_7	_____	_____	_____
_8	_____	_____	_____

Need 10 ug total RNA for protocol (if possible, adjust all samples to the same concentration).

The Poly-A RNA control is a desirable but not vital control to confirm the quality of your prep.

Poly-A RNA control prep (uses Poly-A RNA Control Kit (900433); 1st dilution poly-A control can be stored up to six weeks at -20C with eight freeze-thaws**):**

Poly-A RNA spike	Ratio of copy number	Concentration (stock)
lys	1:100,000	7.6 nM
phe	1:50,000	15.2 nM
thr	1:25,000	30.4 nM
dap	1:7,500	114.0 nM

- Add 2 uL poly-A control stock to 38 uL dilution buffer (1:20). Mix and flash spin.
- Final dilution:
  - For 100 format array (midi), add 2 uL 1st dilution to 38 uL dilution buffer (1:20).
  - For 49 format array (std), add 2 uL 1st dilution to 24 uL dilution buffer (1:13).
- Add 2 uL of 2nd dilution to total RNA sample.

cDNA synthesis:

1. Primer annealing: prepare the following in a nuclease-free 0.5 mL tube.

total RNA	10 ug	
diluted polyA RNA control	2 uL	_____
random primers @ 75 ng/uL	10 uL	_____
nuclease-free water	qs to 30 uL	_____