From Dwyer 2019 correspondence

In the original method, replace **First Strand Synthesis** with the following:

Primer Annealing:

1. Make a primer/template mix for each sample as detailed below:

Reagent	Volume (uL)
5x SSIV buffer	4
1 ug primer (80 nM per primer)	2
10 ug	
ddH2O	to 20 uL
Total	**20**

2. Place samples in thermo-cycler and run the following cycle: **70°C 1 min, 65°C 5 min, 55°C Hold**
3. Make the enzyme dNTP mix for each sample as detailed below (this is following the Invitrogen Superscript IV protocol):

Reagent	Volume (uL)
5X SSIV buffer	4 uL
0.1 M DTT	2 uL
low-dTTP mix [10 mM dATP, 10 mM dCTP, 10 mM dGTP, 6 mM dTTP]	2 uL
1 mM biotin-11-dUTP	8 uL
SSIV MMLV enzyme	2 uL
ddH2O	2 uL

Here the biotin-11-dUTP + TTP concentration is at 10 mM combined.

4. Heat dNTP Enzyme mix to **55°C** by placing it in the thermo-cycler containing the template/primer mix
5. Directly add 20uL dNTP mix to the template/primer mix, ensuring that both samples are kept at **55°C**
    - **Note:** Maintaining a temperature of **50-55°C** is crucial for minimizing non-specific primer

annealing and thus off-target 1st stand products.

6. Allow the 1st strand synthesis reaction to proceed for 10 min at **55°C**
7. Incubate sample at **80°C** for 10 min to inactivate the enzyme
    - **Note:** Sample volume is now 40uL
    - This is a good stopping point for Day 1- put samples at -20°C (or you can go through the RNA hydrolysis step as well and then freeze at -20°C).
    - **Per Zach: Everything else is the same, except the coupling step is unnecessary. Therefore, during the column clean-up after RNA hydrolysis we elute with 50 µL and go straight into the bead purification.**

Things to consider optimizing