Library Synthesis Reaction

Step A:

1. Thaw the library synthesis solution and nuclease free water on ice, (leave the remaining in the -20 degree fridge until ready for use). In a separate eppendorf tube make a master mix of 0.625 ul of library synthesis solution and 1.525 ul of nuclease-free water per sample. (For example, if you have 11 samples, make a master mix that has 11 times the amount of library synthesis solution and nuclease free water).
2. Pipette 2.15 ul of the cocktail into each of the 8 PCR-tube strips.
3. Add, 2ul of the template (RNA+DNA mix 100ng) in each of the tube containing the cocktail. Immediately place the library synthesis solution back in the -20 degree Celsius fridge.
4. Place the caps on and secure it tightly. Spin the tubes down in a picocentrifuge. Incubate the samples in a PCR thermocycler at 95 degrees Celsius for 5 minutes, then let it cool to 18 degrees Celsius.

Step B:

5. Meanwhile, thaw library synthesis buffer, water, and library synthesis enzyme on ice.
6. Mix in a separate tube 0.975 ul of nuclease free water, 0.625 ul of library synthesis buffer and 0.5 ul of library synthesis enzyme for one sample. Make a master mix for the number of samples you will need. Put back the library synthesis buffer and enzyme in the -20 degree fridge.
7. Take out the samples from the thermocycler (step A). Pipette 2 ul of this master mix prepared in step 6 into each of the samples in the 8 strip PCR tubes and mix by pipetting slowly up and down. Place a new cap on the 8 strip PCR tubes and place in the thermocycler again. Proceed to the next step and incubate the sample with the following parameters:

    18 degrees Celsius for 10 minutes
    25 degrees Celsius for 10 minutes
    37 degrees Celsius for 30 minutes
    42 degrees Celsius for 10 minutes
    70 degrees Celsius for 20 minutes

4 degrees Celsius for indefinitely.