RT with SSII (Invitrogen)

Prep: Get ice, set water bath to 65°C

1. Mix RNA, water, random hexamer primers
   1. Volume for 1 µg RNA
   2. 1 µL Primers (50 ng/µL)
   3. ddH2O up to 11 µL total volume
2. Incubate at 65°C for 10 min, quick chill on ice, quick spin
3. Assemble master mix 1x \_\_\_\_\_\_\_\_\_x\_\_\_\_
   1. 5X First Strand Buffer 4 µL \_\_\_\_\_\_\_\_\_\_\_\_\_\_
   2. 0.1 M DTT 2 µL \_\_\_\_\_\_\_\_\_\_\_\_\_\_
   3. RNase Out 1 µL \_\_\_\_\_\_\_\_\_\_\_\_\_\_
   4. dNTPs 1 µL (10 mM) \_\_\_\_\_\_\_\_\_\_\_\_\_\_
4. Add 8 µL of master mix to each sample, incubate at RT for at least 2 min
5. Add 1 µL of SSII RT, flick tubes, quick spin
6. Incubate at 42°C for 1 hr in PCR machine
7. Heat inactivate at 100C for 1 hr, hold at 4°C
8. Add 80 µL ddH2O (final volume = 100 µL) and store at -80°C