PCR

1. Pipette 8µl of 5µM primer (stock: 200µM) pair in each well (Corning round bottom micro titer plate)
2. Add 42µl of PCR mix from a trough (Corning 4870)

PCR mix per 96 well plate:

Water, DEPC treated 3297 µl
10x Taq buffer 525 µl
2.5 mM 4x dNTPs (Pharmacia 27.2035.01) 525 µl
1µg/µl N2 DNA 10.5µl

Taq (Perkin Elmer) 52.5µl

3. Seal with rubber tops (hybaid), incubate in MJ tetrad.

Cycling

92C 1'

34 cycles:
92C 20"
54C 40"
72C 4'

72C 3'
4C hold

4. Aliquot 2µl of the reaction into dye for the gel.
5. Ethanol precipate the PCR samplesEtOH/NaCl mix per 96 well plate:

11 ml EtOH
550µl of 1M NaCl

6. Aliquot 105µl of EtOH/NaCl mix to wells of a pointy-bottom rigid plate (Sarstedt 82.158.001)
7. Transfer and mix PCR reactions with a multipipetter.
8. Precipitate at - 70C for at least 2 hours (optional overnight).
9. Spin at 4K for 7minutes in a plate centrifuge.
10. Lay pad of about 3-4 tissues on plate and invert. Transfer on a second pad of tissues.
11. Add 200µl of 70% EtOH to each well. Leave on ice a few minutes.
12. Spin at 4K for 2 minutes.
13. Vacuum dry for 10 minutes.
14. Add 7µl of To.1E (DEPC) to each well. Spin down and allow to resuspend. Leave in the fridge for at least one hour.

Transcription reactions

Use Ambion T3 and T7 mega kits.

1. Pipette 1.5µl of resuspended PCR reaction into one plate for T3 reaction and one plate for T7 reaction on ice. Use PCR plates that can be capped with a cap mat (ABgene, Surrey, AB-0800).
2. Prepare the following transcription mix on ice:For each plate make one T7 and one T3 mix

105 µl Rnase free water
52.5 µl 10x reaction buffer
210 µl rNTPs
52.5µl enzyme mix

3. Aliquot 4µl of transcription mix into each well. Spin down. Seal with a rubber mat plus parafilm and sticky tape to avoid avaporation. Incubate at 37°C for 4.5 hours.
4. Dilute the T3 and T7 reactions with 47µl of RNase free water.
5. Run 2µl from each reaction on a gel.

Cleaning the RNA

1. Combine the T3 (50µl) and the T7 (50µl) reaction mixes in a plastic Qiagen block.
2. Add 350µl of RLT buffer to each well and close the rack with tape before shaking it back and forth.
3. Add 250µl 96EtOH to each well and mix as before.
4. Load the RNAs into the RNeasy plate (Qiagen).
5. Load 700µl of sample into the plate. Engage vacuum until wells are dry. Turn off vacuum and ventilate the manifold.
6. Load 1ml of RW1 buffer. Engage vacuum until wells are dry. Turn off vacuum and ventilate the manifold.
7. **Empty the waste tray!**
8. Load 1ml of RPE buffer. Engage vacuum until wells are dry. Turn off vacuum and ventilate the manifold.
9. Load 1 ml of RPR buffer. Engage vacuum until wells are dry. Remove plate from holder and shake to remove buffer on the bottom of the tray. Invert and pat dry vigorously on a stack of paper towels. Return to the manifold and leave with vacuum for 10 minutes. Remove the buffer one more time. Return to vacuum manifold and dry additional 5 minutes.
10. **Disengage the vacuum and replace the waste tray with the collection rack, watch the orientation!!!!**
11. Elute 2x with 80µl of Rnase free water. The total volume collected should be 130µl.
12. Take 50µl of RNA and mix with 10µl of 6x injection buffer in a PCR tray. Anneal these in a PCR block (68°C for 10 minutes and then 37°C for 30 minutes). Remove 2µl for assay on 1% agarose, in parallel with single strands. Use Sigma Type 1 gel loading solution. Ds material runs slower.Gel: 100 V, 0.5 h, TBE, 1% Agarose6x injection buffer: 40mM KPO4 pH 7.5, 6mM K-citrate pH 7.5, 4% PEG 6000
13. Freeze the remainder of the non-annealed mixed strands.

Seal the RNAs with a top mat and freeze.