Biomark System Overview

Running a Multi-Array Chip

Preamp Mix (Per Reaction):

Preamp Master Mix: 1 µL
Pooled Primer Mix (500 nm): 0.5 µL
Water: 2.25 µL

1. Assemble the mix as follows and add 3.75 µL of this to 1.25 µL of cDNA.

Preamp

Step 1. 95 °C, 2 min
Step 2. 95 °C, 15 s
Step 3. 60 °C, 4 min ⌝ 10–15 cycles
Step 4. 4 °C, ∞

2. Run the samples through the termocycler under the Preamp parameters.

Exonuclease I Mastermix (Per Reaction)

Water: 1.4 µL
Exonuclease I Reaction Buffer: 0.2 µL
Exonuclease I at 20 Units/µL: 0.4 µL

3. Make up the Exonuclease I Mix and add 2 µL of this to each Preamp reaction.

Exonuclease I Treatment

Step 1, 37 °C, 30 min
Step 2, 80 °C, 15 min
Step 3, 4 °C, ∞

4. Run the samples through the thermocycler under the Exonuclease I Treatment parameters.

10x Assay Mix (Per Reaction, 1 µL Surplus)

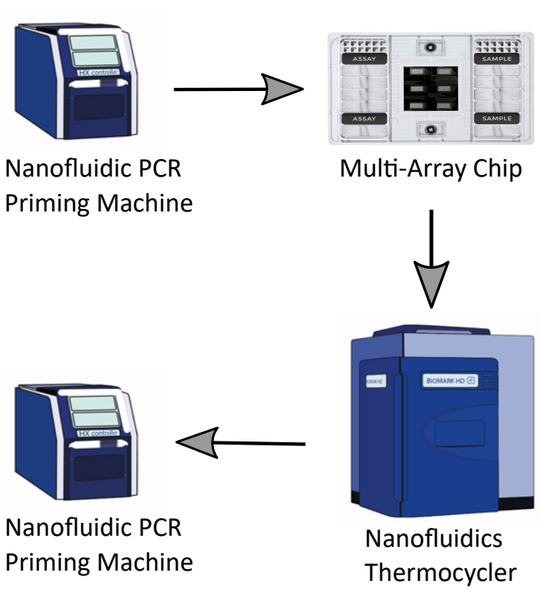
50 µM Forward/Reverse Primers: 0.4 µL
DNA Suspension Buffer: 1.6 µL
2x Assay Loading Reagent: 2.0 µL

5. Make up an Assay Mix for every primer of interest (note this is per inlet).

Sample Mix (Per Reaction, 1 µL Surplus)

Fluorescent Probe Supermix: 2.0 µL
Sample Reagent: 0.2 µL
Preamp and Exo I treated sample: 1.8 µL

6. Make up a Sample Mix for every sample of interest (note this is per inlet).



7. Prime the chip in the Nanofluidic PCR Priming Machine.

8. Load the chip.

9. Run the chip through the Nanofluidics Thermocycler.

10. Run the chip through the Post-run script in the Nanofluidic PCR Priming Machine.

Running a Single-Array Chip

Preamp Mix (Per Reaction):

Preamp Master Mix: 1 µL Pooled
Primer Mix (500nm): 0.5 µL
Water: 2.25 µL

1. Assemble the mix as follows and add 3.75 µL of this to 1.25 µL of cDNA.

Preamp

Step 1. 95 °C, 2 min
Step 2. 95 °C, 15 s
Step 3. 60 °C, 4 min ⌝ 10–15 cycles
Step 4. 4 °C, ∞

2. Run the samples through the termocycler under the Preamp parameters.

Exonuclease I Mastermix (Per Reaction)

Water: 1.4 µL
Exonuclease I Reaction Buffer: 0.2 µL
Exonuclease I at 20 Units/µL: 0.4 µL

3. Make up the Exonuclease I Mix and add 2 µL of this to each Preamp reaction.

Exonuclease I Treatment

Step 1, 37 °C, 30 min
Step 2, 80 °C, 15 min
Step 3, 4 °C, ∞

4. Run the samples through the thermocycler under the Exonuclease I Treatment parameters.

10x Assay Mix (Per Reaction, 1µL Surplus)

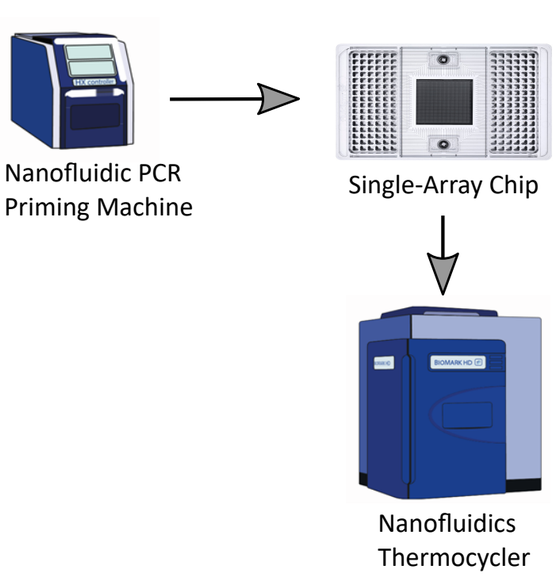
50µM Forward/Reverse Primers: 0.6 µL
DNA Suspension Buffer: 2.4 µL
2x Assay Loading Reagent: 3.0 µL

5. Make up an Assay Mix for every primer of interest (note this is per inlet).

Sample Mix (Per Reaction, 1 µL Surplus)

Fluorescent Probe Supermix: 3.0 µL
Sample Reagent: 0.3 µL
PreAmp and Exo I treated sample: 2.7 µL

6. Make up a Sample Mix for every sample of interest (note this is per inlet).



7. Prime the chip in the Nanofluidic PCR Priming Machine.

8. Load the chip.

9. Run the chip through the Nanofluidics Thermocycler.

10. Run the chip through the Post-run script in the Nanofluidic PCR Priming Machine.