

4. incubate at 30°C for 120 minutes in thermocycler with heated lid
5. keep at +4°C until used
6. microcentrifuge at 14'000g for 10 minutes prior to running on the MITOMI chip so that to prevent clotting of the channels by particles of lysate

Protocol for Klenow extension:

1. for each Klenow extension reaction prepare the following mix in 20 μ L volume taking target oligo in excess so that the final concentration of double-stranded labeled targets is determined by the concentration of primer making it 10 μ M after annealing step and 6.67 μ M after extension step:
 - 0.4 μ L 500 μ M complement primer
 - 2 μ L NEBuffer 2 (10x)
 - 0.6 μ L 500 μ M target oligos
 - 17 μ L mQ water to 20 μ L
2. run the annealing program in thermocycler with heated lid:
 - I. 98°C, 4 minutes
ramp 10%
 - II. 37°C, 3 minutes
 - III. 4°C, hold
3. prepare extension mix and add 10 μ L of it to the result of each annealing reactions:
 - 0.5 μ L Klenow (exo-) enzyme
 - 1 μ L NEBuffer 2 (10x)
 - 3 μ L 10 μ M dNTP mix
 - 5.5 μ L mQ water to 10 μ L
4. run polymerisation program in thermocycler with heated lid:
 - I. 37°C, 90 minutes
ramp 10%
 - II. 80°C, 25 minutes
ramp 100%
 - III. 30°C, 4 minutes
ramp 10%
 - IV. 4°C, 1 minute
ramp 10%
 - V. 4°C, hold