

## Material and equipment

1. Hybridization machine: Immunostainer Ventana Discovery DIS02834  
Kit for DNA-Microarrays: ChipMap Kit, Ventana (100 Hybridizations): Cat Nr. 760-101
2. Commercial Hybridization buffer is mixed to labeled samples up to 200µl (ca. 20µl labeled product + 180µl Hyb-Buffer)
3. Buffer for Prehybridization:
  - a. ChipSpread A:
    - i. 20g Albumin, Bovine Fraction V (Sigma: A3059)
    - ii. 0.5g Sodium Azide (Sigma: S2002)
    - iii. 200ml 20xSSC (Sigma: S6639)
    - iv. ad 1000ml (Deionisiertes Wasser)
    - v. storage maximal 3 month at 4°C
  - b. ChipSpread B:
    - i. 1000 ml Deionized Formamid (Sigma: F9037)
    - ii. 2g SDS (Sigma: L4522)
    - iii. storage maximal 3 month at -20°C

For hybridization, mix ChipSpread A and B in ratio 1:1 enough for 2ml per slide.

Commercial solutions from Ventana (ChipPrep1 and 2, ChipClean) in dispenser are used at RT; Canisters with Buffers could be checked by sensors and filled up.

## Procedure

1. Clean arrays with air pressure
2. Start Ventana Discovery Maschine and choose hybridization program (Array)
3. Print labels for every slide with description of slide and hybridization and put them on the slide with the buffer barrier (the uncutted side) to the spotted area
4. Transfer the dispenser solutions ChipPrep 1 and 2 and ChipClean on the carusell
5. Lay slides up side up on the heating pads with the label outwards
6. Start the program, the run stops after two minutes for prehybridization
7. Add 2ml ChipSpread 1/2 (ratio 1:1) to the slide directly behind the label and continue run. The run stops after 1h30 (prehybridization time) for hybridization
8. Add labeled samples in Hyb-Buffer (200µl) to the surface of the oil behind the label and continue run. Run stops after 8h (Hybridization time)
9. Wash slides 3x 5min and 2x 10min in 1 x SSC at RT
10. Add the slides for 5sec in a serial of 70% Ethanol, 95% Ethanol and incubate them in Isopropanol until drying
11. Remove slides immediately from Isopropanol and dry with air pressure

