

## Protocol for The NimbleGen Array Reuse Kit

### Outline

This protocol describes the use of the NimbleGen Array Reuse Kit for the removal of hybridized nucleic acid samples from NimbleGen DNA microarrays. The method has been developed to remove labeled sample from the microarray surface while leaving microarray probes unaffected. The kit is configured to process up to 40 arrays.

### Kit Contents

Qty	Item	Package Size
2	Strip Buffer I	500ml
1	Strip Buffer II	400ml
2	Strip Buffer III	500ml
2	Plastic Processing Tank	200ml
4	Slide Processing Containers	25ml Capacity
2	Glass Strip Tank	40ml Capacity
1	Array Processing Rack	

### Protocol Information & Safety

- Gloves and safety glasses are required when performing this procedure.
- Strip Buffer II is a corrosive chemical that must be handled in a fume hood.
- In the event of a spill, clean up the solution with absorbent pads, allow the pads to dry in a fume hood and dispose of as regular trash. In the event of skin or eye contact, rinse the affected area with copious amounts of water and seek medical attention where applicable.
- Use caution when handling the arrays to avoid physical contamination of the microarray area. Wear gloves and handle arrays only by the edges.
- Use good laboratory techniques to avoid nuclease contamination of the microarrays, racks, tanks, and tubes. Use sterile labware, wear gloves, and use nuclease-free solutions.
- Discard stripping solutions after each use.

### Required Apparatus & Labware\*

\* See last page for a reagent supplier list

- 45°C water bath
- Purified water – nuclease free sterile high-grade water or autoclaved 18 MΩ purified water such as Milli-Q or E-Pure purified water
- NimbleGen Systems Array-Go-Round centrifugal microarray dryer, compressed high purity argon for drying microarrays, or an ArrayIt Microarray High speed Centrifuge, Cat# MHC110V

## Stripping Procedure – 8 Microarrays

Stripping of NimbleGen Systems microarrays must immediately follow the scanning of the previous hybridization. NimbleGen recommends stripping the arrays as soon after hybridization as possible.

1. Fill one of the provided tanks with 200ml of Stripping Buffer I. Place the tank in a 45°C water bath and allow to equilibrate to temperature for 30 minutes.
2. Fill the second provided tank with 200ml of purified H<sub>2</sub>O.
3. Place the microarrays to be stripped in the provided rack and submerge in a room temperature 200ml H<sub>2</sub>O bath for 5 minutes. Agitate the rack and arrays vigorously for the first minute of incubation.
4. Lift the rack from the water and allow the water to drain off the arrays and rack for 5 seconds.
5. Transfer the rack to the 45°C Strip Buffer I tank and incubate for 2 hours. Agitate the rack and arrays vigorously for the first minute of incubation.
6. Discard the water from the first tank and rinse with purified H<sub>2</sub>O. Re-fill the tank with 200ml of fresh purified H<sub>2</sub>O.
7. Transfer rack and microarrays to the fresh H<sub>2</sub>O bath and incubate for 5 minutes. Agitate the rack and arrays vigorously for the first minute of the incubation.
8. Remove the rack and microarrays from the water and allow to stand for 1 minute to drain off water.
9. In a fume hood, dispense 40 ml of the Strip Buffer II into the two provided 40ml Glass Strip Tanks.
10. Transfer the microarrays from the rack to the slots in the glass tanks, one microarray per slot. Cover the tank with the provided lid and incubate at room temperature for 30 minutes.
11. Discard the Strip Buffer I from the first tank and rinse it and the rack well with purified H<sub>2</sub>O. Re-fill the tank with 200ml Strip Buffer III and insert rack.
12. Transfer the microarrays one at a time to the rack in the Strip Buffer III bath. Incubate for 2 minutes at room temperature. Agitate for the first 20 seconds.
13. Discard the water from the water tank, and rinse with purified H<sub>2</sub>O. Re-fill the tank with 200ml of fresh purified H<sub>2</sub>O.
14. Transfer rack and microarrays to the fresh room temperature purified H<sub>2</sub>O bath and incubate for 2 minutes. Agitate the rack and arrays vigorously for the first minute of incubation.
15. Spin-dry the arrays in the NimbleGen Systems Array-Go-Round for 2 minutes. Alternatively, you can spin-dry the arrays using other centrifugal array drying methods or dry the arrays individually with repeated blasts of purified argon.
16. Store the arrays in their original cases under desiccation at room temperature until rehybridization.

### Important!

Wear gloves and work with Strip Buffer II in a fume hood. Buffer components are corrosive and can cause chemical burns on exposed skin.

## Stripping Procedure – 4 Microarrays

1. Fill the 4 provided Slide Processing Containers with 25ml each of Stripping Buffer I. Place the tubes in a 50ml conical tube rack, place in a 45°C water bath, and equilibrate at 45°C for 30 minutes.
2. Fill a provided tank with 200ml of purified H<sub>2</sub>O.
3. Place microarrays to be stripped in the provided rack and submerge in a room temperature 200ml H<sub>2</sub>O bath for 5 minutes. Agitate the rack and arrays vigorously for the first minute of incubation.
4. Lift the rack from the water and allow the water to drain off the arrays and rack for 5 seconds.
5. Transfer each microarray to a single 45°C Strip Buffer I slide processing container and incubate for 2 hours. Agitate the sleeves gently for the first minute of incubation.
6. Discard the water from the first tank and rinse with purified H<sub>2</sub>O. Re-fill the tank with 200 ml of fresh purified H<sub>2</sub>O and put the rack in the water.
7. Transfer the microarrays to the rack in the fresh H<sub>2</sub>O bath and incubate for 5 minutes. Agitate the rack and arrays vigorously for the first minute of incubation.
8. Remove the rack and microarrays from the water and allow to stand for 1 minute to drain water.
9. In a fume hood, dispense 40 ml of the Strip Buffer II into the provided 40ml Glass Strip Tank.
10. Transfer the microarrays from the rack to the slots in the glass tank, one microarray per slot. Cover the tank with the provided cover and incubate at room temperature for 30 minutes.
11. Discard the Strip Buffer I from the 4 Slide Processing Containers, and rinse with purified H<sub>2</sub>O. Re-fill the containers with 25ml Strip Buffer III.
12. Transfer the microarrays one at a time to the slide processing containers. Incubate for 2 minutes at room temperature.
13. Discard the water from the water tank, and rinse with purified H<sub>2</sub>O. Re-fill the tank with 200ml of fresh purified H<sub>2</sub>O.
14. Transfer the microarrays to the fresh room temperature purified H<sub>2</sub>O bath and incubate for 2 minutes. Agitate the rack and arrays vigorously for the first minute of incubation.
15. Spin-dry the arrays in the NimbleGen Systems Array-Go-Round for 2 minutes. Alternatively, you can spin-dry the arrays using other centrifugal array drying methods or dry the arrays individually with repeated blasts of purified argon.
16. Store the arrays in their original slide cases under desiccation at room temperature until rehybridization.

### Important!

Wear gloves and work with Strip Buffer II in a fume hood. Buffer components are corrosive and can cause chemical burns on exposed skin.

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## Disposal and Cleaning Procedures

- Dispose of all solutions down the drain with copious amounts of water.
- Dispose of Strip Buffer II in a fume hood drain, if possible.
- Racks, tanks, tubes, and glassware should be cleaned with a standard laboratory detergent (e.g: Micro<sup>®</sup> Liquid Laboratory Cleaner or Alconox Detergent), rinsed with purified water and allowed to air dry.

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## Reorder List

Component	Package Size	Item Number
NimbleGen Array Reuse Kit 40	40 Rxn	KIT001-2
NimbleGen Array Reuse Kit 100	100 Rxn	KIT001-3
NimbleGen Array Reuse Kit Refill 40	40 Rxn	KIT004-2
NimbleGen Array Reuse Kit Refill 100	100 Rxn	KIT004-3
NimbleGen Strip Buffer I 500ml	500ml	R10001-2
NimbleGen Strip Buffer I 10L	10L	R10001-4
NimbleGen Strip Buffer II 400ml	400ml	R10002-3
NimbleGen Strip Buffer III 500ml	500ml	R10003-2
NimbleGen Strip Buffer III 10L	10L	R10003-4
Array Processing Rack and Tank	1 each	E10001
Array Processing Rack	1 each	E10002
Array Processing Tank	1 each	E10003
Glass Strip Tank – 40ml	1 each	E10004
Glass Strip Tank – 80ml	1 each	E10005