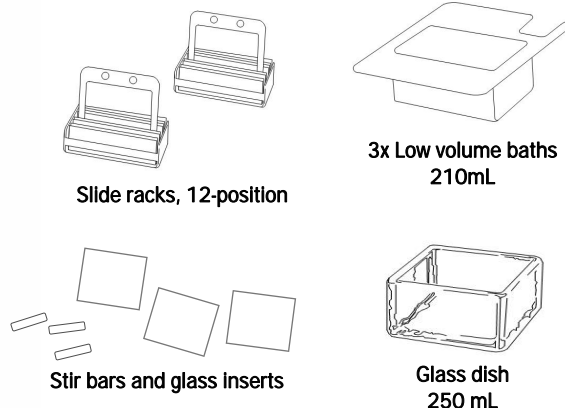
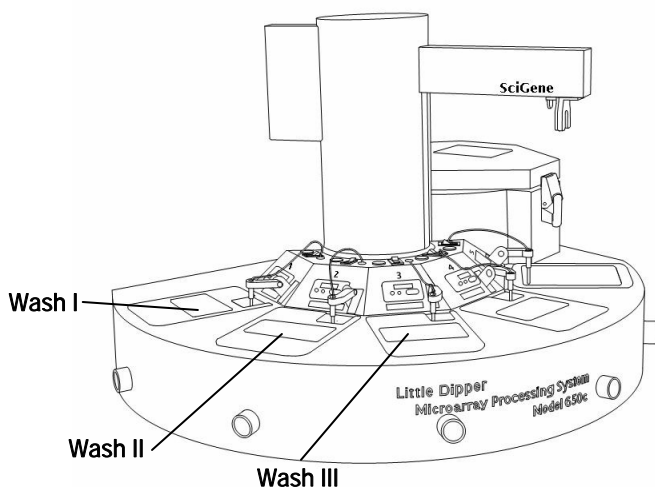


Post-Hyb Processing for Roche NimbleGen Arrays for CGH, Gene Expression & CHIP-chip Assays



Equipment Configuration

- Little Dipper Processor for Roche NimbleGen Arrays*, 115v/230v. (SciGene cat. #1080-80-1/1080-80-2)
*special configuration with longer vertical stroke length
Contact SciGene Technical Service for details.
- 3x Low volume baths (210 ml, non-heatable). (SciGene cat. #1080-10-2)
- 3x Magnetic stir bars for low volume baths. (SciGene cat. #1080-11-1)
- 3x Glass inserts for low volume baths. (SciGene cat. #1080-10-3, 5/pk)
- 2x Slide racks, 12 position for 3 inch slides. (SciGene cat. #1080-20-1)

Required Buffers

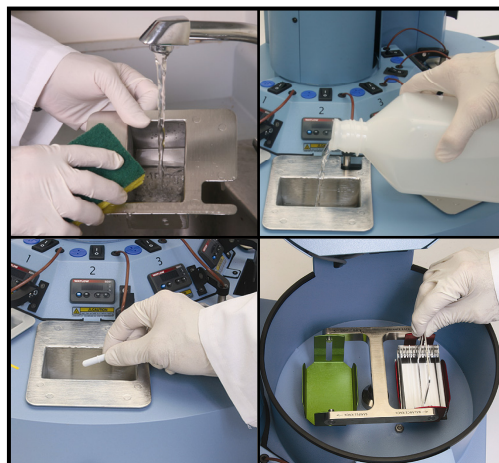
- NimbleGen Wash Buffer Kit (Roche cat. #05584507001)
Kit includes: 10x Wash Buffer I, 10x Wash Buffer III, 10x Wash Buffer II, 1M DTT and Nuclease-free water. Prepare the working volume of buffers as shown in Table 1.

Table 1. Roche NimbleGen Wash Buffer Preparation

Wash	Ingredient	Volume
Wash Buffer I 600 mL	Nuclease-free water	540 mL
	10X Wash Buffer I	60 mL
	1M DTT	60 µL
Wash Buffer II 300 mL	Nuclease-free water	270 mL
	10X Wash Buffer II	30 mL
	1M DTT	30 µL
Wash Buffer III 300 mL	Nuclease-free water	270 mL
	10X Wash Buffer III	30 mL
	1M DTT	30 µL

Instrument Setup

1. Rinse the removable baths, stir bars and the processing rack with 100% ethanol then with de-ionized water three times, and dry with lint-free towels. Do not use detergent.
2. Ensure that all temperature sensors are rotated down and then place clean low volume baths in positions 1 through 3. Add a glass insert and stir bar into each bath.
Note: Any sensor remaining in the "up" position will interfere with the movement of the Little Dipper arm.
3. Fill the baths with 210 ml of each of the solutions shown in Table 2 for the arrays to be processed. Fill the glass dish with 250 ml of Wash Buffer I.
4. Place a balance rack into the red bucket of the centrifuge with the same number of slides to be processed. Consult the *Little Dipper Operations Guide* for details.
5. Turn on main power to the instrument.
6. Activate and set rotation speed of stir bars in all baths so that a vigorous vortex is formed without splashing.



Wash and fill baths, activate stir bars, and then insert balance rack into centrifuge.

Continued on next page...

Load Arrays / Start Protocol

1. Disassemble the mixer-slide assembly as specified in the *NimbleGen Arrays User's Guide*.
2. Place the slides into the 12-position rack in the glass staining dish containing Wash Buffer 1.
3. Once all the arrays to be processed are placed in the slide rack (12 max), move it to Bath #1, and follow the Little Dipper Protocol (NGWash1) below to wash the arrays.

Table 2. Little Dipper Program for Roche NimbleGen Arrays (NGWash1).

Step	Bath	Buffer	Temp	Agitation	Time (sec)
1	1	Wash Buffer I	RT	600	130
2	2	Wash Buffer II	RT	600	60
3	3	Wash Buffer III	RT	600	32
4	C	–	–	–	200
Total Time:					7 min

4. Using the touch screen, select the Roche1 program and start the protocol.
5. Mount the rack on the robot arm as show in the Little Dipper Operations Guide.
6. After completion of the protocols, remove the rack from the centrifuge and place the arrays in a slide box to await scanning.
7. Dispose of wash buffers immediately after use. Wash the baths, stir bars and processing rack with warm water, rinse 3 times with de-ionized water and dry with lint-free towels. Do not use detergents to clean baths. Store the baths in a dust-free environment ready for the next use.
8. Wipe the centrifuge dry.

– End Protocol –

All third party marks are the property of their respective owners.

SciGene
Automating Array Workflows