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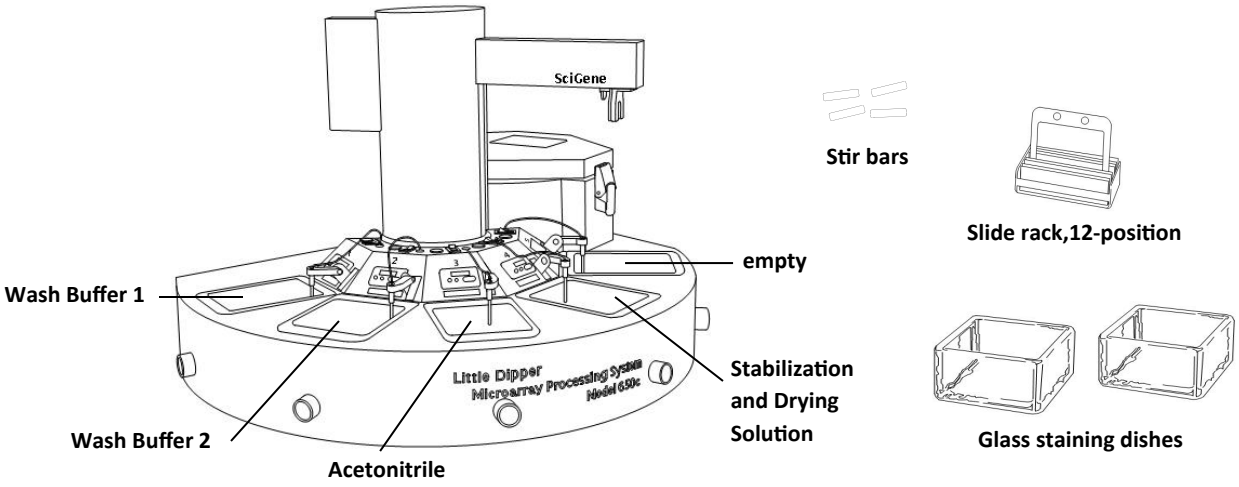
Agilent aCGH, Gene Expression, ChIP-chip and miRNA Arrays



Equipment Configuration

- **Little Dipper Processor for Agilent Arrays**
  SciGene cat. #1080-40-1 (115V) / 1080-40-2 (230V)
- **4x Standard volume baths**
  SciGene cat. #1080-10-1
- **4x Magnetic stir bars**
  SciGene cat. #1080-11-0
- **Slide rack, 12 position for 3 inch slides**
  SciGene cat. #1080-20-1
- **2x Glass staining dish**
  Fisher cat. #08-812 or similar

Required Buffers

aCGH and ChIP-chip Arrays

- **Oligo aCGH Buffer 1**
  SciGene or Agilent cat. #5188-5221
- **Oligo aCGH Buffer 2**
  SciGene or Agilent cat. #5188-5222
- **Acetonitrile***
  Sigma cat. #271004 or similar
- **Stabilization and Drying Solution***
  Agilent cat. #5185-5979

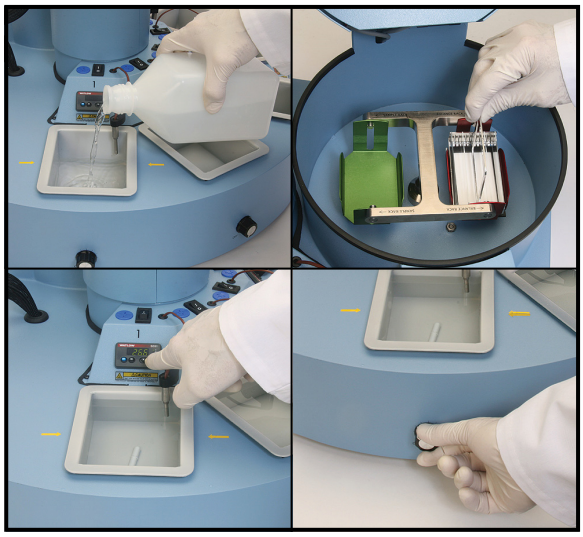
Gene Expression and miRNA Arrays

- **GE Wash Buffer 1**
  Agilent cat. #5188-5325
- **GE Wash Buffer 2**
  Agilent cat. #5188-5326
- **Triton X-102 (10%)**
  Agilent cat. #5185-5975
  Add to Buffers 1 and 2 to a final concentration of 0.005%.
  (Mix 50µl Triton X-102 with 100 ml Buffer.)
  Mandatory for miRNA and optional for Gene Expression.
- **Acetonitrile***
  Sigma cat. #271004 or similar
- **Stabilization and Drying Solution***
  Agilent cat. #5185-5979

**Used in Protocol Version B without ozone control.*

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. Place clean baths in positions 1 through 4 on the processor, add a stir bar to each bath, and rotate all temperature sensors down.
   Note: Any sensor remaining in the "up" position will interfere with the movement of the Little Dipper arm.
3. Fill the baths with ***670 ml*** of each of the solutions shown in Tables 1-4 for the array type to be processed.
4. Turn on main power to the instrument and the individual power switch to Bath #2. Set the temperature on the controller to the correct temperature shown in Tables 1-4 for the type of array to be processed. Wait at least 10 minutes for the temperature of the buffer to stabilize.



Fill baths, insert balance rack and then set temperature and stir bar speed.

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Instrument Setup (continued)

Table 1. Little Dipper Programs for CGH Arrays.

CGH Arrays Processed with Ozone Control (Agilent Protocol A) Program Name: CGH-A					
Step	**Bath**	**Buffer**	**Temp (°C)**	**Agitation**	**Time (sec)**
1	1	aCGH Buffer 1	RT	250	300
2	2	aCGH Buffer 2	37°	250	60
3	C	N/A	N/A	N/A	300
CGH Arrays Processed *without* Ozone Control (Agilent Protocol B) Program Name: CGH-B					
1	1	aCGH Buffer 1	RT	250	300
2	2	aCGH Buffer 2	37°	250	60
3	3	acetonitrile	RT	250	10
4	4	Stabiliz+drying	RT	250	30

Table 2. Little Dipper Programs for miRNA Arrays.

miRNA Arrays Processed with Ozone Control (Agilent Protocol A) Program Name: miRNA-A					
Step	**Bath**	**Buffer**	**Temp (°C)**	**Agitation**	**Time (sec)**
1	1	GE Buffer 1+Triton	RT	250	300
2	2	GE Buffer 2+Triton	37°	250	300
3	C	N/A	N/A	N/A	300

Table 3. Little Dipper Programs for Gene Expression Arrays.

Gene Expression Arrays Processed with Ozone Control (Agilent Protocol A) Program Name: GE-A					
Step	**Bath**	**Buffer**	**Temp (°C)**	**Agitation**	**Time (sec)**
1	1	GE Buffer 1 [S]	RT	250	60
2	2	GE Buffer 2 [S]	37°	250	60
3	C	N/A	N/A	N/A	300
Gene Expression Arrays Processed *without* Ozone Control (Agilent Protocol B) Program Name: GE-B					
1	1	GE Buffer 1[S]	RT	250	60
2	2	GE Buffer 2[S]	37°	250	60
3	3	acetonitrile	RT	250	10
4	4	Stabiliz+drying	RT	250	30

[S] *At your option, you may add Triton X-102 to a final concentration of 0.005% in GE Buffer 1 and 2 for Gene Expression Arrays.*

Table 4. Little Dipper Programs for ChIP-chip Arrays.

ChIP-chip Arrays Processed with Ozone Control (Agilent Protocol A) Program Name: Chip-A					
Step	**Bath**	**Buffer**	**Temp (°C)**	**Agitation**	**Time (sec)**
1	1	aCGH Buffer 1	RT	250	300
2	2	aCGH Buffer 2	31°	250	300
3	C	N/A	N/A	N/A	300
ChIP-chip Arrays Processed *without* Ozone Control (Agilent Protocol B) Program Name: Chip-B					
1	1	aCGH Buffer 1	RT	250	300
2	2	aCGH Buffer 2	31°	250	300
3	3	acetonitrile	RT	250	10
4	4	Stabiliz+drying	RT	250	30

Load Arrays / Start Protocol

1. Fill both glass staining dishes with room temperature aCGH Buffer 1 or GE Wash Buffer 1, consistent with the type of array to be processed. Place the 12-position slide rack into one of the dishes.
2. If running **CGH-A**, **miRNA-A**, **GE-A** or **Chip-A** protocols which incorporate a centrifugal drying step, 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.
3. Disassemble the Agilent SureHyb chamber or SciGene Mai Tai Cassette and sequentially remove the array-gasket slide sandwich and place it in one of the staining dishes. Separate the array from the gasket slide and place the array in the 12 position rack in the second staining dish.
4. Once all the arrays to be processed are placed in the slide rack (12 max), move it to Bath #1**.**
5. Using the touch screen, select the program that matches the array type and protocol version (A or B) shown in Tables 1-4. Start the protocol program.
6. Mount the rack on the robot arm as show in the **Little Dipper Operations Guide**.
7. At the completion of "A" type wash protocols, arrays are recovered from the centrifuge ready for scanning. For "B" type wash protocols, the rack of arrays is slowly withdrawn from the solution after which the rack is released from the gripper through the touch screen.
8. Remove the arrays from the rack and place in a slide box to await scanning.
9. 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.

— End Protocol —

SciGene