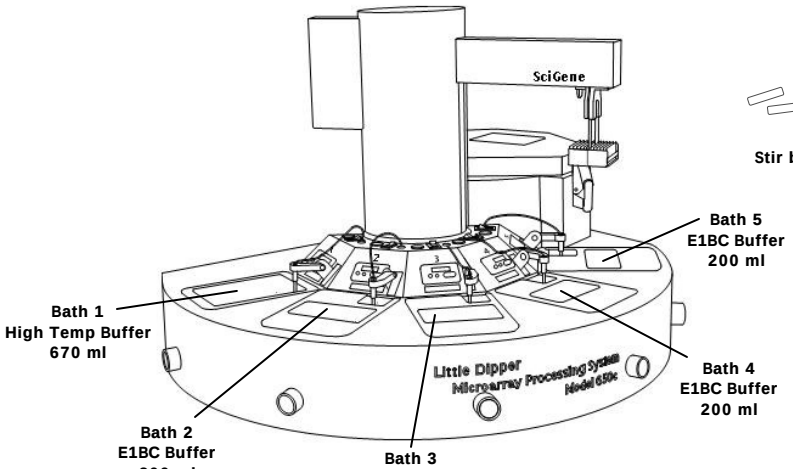
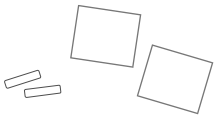
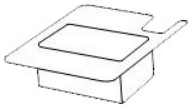
Processing Illumina® BeadChips for Gene Expression

CENTRIFUGAL DRYING METHOD




Stir bars and Glass inserts


Block E1 Buffer
200 ml


Strep-Cy3
200 ml


Staining dish with
E1BC Buffer
200 ml


Slide rack, 12-position
for BeadChips

Equipment Configuration

- **Little Dipper Processor for BeadChips**, 115v /230v.
  (SciGene cat. #1080-30-1/1080-30-2)
- **Standard volume bath**.
  (SciGene cat. #1080-10-1)
- **6x Low volume baths**.
  (SciGene cat. #1080-10-2)
- **Slide Rack, 12-Position for BeadChips**.
  (SciGene cat. #1080-20-2)
- **Glass staining dish**.
  (Fisher cat. #08-812)
- **Absorbent pads for centrifuge bucket.**
  (SciGene cat. #1080-21-1, 25/pk)

Buffer Preparation

- **High Temperature Wash Buffer** (Illumina)
  700 ml working solution – dilute 70 ml 10x stock with 630 ml, RNase-free water
- **E1BC Buffer*** (Illumina)
  800 ml working solution – dilute 2.4 ml, stock buffer with 800 ml, RNase-free water
- **Ethanol, absolute, ACS grade**
  Use fresh from bottle
  (Sigma cat. #459846 or similar)
- **Block E1 Buffer - Large Volume Bottle,** 440ml (Illumina)
  200 ml fresh from bottle
  (Illumina cat. #BD-220-1001)
- **Streptavidin-Cy3 Stain**
  Use a 1mg/ml stock solution: For 200 ml working solution dilute 200 µl of stock solution with 200 ml of Block E1 Buffer.

**Additional E1BC buffer is needed for coverseal removal step as described in Illumina manuals.*

Instrument Setup

1. Rinse the removable baths, glass inserts, stir bars and racks with 100% ethanol, then with de-ionized water three times and dry with lint-free towels. Do not use detergent.
2. Place a standard volume bath in position 1 and low volume baths into positions 2 through 5. Rotate all temperature sensors to the down position. Label two additional low volume baths **Block** and **Stain**. Cover **Stain** bath with aluminum foil. **Note:** Any sensor remaining in the "up" position will interfere with the movement gripper arm.
3. Fill baths and staining dish with the buffers and volumes shown in Table 1. Put a glass insert and stir bar into each low volume bath.
4. Turn on main power to the instrument. Turn on the power switch to Bath #1 and set the temperature to 55°C.
5. Activate and set rotation speed of stir bars in all baths, so that a vigorous vortex is formed, without splashing. Wait 10 minutes for the temperature of the buffer to stabilize.
6. Balance centrifuge. Place a 12-position rack in the red balance bucket containing the same number of arrays to be processed.
7. Place an absorbent pad into the green sample bucket and replace black spacer.

Table 1. Bath Positions, Types and Buffers.

Bath Position	Bath Type	Buffer	Temp (°C)	Volume (ml)
1	Standard	High Temp	55	670
2	Low volume	E1BC	RT	200
3	Low volume	Ethanol	RT	200
4	Low volume	E1BC	RT	200
5	Low volume	E1BC	RT	200
Additional Baths				
Block	Low volume	Block E1	RT	200
Stain	Low volume	Strep-Cy3	RT	200
Pooling Bead-Chips in rack	Glass dish	E1BC	RT	200

Continued on next page...

Load Arrays / Start Protocol

1. Remove BeadArrays from the hybridization cassettes and remove coverseals as specified in the *Illumina Whole Genome Gene Expression Assay Protocol Guide*. Place each array into the 12-position BeadChip processing rack keeping the rack submerged in the glass staining dish containing E1BC.
2. Move rack to Bath #1, start the Bead2 Protocol (Table 2) using the touch screen and load the rack on the gripper as described in the *Little Dipper Operations Guide*.

Table 2. Bead2 Protocol.

Step	Bath Position	Buffer	Agitation Rate	Time (sec)
1	1	High Temp	250	600
2	2	E1 BC	250	300
3	3	Ethanol	250	600
4	4	E1 BC	250☼	120
Instrument Pause / Change Out Baths 2 and 3.				
5	2	Block E1	50	600
6	3	Stain	50	600
7	5	E1 BC	250	300
8	Centrifuge	none	—	300
☼ *Includes user controlled pause in program after Step 4.*			**Total Time:**	57 min

3. Instrument will pause at the completion of step 4. Change bath in position #2 with bath labeled **Block**. Remove the aluminum foil from the **Stain** bath and use it in place of bath #3.
4. Resume instrument operation by the pressing touch screen. At completion of the protocol, remove sample rack from green bucket in centrifuge and store BeadChips in a light-tight box until scanned.
5. Dispose of wash buffers immediately after use. Wash the baths, stir bars and processing rack with warm water, rinse 3 times with de-ionized (DI) water and dry with lint-free towels. Do not use detergents to clean baths. Store baths in a Ziploc bag to protect from dust, ready for the next use.

— End Protocol —