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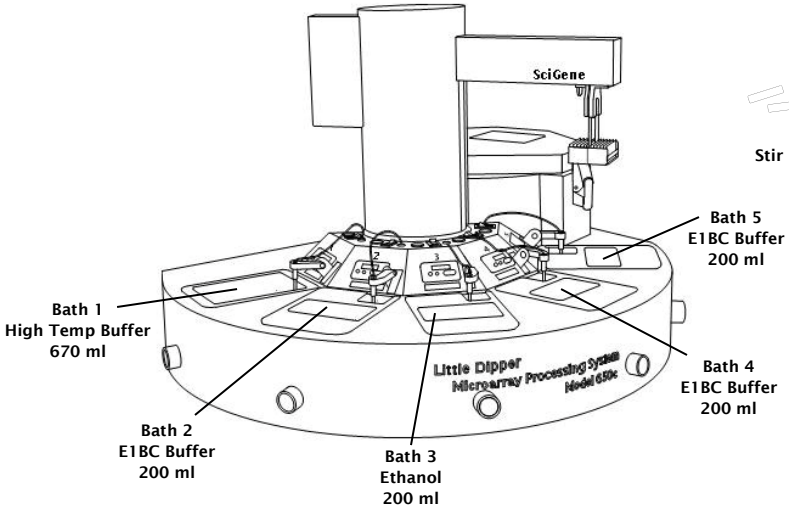
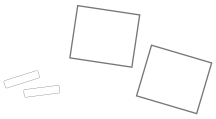
Processing Illumina® BeadChips for Gene Expression

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

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
  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 ml 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.

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

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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 —

SciGene