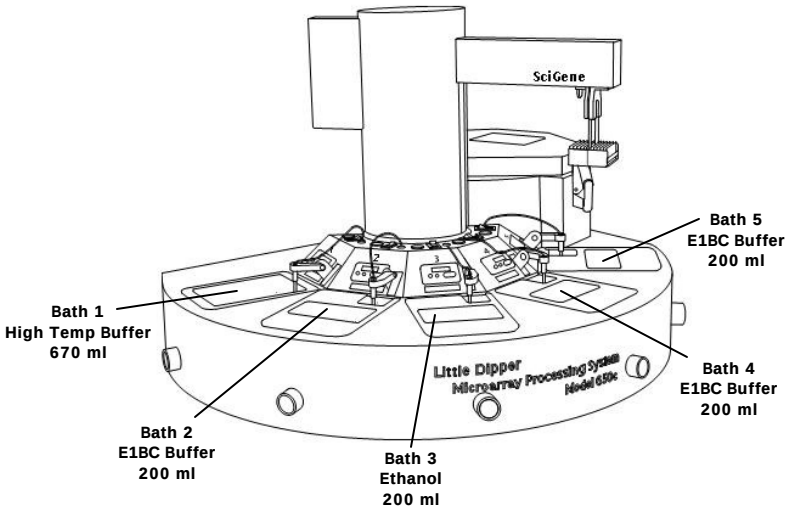
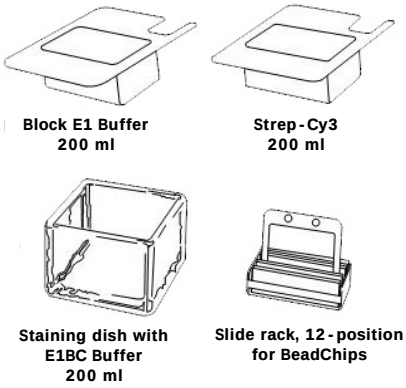
# Processing Illumina® BeadChips for Gene Expression

## CENTRIFUGAL DRYING METHOD




## Equipment Configuration

- **Little Dipper Processor for BeadChips**, 115v /220v.
  (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)
- **Blocker Casein in PPS**
  200 ml fresh from bottle
  (Pierce cat. #37528)
- **Streptavidin-Cy3 Stain**
  (GE Amersham cat. # PA43001)
  use a 1mg/ml stock solution) 200 ml working solution
  dilute 200 µl of stock solution with 200 ml

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

## Instrument Setup

1. Wash all stainless steel baths and the processing rack with warm water, rinse with DI water and dry with lint-free towels. Do not use detergent.
2. Place a standard volume bath in position 1 and low volume baths into the remaining positions. Rotate the temperature sensors to the down position. Label two additional low volume baths **Block** and **Stain**. Cover bath containing stain with aluminum foil.
3. Fill baths and staining dish with the buffers and volumes shown in Table 1.
4. Turn on main power to the instrument and the individual power switch to Bath #1 and set the temperature on the controller to 55°C. Wait approximately 10 minutes for the temperature of the buffer to stabilize.
5. Add stir bars and adjust speed for good vortex mixing without splashing.
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 | Volume (ml) |
|---|---|---|---|
| 1 | Standard | High Temp | 670 |
| 2 | Low volume | E1BC | 200 |
| 3 | Low volume | Ethanol | 200 |
| 4 | Low volume | E1BC | 200 |
| 5 | Low volume | E1BC | 200 |
| Additional Baths | | | |
| Block | Low volume | Block E1 | 200 |
| Stain | Low volume | Strep-Cy3 | 200 (*) |
| Pooling Bead-Chips in rack | Glass dish | E1BC | 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 (cycles/min) | 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. 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. Instrument will pause at the completion of step 4. Resume instrument operation by pressing touch screen.
4. At completion of protocol, remove sample rack from green centrifuge bucket and store BeadChips in a light-tight box until scanned.

— End Protocol —