

Hybridization Control Kit

Each commercially available eukaryotic probe array contains probe sets for several prokaryotic genes as controls. These probe sets are readily identified by the AFFX prefix in the probe set name. The .chp data for these control probe sets can be examined in the Summary Report File (.rpt).

Control Oligo B2

Control Oligo B2 hybridizes to features along the outer edge of all expression arrays and to the checkerboard pattern in each corner. These predefined patterns provide signals for the Affymetrix® Microarray Suite software to perform automatic grid alignment during image analysis. They can also be used to align the grid manually. The fluorescence intensities for Control Oligo B2 are not used for analyzing data.

A 60X stock of the B2 oligo is provided as part of the GeneChip Eukaryotic Hybridization Control Kit (P/N 900299 or 900362, for 30 or 150 reactions, respectively), or can be purchased alone (P/N 900301). Please refer to the instructions in Section 2, Chapter 3 for detailed information on including the B2 oligo in preparing the hybridization cocktail.

Biotinylated Hybridization Controls: *bioB*, *bioC*, *bioD*, and *cre*

BioB, *bioC*, and *bioD* are genes of the biotin synthesis pathway from the bacteria *E. coli*, and *cre* is the recombinase gene from P1 bacteriophage. A ready-prepared mixture of these biotinylated controls at staggered concentrations can be added with labeled eukaryotic cRNA samples to hybridize onto GeneChip probe arrays. Signal intensities obtained on these genes provide information on how well the hybridization, washing, and staining procedures have performed.

Affymetrix provides a kit that contains a 20X pre-mixed control reagent (P/N 900299 or 900362) and the final concentrations in the hybridization cocktail are 1.5 pM, 5 pM, 25 pM, and 100 pM for the four transcripts *bioB*, *bioC*, *bioD*, and *cre*, respectively.

Poly-A Spike Controls

Five poly-A-tailed control clones encoding *B. subtilis* genes (*dap*, *thr*, *trp*, *phe*, *lys*) are cloned into pBluescript as an *Xho I* to *Not I* insert, 5' to 3', respectively.



Poly-A-tailed constructs (*dap*, *thr*, *trp*, *phe*, *lys*)

These clones can be cut with different restriction enzymes to produce template DNA for either sense strand RNA synthesis or antisense RNA synthesis. The antisense control RNA for each *B. subtilis* gene is synthesized from linearized plasmid using T7 RNA polymerase with biotinylated nucleotides. The sense RNA for each *B. subtilis* gene is synthesized from linearized plasmid using T3 RNA polymerase with unlabeled nucleotides. For detailed preparation of sense RNA controls, please refer to Section 3, Chapter 2.

The antisense strand *B. subtilis* RNA controls are used as described above for *bioB*, *bioC*, and *bioD* genes. The sense strand RNA controls can be spiked into samples during mRNA preparation to monitor the efficiency of target preparation, hybridization, wash, and stain.

Bacteria containing these recombinant plasmids can be obtained from the American Type Culture Collection (ATCC). See *Reagents and Materials Required* on page 2.2.5 for details.