

## Normalization Protocol

Open your BAGEL input file. Open the file entitled "NormTemplateGPX.X", where X.X represents the version of GenePix that produced your .GPR file. Open your filename.GPR file in Excel.

In the *filename.GPR* spreadsheet:

SORT all data by ID. Use the default setting for ascending or descending order.

COPY all data (from upper left-hand cell to the bottom right-hand-most cell of the data matrix)

In the *NormTemplateX.X* spreadsheet:

PASTE data into the upper left cell of the NormTemplate spreadsheet.

Examine the "All Points", "StrictPass Points", and "Kept Points" scatter plots to see the quality of the hybridization. Generally, but not always, a high R-squared and a regression close to  $y=x$  indicate a high quality hybridization.

COPY all data and results (from the left-most cell below the header line to the bottom right-most cell of the data & results matrix).

PASTE SPECIAL...VALUES all data and results *into the same spreadsheet cells*. This converts all the formulas into values and is *absolutely essential*.

SORT all data & results by KEEP column in descending order.

SAVE AS *filename.xls*.

In the BAGEL input spreadsheet:

In the top cell below the header rows in the appropriate column, type:

`=VLOOKUP(lookup_value, table_array, 78, FALSE)`

- *lookup\_value* is the cell address of the far left of the BAGEL input file, which should have gene Names.
- *table\_array* is the matrix address, from the ID column on the left to the column of ratios on the right, from just below the header row on the top to the bottom row of the data matrix that has a value of KEEP = 1.

Select this cell and FILL DOWN to the bottom of the BAGEL input spreadsheet.

COPY this column.

PASTE SPECIAL...VALUES into the very same column. Again, this converts all the formulas into values and is *essential*.

SAVE your BAGEL input spreadsheet as a tab-delimited text file.