METHODS (Supplementary Data)

RNA preparation and quantification. Total cellular RNAs were extracted from BT474 and four BT/Her^R subclones, two that were originally selected in 1.0 μM Herceptin and two that were originally selected in 0.2 μM Herceptin (1). Total RNA was purified using the RNeasy kit purchased from Qiagen (Valencia, CA) and the integrity of the RNA was verified using the Agilent Technologies Bioanalyzer 2100 (Santa Clara, CA). Synthesis and labelling of cRNA, microarray hybridization, and signal detection were carried out by the Microarray Core Facility at the City of Hope. Briefly, biotinylated cRNA was generated from 5 μg total RNA by T7 RNA polymerase. The Affymetrix GeneChip Human Genome U133 plus v2.0 array (HGU133A2) (Affymetrix, Santa Clara, CA) was used to analyze gene expression profiles. For microarray hybridization, the GeneChip arrays were hybridized with 20 μg of fragmented cRNA. Staining was performed with streptavidin-PE. Affymetrix GeneChip images were scanned at 11 μm resolution using a high resolution GeneChip Scanner 3000 (Hewlett-Packard). Quality assessment and statistical analysis of gene expression data were performed using the R/Bioconductor packages (2). To ensure the quality of the microarray assay, a set of quality assessment steps implemented in Bioconductor package "AffyExpress" were applied to the data. Raw intensity measurements of all probe sets were converted into expression measurements using the "RMA" method. The "LIMMA" package was then used to identify genes that were differentially expressed between each of the BT/Her^R clones and the wildtype BT474 cells (3). The corresponding p-values were adjusted using the false discovery rate (FDR) method to control the multiple comparisons of a large number of genes. Genes were defined as significantly up- or down-regulated if their expression by a BT/Her^R clone was statistically different from that in parent BT474 cells ($p < 0.05$) and the log2 of its expression ratio between a BT/Her^R clone and the parent BT474 cells was greater than 1 or less than -1. Hierarchical clustering with Pearson correlation and average linkage was done using Cluster V2.11 (4) and visualized by Java TreeView V1.1 (5).

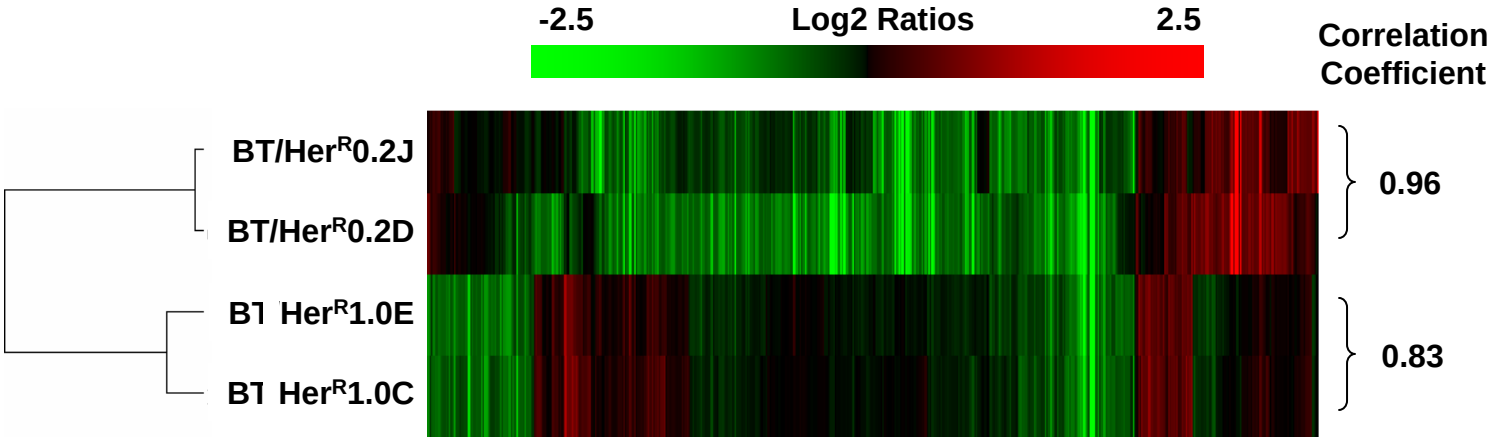
REERENCES (Supplementary Data)

1. Chan CT, Metz MZ, Kane SE. Differential sensitivities of trastuzumab (Herceptin)-resistant human breast cancer cells to phosphoinositide-3 kinase (PI-3K) and epidermal growth factor receptor (EGFR) kinase inhibitors. Breast Cancer Res Treat 2005;91:187-201.
2. Dudoit S, Gentleman RC, Quackenbush J. Open source software for the analysis of microarray data. Biotechniques 2003;Suppl:45-51.
3. Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 2004;3:Article 3.
4. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998;95:14863-8.
5. Saldanha AJ. Java Treeview--extensible visualization of microarray data. Bioinformatics 2004;20:3246-8.

FIGURE LEGEND (Supplementary Data)

Figure S1. Microarray analysis of gene expression in BT/Her^R clones. Heat map depicting the relatedness of four BT/Her^R clones based on the expression of 506 genes that are significantly down- or up-regulated in at least one BT/Her^R clone in comparison to the expression level in parent BT474 cells. The down- (green) or up-regulation (red) of those genes is expressed as log2 ratios between their expression levels in a BT/Her^R clone vs. parent BT474 cells and analyzed by hierarchical clustering using Cluster V2.11 (4) and Java TreeView V1.1 (5).

Fig. S1


-2.5
Log2 Ratios
2.5
Correlation Coefficient
BT/HerR0.2J
BT/HerR0.2D
BT HerR1.0E
BT HerR1.0C
0.96
0.83