

**Analysis Consulting
Service Specifications
Basic Analysis****A. General Analysis Procedure:**

1. **Data reduction:** Raw intensity signals for each microarray are captured using a Molecular Dynamics™ Axon 4100A scanner, measured using GenePixPro™ Software, and stored in GPR format. The data from all microarrays in each experimental set is then passed to Omicsoft Array Studio software, control and missing features are removed, and the remaining signals are quantile normalized and transformed to log₂ values.
2. **Hypothesis testing & generation of fold change values:** Testing is performed by combining technical replicates and performing a standard student's T-test to calculate raw P-values. Adjusted P-values are calculated using the Benjamini and Hochberg method with a false discovery rate α -value of 0.05. Fold changes calculated based on the mean values of the technical replicates for each probe.
3. **Data filtering & selection of affected genes:** A subset of genes is also generated based on an internal intensity stringency filter.

B. Basic Analysis:

1. **Selection of gene set:** The gene sets used for the basic analysis include a) the complete dataset (all probes) and b) a filtered set based on an intensity stringency filter.
2. **Output file description:**
 - a. An Excel workbook, REPORT_X, is generated for each sample comparison. Each workbook contains two pages. 'Normalized Report' contains data test results for all probes in the gene set. 'Filtered Report' contains data and test results of a subset of genes that pass an intensity stringency filter. Each page contains the following columns:
 - i. **ID:** The Phalanx Biotech probe ID for each gene probe.
 - ii. **HOXX.C1:** Data from each microarray (including technical replicates) of the normalized intensity for each probe. The number of columns with this format varies depending upon the number of technical replicates and the experimental design.
 - iii. **HOXX:** Data from each microarray (including technical replicates) of the log₂ value of the normalized intensity for each probe.
 - iv. **Effect:** The log₂ value of the fold change for each probe. For example, a log₂ value of 1 corresponds to a two-fold upregulation, a log₂ value of -2 corresponds to a four-fold downregulation, etc. (NOTE: Some specialized analyses may require an ANOVA analysis, and an additional column entitled 'Fold Change' is inserted that contains the fold change values, converted from log₂)
 - v. **Raw PValue:** The raw P-value based on the analysis described in Step A2 above.
 - vi. **Adjusted PValue:** The adjusted P-value based on the analysis described in Step A2 above.
 - vii. **Annotation columns:** The gene-related information for each probe based on Phalanx Biotech annotation (See details in C, below).

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- b. A second Excel workbook, REPORT_RAW, is generated that contains summary data. It contains three pages:
- i. Raw Data: Raw signal intensities for all features (gene probes, control probes, and empty regions) on each array.
 - ii. R Table: A correlation matrix for all the microarrays in the experiment, including technical replicates.
 - iii. Design Table: A summary table that contains a list of the array names and corresponding sample names that can be used as a design table with OneArray Studio software.

C. Annotation Information:

1. **General description:** Our bioinformatics pipeline derives the gene annotations from a whole genome sequence alignment. The details of our annotation can be found in the attached README file and can also be downloaded from our support web page at <http://www.phalanxbiotech.com/Support/Files.html>. This web page also includes probe sequences and chromosomal alignment information. Probes annotated 'NA' fall into gene groups that do not correlate to a single gene exon or align to a region to which no known gene is assigned in the Ensembl database (pseudogenes, for example).
2. **Ensembl-derived annotation:** Ensembl_Gene_Symbol, Ensembl_Gene_Description, Ensembl, Ensembl_Transcript, GO_Term_Names, Chromosome.
3. **Phalanx Probe Information:** Gene group.