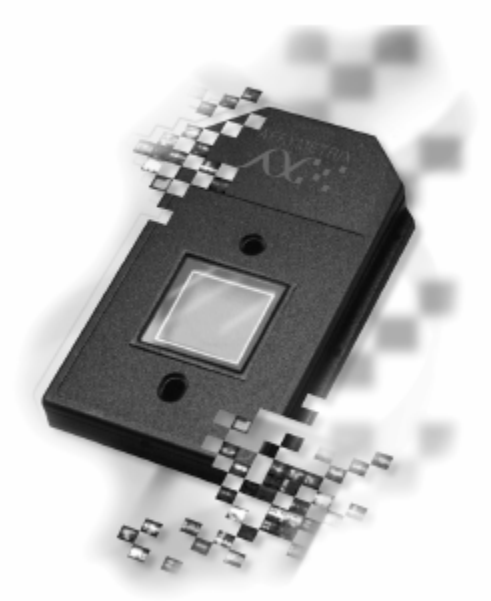
Affymetrix®

GeneChip® Chromosome Copy Number Tool



User's Guide

Version 1.1

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Introduction

Changes in DNA copy number are one of the hallmarks of genetic instability common to most human cancers. Previous microarray-based methods have been used to identify chromosomal gains and losses; however they are unable to genotype alleles at the level of single nucleotide polymorphisms (SNPs).

The Affymetrix® GeneChip® Chromosome Copy Number Tool implements a novel algorithm that uses a recently developed high-density oligonucleotide array-based SNP genotyping method, whole genome sampling analysis (WGSA), to identify genome-wide chromosomal gains and looses at high resolution. The tool compares values from your sample to SNP-specific distributions derived from a reference set containing over one hundred normal individuals.

Support for this tool is provided only by this documentation and through the Affymetrix forums. Use of this tool is offered to those customers who understand and accept these support limitations and wish to take advantage of this high value package for their analysis.

Installation

Prerequisites to installing the Chromosome Copy Number Tool are the installation of the Affymetrix GCOS and GDAS software.

To install the software, extract the contents of the downloaded "ChromosomeCopyNumberTool_Install-v1.1.ZIP" archive file. This file contains two separate installation programs.

Run the "GdacFilesRuntimeInstall-v4.exe" installation program. Follow the directions displayed on-screen. This installation program will install the parsers for the Affymetrix data files used by the Chromosome Copy Number Tool.

The next step is to run the "ChromosomeCopyNumberTool_Install-v1.1.exe" installation program. Follow the directions displayed on-screen. This installation program will install the Chromosome Copy Number Tool and create a "Start->Programs->Affymetrix-> Chromosome Copy Number Tool" menu item.

NOTE: You must have administrative rights on the Windows machine to install the software.

Running an Analysis

Use the "File->Open …" menu item to select the GDAS analysis results (CHP) to analyze. You may select one or more CHP files from the file chooser dialog. The list of available files may be filtered using the "Filter>>" button.

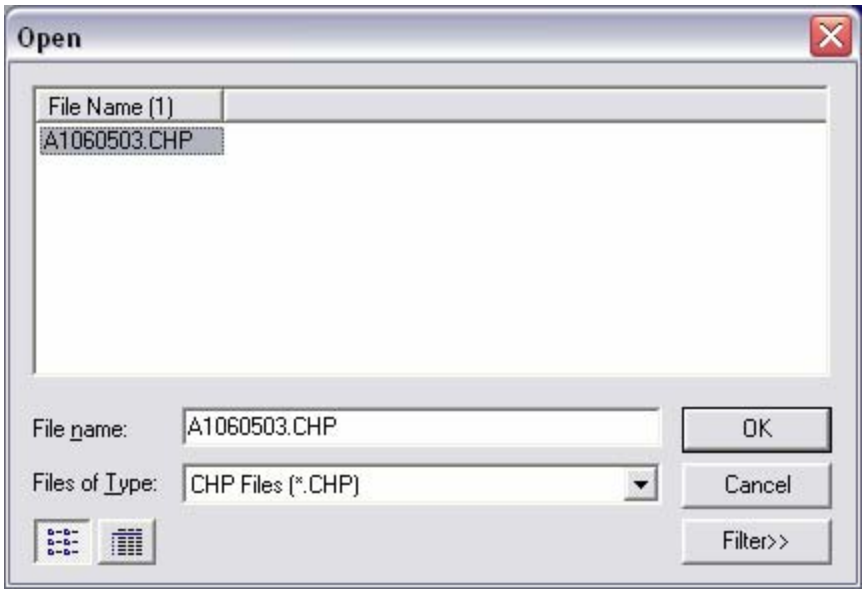

Figure 1 - File Open Dialog

Note: You must set the Assay Type filter to "Mapping" for the CHP files to be listed in the file open dialog.

Analysis Results

For each analysis selected the software will generate 4 columns of results. These columns are:

1. Copy Number – The estimate of the genetic copy number changes.
2. +-log10(p-value) – The significance of the copy number variation. When the value from the target cell line is higher than the reference mean, we use –log10(p-value), which is a positive value, to represent the significance. When the value from the target cell line is lower than the reference mean, we use log10(p-value), which is a negative value, to represent the significance.
3. +-log10(meta p-value) – The meta analysis significance. When the value from the target cell line is higher than the reference mean, we use –log10(p-value), which is a positive value, to represent the significance. When the value from the target cell line is lower than the reference mean, we use log10(p-value), which is a negative value, to represent the significance.
4. LOH – The -log10 of the probability of the long stretch of homozygous calls, for which the SNP is located in, happen in random.

The analysis results are ordered by chromosome then by position within the chromosome. SNPs that are not mapped are shown at the top of the list.

Graphing Results

Use the "Graph->Graph Data…" menu item to specify the analyses and chromosomes result values to plot.

The “Select Graphs” dialog will appear prompting for the analysis and chromosomes to plot. You may select one or more analyses and one or more chromosomes.

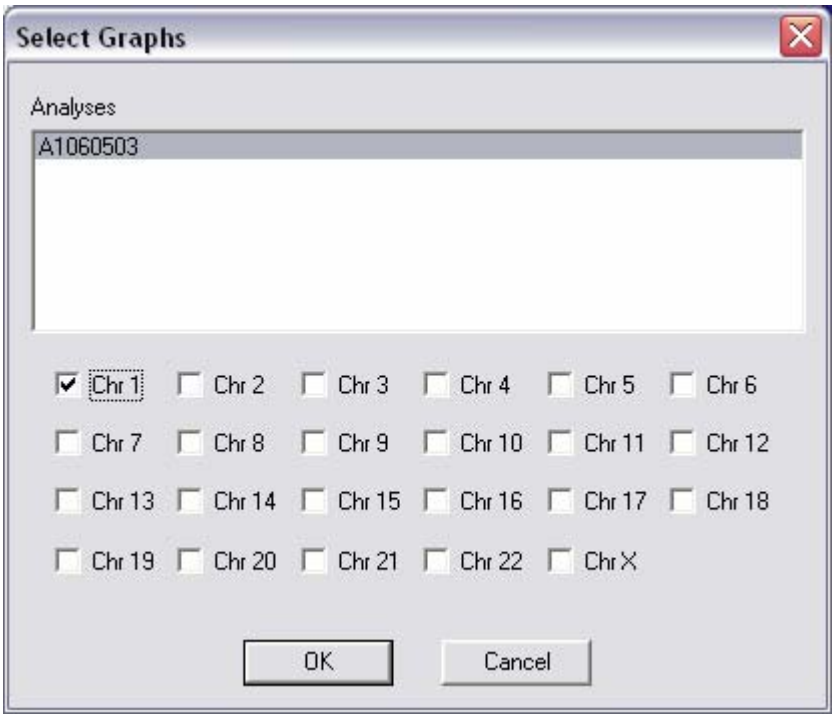

Figure 2 – Select Graph Dialog

For each selected analysis and chromosome a series of 4 graphs will be displayed, one for each results column. The axes on the graph are Y – value, X – SNP (index). The SNPs on the X axis are ordered by position within the chromosome.

The significance values are plotted using two colors – one for positive values and one for negative values.

Figure 3 - Significance Graph

The copy number results are plotted using two colors – one for values above the threshold (2) and one for values below the threshold. Included in the graph, at the bottom of the display, is a representation of the genotype calls associated with the SNPs. Green, or color 1, represents heterozygous calls while red, or color 2, represents homozygous calls.

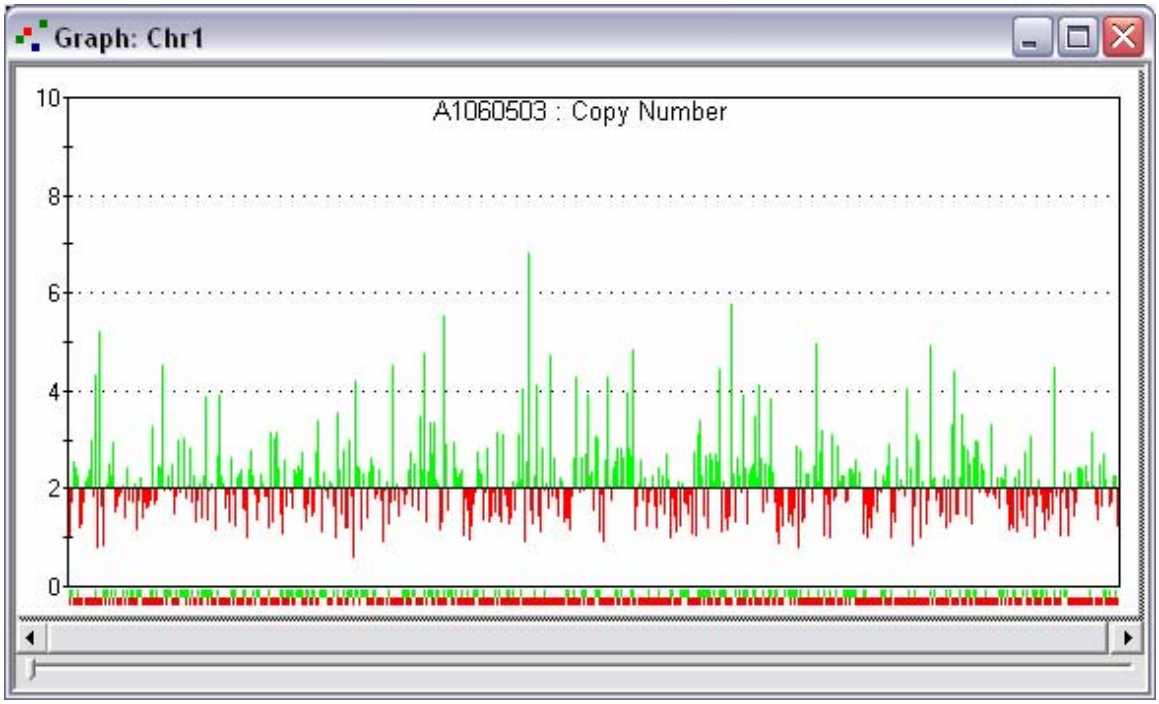

Figure 4 - Copy Number Graph

The LOH values are plotted using a single color. Included in the graph, at the bottom of the display, is a representation of the genotype calls associated with the SNPs. Green, or color 1, represents heterozygous calls while red, or color 2, represents homozygous calls.

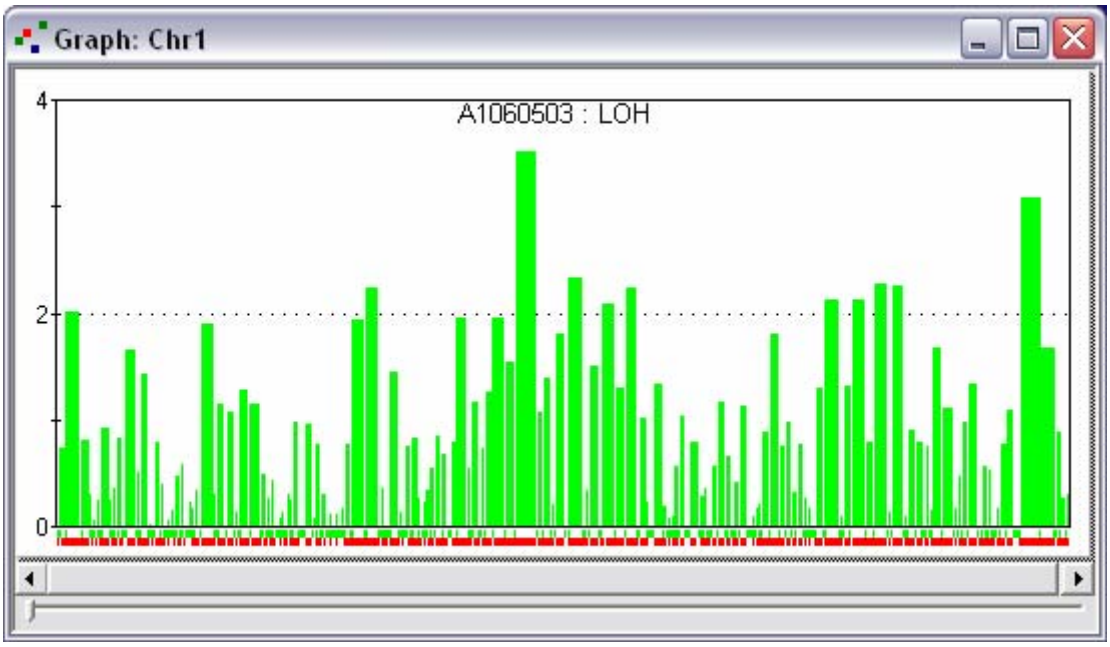

Figure 5 - LOH Graph

The slider at the bottom of the graph window is used to change the horizontal scale. The scroll bar above the slider is used to move the horizontal position of the graph.

Changing Graph Colors

Colors for the graphs may be changed using the "Edit->Choose Color 1" and "Edit->Choose Color 2" menu items.

The standard Windows color chooser will be displayed prompting to select the desired color.

Colors selected will be applied to new graphs only; existing graphs will not be updated.

Exporting Results

Use the "File->Save" menu item to export the tabular results to a tab delimited ASCII text file.

Appendix I – Algorithm Details

- Feature Extraction:
    1. Log intensity as basic measurement with appropriate chip-wise normalization

- Significance Calculation
    1. Derive reference distribution from 110 normal samples
    2. Get significance by comparing cancer to reference
    3. The comparison is genotype specific

- Copy Number Estimation
    1. Very strong dosage response on 1X to 5X
    2. Log-log linear relationship fits in general

- Meta Analysis: Improve detection rate in moderate alteration
    1. Basic Assumption: the greater the number of consecutive SNPs which display the same type of alteration (gain or loss) leads to increased confidence

- LOH: Focus on long stretches of homozygous calls
    1. Get the probability of being homozygous for each SNP using the large reference set
    2. For any given stretch of homozygous calls, calculate the probability of such stretch happen in random using the probability derived from "1". Each SNP is treated independently.

Appendix II – Version History

Version 1.0 – Initial version which supported following probe array types:
Mapping10K_Xba131

Version 1.1 – Added support for the following probe array types:
Mapping10K_Xba142
Mapping50K_Xba240
Mapping50K_Hind240