

# Quick Reference Card

## GeneChip® Sequence Analysis Software 4.1



### I. GSEQ Introduction

GeneChip Sequence Analysis Software (GSEQ) is used to analyze data from the Resequencing Arrays

GSEQ allows you to:

- Analyze CEL intensity data to generate sequence data
- View the CHP analysis results
- Create reports
- Export data in formats that can be used by other software applications

GSEQ uses two different algorithms to analyze data:

- Resequencing Algorithm Version 1 for 20 x 25 um arrays
- Resequencing Algorithm Version 2 for 8 um arrays

To Start GSEQ:

- Click the Windows Start button and select **Programs** → **Affymetrix** → **GSEQ 4.1**.

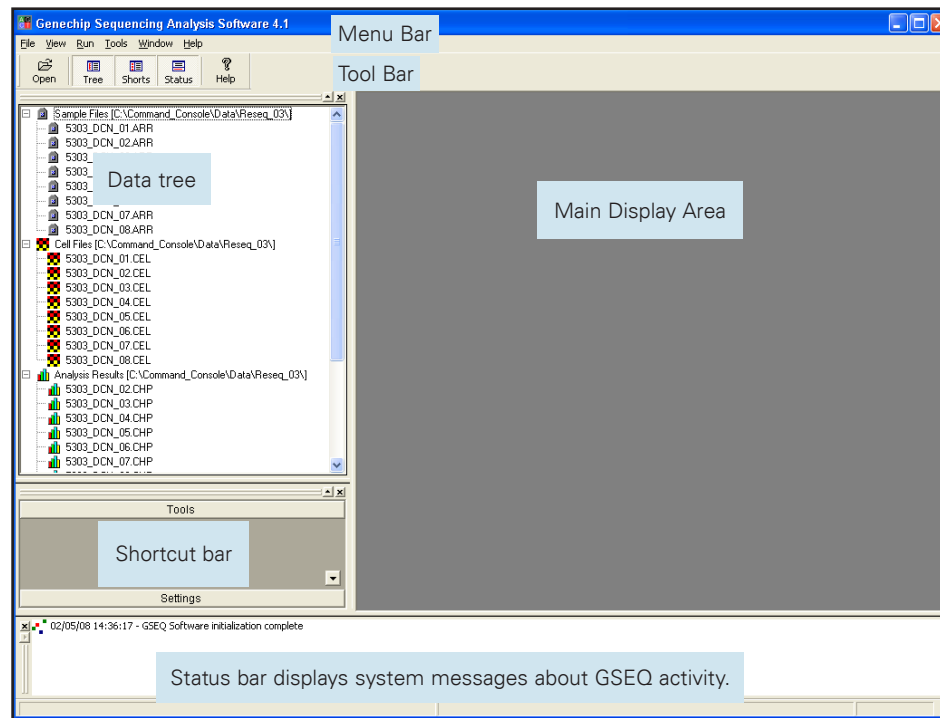
#### GSEQ User Interface at Startup

The Main Display Area displays:

- Resequencing Analysis window (See Section II)
- Analysis Results (See Section IV)
- Probe Intensity window (See Section V)

The Data tree lists:

- Sample Files (ARR)
- Cell Intensity Files (CEL)
- Probe Analysis Data Files (CHP)
- FileSets (GFS)



The Shortcut bar provides quick access to:

- Windows in the main display area
- Dialog boxes for setting parameters

See the *AGCC User's Guide* for information about viewing CEL intensity data in the Image window

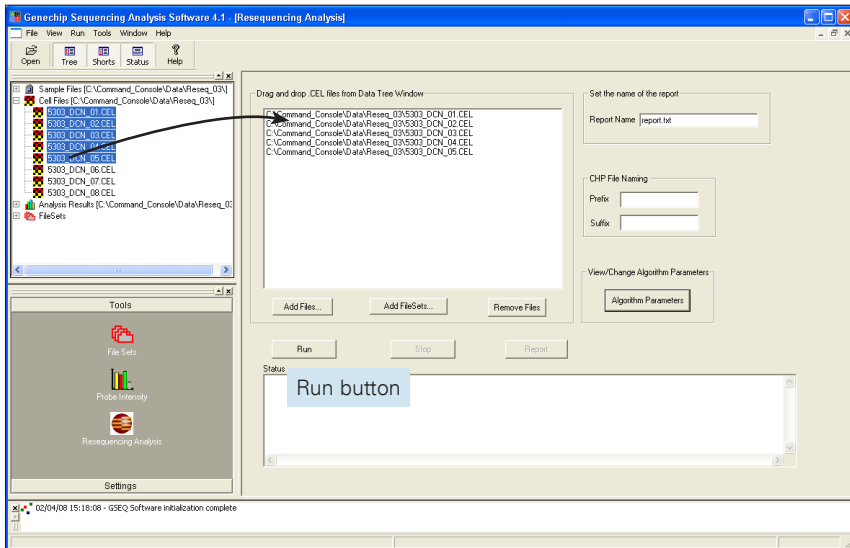
## II. Analyzing Cell Intensity Data

You can select Resequencing Cell intensity data (CEL) files for analysis using the Resequencing Analysis window.

The Resequencing Analysis window automatically selects the proper algorithm for the selected array types.

1. Click the Resequencing Analysis button in the Shortcut bar, or select **Run** → **Resequencing Analysis** from the Menu bar.

The Analysis window opens.

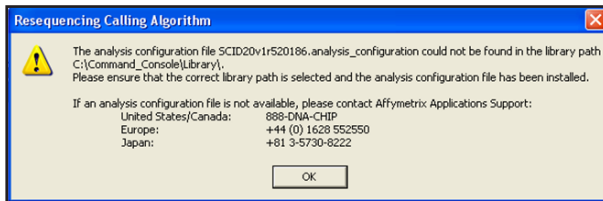


2. Select cell intensity data in the data tree and drag into the Resequencing Analysis window.

**NOTE:** When performing a resequencing analysis, you should select at least 15 cell intensity files for best results.

3. Click the Run button in the Resequencing Analysis window toolbar to generate genotype or base calls and associated quality information.

**NOTE:** If you do not have the analysis configuration file for your CustomSeq array, GSEQ will not be able to run the analysis and the following message will appear:



Follow the instructions in the notice before proceeding with the analysis.

When the analysis is finished, the Resequencing Algorithm report opens and the analysis results files are listed in the data tree (see section IV).

See Chapter 3 of the *GSEQ User's Guide* for more information about analyzing CEL Intensity data.

## Resequencing Algorithm Report

Contains a summary of information about the Resequencing analysis.

Sample	Total	Called	CallRate	NS	WS	Se
21 WTest_MitoSTDa2_102105_6G	37756	26417	69.97%	0	0	0
22 WTest_MitoSTDb2_102105_6G	37756	26590	70.43%	0	0	0
23 WTest_MitoTri1a_102105_6G	37756	26312	69.69%	0	0	0
24 WTest_MitoTri1a_rep3_102105_6G	37756	26529	70.26%	0	0	0
25 WTest_MitoTri1a_rep3_102105_6G	37756	26931	71.33%	0	0	0
26 WTest_MitoTri1b_102105_6G	37756	26761	70.88%	0	17	0
27 WTest_MitoTri1c_102105_6G	37756	26677	70.66%	0	0	0
28 WTest_MitoTri2a_102105_6G	37756	27077	71.72%	0	0	0
29 WTest_MitoTri2b_102105_6G	37756	26126	69.20%	0	0	0
30 WTest_MitoTri2c_102105_6G	37756	26987	71.48%	0	0	0
31 WTest_MitoTri3a_102105_6G	37756	27153	71.92%	0	0	0
32 WTest_MitoTri3b_rep2_102105_6G	37756	26802	70.99%	0	0	0
33 WTest_MitoTri3b_rep3_102105_6G	37756	26477	70.13%	0	0	0
34 WTest_MitoTri3c_102105_6G	37756	26370	69.84%	0	0	0

Fragment	NumBases	CallRate	RefCalls	CallRate	RefCalls	CallRate	RefCalls
39 AFFX-TagIO-EX	814	97.70%	100.00%	97.70%	100.00%		
40 human_mtDNA_RCRC	16544	96.70%	99.80%	96.90%	99.90%		
41 mt105ref49-97	26	80.80%	100.00%	84.50%	100.00%		
42 mt1059ref49-97	26	61.50%	100.00%	89.50%	100.00%		
43 mt1059ref49-99	27	77.80%	100.00%	81.50%	100.00%		
44 mt106ref49-113	41	73.20%	97.60%	65.90%	96.10%		
45 mt106ref49-119	47	60.00%	93.60%	61.70%	93.60%		
46 mt1064ref49-119		60%	76.60%	93.60%	96.70%		
47 mt1066ref49-119	56	87.50%	93.60%	95.70%	96.70%		
48 mt1068ref49-134	56	87.20%	92.90%	94.50%	94.50%		
49 mt234ref49-113	41	80.50%	97.60%	75.60%	95.10%		

See Chapter 6 of the *GSEQ User's Guide* for more information about the Resequencing report.

### III. Opening Analysis Results

The analysis results (CHP) files are listed in the Data tree.

To open analysis results files:

- Double-click on selected analysis results files in the data tree.
  - To select adjacent files, press and hold the Shift key while you click the first and last file in the selection.

To select non-adjacent files, press and hold the Ctrl key while you click the files.

**TIP:** You can also display analysis results by selecting **File** → **Open** from the main menu and using the Open dialog box. See the ***GSEQ User's Guide*** for more information.

### IV. Viewing Resequencing Analysis Results

The Resequencing Analysis window displays Resequencing analysis results.

You can choose from:

- Table View
- Sequence View
- SNP Table

#### Resequencing Window: Table View

The Table view displays information on:

- Reference fragment
- Sample base calls

See Chapter 4 of the *GSEQ User's Guide* for information on:

- Displaying forced calls and fail reasons
- Editing calls and saving the edits in a new analysis results file
- Sorting, searching, and exporting the data in the table

Fragment Information						First Sample	Second Sample	Third Sample			
Fragment	Frag Pos	Tiling Pos	Ref	Heterozygosity	Call	Quality	Call	Quality			
1	IQ.EX	12	1	c	0.000	c	58.297791	c	157.898926	c	110.860352
2	IQ.EX	13	2	c	0.000	c	115.063477	c	134.090637	c	90.927734
3	IQ.EX	14	3	c	0.000	c	155.800842	c	159.785625	c	125.999023
4	IQ.EX	15	4	a	0.000	a	134.784058	a	160.355957	a	128.160095
5	IQ.EX	16	5	g	0.000	g	191.207275	g	200.678589	g	209.751282
6	IQ.EX	17	6	t	0.000	t	91.784424	t	92.077637	t	81.060791
7	IQ.EX	18	7	c	0.000	c	206.352295	c	241.359558	c	198.592529
8	IQ.EX	19	8	a	0.000	a	140.348877	a	144.022400	a	157.048340
9	IQ.EX	20	9	c	0.000	c	284.202332	c	294.578186	c	269.084412
10	IQ.EX	21	10	g	0.000	g	259.529724	g	274.564697	g	235.609680
11	IQ.EX	22	11	a	0.000	a	119.006958	a	113.611226	a	115.461050

Sequence Table SNP Viewer

Click the tabs to display the Sequence and SNP View  
The selected analysis results are displayed in the Resequencing window

Fragment Information:

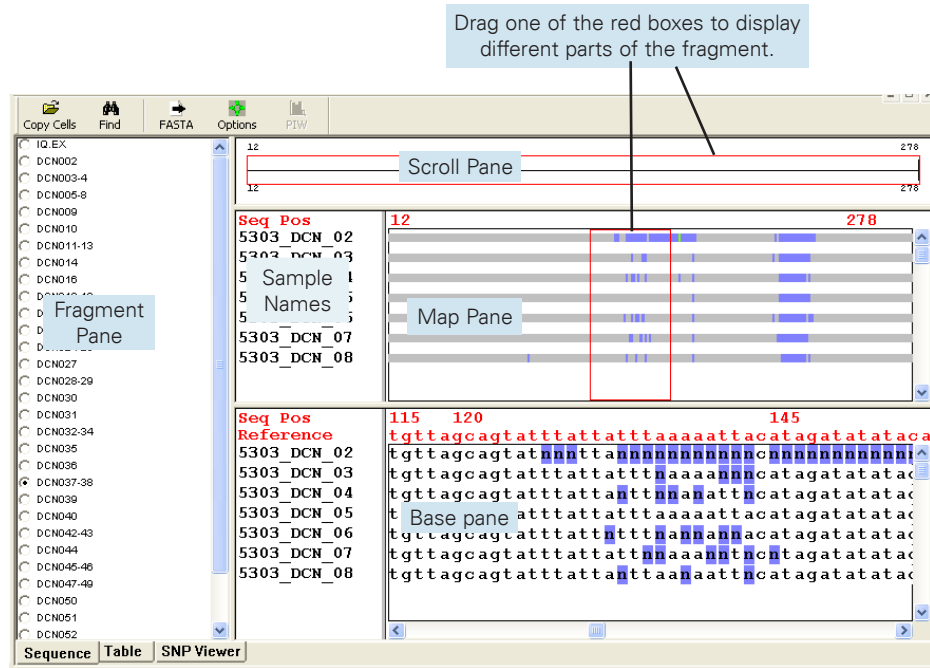
- **Fragment:** Reference fragment name
- **Fragment Position:** The Sequence position of the reference base in the reference fragment
- **Tiling Position:** The tile position number on the resequencing array of the probes for the reference base
- **Reference Base:** The reference base at the fragment position
- **Heterozygosity:** the relative frequency of mutations and reference base at a particular site in the sample.

Sample Information:

- **Call:** The base call for the sample at the queried site
- **Forced Call:** For a no call, the call that would have been made if the fail reason was ignored (Optional for Algorithm Version 2 only)
- **Fail Reason:** A code giving the fail reason a no call occurred (Optional for Algorithm Version 2 files only)
- **Quality score:** The total quality score computed for the base call

## Resequencing window: Sequence View

The Sequence view displays the sequence of the selected reference fragment and the base calls for selected samples.



**Fragment Pane:** List of the reference fragments (click to select a fragment for display)

**Scroll Pane:** Overview of the selected reference fragment

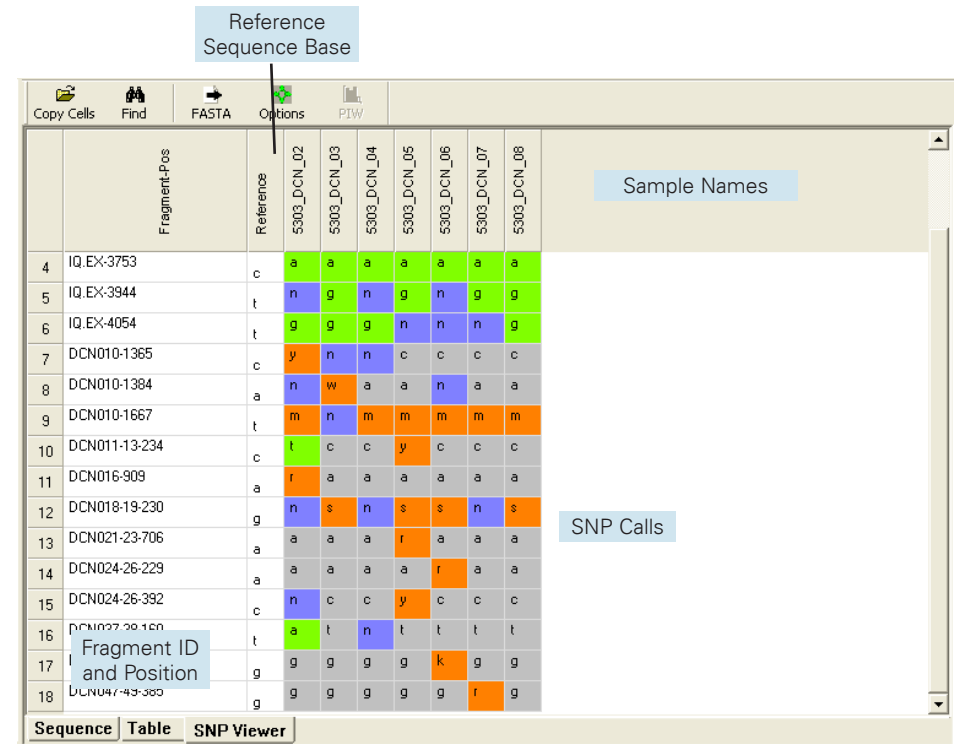
**Map Pane:** Color-coded sequence maps of the base calls for the samples

**Base pane:** Detailed views of the base calls for the samples

See Chapter 4 of the *GSEQ User's Guide* for more information about the Resequencing window.

## Resequencing window: SNP View

Displays the bases and samples with SNPs.



**Fragment ID and Position:** The fragment name and sequence position of the reference base in the reference fragment

**Reference Sequence Base:** The reference base call at the fragment position.

**Sample Name:** The analysis results with the SNP

**SNP Calls:** The base call at the position for the sample

## V. Viewing Probe Intensity

You can use the Probe Intensity window to evaluate the cell intensity data for probe cells used to call particular features.

To display cell intensity data in the Probe Intensity window:

1. Click the Probe Intensity window button in the Genotyping Views shortcut bar, or select **Run → Probe Intensity** from the menu bar.  
The Add Cell Intensity Data dialog box opens.

2. Select the cell intensity data for the samples that you want to view and click **OK**.



**Intensity Plot:** Displays the intensity information for the probe cells of the selected feature as a:

- Trace Plot (Shown)
- Bar Graph
- Line Graph

**Probe Intensity table:** Displays a list of the probe sets used in the selected assay and probe array type with cell intensity values

See Chapter 5 of the *GSEQ User's Guide* for more information.