

# Gene Net™ siRNA Library Data Analysis Software and Gene List Tutorial

## Technical Requirements:

- Windows 2000 or XP
- Affymetrix GeneChip® Mouse Genome 430 2.0 Array or
- Affymetrix GeneChip® Human Genome U133 Plus 2 Array
- \*.cel file generated from GCOS (Command Console is not currently supported)

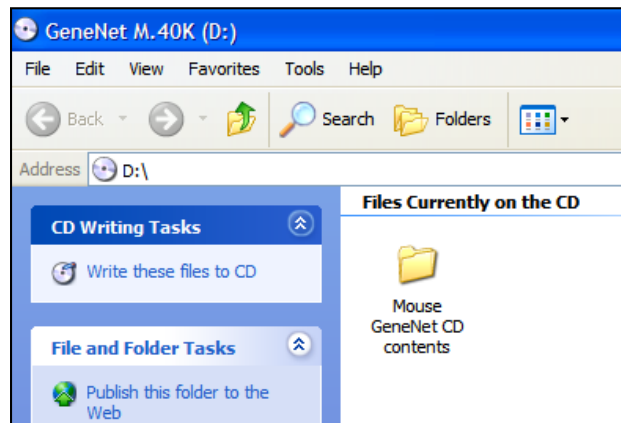
## Convert Command Console file to GCOS

- 1) Open the Data Exchange Console (Start à All Programs à Affymetrix à Data Exchange Console)
- 2) Choose the “Export Data Out of Command Console” radio button.
- 3) Select the files you want to convert and choose a location to save the DTT file.
- 4) The DTT file is a zipped file containing all the converted cel files. Use Winzip to open the DTT file and extract your cel files for use with the analysis software.

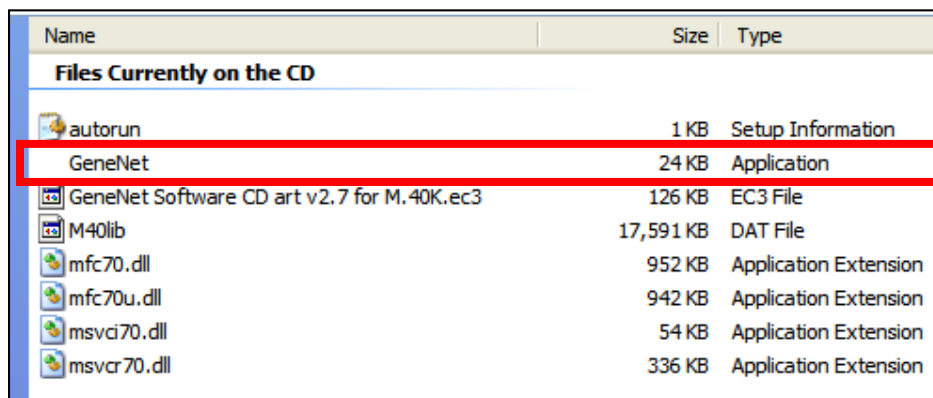
The function of exporting the files converts them from AGCC to GCOS.

## Steps

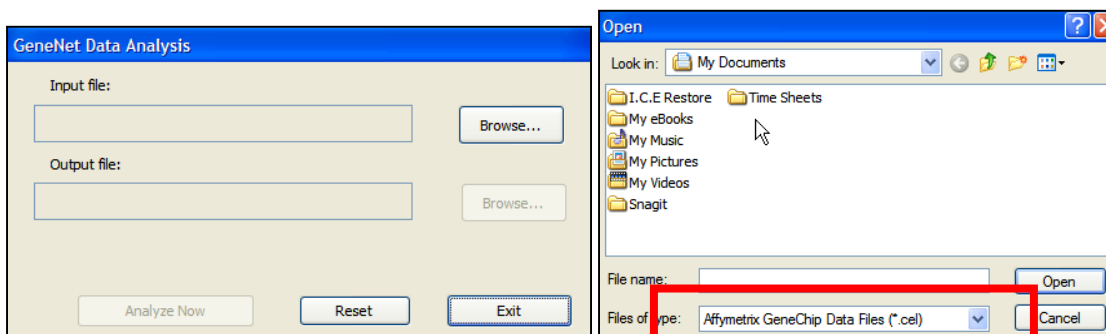
1. Insert the CD into your computer. If it does not automatically run, follow these steps.
2. Open Windows Explorer and double-click the GeneNet CD contents to open the folder.



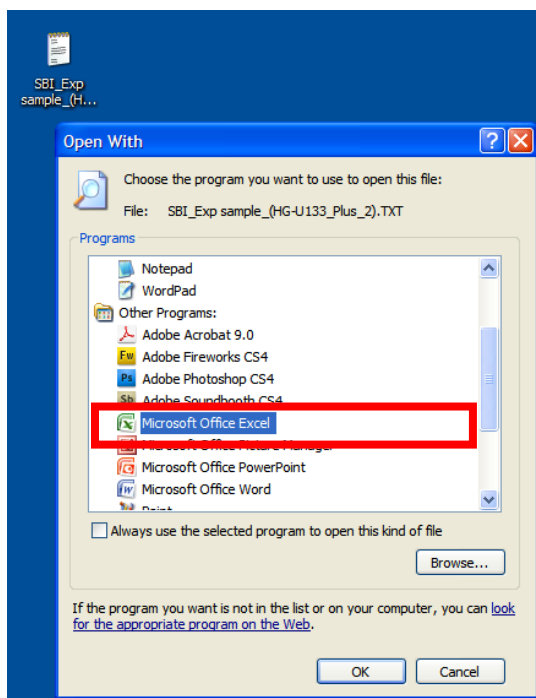
3. Double-click the GeneNet Application (.exe file).



4. The following window opens. Click on Browse... and browse for the Affymetrix GeneChip Data File generated from GCOS (\*.cel). Click on Open to select the file. Then click Analyze Now.



5. The analyzed file will be saved in the location that is set in the Output file, and will be saved as a .txt file. Use Microsoft Excel to open the .txt file.





A	B	C	D
Value	GeneBank#	Gene name	siRNA sense sequence
58	BC024686	Mus musculus, coatomer protein complex, subunit gamn	TACTATCAAGACACTCGAAGAGGCTGT
69	BC024686	Mus musculus, coatomer protein complex, subunit gamn	GAGGAAACGTTACCCTGTCTACTATC
54	BC024686	Mus musculus, coatomer protein complex, subunit gamn	GAATGCATCCTGTGAGAGGTCAGACA
52	BC024686	Mus musculus, coatomer protein complex, subunit gamn	TATCTTGCCAACACTACGTCGAAGCC
58	NM_013477	Mus musculus ATPase, H+ transporting, lysosomal 38kDa,	GTTTACAGCCACTGACATGTCTAAGAA
61	NM_013477	Mus musculus ATPase, H+ transporting, lysosomal 38kDa,	TCACCTTGCTGACCTGGTGAGGTGTAG
68	NM_013477	Mus musculus ATPase, H+ transporting, lysosomal 38kDa,	TAGCATTGCTCTGGGATTGTTGCCCA
50	NM_013477	Mus musculus ATPase, H+ transporting, lysosomal 38kDa,	ACATCGTATGGATTGCTGAGTGCATTG
243	NM_020585	Mus musculus hypothetical protein, MNCb-1213 (AB0415	TTGGCTCCGTGTAACCTCAAGTTAAA
46	NM_020585	Mus musculus hypothetical protein, MNCb-1213 (AB0415	TTGTAACCTACTTTAAACTCTGATTTA
59	NM_020585	Mus musculus hypothetical protein, MNCb-1213 (AB0415	TCACATACAGTATTCAGGGCACAGAAA

6. **Data Analysis Summary:** One approach to generate a list of enriched shRNAs: compare the signal values for each shRNA in treated versus control samples, and look for those shRNAs with significant control values ( $> \sim 100$ ) which are 3-10 times higher in the treated population. Significant values are usually considered 1.5 - 2 times background, or  $\sim 100$ . The software includes a calculation of background on the last line of the GeneNet output .txt file, or background can be calculated as the average value of the lowest 10% of signals. To further restrict the list of hits, you can use a cutoff for significant control values of 100 or greater. The 10X enrichment is typically the cutoff for meaningful hits... enrichment above this level often represents noise or a spot/dust on the chip.

This analysis can be performed by merging the data sets for the two conditions, and applying a conditional sort to look for the enriched shRNAs. More advanced analysis may involve comparing replicates or other treated conditions. While some people filter the list to look for multiple enriched shRNAs against a given gene target, it is important to recognize that a single shRNA hit for a given gene may be significant, as it is possible the other shRNAs were not enriched for other reasons (such as loss of representation during the screen, low knockdown efficacy, etc.). One successful and recommended strategy is to look for those hits which are enriched in multiple biological replicates.