GSEA 分析軟體操作與分析步驟-姵蓁

1.註冊與下載軟體

GSEA home page 以信箱進行註冊

http://software.broadinstitute.org/gsea/index.jsp

註冊後至 download 頁面安裝 GSEA 軟體

http://software.broadinstitute.org/gsea/downloads.jsp

目前是 GSEA v4.0.2 版本

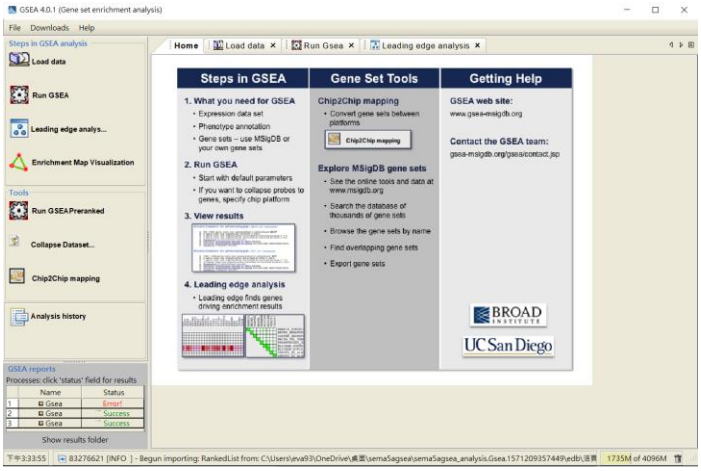
2.準備分析檔案

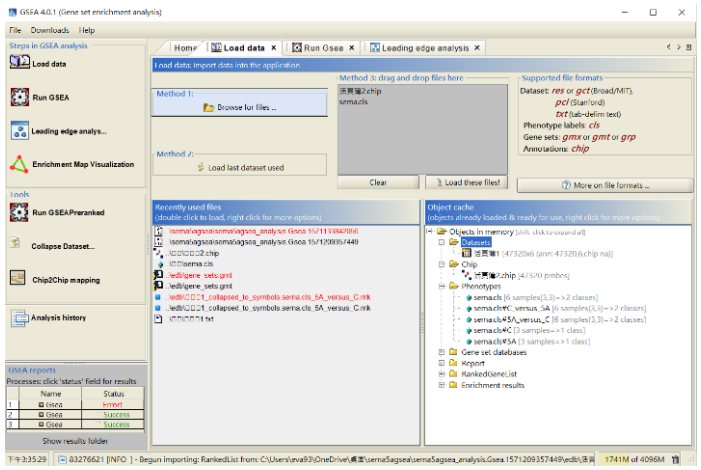
軟體中 Home 頁面左側有分析所需的功能列表，基本上是由上往下依序進行

Load data->Run GSEA->Leading edge analysis

擊點 Load data 出現分頁，右上小方框有 GSEA 分析所需的特定格式

須將檔案依照規定存成 dataset/phenotype/annotation 三種不同的檔案上傳

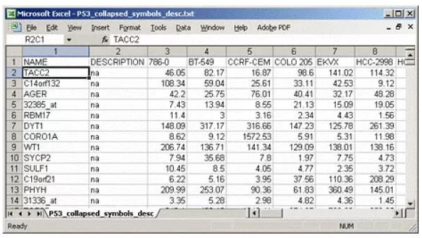




Load data

分析檔案格式參考說明：https://www.jianshu.com/p/aab52528c1e2

① Dataset：一般分析的 dataset 用.txt 檔最簡單



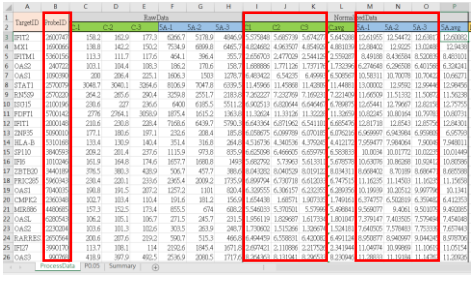
第一列基因名(必須大寫字母)或是 probe ID (避免基因重複認到)

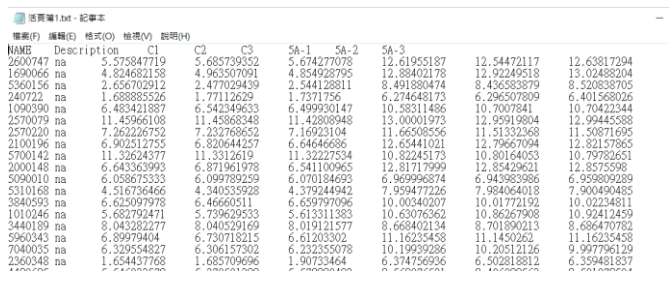
第二列 DESCRIPTION 都打 na 就好，後面幾列是不同組別分析結果

以 overexpress sema5a 與 control 組做 microarray 的 data 為例

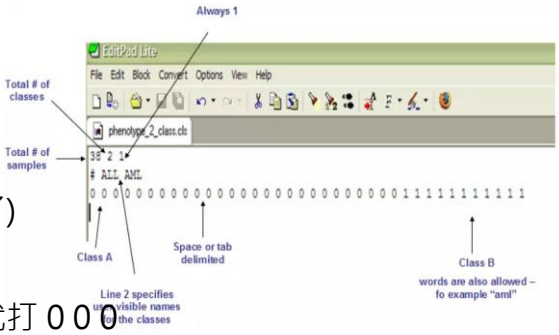
另開一個 excel 活頁簿貼上紅框中要選取的 data 範圍再轉存成.txt 檔

Ex.





② Phenotype：用來做類別分類，需存成.cls 檔



先在記事本裡面打完存檔再改附檔名成.cls 檔

第一行 total sample 數(空格)類別數目(空格)1(打 1 就對了)

第二行#(空格)分類 0 的名稱(空格)分類 1 的名稱

第三行跟 dataset 相對應的分類分組 Ex.分類 0 有三重複就打 0 0 0

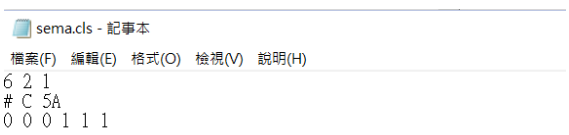
以 overexpress sema5a 與 control 組做 microarray 的 data 為例

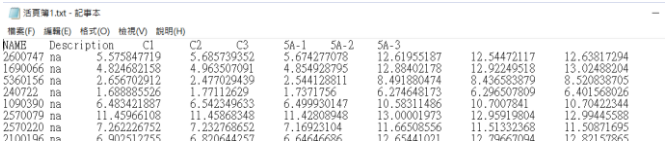
第一行 Total 6 組(control 3 組+overexpress 5a 3 組)/分 control 跟 overexpress 2 組/就打 1

第二行#/control 組 C /overexpress 組 5A

第三行 0 代表 control 組 1 代表 overexpress 5a 組 依照 dataset 排序所以打 0 0 0 1 1 1

Ex.





③ Gene sets：連網路的話 GSEA 軟體裡面就有，不用弄

④ Annotations：對 dataset 的說明，存成.chip 檔

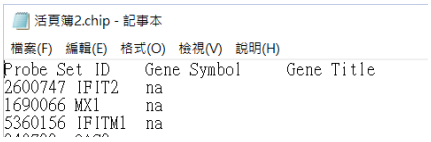
先在 excel 中打完轉記事本存檔再改成.chip 附檔名

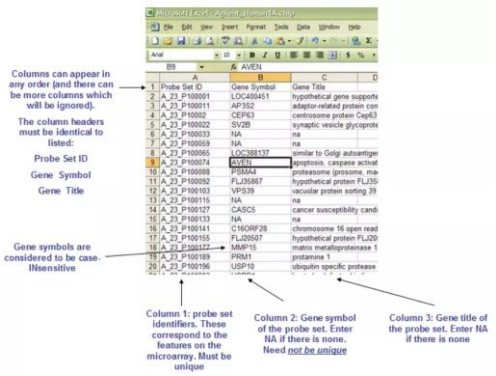
第一行是每一行的名稱

必須是 Probe Set ID/Gene Symbol/Gene Title 三列

第一列依 dataset/第二列打 na 就好/第三列沒用不用打

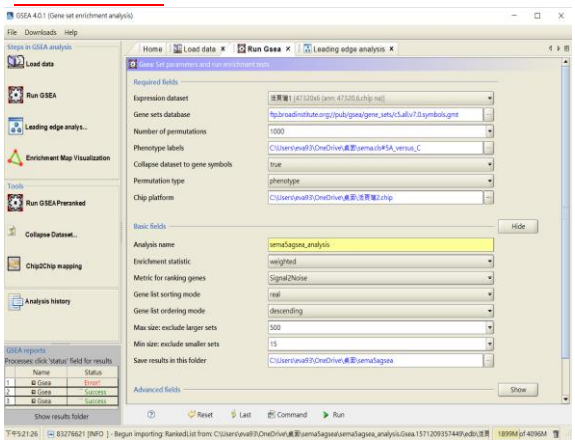
Ex.





⑤ 三個不同格式的檔案存好之後上傳到 GSEA 軟體，軟體自己會認哪個檔是幹嘛的

Run GSEA



Expression dataset:基本上軟體自己會選

Gene set database: c5 的都可以選

Permutations: data 打亂重排次數 >100

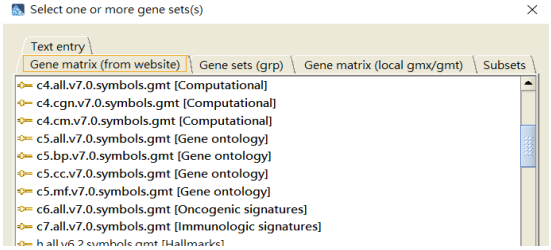
Phenotype:選讓誰當基準

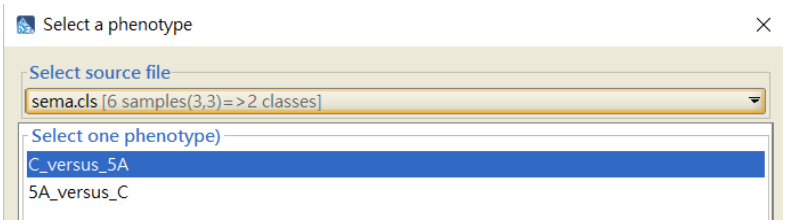
Chip: 選剛才存的 chip 檔

Max/Min size:設定要分析的 gene set 的大小

數字代表基因數目

分析檔命名跟存檔位置記得設定 →Run

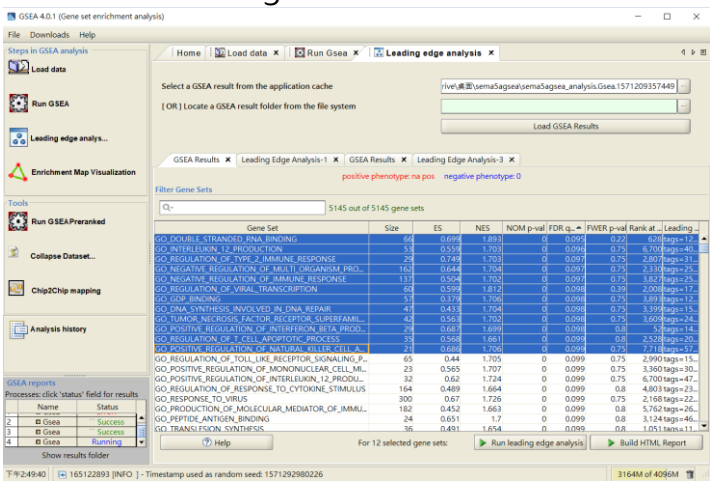


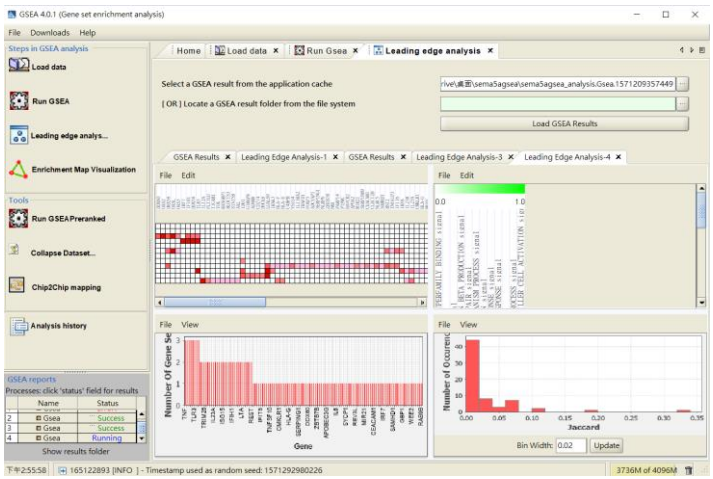


Leading edge analysis

選取想看的 gene set ->Run

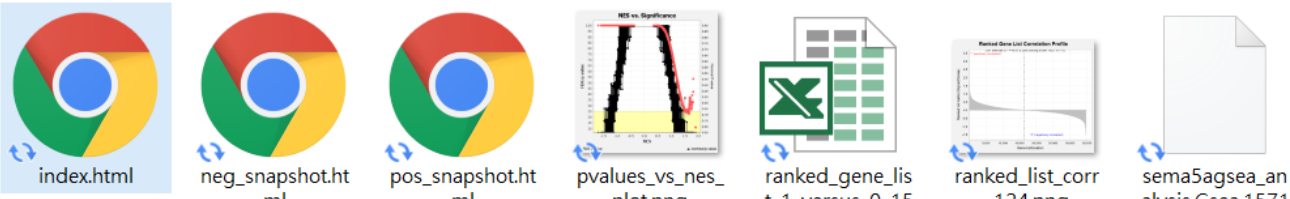
看細部基因跟 gene set 分析結果



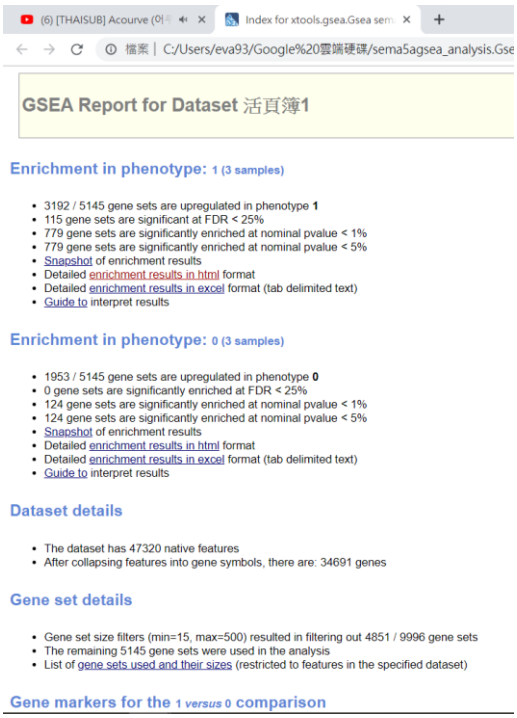


分析結果的資料夾中只有前 20 名的 GSEA 圖

裡面有一個 index.html 連結



點進去有其他詳細資料



GSEA Report for Dataset 活頁簿1

Enrichment in phenotype: 1 (3 samples)

- 3192 / 5145 gene sets are upregulated in phenotype **1**
- 115 gene sets are significant at FDR < 25%
- 779 gene sets are significantly enriched at nominal pvalue < 1%
- 779 gene sets are significantly enriched at nominal pvalue < 5%
- Snapshot of enrichment results
- Detailed enrichment results in html format
- Detailed enrichment results in excel format (tab delimited text)
- Guide to interpret results

Enrichment in phenotype: 0 (3 samples)

- 1953 / 5145 gene sets are upregulated in phenotype **0**
- 0 gene sets are significantly enriched at FDR < 25%
- 124 gene sets are significantly enriched at nominal pvalue < 1%
- 124 gene sets are significantly enriched at nominal pvalue < 5%
- Snapshot of enrichment results
- Detailed enrichment results in html format
- Detailed enrichment results in excel format (tab delimited text)
- Guide to interpret results

Dataset details

- The dataset has 47320 native features
- After collapsing features into gene symbols, there are: 34691 genes

Gene set details

- Gene set size filters (min=15, max=500) resulted in filtering out 4851 / 9996 gene sets
- The remaining 5145 gene sets were used in the analysis
- List of gene sets used and their sizes (restricted to features in the specified dataset)

Gene markers for the 1 *versus* 0 comparison