RNA seq analysis
with reference genome

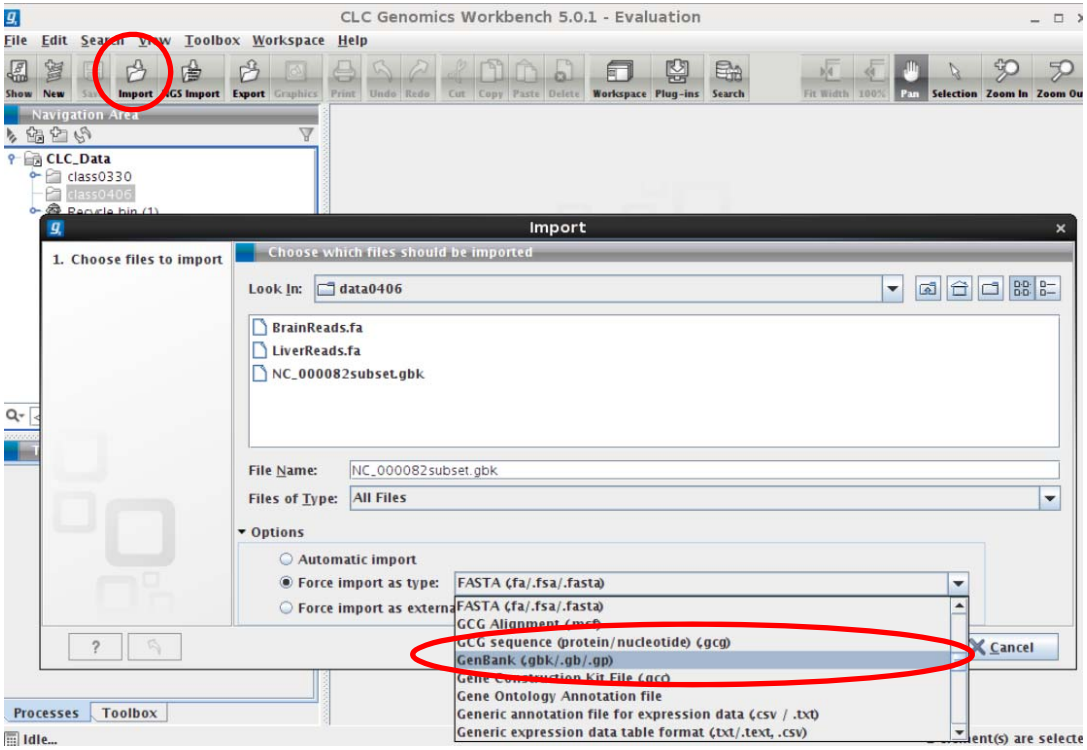
Dataset

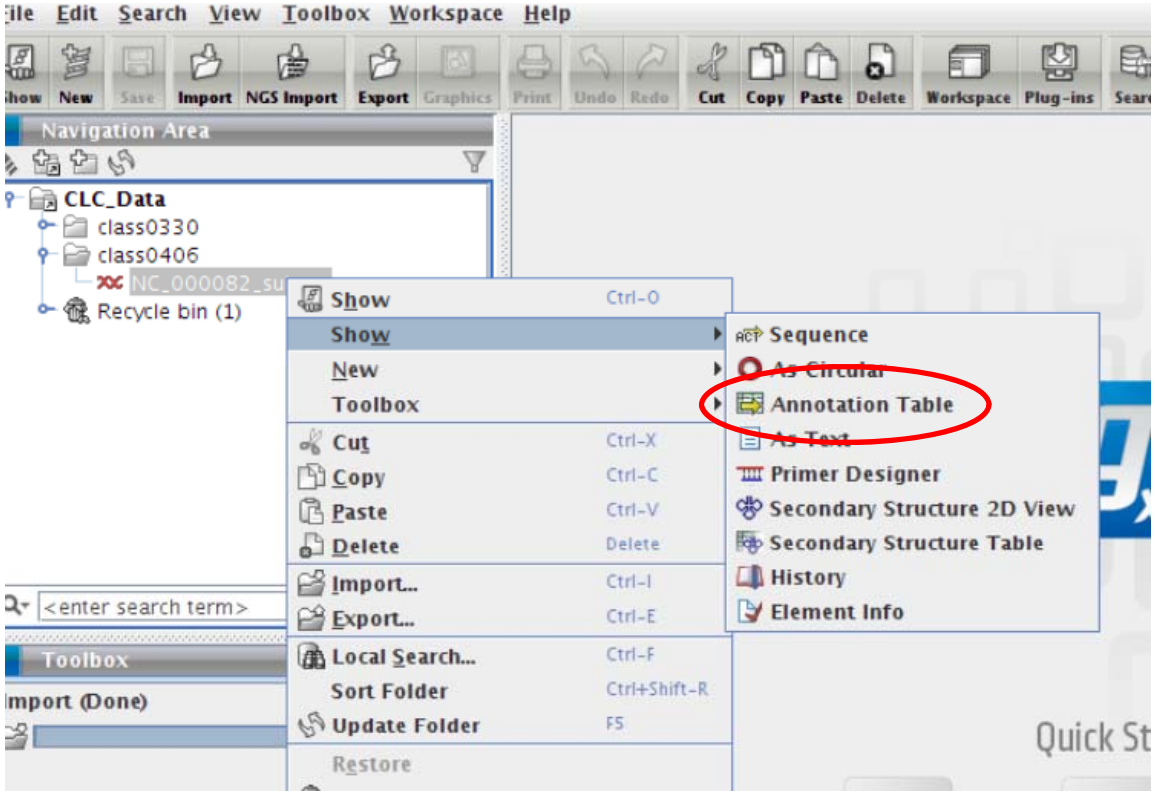
- Reference genome
  - mouse chromosome 16 subset, 13Mbp (complete chr16, 98Mbp)
- Reads
  - Brain
  - Liver

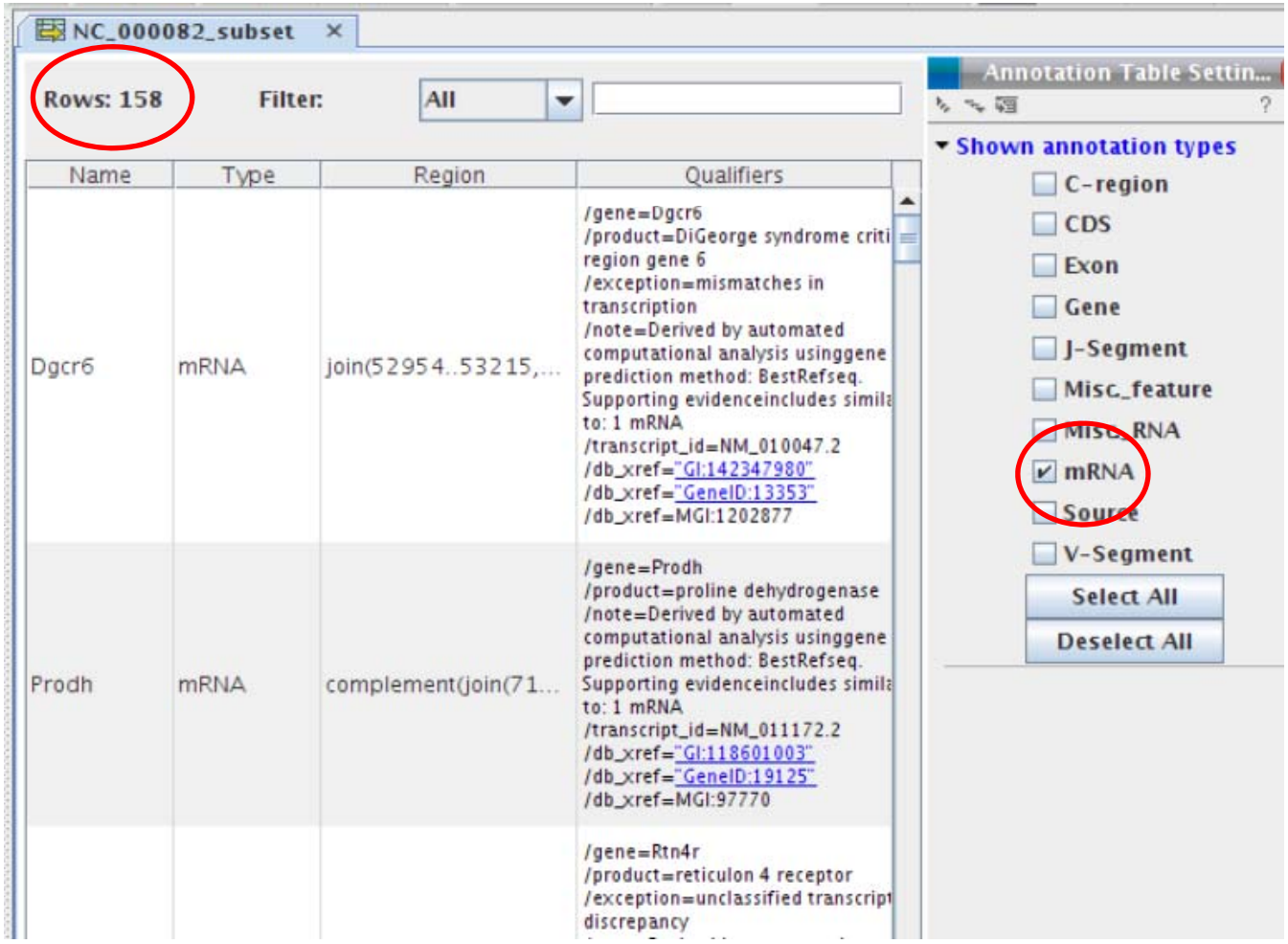
Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L., and Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by rna-seq. *Nat Methods*, 5(7):621-628.

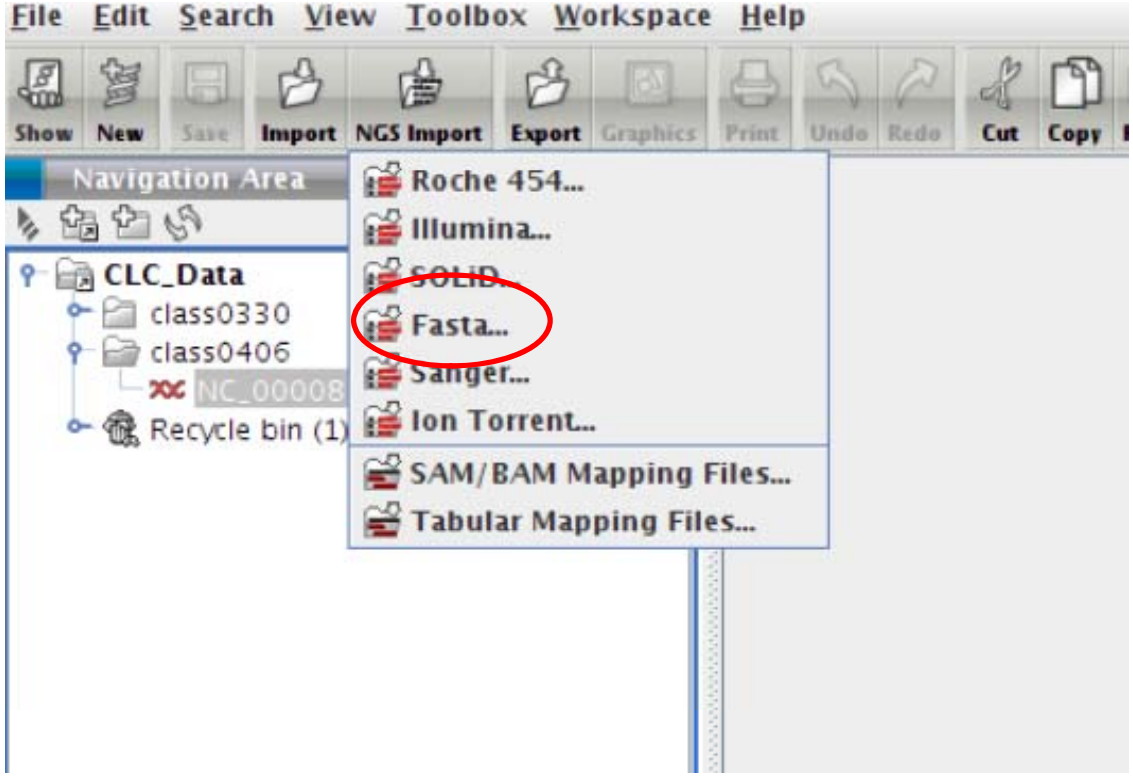
Import Genome information

- NC000082subset.gbk

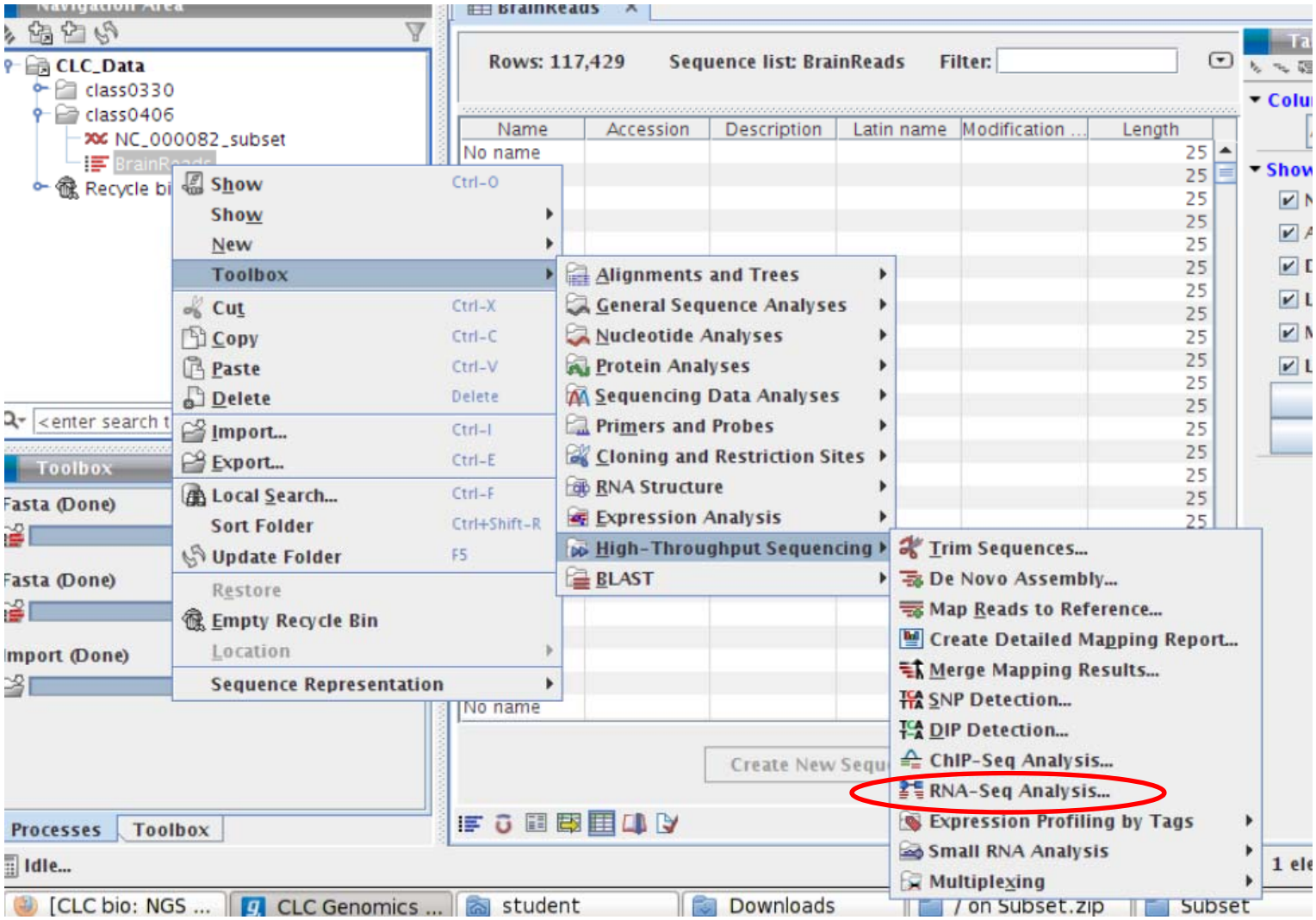



File Edit Search View Toolbox Workspace Help
Show New Save Import NGS Import Export Graphics Print Undo Redo Cut Copy Paste Delete Workspace Plug-ins
Navigation Area
CLC_Data
class0330
class0406
Recycle bin (1)
Show Ctrl-O
Show
New
Toolbox
Cut Ctrl-X
Copy Ctrl-C
Paste Ctrl-V
Delete Delete
Import... Ctrl-I
Export... Ctrl-E
Local Search... Ctrl-F
Sort Folder Ctrl+Shift-R
Update Folder F5
Restore
Sequence
As Circular
Annotation Table
As Text
Primer Designer
Secondary Structure 2D View
Secondary Structure Table
History
Element Info
<enter search term>
Toolbox
Import (Done)
Quick St

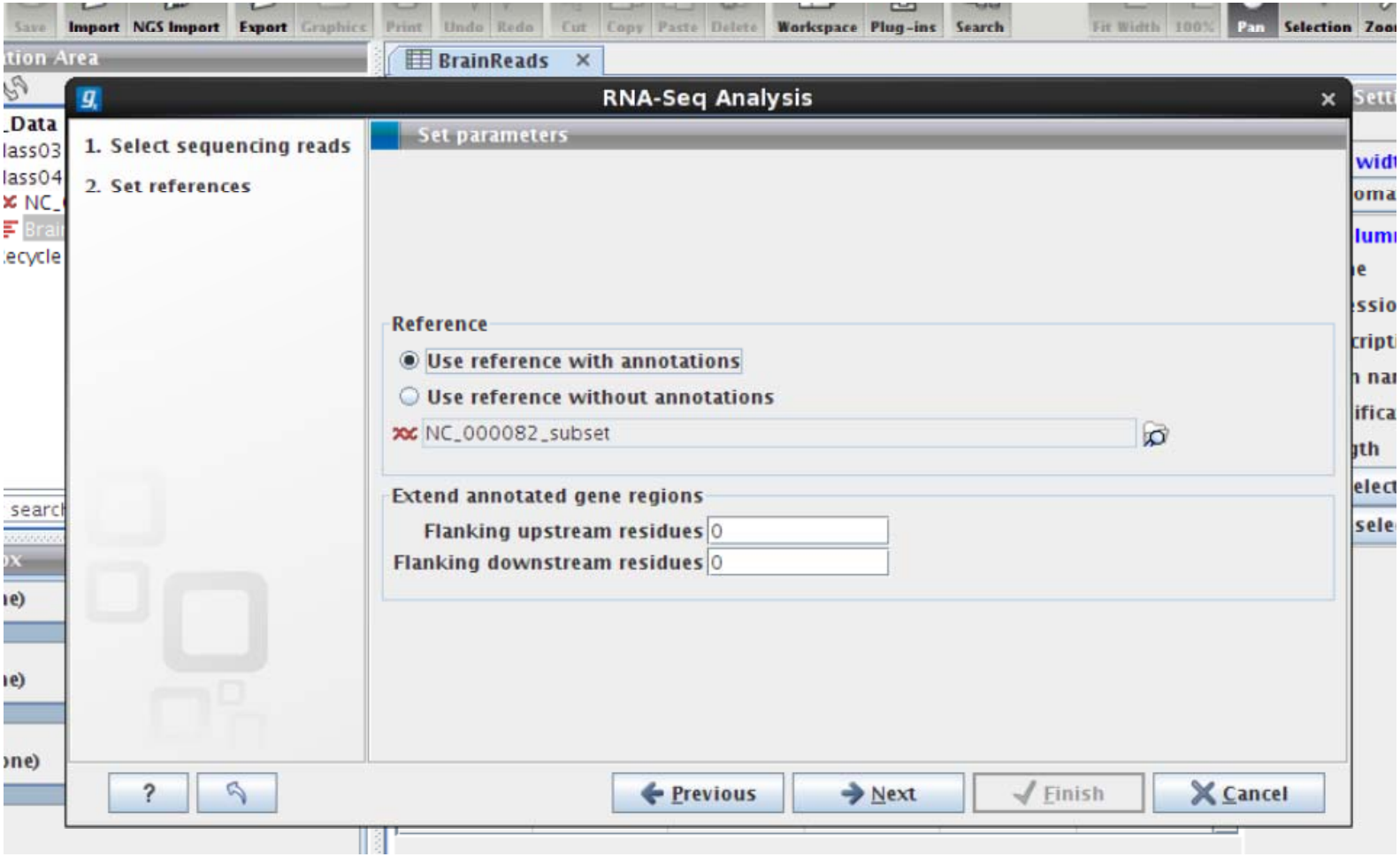

NC_000082_subset
Rows: 158
Filter:
All
Name
Type
Region
Qualifiers
Dgcr6
mRNA
join(52954..53215,...
/gene=Dgcr6
/product=DiGeorge syndrome criti region gene 6
/exception=mismatches in transcription
/note=Derived by automated computational analysis usinggene prediction method: BestRefseq. Supporting evidenceincludes simila to: 1 mRNA
/transcript_id=NM_010047.2
/db_xref="GI:142347980"
/db_xref="GeneID:13353"
/db_xref=MGI:1202877
Prodh
mRNA
complement(join(71...
/gene=Prodh
/product=proline dehydrogenase
/note=Derived by automated computational analysis usinggene prediction method: BestRefseq. Supporting evidenceincludes simila to: 1 mRNA
/transcript_id=NM_011172.2
/db_xref="GI:118601003"
/db_xref="GeneID:19125"
/db_xref=MGI:97770
/gene=Rtn4r
/product=reticulon 4 receptor
/exception=unclassified transcript discrepancy
Annotation Table Settin...
Shown annotation types
C-region
CDS
Exon
Gene
J-Segment
Misc_feature
mRNA
V-Segment
Select All
Deselect All


File Edit Search View Toolbox Workspace Help
Show New Save Import NGS Import Export Graphics Print Undo Redo Cut Copy
Navigation Area
CLC_Data
class0330
class0406
NC_00008
Recycle bin (1)
Roche 454...
Illumina...
SOLiD...
Fasta...
Sanger...
Ion Torrent...
SAM/BAM Mapping Files...
Tabular Mapping Files...

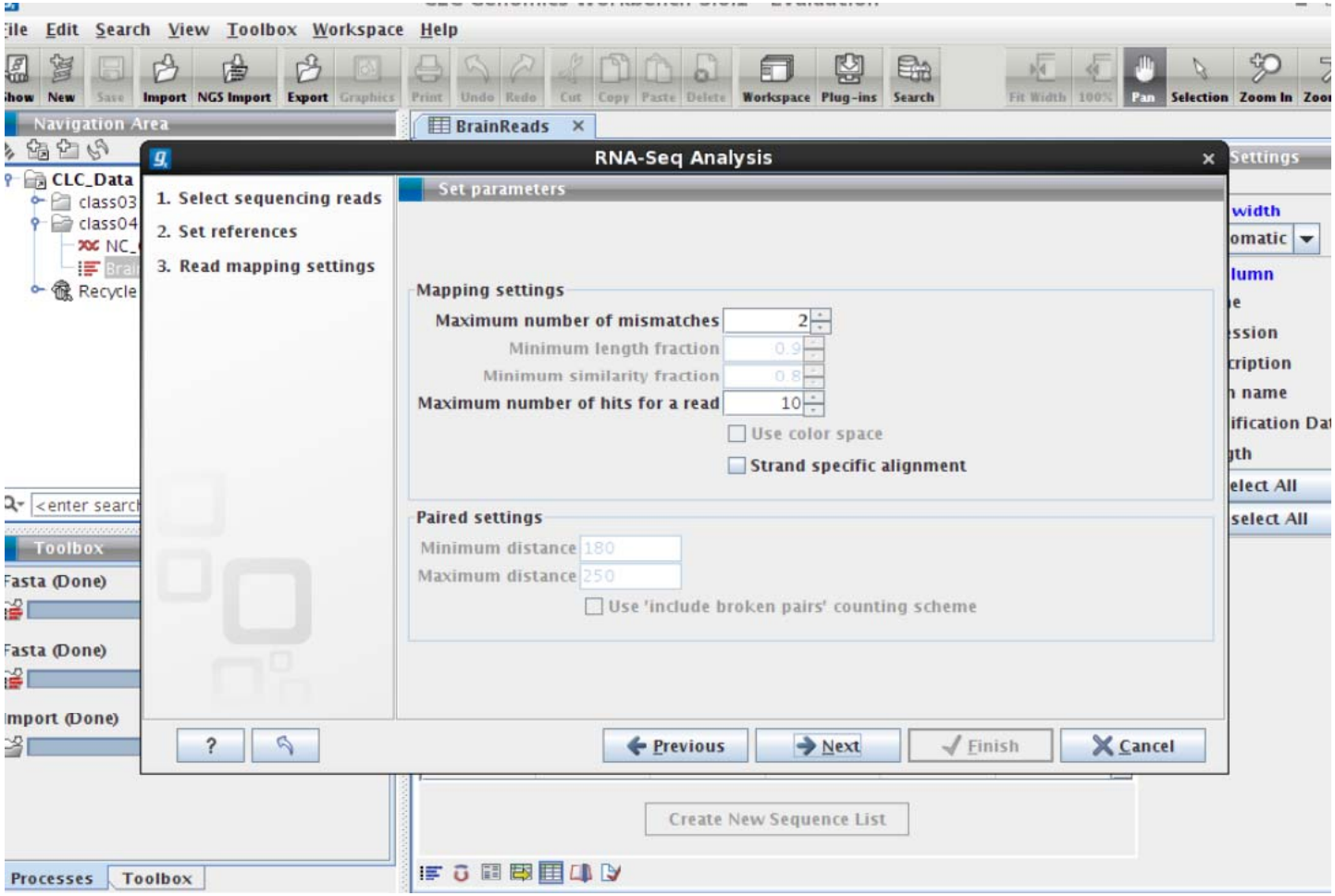
Start RNA seq analysis



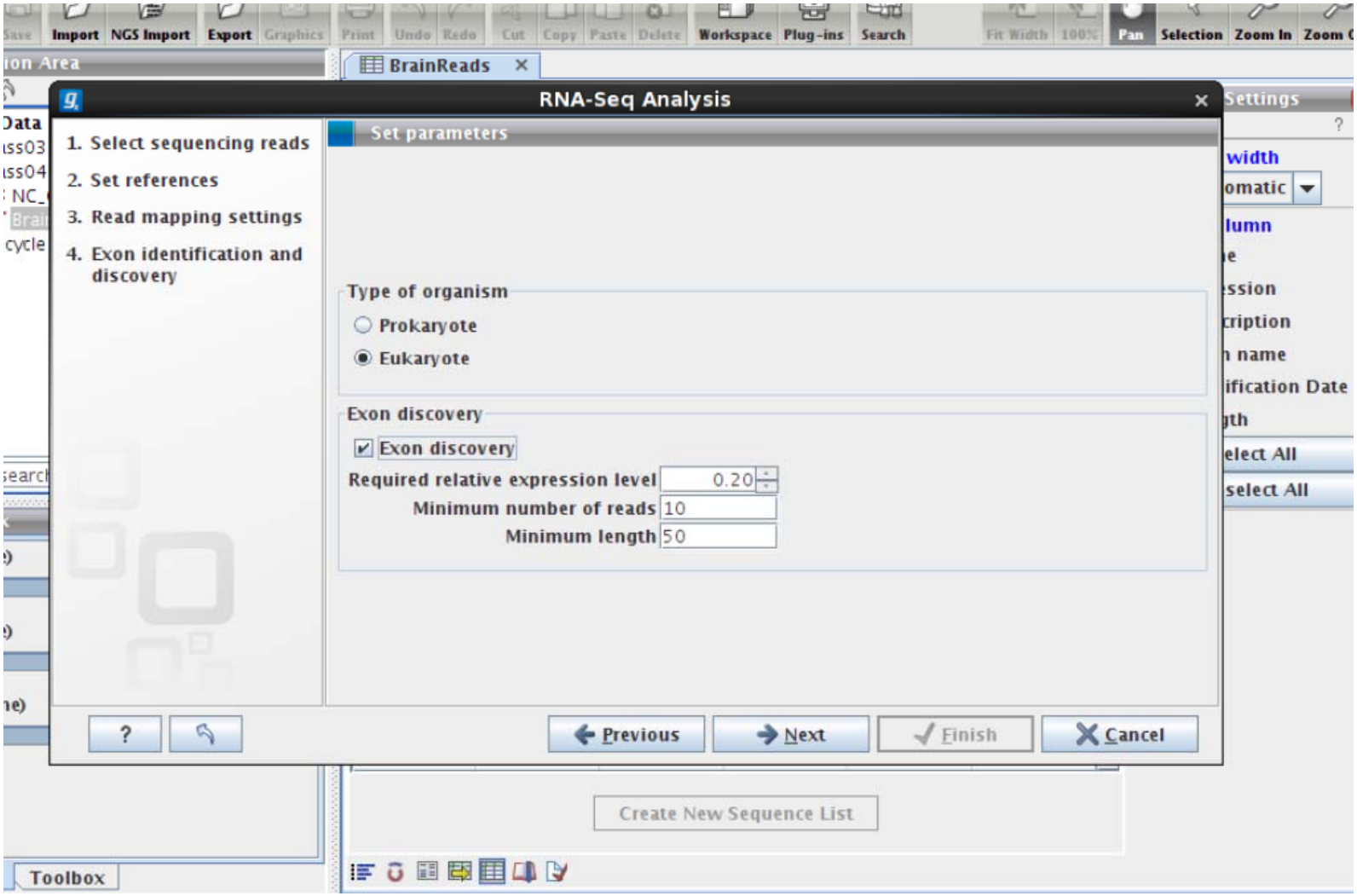
Select Reference with annotation

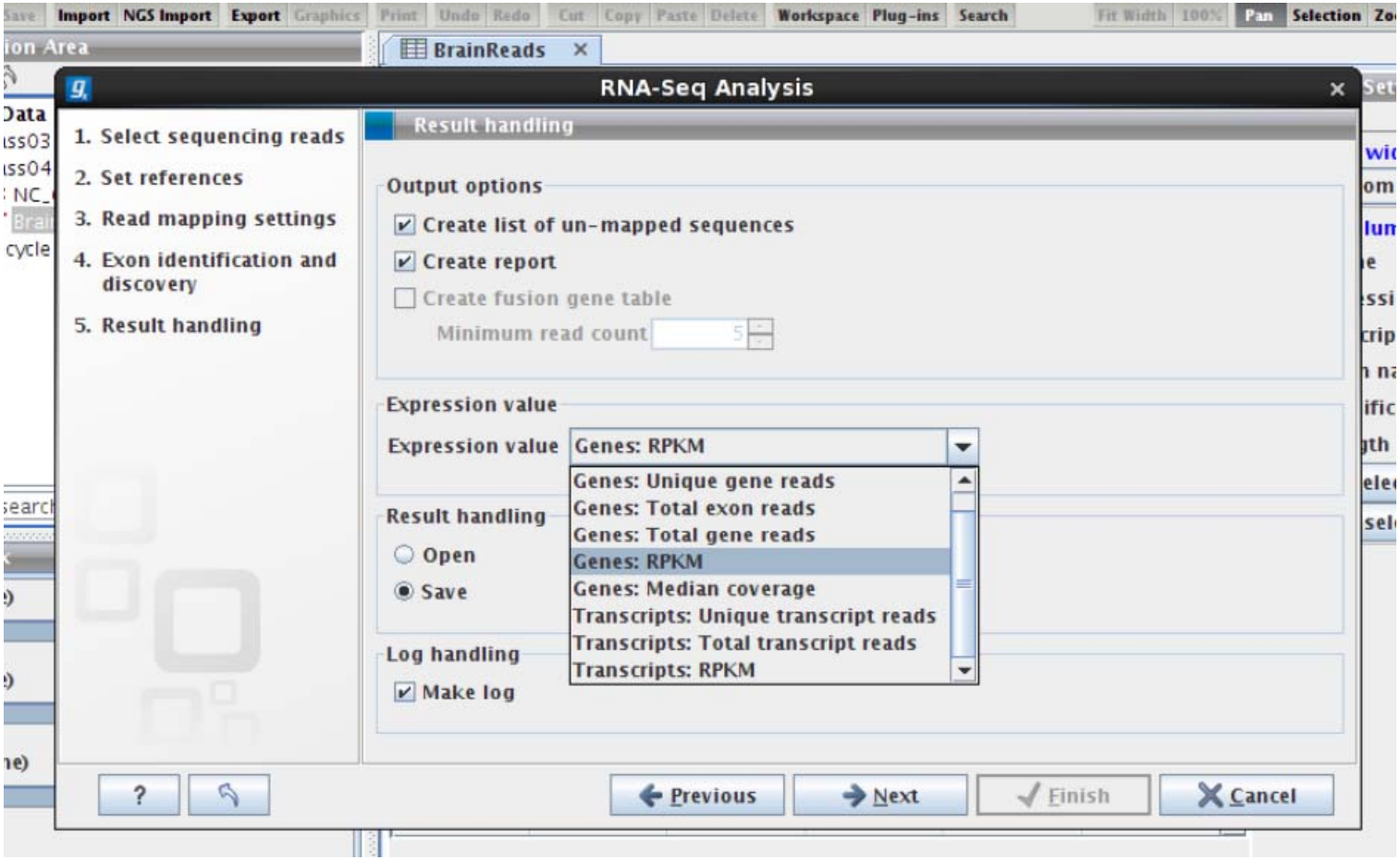


Setting mapping argument



setting Exon argument




Import
NGS Import
Export
Graphics
Print
Undo
Redo
Cut
Copy
Paste
Delete
Workspace
Plug-ins
Search
Fit Width
100%
Pan
Selection
BrainReads
RNA-Seq Analysis
1. Select sequencing reads
2. Set references
3. Read mapping settings
4. Exon identification and discovery
5. Result handling
Result handling
Output options
Create list of un-mapped sequences
Create report
Create fusion gene table
Minimum read count
5
Expression value
Expression value
Genes: RPKM
Genes: Unique gene reads
Genes: Total exon reads
Genes: Total gene reads
Genes: RPKM
Genes: Median coverage
Transcripts: Unique transcript reads
Transcripts: Total transcript reads
Transcripts: RPKM
Result handling
Open
Save
Log handling
Make log
Previous
Next
Finish
Cancel

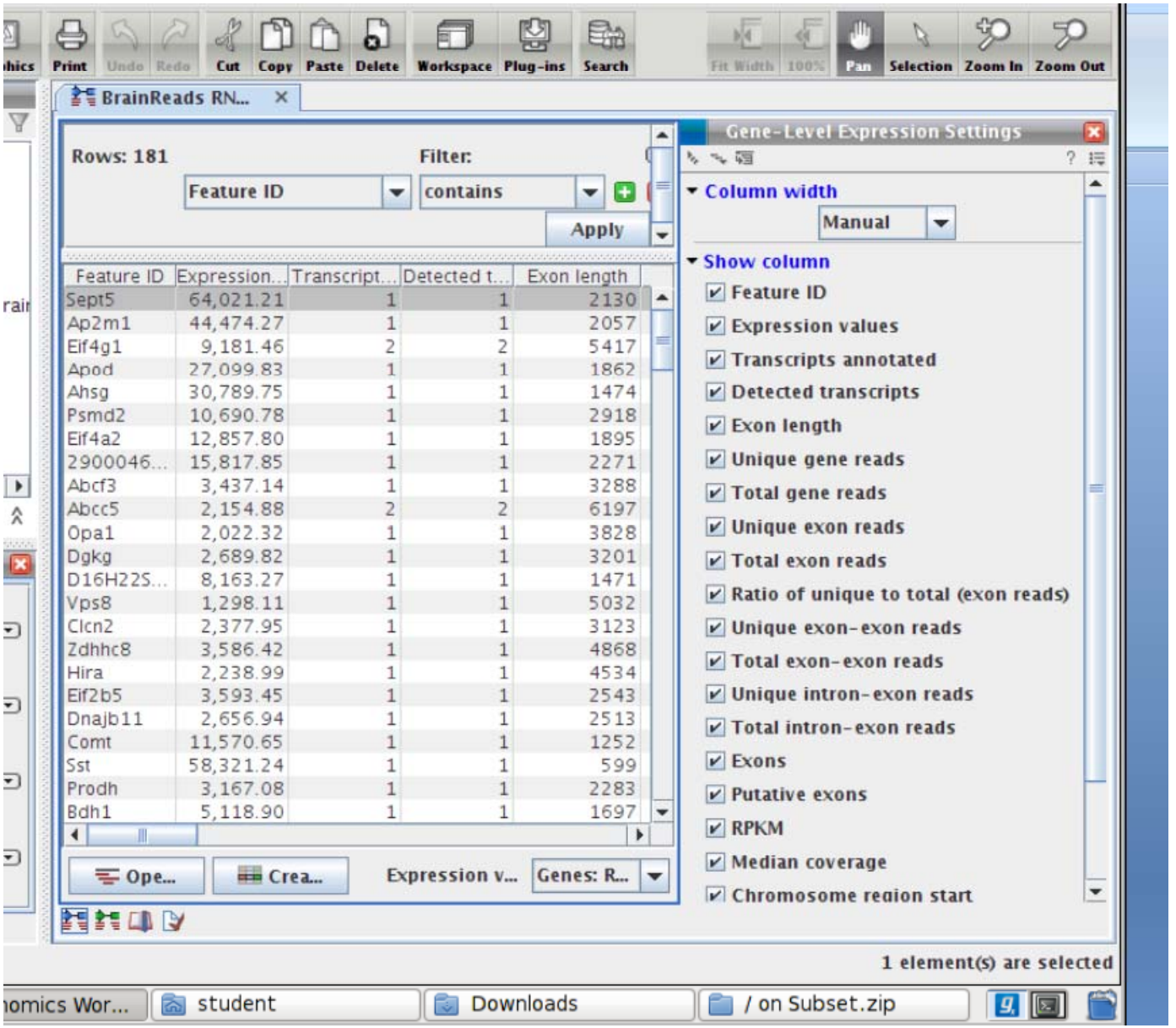
RPKM

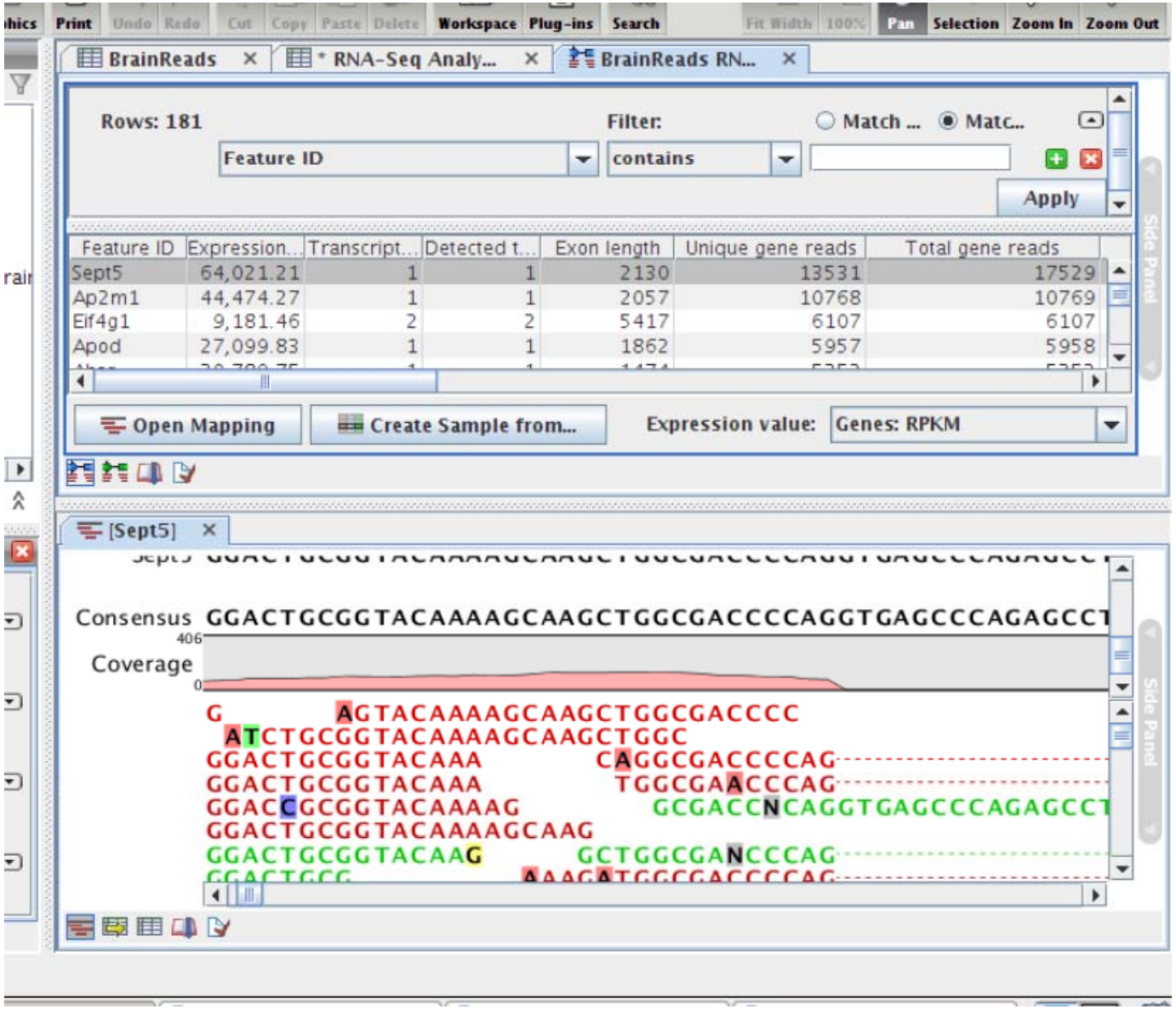
- Read per kilobase of exon model per million mapped reads

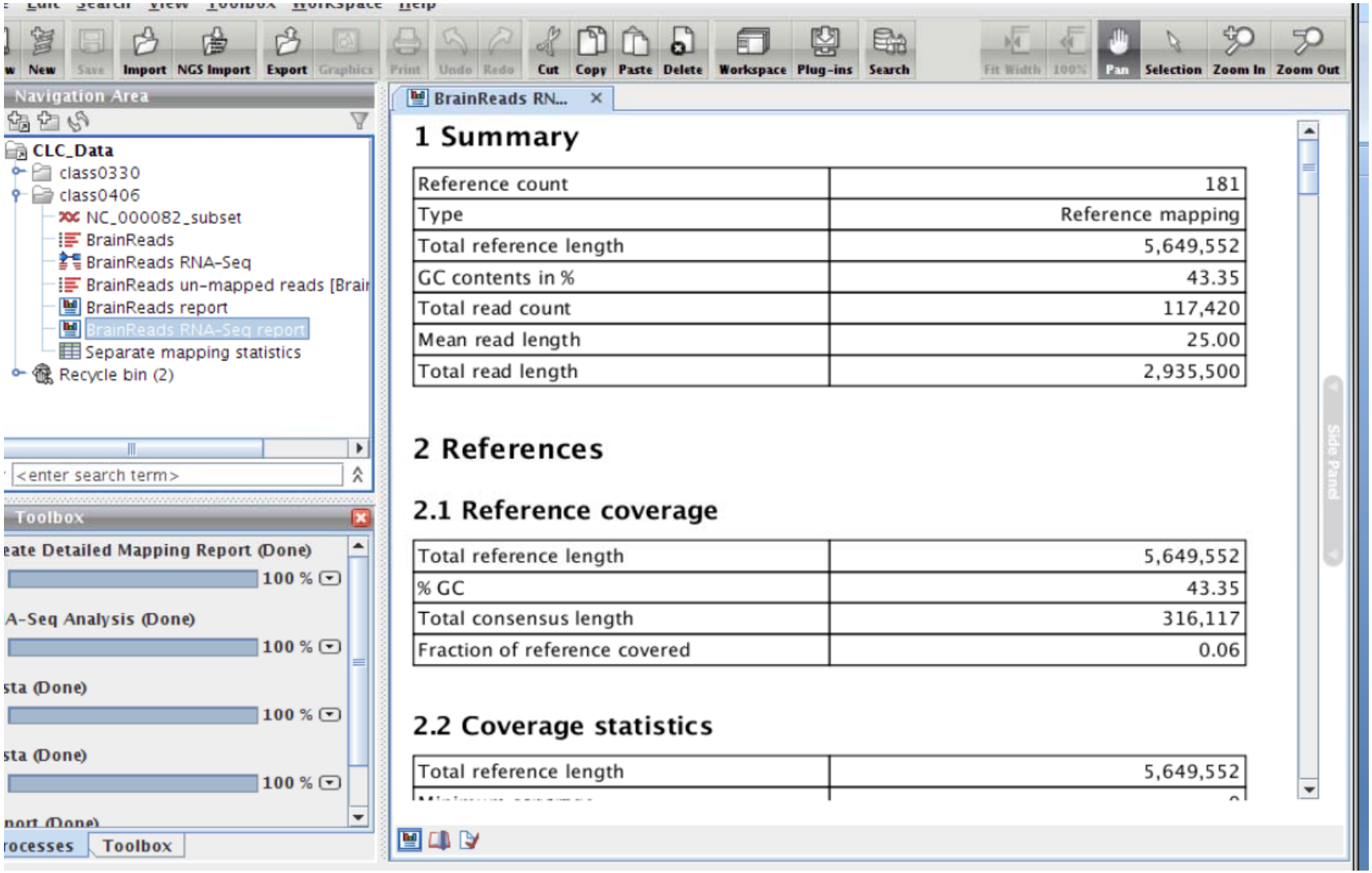
$$\text{RPKM} = \frac{total\ exon\ reads}{mapped\ reads\ (millions) * exon\ length\ (KB)}$$

- # total mapped reads
- Mapped contig/exon length

Mortazavi, A. et.al. (2008). Mapping and quantifying mammalian transcriptomes by rna-seq. *Nat Methods, 5(7):621--628.*


Print
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Workspace
Plug-ins
Search
Fit Width
100%
Pan
Selection
Zoom In
Zoom Out
BrainReads RN...
Rows: 181
Filter:
Feature ID
contains
Apply
Feature ID | Expression... | Transcript... | Detected t... | Exon length
Sept5 | 64,021.21 | 1 | 1 | 2130
Ap2m1 | 44,474.27 | 1 | 1 | 2057
Eif4g1 | 9,181.46 | 2 | 2 | 5417
Apod | 27,099.83 | 1 | 1 | 1862
Ahsg | 30,789.75 | 1 | 1 | 1474
Psmd2 | 10,690.78 | 1 | 1 | 2918
Eif4a2 | 12,857.80 | 1 | 1 | 1895
2900046... | 15,817.85 | 1 | 1 | 2271
Abcf3 | 3,437.14 | 1 | 1 | 3288
Abcc5 | 2,154.88 | 2 | 2 | 6197
Opa1 | 2,022.32 | 1 | 1 | 3828
Dgkg | 2,689.82 | 1 | 1 | 3201
D16H22S... | 8,163.27 | 1 | 1 | 1471
Vps8 | 1,298.11 | 1 | 1 | 5032
Clcn2 | 2,377.95 | 1 | 1 | 3123
Zdhhc8 | 3,586.42 | 1 | 1 | 4868
Hira | 2,238.99 | 1 | 1 | 4534
Eif2b5 | 3,593.45 | 1 | 1 | 2543
Dnajb11 | 2,656.94 | 1 | 1 | 2513
Comt | 11,570.65 | 1 | 1 | 1252
Sst | 58,321.24 | 1 | 1 | 599
Prodh | 3,167.08 | 1 | 1 | 2283
Bdh1 | 5,118.90 | 1 | 1 | 1697
Ope...
Crea...
Expression v...
Genes: R...
Gene-Level Expression Settings
Column width
Manual
Show column
Feature ID
Expression values
Transcripts annotated
Detected transcripts
Exon length
Unique gene reads
Total gene reads
Unique exon reads
Total exon reads
Ratio of unique to total (exon reads)
Unique exon-exon reads
Total exon-exon reads
Unique intron-exon reads
Total intron-exon reads
Exons
Putative exons
RPKM
Median coverage
Chromosome region start
1 element(s) are selected
student
Downloads
/ on Subset.zip

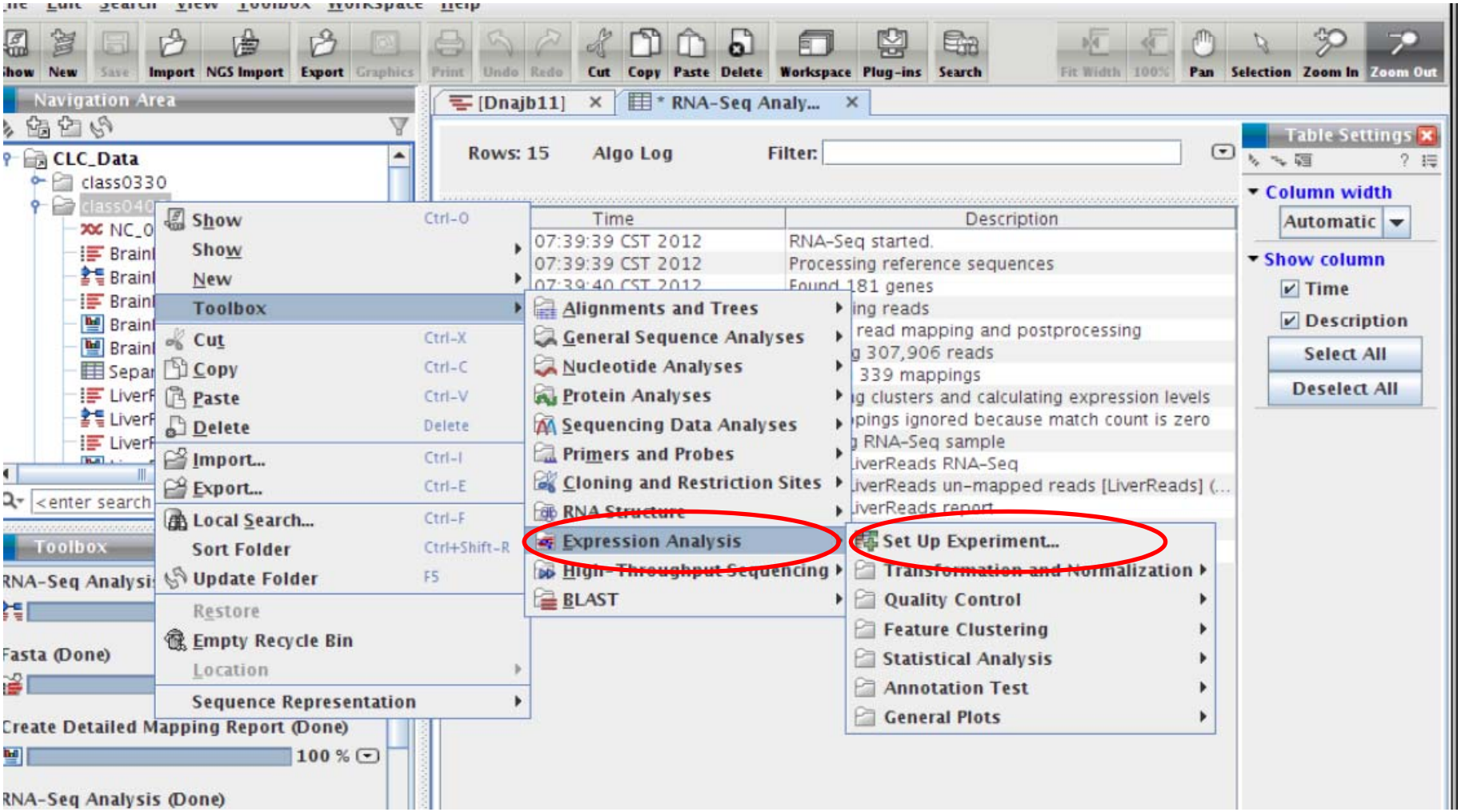

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Plug-ins
Search
Fit Width
100%
Pan
Selection
Zoom In
Zoom Out
BrainReads
* RNA-Seq Analy...
BrainReads RN...
Rows: 181
Filter:
Match ...
Matc...
Feature ID
contains
Apply
Feature ID | Expression... | Transcript... | Detected t... | Exon length | Unique gene reads | Total gene reads
Sept5 | 64,021.21 | 1 | 1 | 2130 | 13531 | 17529
Ap2m1 | 44,474.27 | 1 | 1 | 2057 | 10768 | 10769
Eif4g1 | 9,181.46 | 2 | 2 | 5417 | 6107 | 6107
Apod | 27,099.83 | 1 | 1 | 1862 | 5957 | 5958
Open Mapping
Create Sample from...
Expression value:
Genes: RPKM
[Sept5]
Consensus
GGACTGCGGTACAAAAGCAAGCTGGCGACCCCAGGTGAGCCCAGAGCCT
406
Coverage
0
Side Panel

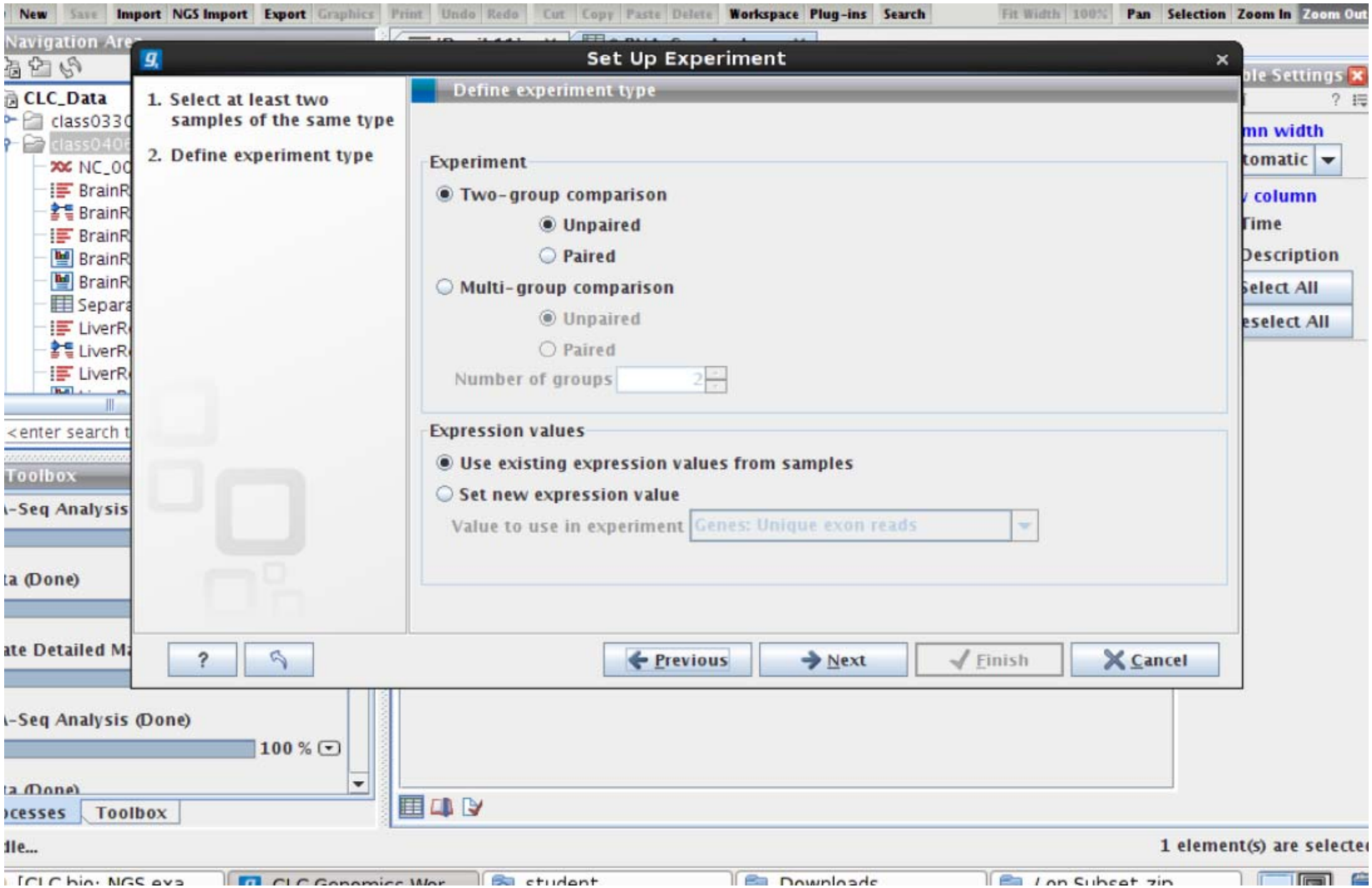

New
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Workspace
Plug-ins
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Fit Width
100%
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Selection
Zoom In
Zoom Out
Navigation Area
CLC_Data
class0330
class0406
NC_000082_subset
BrainReads
BrainReads RNA-Seq
BrainReads un-mapped reads [Brai
BrainReads report
BrainReads RNA-Seq report
Separate mapping statistics
Recycle bin (2)
<enter search term>
Toolbox
eate Detailed Mapping Report (Done)
100 %
A-Seq Analysis (Done)
100 %
sta (Done)
100 %
sta (Done)
100 %
port (Done)
rocesses
Toolbox
BrainReads RN...
1 Summary
Reference count 181
Type Reference mapping
Total reference length 5,649,552
GC contents in % 43.35
Total read count 117,420
Mean read length 25.00
Total read length 2,935,500
2 References
2.1 Reference coverage
Total reference length 5,649,552
% GC 43.35
Total consensus length 316,117
Fraction of reference covered 0.06
2.2 Coverage statistics
Total reference length 5,649,552
Side Panel

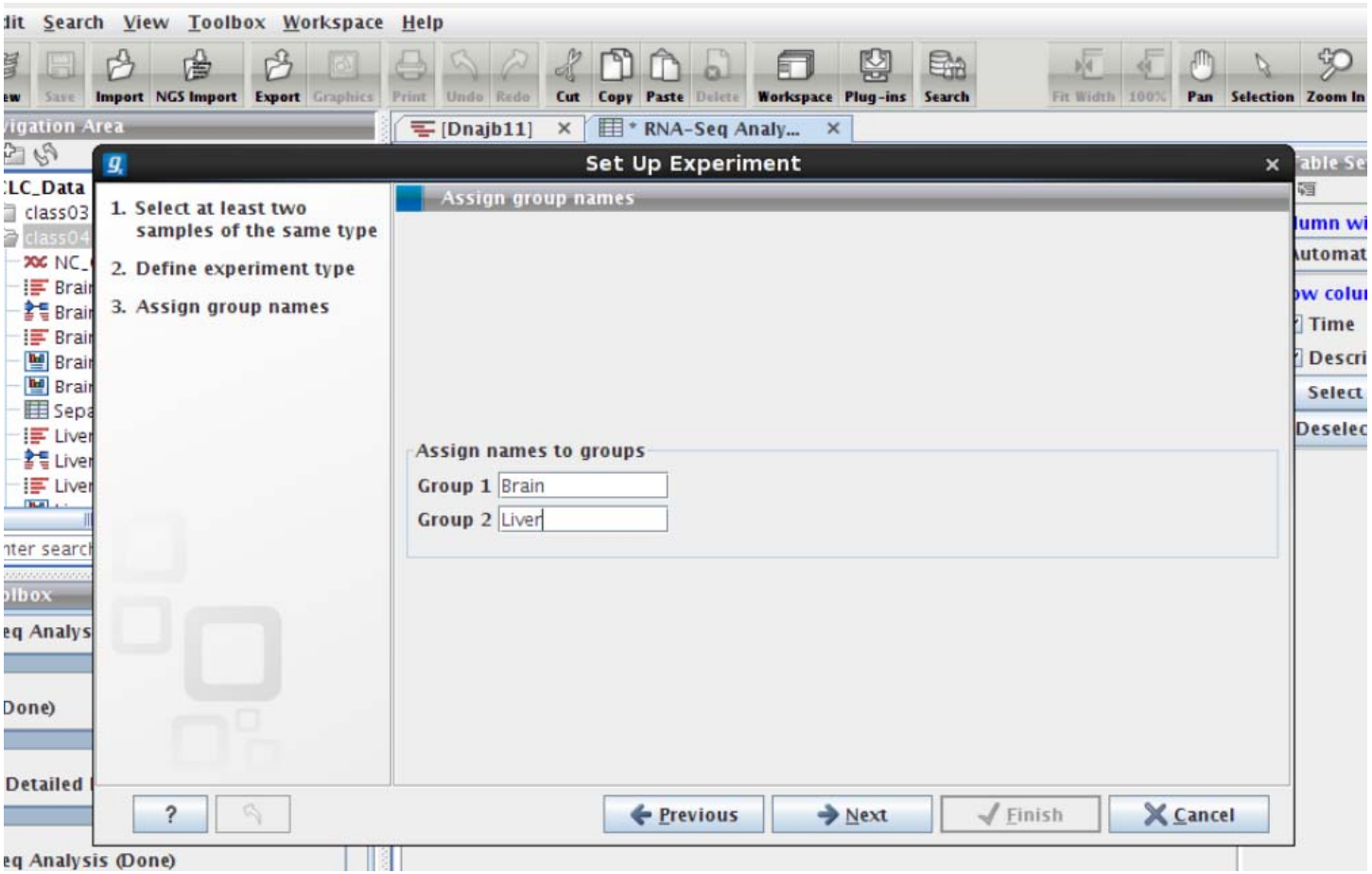
Exercise:

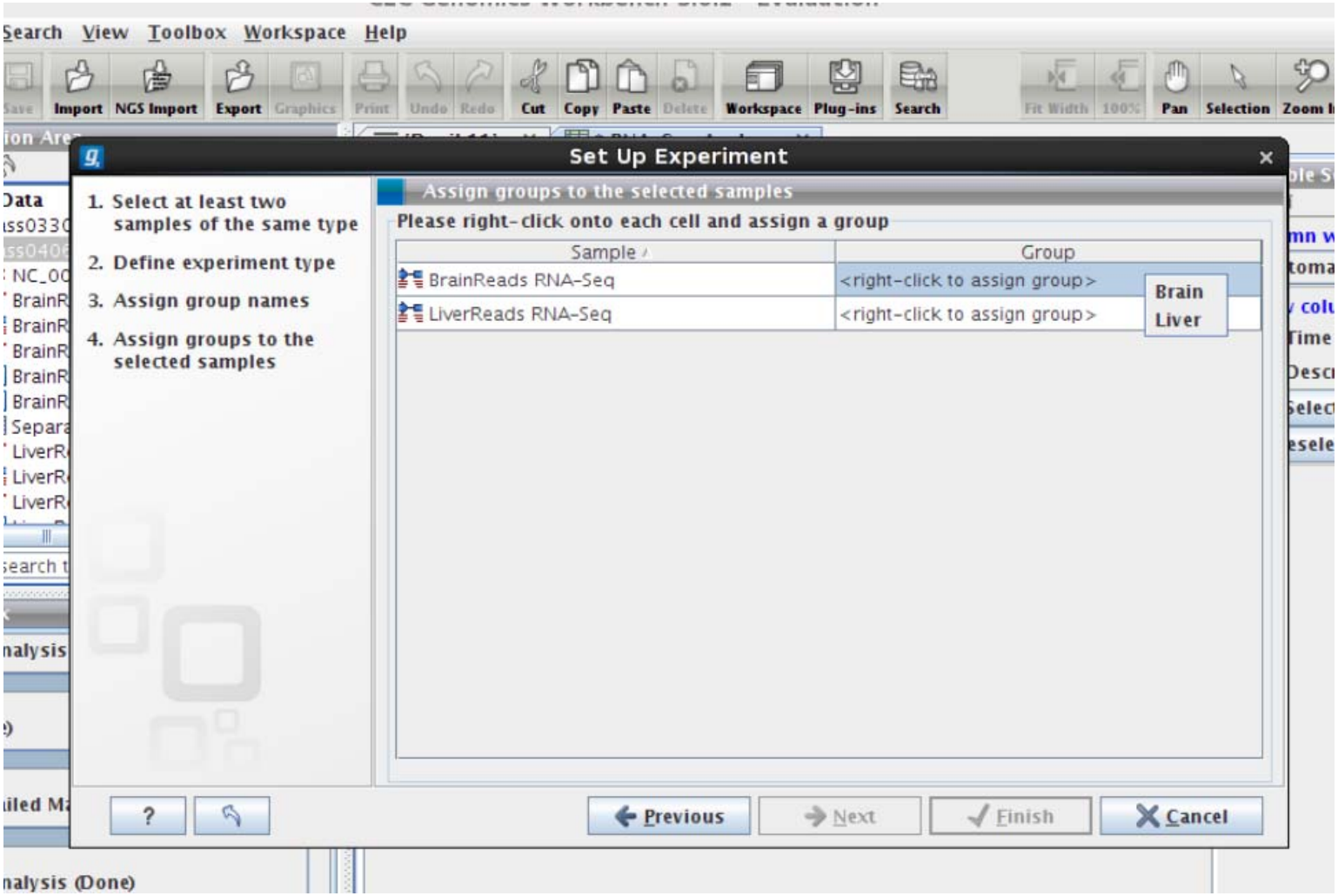
- Import Liver Reads
- do above steps

Expression analysis

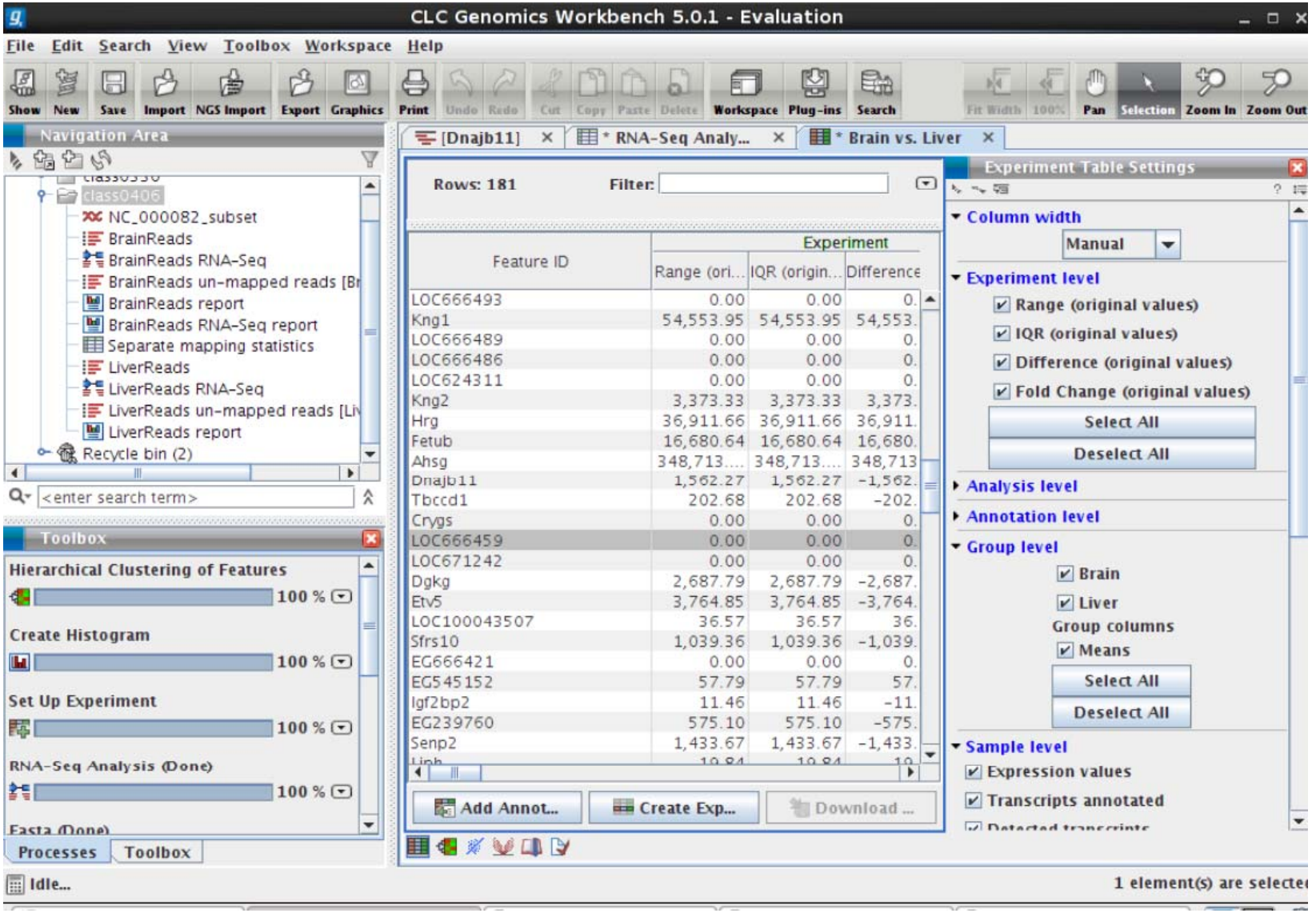



Set Up Experiment
1. Select at least two samples of the same type
2. Define experiment type
Define experiment type
Experiment
Two-group comparison
Unpaired
Paired
Multi-group comparison
Unpaired
Paired
Number of groups
Expression values
Use existing expression values from samples
Set new expression value
Value to use in experiment
Genes: Unique exon reads
Previous
Next
Finish
Cancel
Toolbox
CLC_Data
1 element(s) are selected


Search View Toolbox Workspace Help
Import NGS Import Export Graphics Print Undo Redo Cut Copy Paste Delete Workspace Plug-ins Search Fit Width 100% Pan Selection Zoom In
[Dnajb11]
* RNA-Seq Analy...
Set Up Experiment
1. Select at least two samples of the same type
2. Define experiment type
3. Assign group names
Assign group names
Assign names to groups
Group 1 Brain
Group 2 Liver
Previous
Next
Finish
Cancel
Done)
Detailed
eq Analysis (Done)


Search View Toolbox Workspace Help
Import NGS Import Export Graphics Print Undo Redo Cut Copy Paste Delete Workspace Plug-ins Search Fit Width 100% Pan Selection
Set Up Experiment
1. Select at least two samples of the same type
2. Define experiment type
3. Assign group names
4. Assign groups to the selected samples
Assign groups to the selected samples
Please right-click onto each cell and assign a group
Sample
Group
BrainReads RNA-Seq
<right-click to assign group>
LiverReads RNA-Seq
<right-click to assign group>
Brain
Liver
Previous
Next
Finish
Cancel

Experiment Table for Comparision



- Comparative analysis for denovo transcriptome analysis?