BV-BRC Test Report

A14. Service – Tn-seq Analysis - Bacteria

Item to test	Tn-seq Analysis Service using bacterial read files
URL	https://www.bv-brc.org/app/Tnseq
Prerequisites	Bacterial read files in Workspace
References	https://www.bv-brc.org/docs/quick_references/services/tn_seq_analysis_service.html https://www.bv-brc.org/docs/tutorial/tn-seq/tn-seq.html
Tester(s)	Rebecca Wattam, Maulik Shukla
Test date	08-May-2022 (follow-up from original test)
Test result	**Passed**

Overview

- Test the Tn-seq Analysis Service using exemplar reads sets for bacterial genomes.
- Test different strategies, i.e., essential genes and conditionally essential genes.
- For each job submitted, verify successful completion of the job, presence of output files, including bam, wig, counts, transit stats files. Verify the quality of the results.
- Review the results in the genome browser.

Test Data

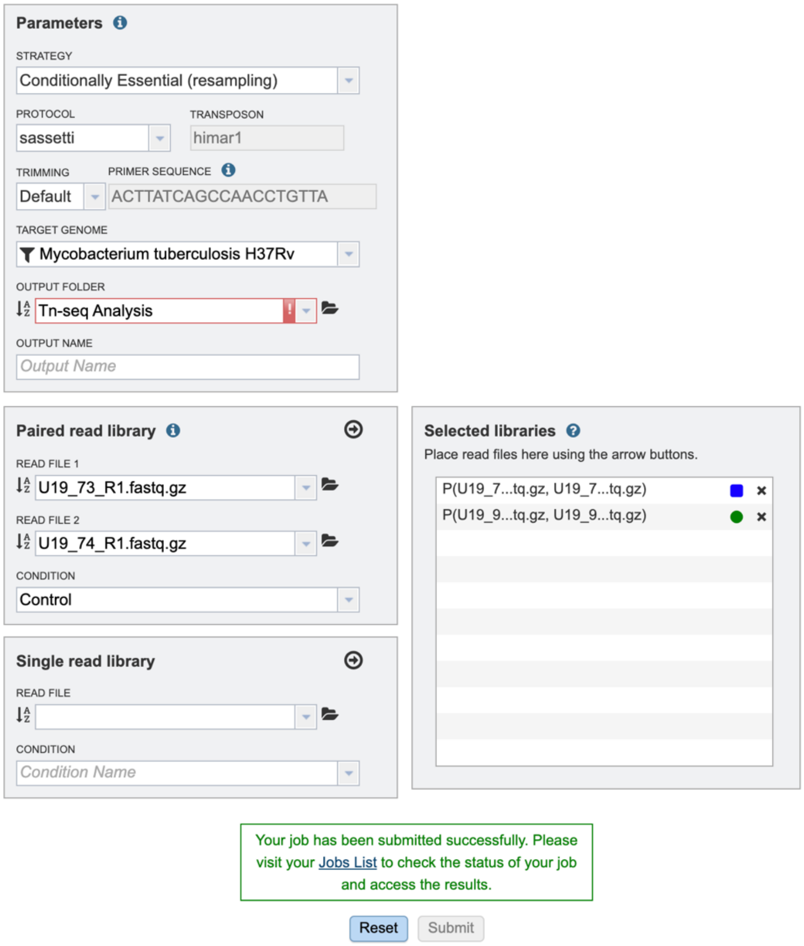
Dataset	Rational	Input Format	Input
Mycobacterium tuberculosis genomes	Workshop example	FASTQ files	U19_73_R1.fastq.gz U19_74_R1.fastq.gz U19_91_R1.fastq.gz U19_92_R1.fastq.gz

- All test datasets and corresponding job results are available in the following public workspace: https://www.bv-brc.org/workspace/BVBRC@patricbrc.org/BVBRC%20Tests/Tn-seq%20Analysis

Test Results

- All Tn-seq analysis jobs completed successfully, without errors.
- All jobs resulted in expected output files in corresponding job output directory, including bam, wig, read count and tn-stats files per sample, and a summary report.
- The transit summary report was informative and provided A summary of the statistical data produced by the TRANSIT software, including comparison of the control and treatment samples, log FC, and P-value.
- The link to genome browser worked and loaded all bam files as separate tracks as expected.
- All test datasets and corresponding job results are available in the following public workspace: https://www.bv-brc.org/workspace/BVBRC@patricbrc.org/BVBRC%20Tests/Tn-seq%20Analysis

- Below are a series of screenshots showing the successful completion of the jobs, the files in the job output directory, the transit report, and the genome browser.



Parameters

STRATEGY: Conditionally Essential (resampling)

PROTOCOL: sassetti

TRANSPOSON: himar1

TRIMMING: Default

PRIMER SEQUENCE: ACTTATCAGCCAACCTGTTA

TARGET GENOME: Mycobacterium tuberculosis H37Rv

OUTPUT FOLDER: Tn-seq Analysis

OUTPUT NAME: Output Name

Paired read library

READ FILE 1: U19_73_R1.fastq.gz

READ FILE 2: U19_74_R1.fastq.gz

CONDITION: Control

Single read library

READ FILE:

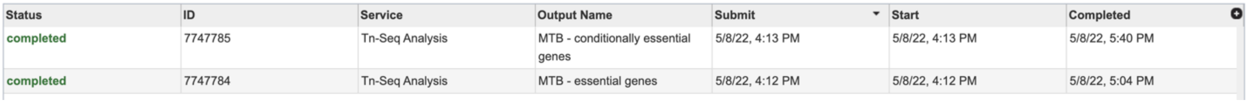
CONDITION: Condition Name

Selected libraries

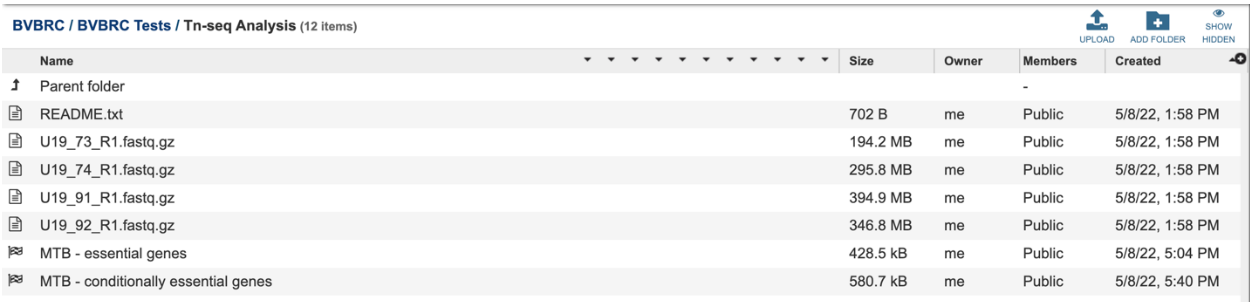
Place read files here using the arrow buttons.

P(U19_7...tq.gz, U19_7...tq.gz) 🟦 ×
P(U19_9...tq.gz, U19_9...tq.gz) ● ×

Your job has been submitted successfully. Please visit your Jobs List to check the status of your job and access the results.



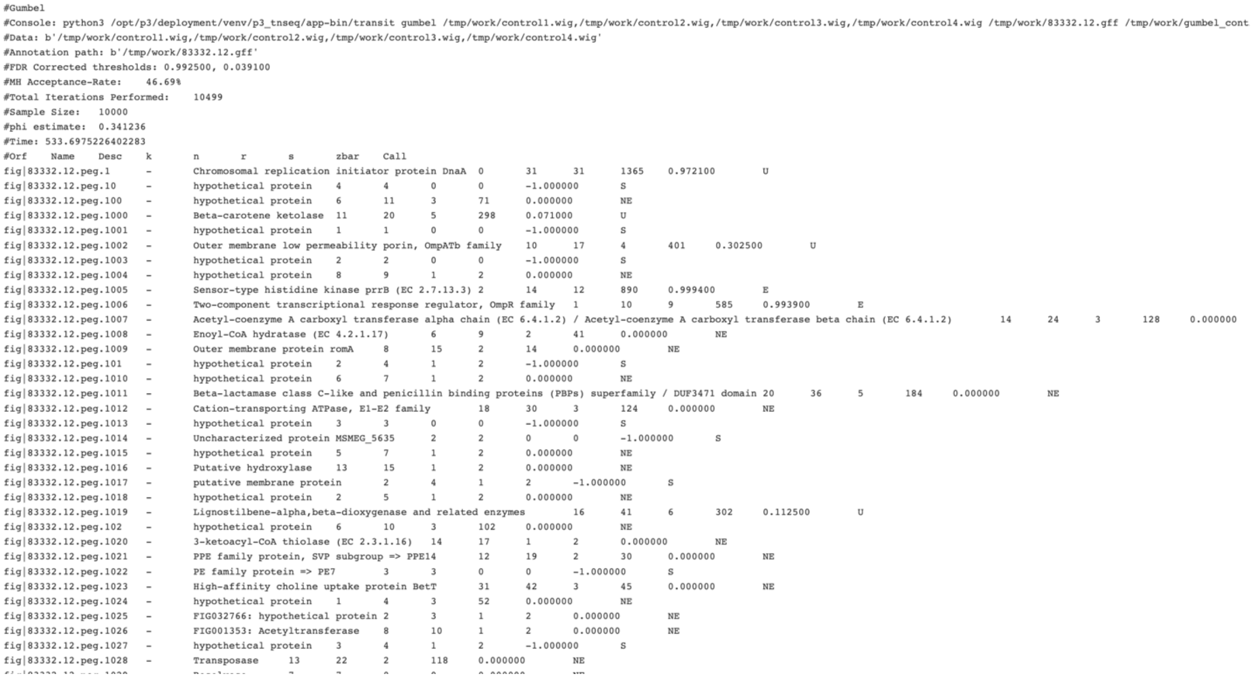
Status	ID	Service	Output Name	Submit	Start	Completed
completed	7747785	Tn-Seq Analysis	MTB - conditionally essential genes	5/8/22, 4:13 PM	5/8/22, 4:13 PM	5/8/22, 5:40 PM
completed	7747784	Tn-Seq Analysis	MTB - essential genes	5/8/22, 4:12 PM	5/8/22, 4:12 PM	5/8/22, 5:04 PM



BVBRC / BVBRC Tests / Tn-seq Analysis (12 items)

Name	Size	Owner	Members	Created
Parent folder			-	
README.txt	702 B	me	Public	5/8/22, 1:58 PM
U19_73_R1.fastq.gz	194.2 MB	me	Public	5/8/22, 1:58 PM
U19_74_R1.fastq.gz	295.8 MB	me	Public	5/8/22, 1:58 PM
U19_91_R1.fastq.gz	394.9 MB	me	Public	5/8/22, 1:58 PM
U19_92_R1.fastq.gz	346.8 MB	me	Public	5/8/22, 1:58 PM
MTB - essential genes	428.5 kB	me	Public	5/8/22, 5:04 PM
MTB - conditionally essential genes	580.7 kB	me	Public	5/8/22, 5:40 PM

Name	Size	Owner	Members	Created
Parent folder			-	
control1.bam	43.6 MB	me	Public	5/8/22, 5:04 PM
control1.bam.bai	11.9 kB	me	Public	5/8/22, 5:04 PM
control1.counts	75 B	me	Public	5/8/22, 5:04 PM
control1.tn_stats	1.6 kB	me	Public	5/8/22, 5:04 PM
control1.wig	756.4 kB	me	Public	5/8/22, 5:04 PM
control2.bam	64.3 MB	me	Public	5/8/22, 5:04 PM
control2.bam.bai	12.4 kB	me	Public	5/8/22, 5:04 PM
control2.counts	75 B	me	Public	5/8/22, 5:04 PM
control2.tn_stats	1.6 kB	me	Public	5/8/22, 5:04 PM
control2.wig	762.6 kB	me	Public	5/8/22, 5:04 PM
control3.bam	83.8 MB	me	Public	5/8/22, 5:04 PM
control3.bam.bai	12.1 kB	me	Public	5/8/22, 5:04 PM
control3.counts	75 B	me	Public	5/8/22, 5:04 PM
control3.tn_stats	1.6 kB	me	Public	5/8/22, 5:04 PM
control3.wig	751.4 kB	me	Public	5/8/22, 5:04 PM
control4.bam	75.4 MB	me	Public	5/8/22, 5:04 PM



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#Gumbel
#Console: python3 /opt/p3/deployment/venv/p3_tnseq/app-bin/transit gumbel /tmp/work/control1.wig,/tmp/work/control2.wig,/tmp/work/control3.wig,/tmp/work/control4.wig /tmp/work/83332.12.gff /tmp/work/gumbel_cont
#Data: b'/tmp/work/control1.wig,/tmp/work/control2.wig,/tmp/work/control3.wig,/tmp/work/control4.wig'
#Annotation path: b'/tmp/work/83332.12.gff'
#FDR Corrected thresholds: 0.992500, 0.039100
#MH Acceptance-Rate:    46.69%
#Total Iterations Performed:    10499
#Sample Size:   10000
#phi estimate:  0.341236
#Time: 533.6975226402283
#Orf    Name    Desc    k       n       r       s       zbar    Call
fig|83332.12.peg.1      -       Chromosomal replication initiator protein DnaA  0       31      31      1365    0.972100        U
fig|83332.12.peg.10     -       hypothetical protein    4       4       0       0       -1.000000       S
fig|83332.12.peg.100    -       hypothetical protein    6       11      3       71      0.000000        NE
fig|83332.12.peg.1000   -       Beta-carotene ketolase  11      20      5       298     0.071000        U
fig|83332.12.peg.1001   -       hypothetical protein    1       1       0       0       -1.000000       S
fig|83332.12.peg.1002   -       Outer membrane low permeability porin, OmpATb family    10      17      4       401     0.302500        U
fig|83332.12.peg.1003   -       hypothetical protein    2       2       0       0       -1.000000       S
fig|83332.12.peg.1004   -       hypothetical protein    8       9       1       2       0.000000        NE
fig|83332.12.peg.1005   -       Sensor-type histidine kinase prrB (EC 2.7.13.3) 2       14      12      890     0.999400        E
fig|83332.12.peg.1006   -       Two-component transcriptional response regulator, OmpR family   1       10      9       585     0.993900        E
fig|83332.12.peg.1007   -       Acetyl-coenzyme A carboxyl transferase alpha chain (EC 6.4.1.2) / Acetyl-coenzyme A carboxyl transferase beta chain (EC 6.4.1.2)       14      24      3       128     0.000000
fig|83332.12.peg.1008   -       Enoyl-CoA hydratase (EC 4.2.1.17)       6       9       2       41      0.000000        NE
fig|83332.12.peg.1009   -       Outer membrane protein romA     8       15      2       14      0.000000        NE
fig|83332.12.peg.101    -       hypothetical protein    2       4       1       2       -1.000000       S
fig|83332.12.peg.1010   -       hypothetical protein    6       7       1       2       0.000000        NE
fig|83332.12.peg.1011   -       Beta-lactamase class C-like and penicillin binding proteins (PBPs) superfamily / DUF3471 domain 20      36      5       184     0.000000        NE
fig|83332.12.peg.1012   -       Cation-transporting ATPase, E1-E2 family        18      30      3       124     0.000000        NE
fig|83332.12.peg.1013   -       hypothetical protein    3       3       0       0       -1.000000       S
fig|83332.12.peg.1014   -       Uncharacterized protein MSMEG_5635      2       2       0       0       -1.000000       S
fig|83332.12.peg.1015   -       hypothetical protein    5       7       1       2       0.000000        NE
fig|83332.12.peg.1016   -       Putative hydroxylase    13      15      1       2       0.000000        NE
fig|83332.12.peg.1017   -       putative membrane protein       2       4       1       2       -1.000000       S
fig|83332.12.peg.1018   -       hypothetical protein    2       5       1       2       0.000000        NE
fig|83332.12.peg.1019   -       Lignostilbene-alpha,beta-dioxygenase and related enzymes        16      41      6       302     0.112500        U
fig|83332.12.peg.102    -       hypothetical protein    6       10      3       102     0.000000        NE
fig|83332.12.peg.1020   -       3-ketoacyl-CoA thiolase (EC 2.3.1.16)   14      17      1       2       0.000000        NE
fig|83332.12.peg.1021   -       PPE family protein, SVP subgroup => PPE14       12      19      2       30      0.000000        NE
fig|83332.12.peg.1022   -       PE family protein => PE7        3       3       0       0       -1.000000       S
fig|83332.12.peg.1023   -       High-affinity choline uptake protein BetT       31      42      3       45      0.000000        NE
fig|83332.12.peg.1024   -       hypothetical protein    1       4       3       52      0.000000        NE
fig|83332.12.peg.1025   -       FIG032766: hypothetical protein 2       3       1       2       0.000000        NE
fig|83332.12.peg.1026   -       FIG001353: Acetyltransferase    8       10      1       2       0.000000        NE
fig|83332.12.peg.1027   -       hypothetical protein    3       4       1       2       -1.000000       S
fig|83332.12.peg.1028   -       Transposase     13      22      2       118     0.000000        NE
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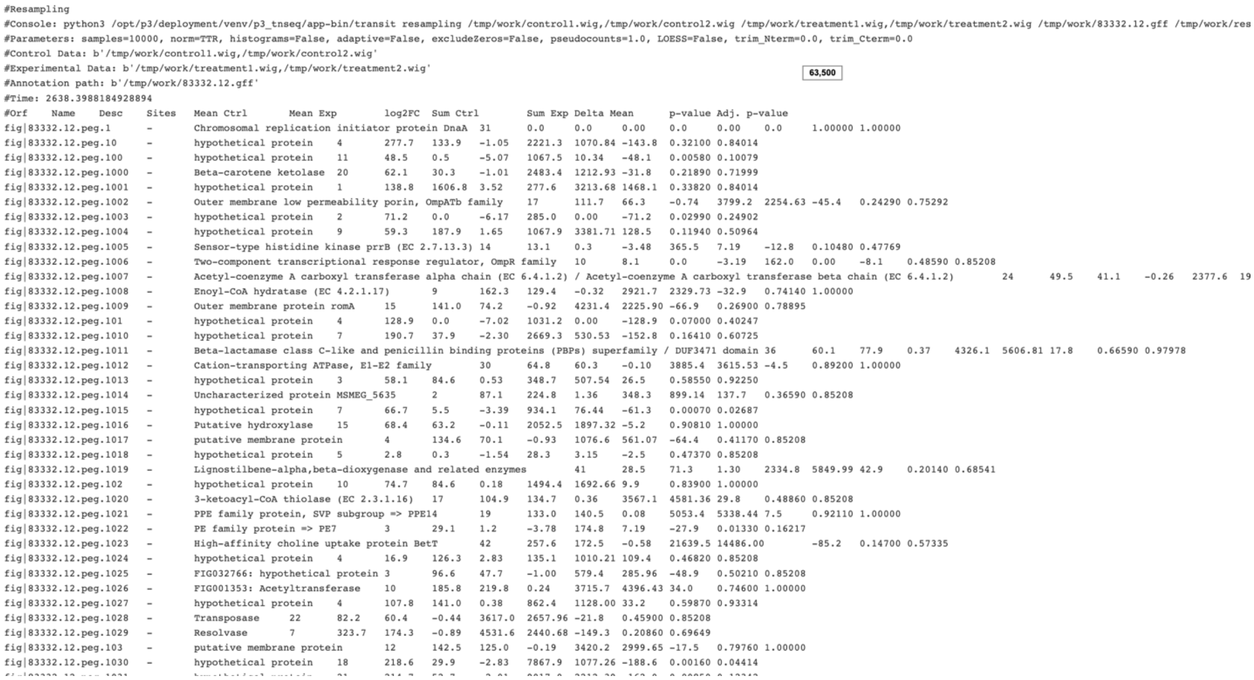

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#Resampling
#Console: python3 /opt/p3/deployment/venv/p3_tnseq/app-bin/transit resampling /tmp/work/control1.wig,/tmp/work/control2.wig /tmp/work/treatment1.wig,/tmp/work/treatment2.wig /tmp/work/83332.12.gff /tmp/work/res
#Parameters: samples=10000, norm=TTR, histograms=False, adaptive=False, excludeZeros=False, pseudocounts=1.0, LOESS=False, trim_Nterm=0.0, trim_Cterm=0.0
#Control Data: b'/tmp/work/control1.wig,/tmp/work/control2.wig'
#Experimental Data: b'/tmp/work/treatment1.wig,/tmp/work/treatment2.wig'
#Annotation path: b'/tmp/work/83332.12.gff'
#Time: 2638.3988184928894
#Orf    Name    Desc    Sites   Mean Ctrl       Mean Exp        log2FC  Sum Ctrl        Sum Exp Delta Mean      p-value Adj. p-value
fig|83332.12.peg.1      -       Chromosomal replication initiator protein DnaA  31      0.0     0.0     0.00    0.0     0.00    0.0     1.00000 1.00000
fig|83332.12.peg.10     -       hypothetical protein    4       277.7   133.9   -1.05   2221.3  1070.84 -143.8  0.32100 0.84014
fig|83332.12.peg.100    -       hypothetical protein    11      48.5    0.5     -5.07   1067.5  10.34   -48.1   0.00580 0.10079
fig|83332.12.peg.1000   -       Beta-carotene ketolase  20      62.1    30.3    -1.01   2483.4  1212.93 -31.8   0.21890 0.71999
fig|83332.12.peg.1001   -       hypothetical protein    1       138.8   1606.8  3.52    277.6   3213.68 1468.1  0.33820 0.84014
fig|83332.12.peg.1002   -       Outer membrane low permeability porin, OmpATb family    17      111.7   66.3    -0.74   3799.2  2254.63 -45.4   0.24290 0.75292
fig|83332.12.peg.1003   -       hypothetical protein    2       71.2    0.0     -6.17   285.0   0.00    -71.2   0.02990 0.24902
fig|83332.12.peg.1004   -       hypothetical protein    9       59.3    187.9   1.65    1067.9  3381.71 128.5   0.11940 0.50964
fig|83332.12.peg.1005   -       Sensor-type histidine kinase prrB (EC 2.7.13.3) 14      13.1    0.3     -3.48   365.5   7.19    -12.8   0.10480 0.47769
fig|83332.12.peg.1006   -       Two-component transcriptional response regulator, OmpR family   10      8.1     0.0     -3.19   162.0   0.00    -8.1    0.48590 0.85208
fig|83332.12.peg.1007   -       Acetyl-coenzyme A carboxyl transferase alpha chain (EC 6.4.1.2) / Acetyl-coenzyme A carboxyl transferase beta chain (EC 6.4.1.2)        24      49.5    41.1    -0.26   2377.6  19
fig|83332.12.peg.1008   -       Enoyl-CoA hydratase (EC 4.2.1.17)       9       162.3   129.4   -0.32   2921.7  2329.73 -32.9   0.74140 1.00000
fig|83332.12.peg.1009   -       Outer membrane protein romA     15      141.0   74.2    -0.92   4231.4  2225.90 -66.9   0.26900 0.78895
fig|83332.12.peg.101    -       hypothetical protein    4       128.9   0.0     -7.02   1031.2  0.00    -128.9  0.07000 0.40247
fig|83332.12.peg.1010   -       hypothetical protein    7       190.7   37.9    -2.30   2669.3  530.53  -152.8  0.16410 0.60725
fig|83332.12.peg.1011   -       Beta-lactamase class C-like and penicillin binding proteins (PBPs) superfamily / DUF3471 domain 36      60.1    77.9    0.37    4326.1  5606.81 17.8    0.66590 0.97978
fig|83332.12.peg.1012   -       Cation-transporting ATPase, E1-E2 family        30      64.8    60.3    -0.10   3885.4  3615.53 -4.5    0.89200 1.00000
fig|83332.12.peg.1013   -       hypothetical protein    3       58.1    84.6    0.53    348.7   507.54  26.5    0.58550 0.92250
fig|83332.12.peg.1014   -       Uncharacterized protein MSMEG_5635      2       87.1    224.8   1.36    348.3   899.14  137.7   0.36590 0.85208
fig|83332.12.peg.1015   -       hypothetical protein    7       66.7    5.5     -3.39   934.1   76.44   -61.3   0.00070 0.02687
fig|83332.12.peg.1016   -       Putative hydroxylase    15      68.4    63.2    -0.11   2052.5  1897.32 -5.2    0.90810 1.00000
fig|83332.12.peg.1017   -       putative membrane protein       4       134.6   70.1    -0.93   1076.6  561.07  -64.4   0.41170 0.85208
fig|83332.12.peg.1018   -       hypothetical protein    5       2.8     0.3     -1.54   28.3    3.15    -2.5    0.47370 0.85208
fig|83332.12.peg.1019   -       Lignostilbene-alpha,beta-dioxygenase and related enzymes        41      28.5    71.3    1.30    2334.8  5849.99 42.9    0.20140 0.68541
fig|83332.12.peg.102    -       hypothetical protein    10      74.7    84.6    0.18    1494.4  1692.66 9.9     0.83900 1.00000
fig|83332.12.peg.1020   -       3-ketoacyl-CoA thiolase (EC 2.3.1.16)   17      104.9   134.7   0.36    3567.1  4581.36 29.8    0.48860 0.85208
fig|83332.12.peg.1021   -       PPE family protein, SVP subgroup => PPE14       19      133.0   140.5   0.08    5053.4  5338.44 7.5     0.92110 1.00000
fig|83332.12.peg.1022   -       PE family protein => PE7        3       29.1    1.2     -3.78   174.8   7.19    -27.9   0.01330 0.16217
fig|83332.12.peg.1023   -       High-affinity choline uptake protein BetT       42      257.6   172.5   -0.58   21639.5 14486.00        -85.2   0.14700 0.57335
fig|83332.12.peg.1024   -       hypothetical protein    4       16.9    126.3   2.83    135.1   1010.21 109.4   0.46820 0.85208
fig|83332.12.peg.1025   -       FIG032766: hypothetical protein 3       96.6    47.7    -1.00   579.4   285.96  -48.9   0.50210 0.85208
fig|83332.12.peg.1026   -       FIG001353: Acetyltransferase    10      185.8   219.8   0.24    3715.7  4396.43 34.0    0.74600 1.00000
fig|83332.12.peg.1027   -       hypothetical protein    4       107.8   141.0   0.38    862.4   1128.00 33.2    0.59870 0.93314
fig|83332.12.peg.1028   -       Transposase     22      82.2    60.4    -0.44   3617.0  2657.96 -21.8   0.45900 0.85208
fig|83332.12.peg.1029   -       Resolvase       7       323.7   174.3   -0.89   4531.6  2440.68 -149.3  0.20860 0.69649
fig|83332.12.peg.103    -       putative membrane protein       12      142.5   125.0   -0.19   3420.2  2999.65 -17.5   0.79760 1.00000
fig|83332.12.peg.1030   -       hypothetical protein    18      218.6   29.9    -2.83   7867.9  1077.26 -188.6  0.00160 0.04414
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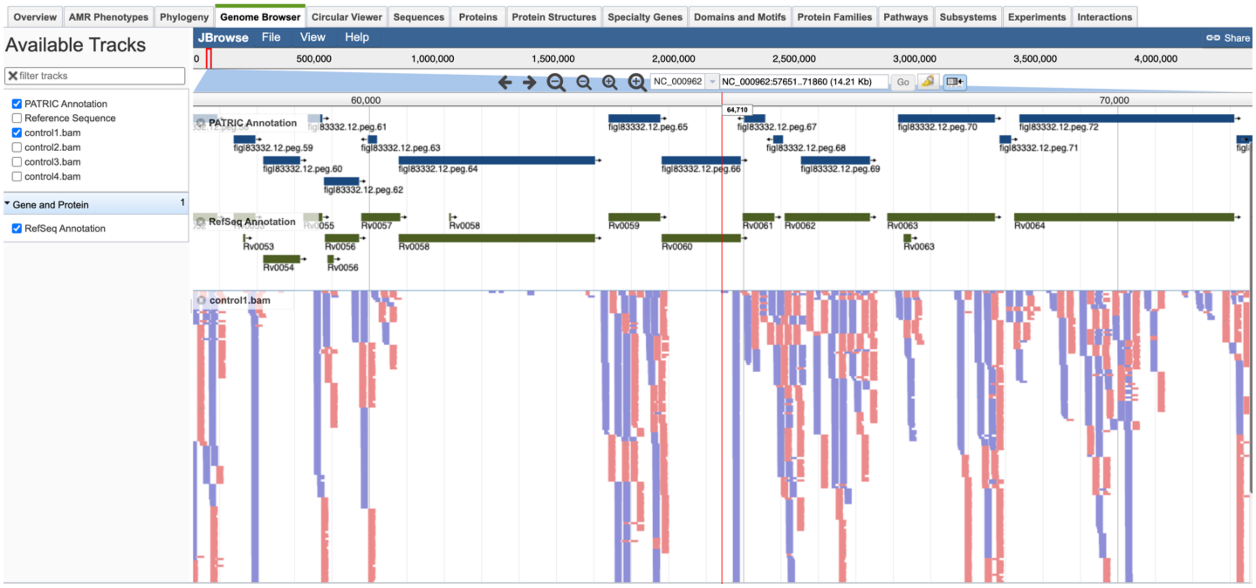

References

- [Tn-seq Analysis Service Quick Reference Guide]
- [Tn-seq Analysis Service Tutorial]