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A sequence comparison and gene expression data integration add-on for the Pathway Tools software

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Toolbox Features

- Multiple sequence alignment of orthologs
- Mapping of gene expression data
- Annotation and cross-species comparison of IS Elements
- Plug-In API

- Seamless integration into Pathway Tools user interface
- Easy to install: Automated installer

Multiple Sequence Alignment

Feature Description

- Expanded CLUSTAL W alignment of orthologous genes
  - Complements Pathway Tools’ sequence viewers and Multiple Genome Browser
- User defined additional 5’ region to compare
  - Promoters
  - Regulator binding sites
  - …
- Highlighting of
  - 5’ region – lower case letters
  - Coding region – upper case letters
- Additional legend and position information

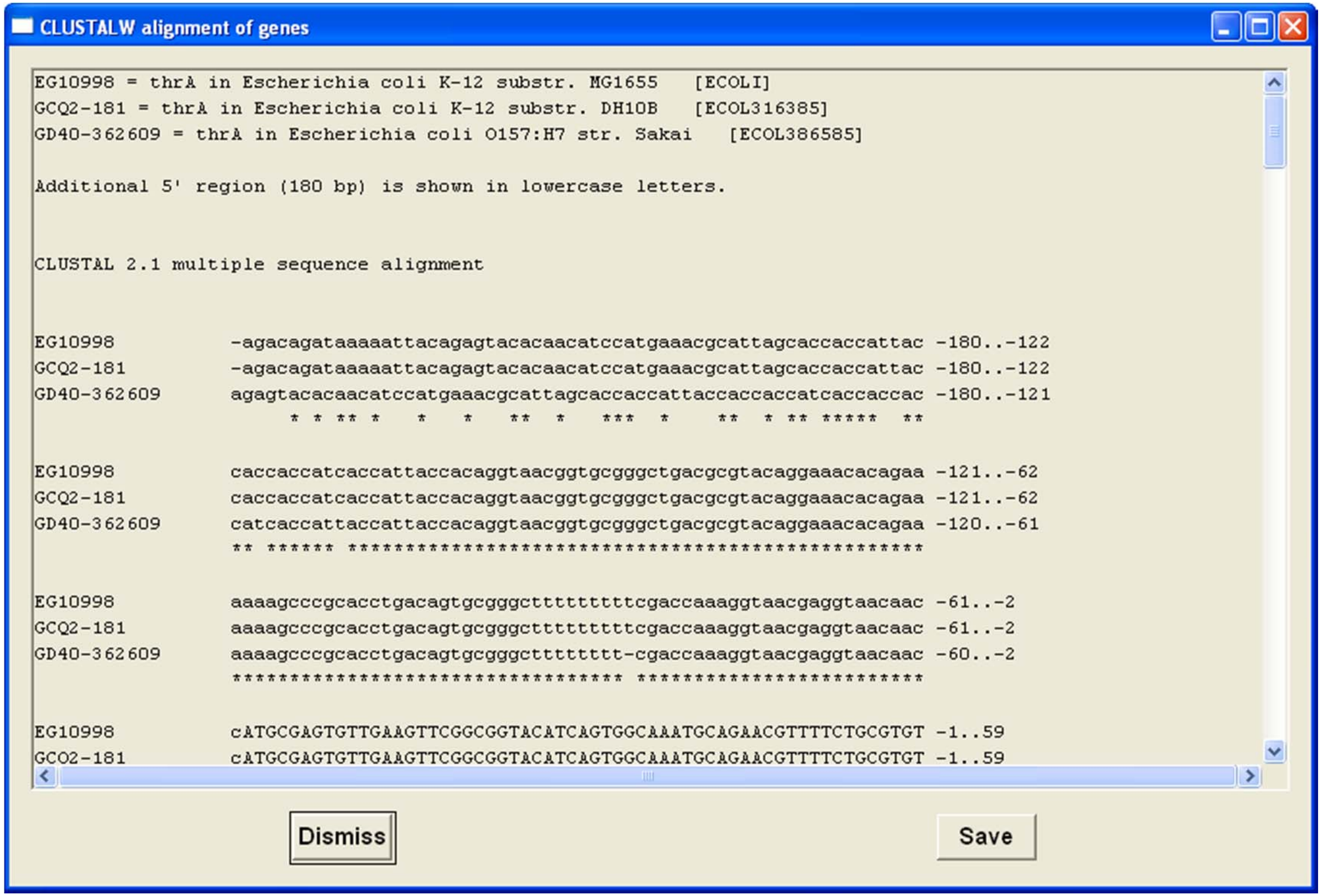
Multiple Sequence Alignment

HowTo

- Select gene
  - Gene or protein page
  - Ortholog links must be present
- Select organisms to compare
  - Like for Pathway Tools Multiple Genome Browser
  - …or using organism selection dialog of the toolbox
- Select from menu:
  - ➢ `ACIB-Toolbox`
    - ➢ `Species Comparison`
      - ➢ `Sequence alignment of orthologous genes`
- Enter length of 5’ region

Multiple Sequence Alignment

Example Results



```
EG10998 = thrA in Escherichia coli K-12 substr. MG1655   [ECOLI]
GCQ2-181 = thrA in Escherichia coli K-12 substr. DH10B   [ECOL316385]
GD40-362609 = thrA in Escherichia coli O157:H7 str. Sakai   [ECOL386585]

Additional 5' region (180 bp) is shown in lowercase letters.


CLUSTAL 2.1 multiple sequence alignment


EG10998          -agacagataaaaattacagagtacacaacatccatgaaacgcattagcaccaccattac -180..-122
GCQ2-181         -agacagataaaaattacagagtacacaacatccatgaaacgcattagcaccaccattac -180..-122
GD40-362609      agagtacacaacatccatgaaacgcattagcaccaccattaccaccaccatcaccaccac -180..-121
                      * * ** *   *   *   **  *   ***  *    **  * ** *****  **

EG10998          caccaccatcaccattaccacaggtaacggtgcgggctgacgcgtacaggaaacacagaa -121..-62
GCQ2-181         caccaccatcaccattaccacaggtaacggtgcgggctgacgcgtacaggaaacacagaa -121..-62
GD40-362609      catcaccattaccattaccacaggtaacggtgcgggctgacgcgtacaggaaacacagaa -120..-61
                 ** ****** **************************************************

EG10998          aaaagcccgcacctgacagtgcgggcttttttttttcgaccaaaggtaacgaggtaacaac -61..-2
GCQ2-181         aaaagcccgcacctgacagtgcgggcttttttttttcgaccaaaggtaacgaggtaacaac -61..-2
GD40-362609      aaaagcccgcacctgacagtgcgggcttttttttt-cgaccaaaggtaacgaggtaacaac -60..-2
                 ********************************** *************************

EG10998          cATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGT -1..59
GCO2-181         cATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGT -1..59
```

Gene Expression Data Integration

Feature Description

- Import of probe libraries or array designs from different file formats:
  - Tab-delimited text (with header row)
  - MAGE-TAB Array Design Format (ADF)
  - GenePix Array List (GAL)
- Sequence-based mapping of microarray probes to target PGDB genomes
- Conversion of expression data into input for
  - GFF tracks for genome browser
  - Overlay of gene expression data in
    - Cellular overview
    - Genome overview
    - Regulatory overview

Gene Expression Data Integration

Why Sequence-Based Probe Mapping?

- Name matching depends on:
  - Complete and accurate target gene annotation of probe library / array design
  - Extensive annotation of gene names, synonyms and accessions in target PGDBs
- Common errors using name matching:
  - Incomplete annotation
    ➔ low mapping efficiency
  - Annotation of probes and PGDB not compatible
    ➔ low mapping efficiency
  - Ambiguous or misleading synonyms in either annotation
    ➔ false positive assignments

Gene Expression Data Integration

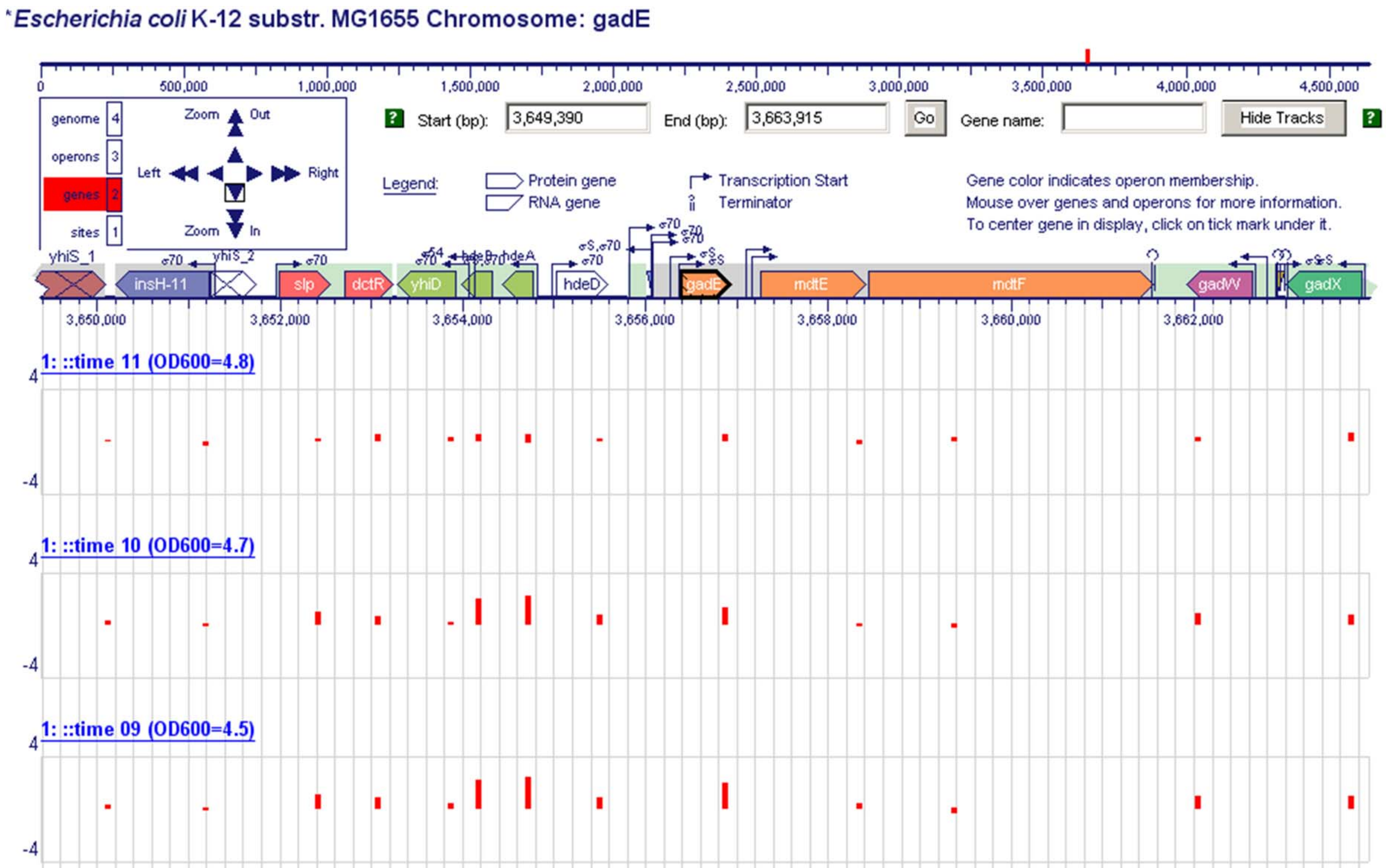
Why Sequence-Based Probe Mapping?

Sequence based probe mapping

- Circumvents drawbacks of name matching
- Provides unambiguous matching of probes to their exact target genes
  - No false-positive matches due to ambiguous gene synonyms
- Allows detection of cross-talking probe matches
- Provides stable mapping efficiency regardless of annotation of probes and target PGDBs

Gene Expression Data Integration

Example: GFF Tracks in Genome Browser



Escherichia coli K-12 substr. MG1655 Chromosome: gadE

Start (bp): 3,649,390

End (bp): 3,663,915

Gene name:

Gene color indicates operon membership.
Mouse over genes and operons for more information.
To center gene in display, click on tick mark under it.

1: ::time 11 (OD600=4.8)

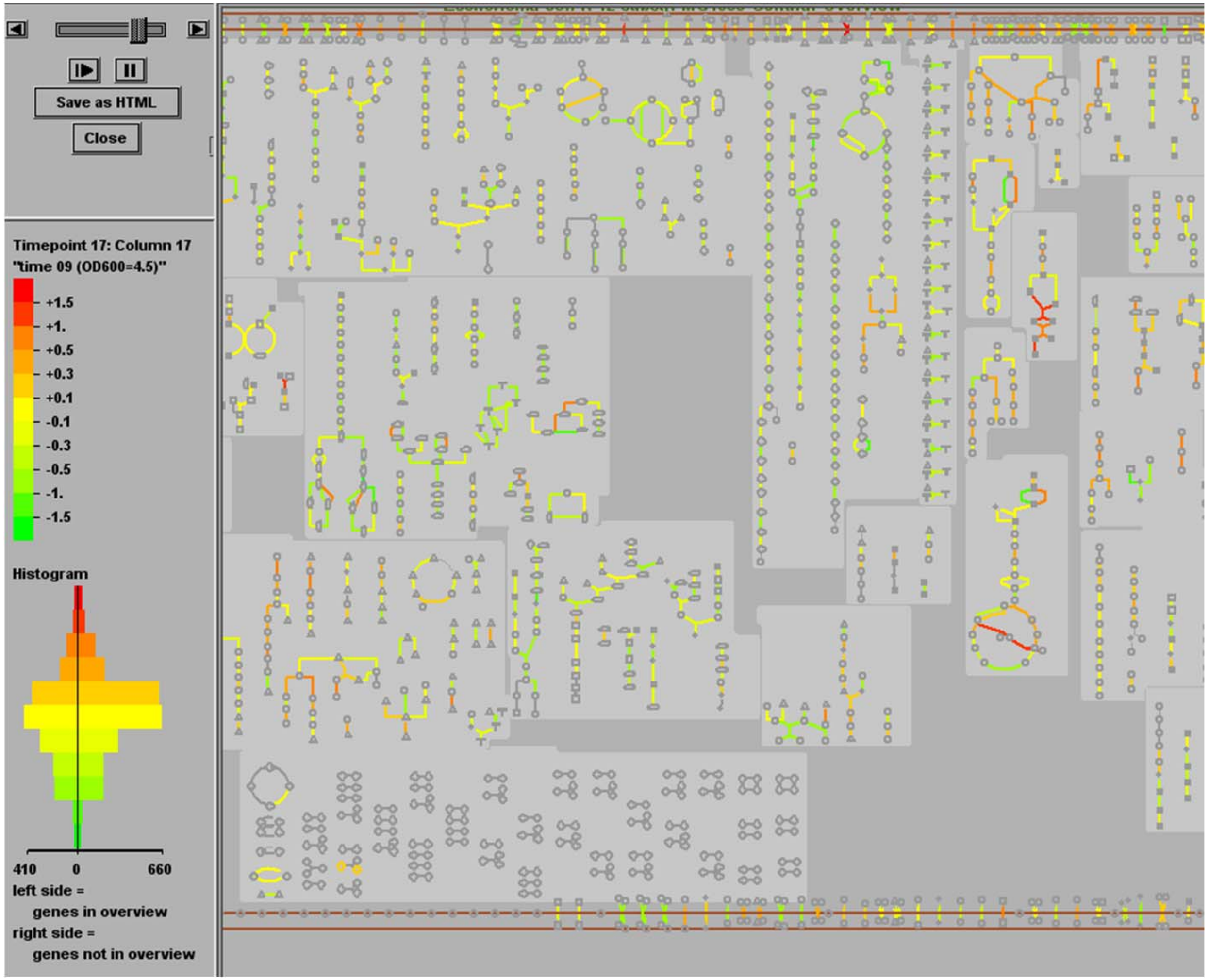
1: ::time 10 (OD600=4.7)

1: ::time 09 (OD600=4.5)

Experimental data: Reference Design time-course, *E.coli* (Takahashi et al., 2011); ArrayExpress Accession E-GEOD-6033

Gene Expression Data Integration

Example: Visualization in Cellular Overview



Timepoint 17: Column 17
"time 09 (OD600=4.5)"

left side =
genes in overview
right side =
genes not in overview

Experimental data: Reference Design time-course, *E.coli* (Takahashi et al., 2011); ArrayExpress Accession E-GEOD-6033

Annotation of IS Elements

Feature Description

Insertion Sequence (IS) Elements are:

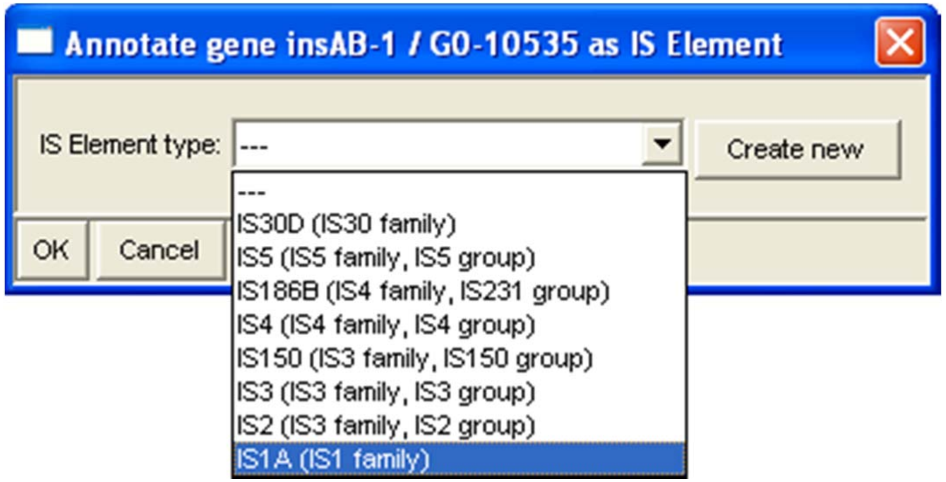
- short mobile genetic elements
- a major cause of genomic modifications

ACIB PGDB Toolbox provides:

- Systematic annotation and browsing of the IS Element taxonomy
  - IS Element types as Paralogous Gene Groups
- Species comparison features
  - Check for correct ortholog links between strains
  - Detection of shared and singleton IS Element loci

Annotation of IS Elements

Annotation



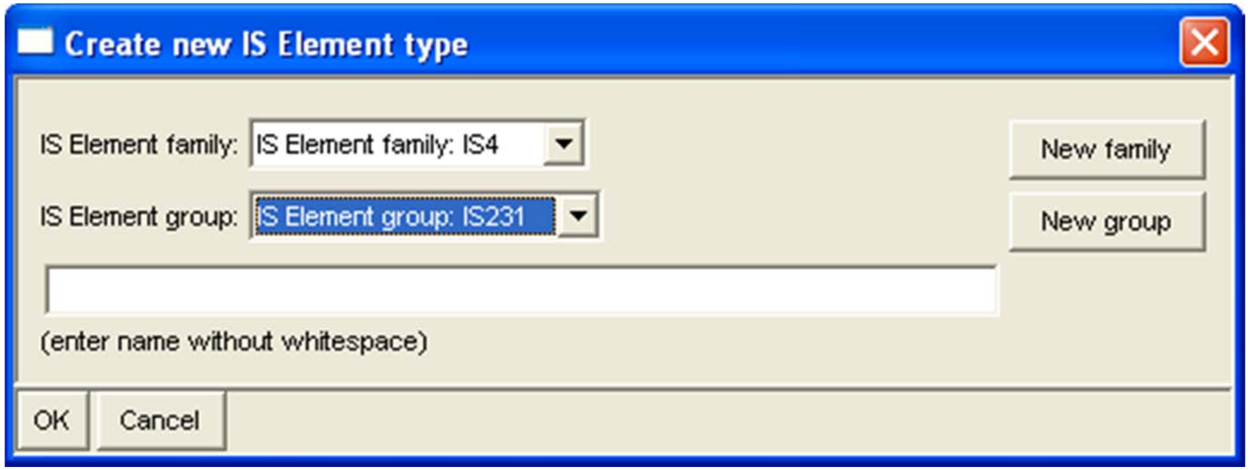
Annotate gene insAB-1 / GO-10535 as IS Element

IS Element type: ---

- ---
- IS30D (IS30 family)
- IS5 (IS5 family, IS5 group)
- IS186B (IS4 family, IS231 group)
- IS4 (IS4 family, IS4 group)
- IS150 (IS3 family, IS150 group)
- IS3 (IS3 family, IS3 group)
- IS2 (IS3 family, IS2 group)
- IS1A (IS1 family)

...annotate genes

create new IS Element families, groups and types...



Create new IS Element type

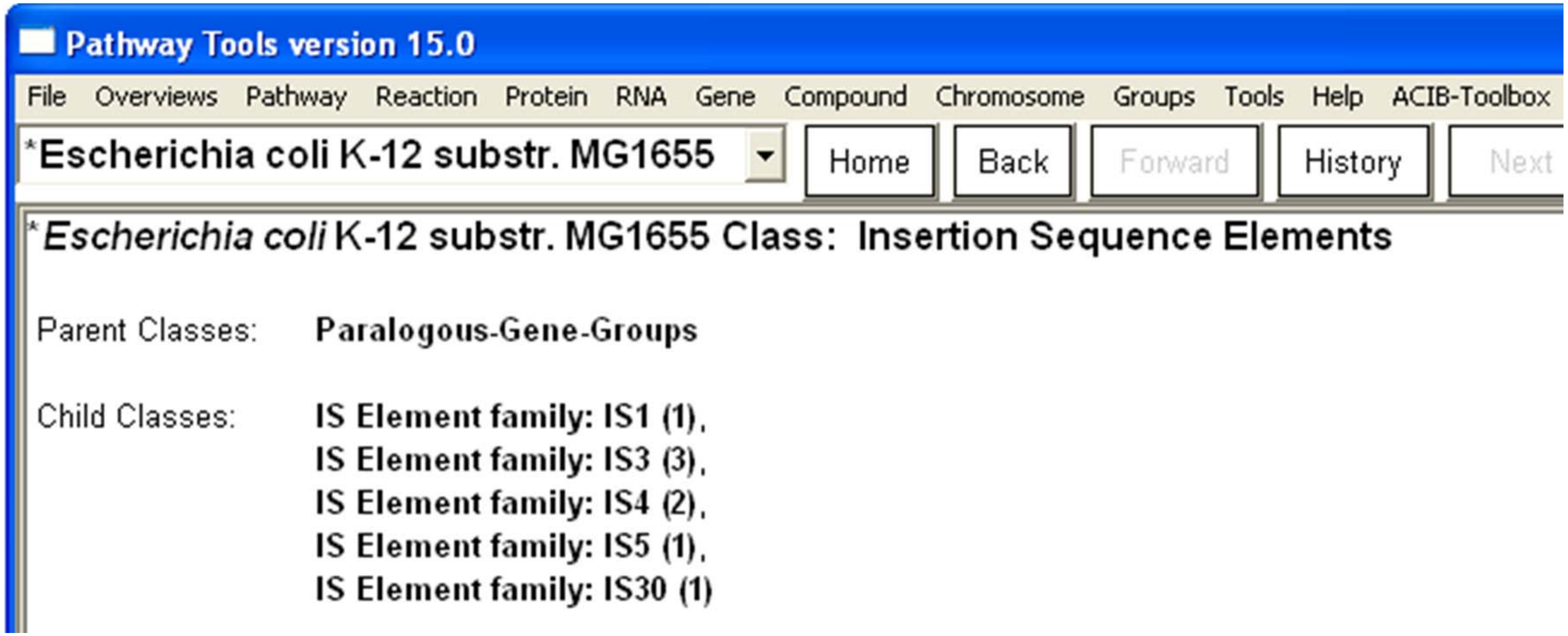
IS Element family: IS Element family: IS4

IS Element group: IS Element group: IS231

(enter name without whitespace)

Annotation of IS Elements

Browsing: Root of Taxonomy Tree



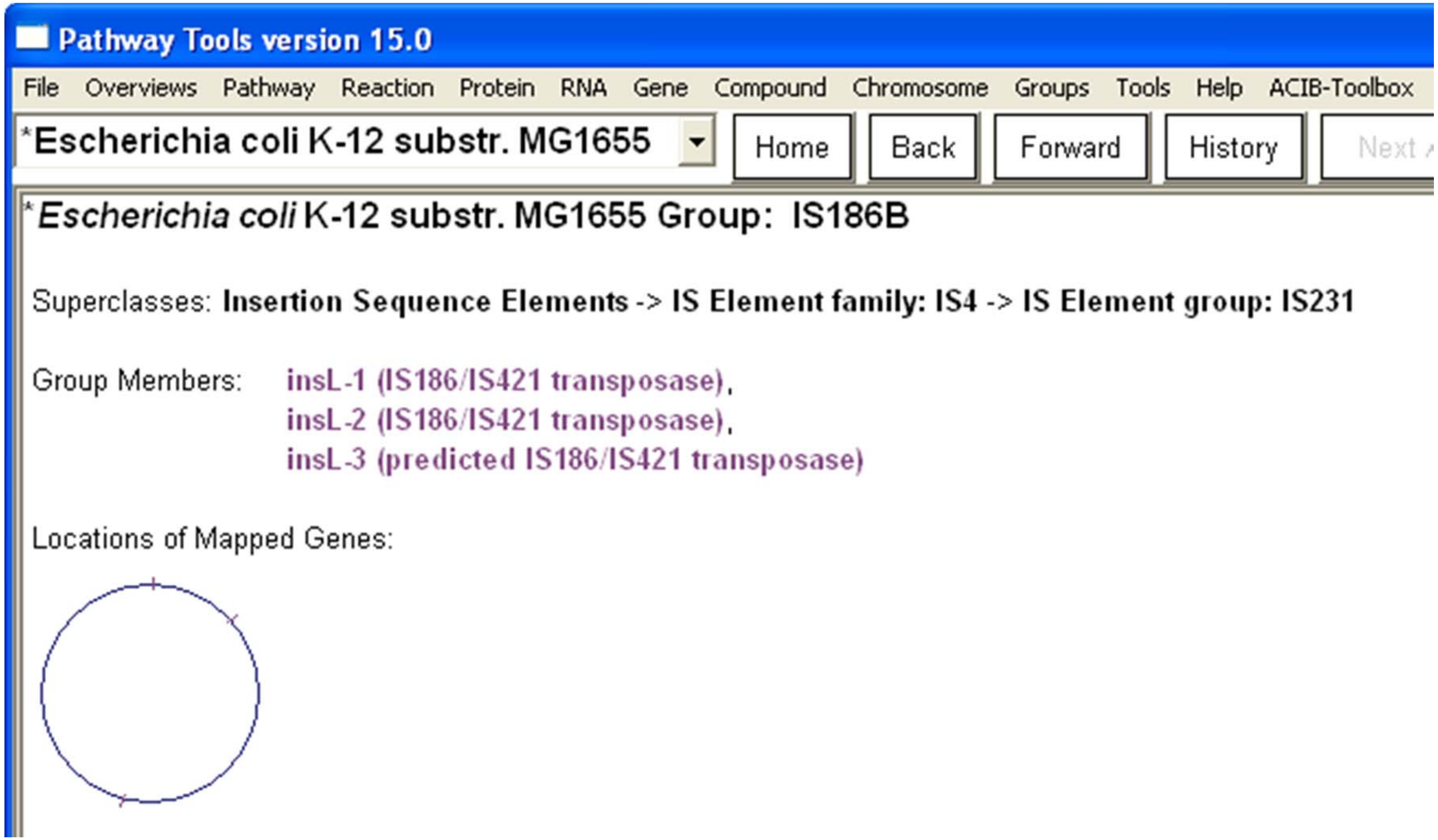
Escherichia coli K-12 substr. MG1655 Class: Insertion Sequence Elements

Parent Classes: **Paralogous-Gene-Groups**

Child Classes:
IS Element family: IS1 (1),
IS Element family: IS3 (3),
IS Element family: IS4 (2),
IS Element family: IS5 (1),
IS Element family: IS30 (1)

Annotation of IS Elements

Browsing: IS Element Type Overview Page



Escherichia coli K-12 substr. MG1655 Group: IS186B

Superclasses: **Insertion Sequence Elements -> IS Element family: IS4 -> IS Element group: IS231**

Group Members: insL-1 (IS186/IS421 transposase),
insL-2 (IS186/IS421 transposase),
insL-3 (predicted IS186/IS421 transposase)

Locations of Mapped Genes:

Annotation of IS Elements

Export and Analysis Features

- Export of IS Element annotation in GFF v2 format
- Cross-species comparison
  - Selection of organisms: like for multiple sequence alignment feature
  - Tabular output of orthologous and singleton IS Element loci
  - For each strain: Tabular output of
    - IS Element genes
    - Adjacent genes
    - % matching identity of +/- 5000 bp region in other strains
      - Detection of missing ortholog links
    - Orthologous IS Element genes in other strains

Plug-In API

Feature Description

- Easy-to-use API to integrate user-defined tools into the Pathway Tools graphical user interface
- Direct integration of own commands into the application’s menu
- Support for creation of GUI dialog windows
- Useful for
  - Pathway Tools users - to integrate their own queries and tools
  - Third-party developers - to provide user-friendly access to their Pathway Tools enhancements
- Detailed documentation in toolbox user guide

Plug-In API

HowTo: Menu integration

- Write your analysis function:

```
(defun my-function … )
```

- Wrap your function in a CLIM command:

```
(clim:define-command com-my-command-name () (my-function))
```

- Use the plug-in API to place it in the menu:

```
(acib::register-plugin-menu-command
  “Menu Command Name” ‘com-my-command-name
  :submenu ‘(“First Level Submenu” “Second Level Submenu”))
```

- That’s all!

Plug-In API

Example: Menu integration

Toolbox Availability

- http://genome.tugraz.at/PGDBToolbox
- Contact: ptools@acib.at
- Free of charge for academic/non-commercial use
  - Registration required
- User guide (freely available):
  http://genome.tugraz.at/PGDBToolbox/documentation.shtml
- Reference – please cite:
  Krempl PM, Mairhofer J, Striedner G, Thallinger GG.
  A Sequence Comparison and Gene Expression Data Integration Add-on for the Pathway Tools Software.
  Bioinformatics. 2012 Sep 1;28(17):2283-4.
  doi: 10.1093/bioinformatics/bts431
  PMID: 22772948

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