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Computational annotation of UTR cis-regulatory modules using the EGEE (gLite) infrastructure

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This work is carried out within the LIBI project exploiting the EGEE production infrastructure

From gene to protein

Gene

yes/not
..at what extend?

DNA

TRANSCRIPTION

mRNA

when/where
..at what extend?

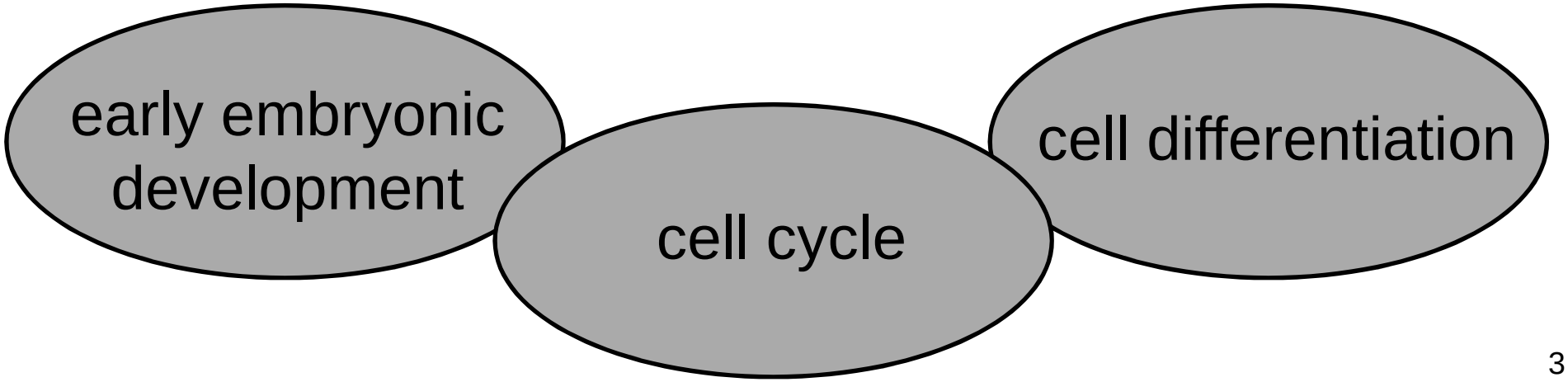
TRANSLATION

PROTEIN

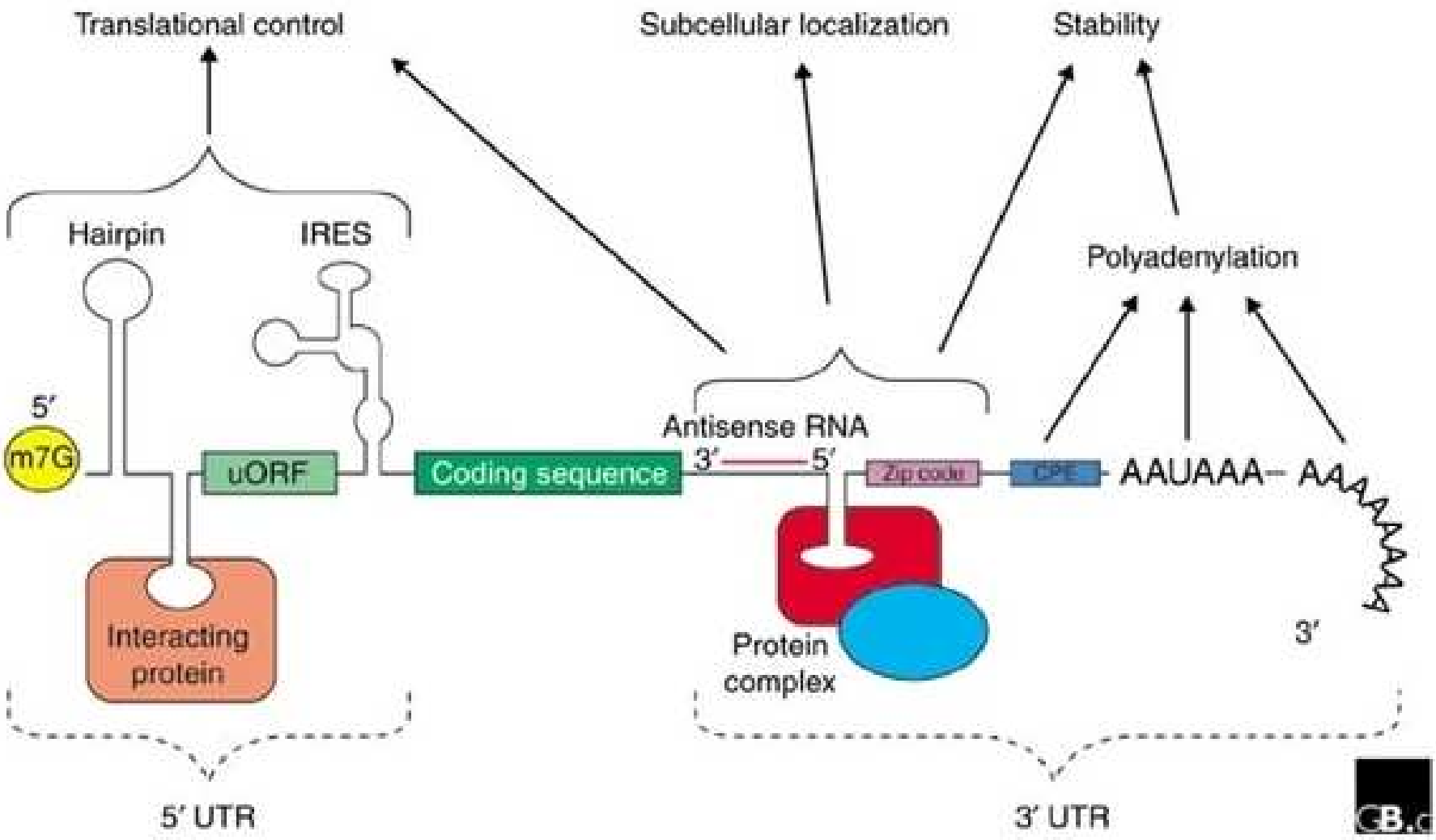
Translational control fine-tunes protein levels in time and space

Translation control modes:

- Global control
  - mainly occurs by the modification of translation-initiation factors
- mRNA specific-control
  - is driven by regulatory proteins that recognize functional motifs in the 5' and 3' UTRs



Different types of regulatory motifs involved in translational control (mRNA -> Protein)



Genome Biol. 2002; 3(3): reviews0004.1–reviews0004.10.
Published online 2002 February 28.

Research Goal

Annotation of UTRs cis-regulatory modules

The computational approach aim:

- extraction of implicit knowledge (relations) not explicitly annotated in current biological databases in order to discover **Frequent Sequential Patterns (FSP)** of Translation Regulatory Motifs **by using data mining techniques**

Biological Issue

The underlying biological assumption is that:

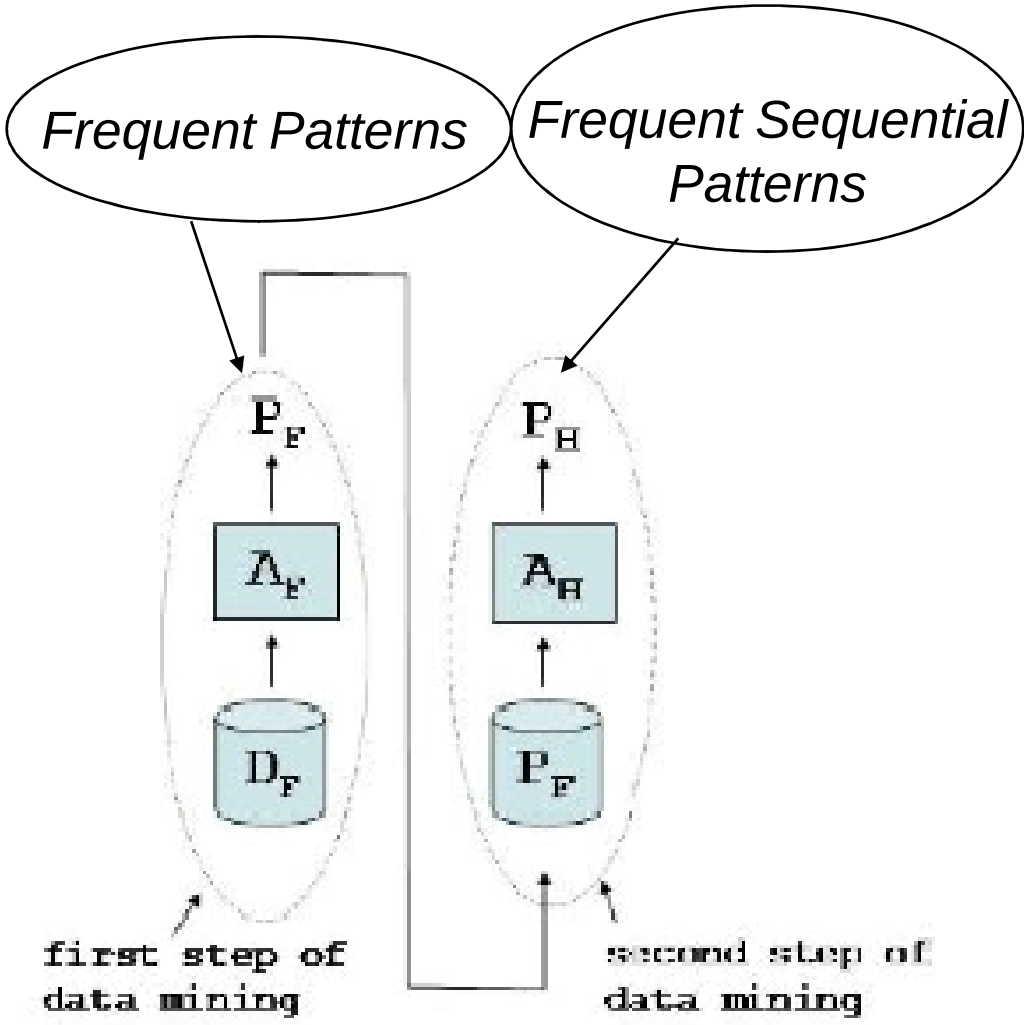
- a **pattern of regulatory motifs** which **appears with high frequency** in a set of biological sequences has been preserved for longer time during evolution and thus it **is expected to be biologically important** from a functional point of view

- **mutual distance** among motifs frequently co-occurring along a UTR sequence **may play a key role** for their functional interaction

Data Mining Approach

The approach is based on a two-stepped procedure using a **sequential pattern mining algorithm**

- The first step (A_F) generates frequent patterns of motifs (P_F) from a specific set of motifs previously mapped, without taking into account their spatial displacement
- The second step exploits a technique of sequence mining (A_H) on annotated motifs (P_F) to generate FSP of motifs by taking into account their spatial displacement and conservation of spacers



First mining step

Generation of Frequent Patterns (FPs):

- the search is based on the levelwise method by Mannila and Toivonen, i.e. a breadth-first search is performed in the lattice of sets of motifs
- starts from the smallest element (sets with a single motif) and proceeds from smaller to larger sets
- the set of motifs which are frequent at the i-th level are considered to generate candidate sets of motifs at the (i+1)-th level. Candidates are then evaluated against the input data in order to prune those that are infrequent

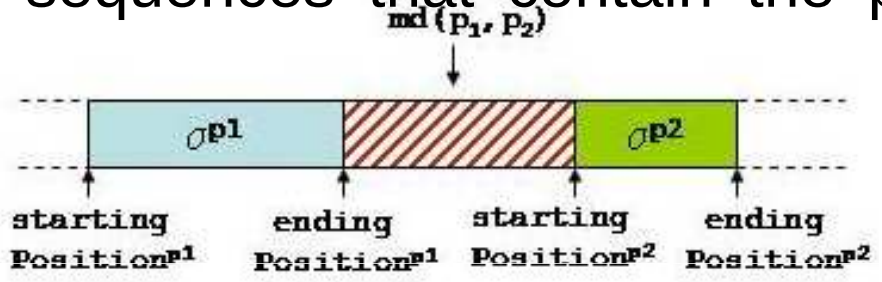
Advantage:

- timing the high computational complexity of the FSP generation process

Second mining step

Generation of Frequent Sequential Patterns (FSPs) by using GSP*:

- We are interested in **sequential patterns** which begin and end with a motif and have a greater support than an input threshold (*minsup*). The support of a sequential pattern is computed as the percentage of sequences that contain the pattern in the same order.



- Given 2 annotated motifs p_1 and p_2, they can be projected into a sequence of motifs ordered according to the values of the spacers between them $md(p_i, p_{i+1})$

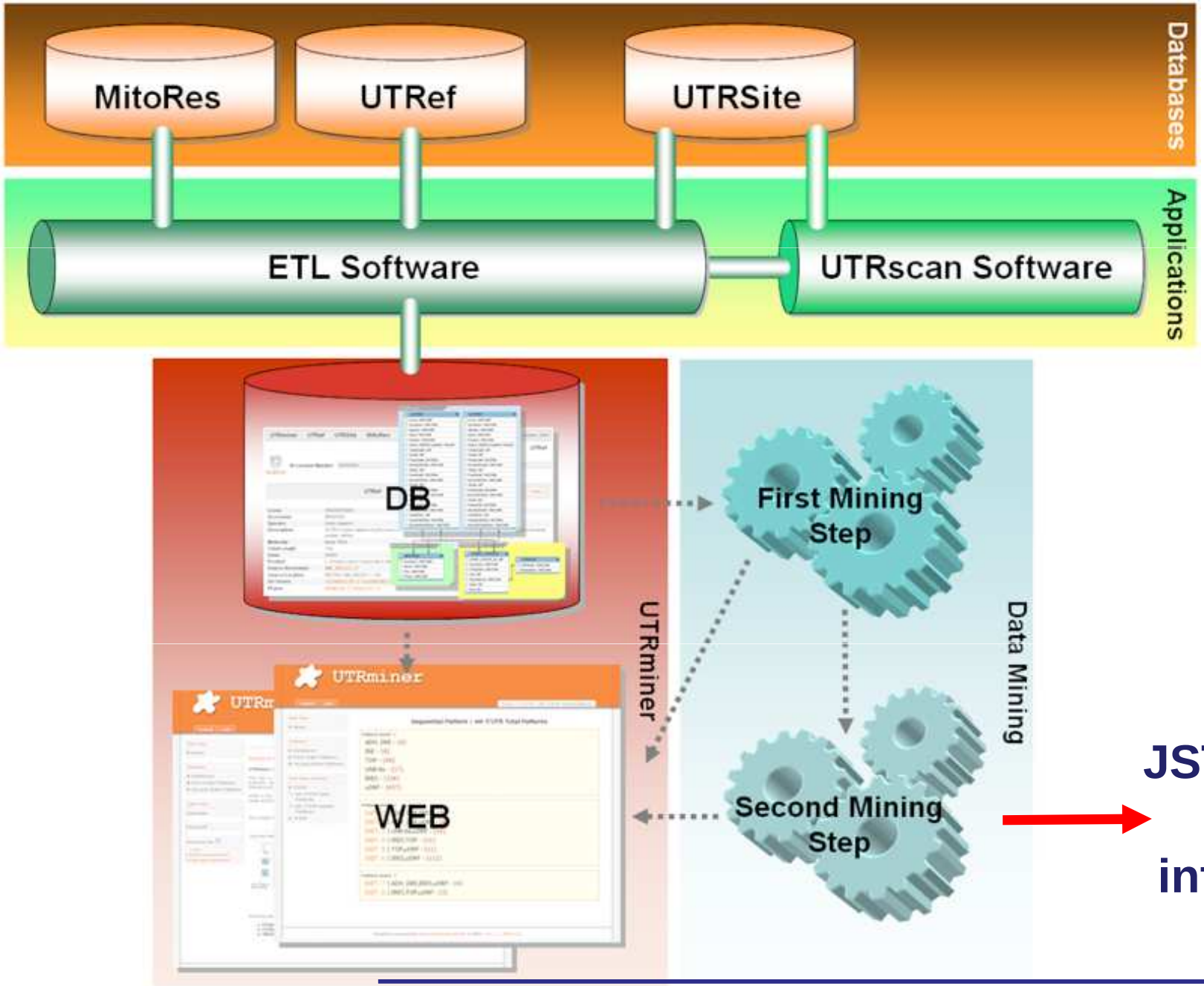
* *Generalized Sequential Pattern miner (Weka data mining tool)*

Second mining step

- the original numerical spacers (*Z) are mapped* into a finite set of bins/intervals (*Ψ = 6, 9, 12 …bins)* using an unsupervised equal frequency discretization method
- in this way each bin has a different width from each other and the initial values of each bin are distributed approximately in a uniform way
- at the end of the discretization each spacer is mapped into an interval and **GSP** can mine data and extract FSPs.

- The **number of FSPs** that can be detected **depends on:**
  - ✓input threshold set for minimum pattern support
  - ✓granularity of spacer length discretization
- **By varying** these **two parameters** it is possible to **flexible customize** the tool in order improve the quality of the computational analysis

The UTRminer resource



JST application LIBI GRID infrastructure

First mining step: experimentation and results

1. M-dataset (1944 5’UTR and 1952 3’UTR sequences from mitochondrial targeted mRNA of metazoan org.)

   FPs found:

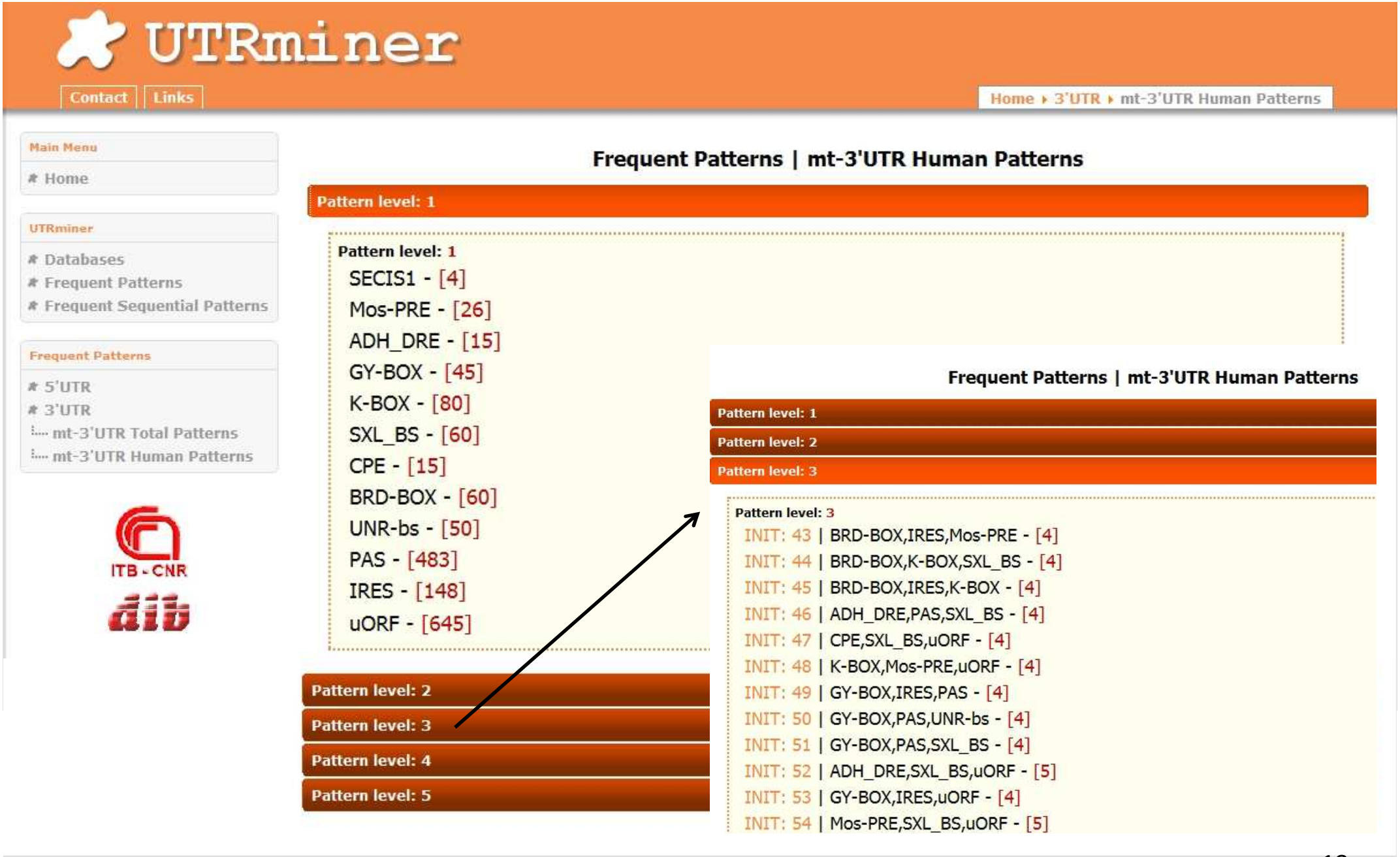
   - 8 up to three motifs in 5’UTR
   - 211 up to five motifs in 3’UTR

2. H-dataset (728 5’UTR and 728 3’UTR sequences from the human subset)

   FPs found:

   - 5 up to three motifs in 5’UTR
   - 135 up to five motifs in 3’UTR

First mining step results



First mining step results

UTRminer

Contact | Links

Home ▸ 3'UTR ▸ mt-3'UTR Human Patterns

Main Menu
- Home

UTRminer
- Databases
- Frequent Patterns
- Frequent Sequential Patterns

Frequent Patterns
- 5'UTR
- 3'UTR
  - mt-3'UTR Total Patterns
  - mt-3'UTR Human Patterns

Frequent Pattern | mt-3'UTR Human Patterns | INIT: 88

Pattern level: 3

INIT: 88 | IRES,PAS,uORF - Support items: 111

Chi-square: 6.622 | Significant (alpha=0.05)

COMPACT VIEW | NORMAL VIEW | FULL VIEW

POSITIONAL PATTERNS: COMPACT VIEW

- IRES uORF PAS (1)
- uORF IRES PAS (107)
- uORF IRES uORF PAS (3)

Pattern level: 3

INIT: 88 | IRES,PAS,uORF - Support items: 111

Chi-square: 6.622 | Significant (alpha=0.05)

COMPACT VIEW | NORMAL VIEW | FULL VIEW

POSITIONAL PATTERNS: NORMAL VIEW

- IRES uORF PAS (1)
- uORF IRES PAS (24)
- uORF IRES uORF PAS (1)
- uORF[2] IRES PAS (12)
- uORF[3] IRES PAS (10)
- uORF[4] IRES PAS (8)
- uORF[5] IRES PAS (5)

Pattern level: 3

INIT: 88 | IRES,PAS,uORF - Support items: 111

Chi-square: 6.622 | Significant (alpha=0.05)

COMPACT VIEW | NORMAL VIEW | FULL VIEW

POSITIONAL PATTERNS: FULL VIEW

- uORF (81:-52..28) 30 | uORF (111:57..167) 19 | uORF (216:185..400) 88 | uORF (135:487..621) 88 | uORF (351:708..1058) 142 | uORF (129:1199..1327) 12 | uORF (120:1338..1457) 2 | uORF (93:1458..1550) 166 | uORF (225:1715..1939) 24 | uORF (87:1962..2048) 33 | uORF (117:2080..2196) 66 | uORF (69:2261..2329) 18 | uORF (72:2346..2417) 6 | uORF (129:2422..2550) 9 | uORF (78:2558..2635) 30 | uORF (108:2664..2771) 171 | uORF (108:2941..3048) 38 | uORF (165:3085..3249) 46 | uORF (114:3294..3407) -5 | IRES (95:3401..3495) -35 | PAS (37:3459..3495) [CR144407]
- uORF (207:33..239) 11 | IRES (96:249..344) -20 | PAS (22:323..344) [CR655030]
- uORF (123:4..126) -99 | IRES (103:26..128) -22 | PAS (24:105..128) [CR036012]

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Second mining step: experimentation

- 346 **FPs** analised
- varying **number of bins (intervals)** for spacer length discretization (6, 9 and 12 bins)
- varying **the threshold** (minsup=0.2 and 0.3) for **FSP** support
- **6 runs for each set of sequences containing a FP**

Second mining step: computational issue

- the second step of mining is quite **CPU intensive**
- it is not possible to solve the problem using typical department computational resources
- in order to solve this problem **we have used the EGEE Grid infrastructure**
- since the application was written in Java, **the Java Virtual Machine was required** on each WN
- the **running time** of a single job is **quite long** (it could be also > 60 hours)

Job Submission Tool

- In order to simplify the jobs submission we have used a tool developed by the LIBI project called Job Submission Tool (JST).
- JST allows to:
  - ✓ track the status of a large amount of jobs
  - ✓ automatic resubmit the failing jobs
  - ✓ collect the output files produced by all the jobs

*Poster: “A web interface to Job Submission Tool (JST)”

(Poster Board: 30)

Second mining step: issues

Using Java Virtual Machine:

- **many failures** due to missing or wrong Java configuration

  - we decided to **install** our Java Virtual Machine **on the fly** just before running each job

    ✓ this increased the job success rate by a factor 2 (more than 90%)

- **large memory required** by the application
- **many sites do not support long jobs**
- only a **small set of resources** were **usable**

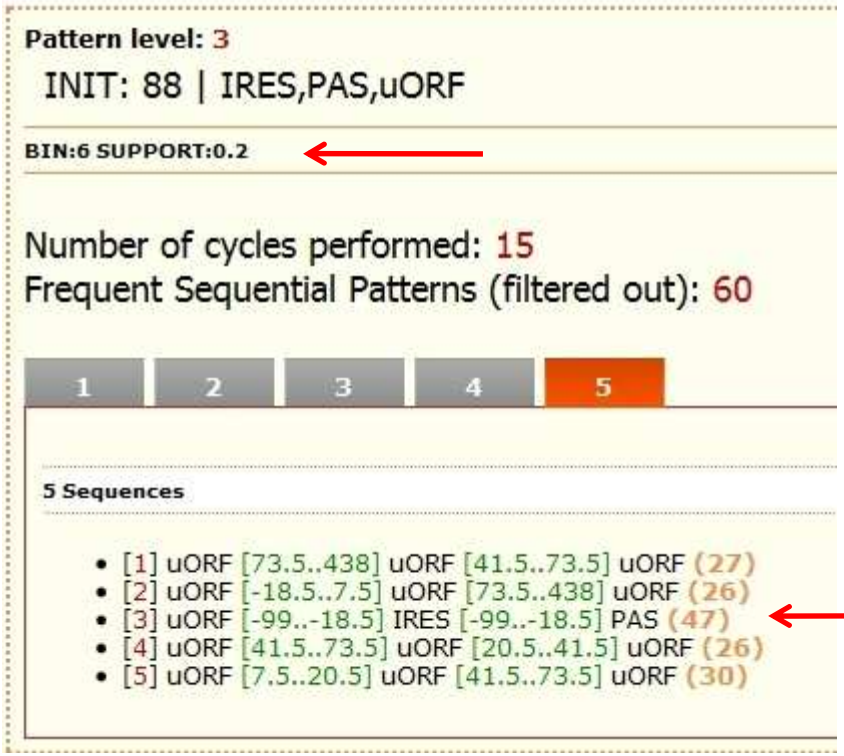
Automatic resubmission in case of failure was of great help!

Second mining step: results

- **Results** from the second mining step on FPs may **refine the prediction** by more than **two folds** compared to the first step of the mining process, where no evaluation about structural features of spacers is done.

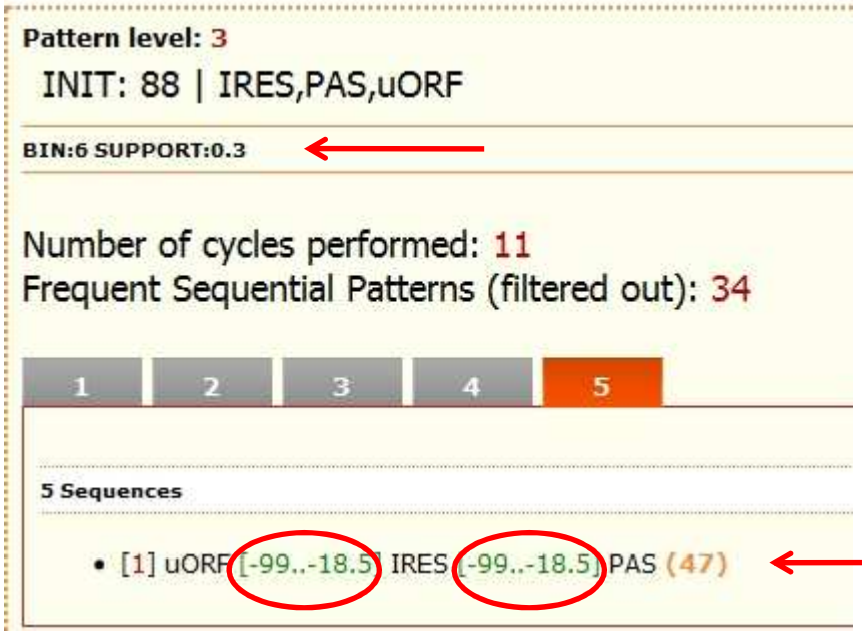
- **Grid execution numbers**:
  - ✓ jobs: 2076
  - ✓ time required on a single CPU: ~ 7 years
  - ✓ run time: about 10 days (EGEE Grid infrastructure)
  - ✓ speedup factor: 255

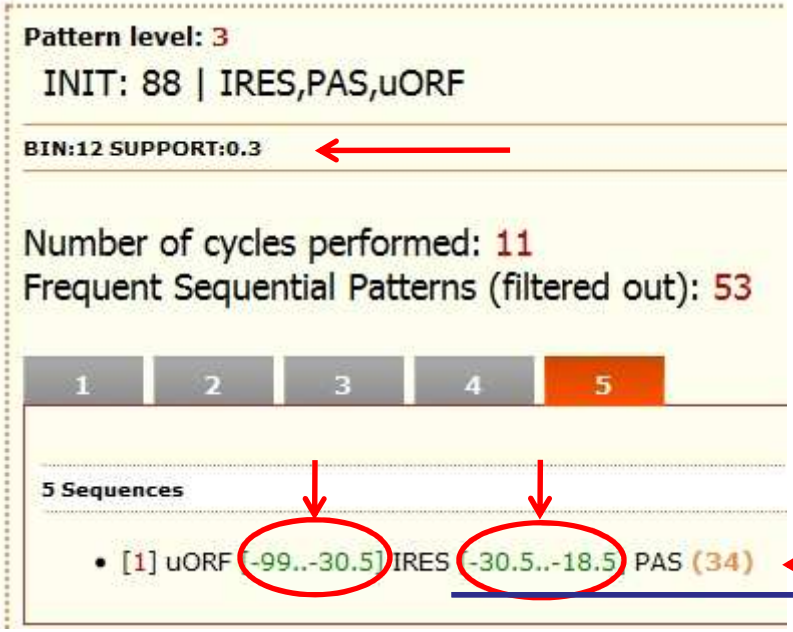
Second mining step: results on INIT 88



FP support = 111

bin 6, minsup 0.2 -> FSP support **= 47**
bin 6, minsup 0.3 -> FSP support **= 47**





bin 12, minsup 0.3 -> FSP support at **= 34**

Conclusions

- **Sequential pattern miners** allow to overcome some limits of other competitive pattern finder algorithms, i.e.:
  - need of a priori knowledge
  - pattern size
  - fixed width of spacers

  ..but are CPU expensive

- The **gLite infrastructure was a key element** for this preliminary study

- The **main problems** we faced were:
  - few resources for the execution of long job
  - non uniform Java environment
  - memory reservation on WNs

- By a **biological** point of view **preliminary results are promising** since they support experimental evidences reported in literature

- **Future developments** will include the improvement of the FSP mining process and the enlargement of analysis to whole transcript collections of different organisms for comparative analysis

Reference and links

UTRminer: http://utrminer.ba.itb.cnr.it/

Job Submission Tool: http://webcms.ba.infn.it/cms.software/index.html/index.php/Main/JobSubmissionTool

JST Web Page: http://webcms.ba.infn.it/~pierro/JST/

Acknowledgements

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