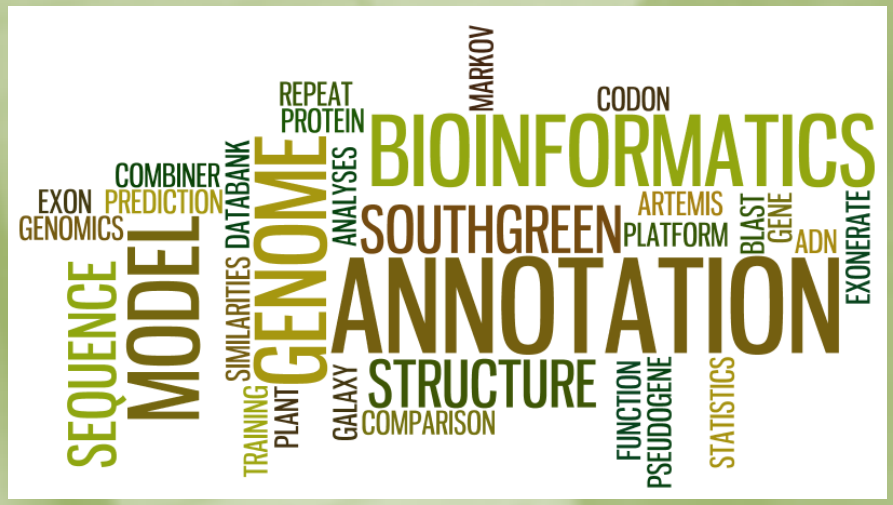
# GENOME ANNOTATION

## INTRODUCTION TO CONCEPTS AND METHODS

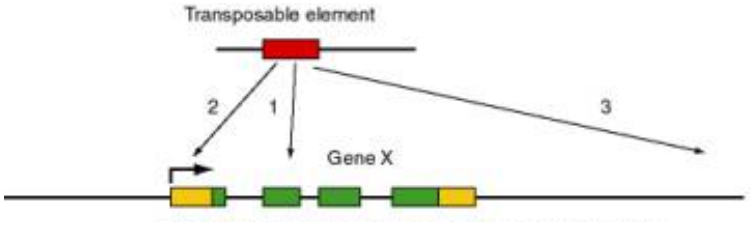


# Introduction

two main concepts:

- Identify the different elements of the genome, (location and stucture) :

structural annotation

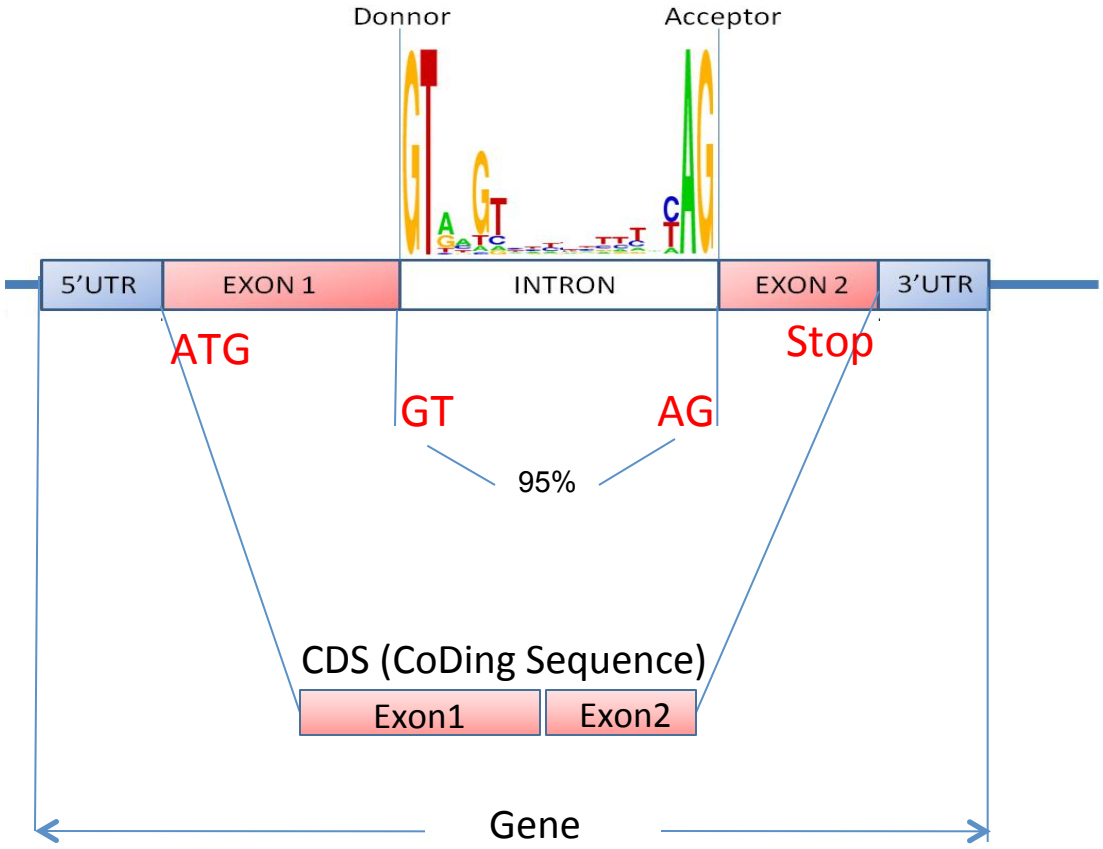



- Attribute a biological information to these elements : functional annotation

- Amino acid biosynthesis
- Biosynthesis of cofactors, prosthetic groups, and carriers
- Cell envelope
- Hypothetical proteins
- Hypothetical proteins (conserved)
- Mobile and extrachromosomal element functions
- Transcription
- Unknown function
- Cellular processes
- Central intermediary metabolism
- Disrupted reading frame
- Pathogen responses
- Protein fate
- Protein synthesis
- Transport and binding proteins
- Viral functions
- DNA metabolism
- Energy metabolism
- Fatty acid and phospholipid metabolism
- Purines, pyrimidines, nucleosides, and nucleotides
- Regulatory functions
- Signal transduction
- Unclassified

# Gene structure

- Where are the genes on the sequence ?
- Genes predictions (gene-finder softwares) are based on the structure (intron, exon, splice site, UTR).




**Predicted functional gene**
→ Structure is complete

Starts with M (ATG)
Stop codon TAA, TAG or TGA
GT (GC) / AG splicing site
No stop in exon frame

**Pseudogene (not functional)**
→ Structure is not complete

missing_acceptor
missing_donor
missing_start_codon
missing_stop_codon
multiple_stop_in_frame

# Automatic annotation

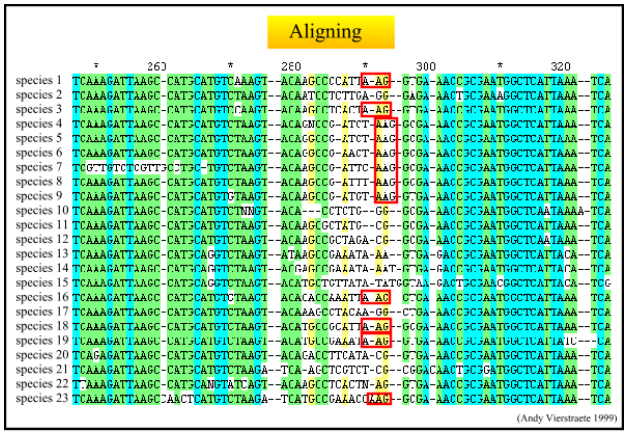
Two methods for automatic predictions

- Intrinsec methods (ab-initio)

*statistics*

- Extrinsec methods

*Sequence comparisons*



# Intrinsic methods

(ab-initio)

- Only based on computational analysis using statistical models.
- Probabilistic models like *Hidden Markov models* (HMM) for discriminating coding and non-coding region of the genome.

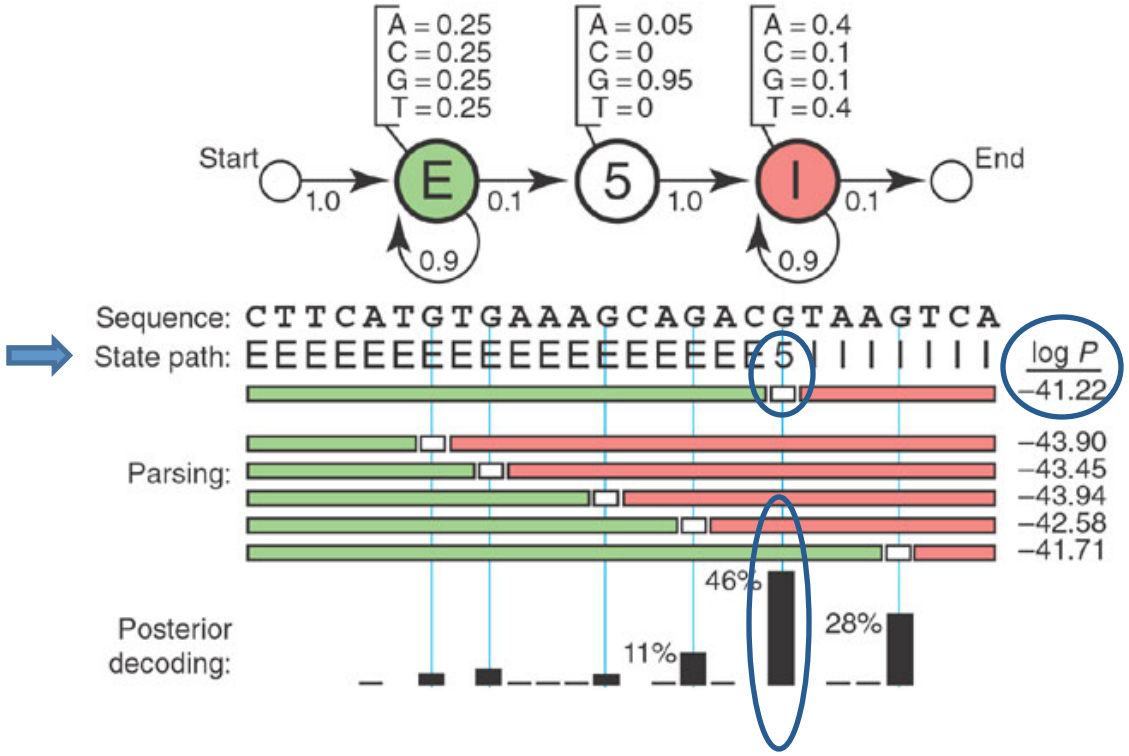
-Need a training set genes. (learning)

The learning set is composed of several hundred of gene-sequences manually annotated derived from cDNAs / genomic alignments. Ideally, these genes represent the diversity of the genes that can be found in the genome.

# Intrinsic methods : Hidden Markov Model = HMM

The sequences of exons, splice sites and introns have different statistical properties, such as GC%. Introns are AT rich and splicing site consensus is almost GT (95%).

Probabilities of moving from a state to another state : Intron ←→ Exon

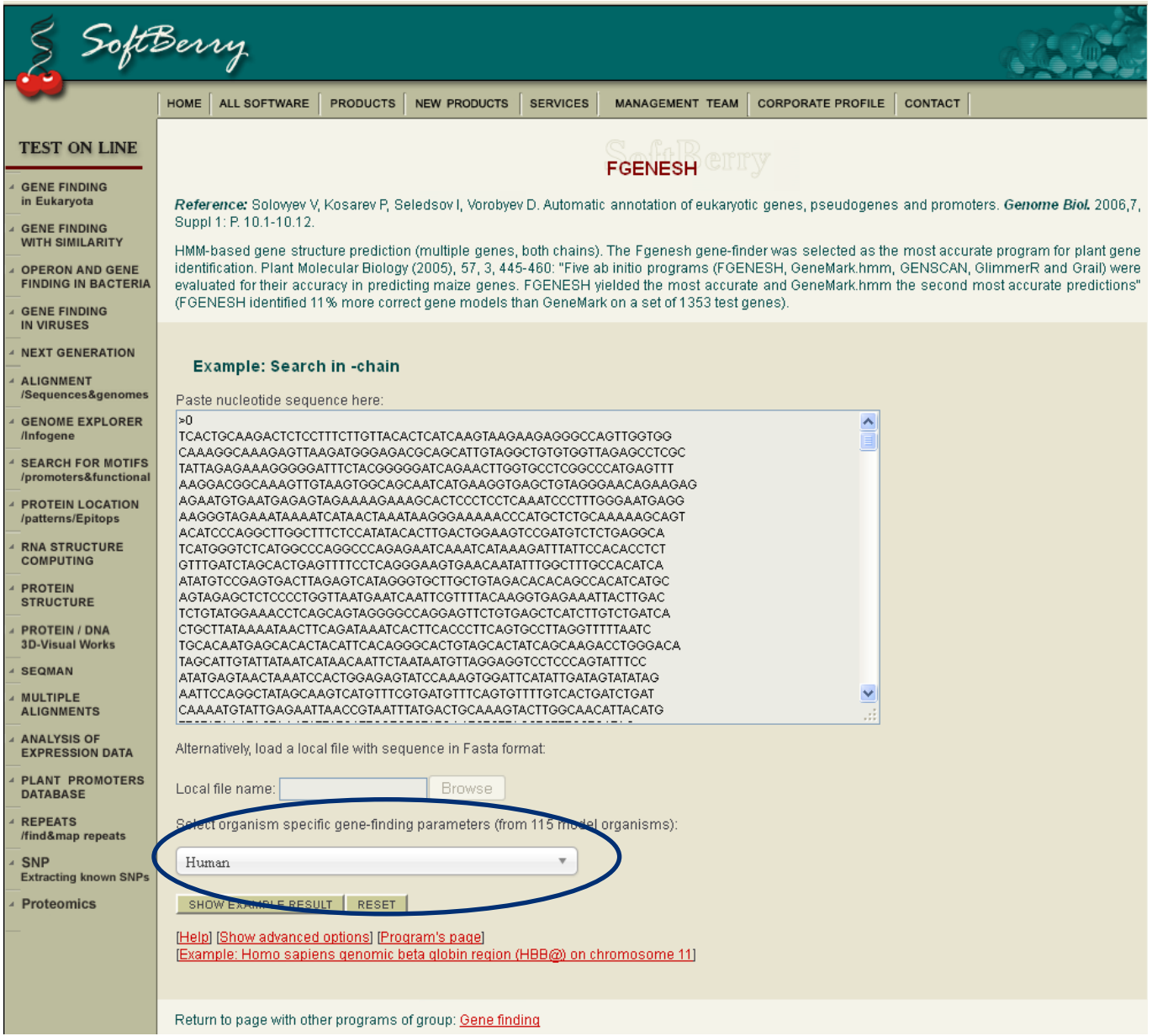



3 states : E, I, and 5 transition between the exon (E) and the intron (I).

The transition probabilities describe the linear order in which we expect the states to occur: Exons, Introns, Splicing site.

# HMM based gene structure prediction : FGENESH

http://linux1.softberry.com/berry.phtml?topic=fgenesh&group=programs&subgroup=gfind



# Automatic annotation : tools

Example of prediction softwares

Plants

GeneMark.HMM
GeneFinder
Eugene-HMM
FgeneSH
GlimmerA
Augustus

Animals

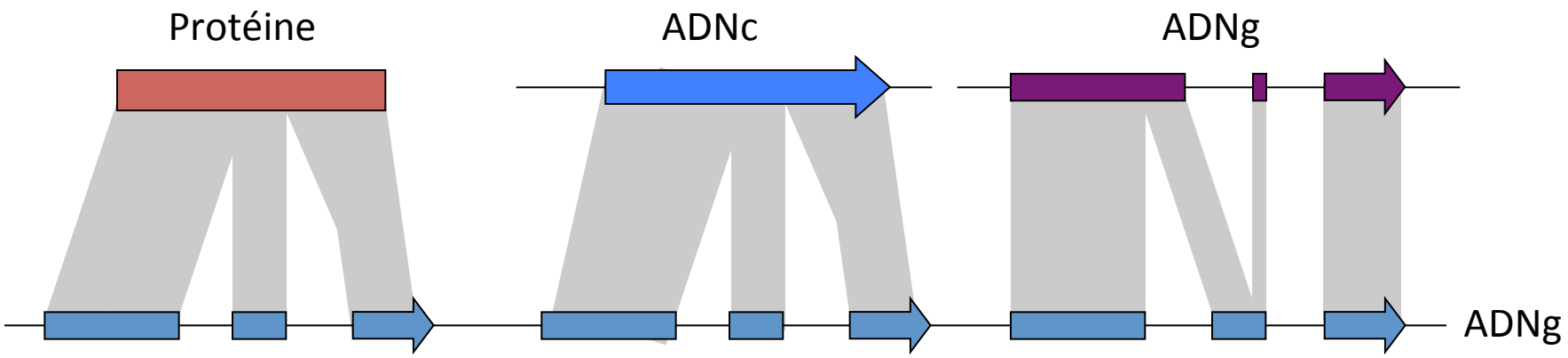
Genie
HMMgene
MagPie
GeneID
Grail

Human

GeneScan
GeneFinder
GeneWise

# Extrinsic methods

Comparative approach based on sequence similarities.
The sequence to annotate is compared with databases.




Alignement
ADNg - Protéine

Alignement
ADNg - ADNc

Alignement
ADNg - ADNg

# Extrinsic methods

| | sequence (query) | target (Subject) | database (example) |
|---|---|---|---|
| BLASTN | nucleotide | nucleotide | NR , EST, genomes |
| BLASTX | translated nucleotide | protein | Swissprot-Trembl |
| BLASTP | protein | protein | Swissprot-Trembl |
| TBLASTX | translated nucleotide | translated nucleotide | NR , EST, genomes |
| TBALSTN | protein | translated nucleotide | NR , EST, genomes |

**Comparisons with existing genes or proteins is helpful to refine the structure of predictions.**

**The Functional annotation will be always deduced of the similarities (homology) of the predicted elements with databases.**

# alignment tools to help gene structure prediction

Genome Threader

Use a similarity-based approach where cDNA/EST and/or protein sequences are compared to the genomic sequence

Useful to predict the splice sites

*www.genomethreader.org/*

Exonerate

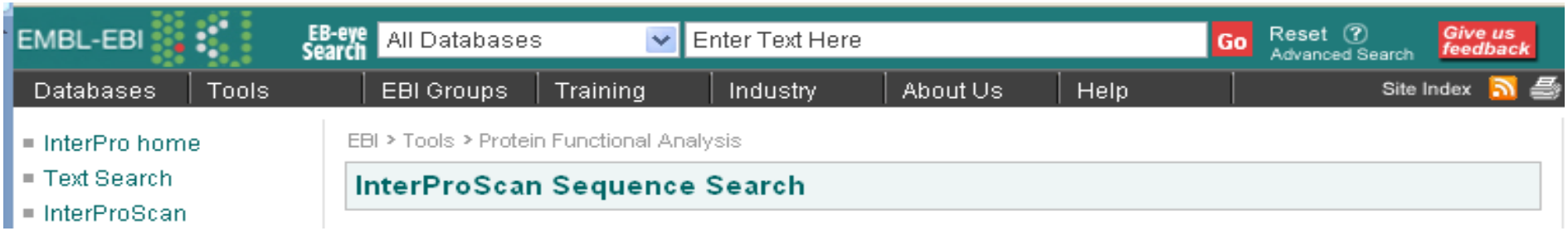
This is a generic tool for pairwise sequence comparison using a many alignment models

This tool is used to refine the structure of gene models (based on cDNA alignments)

*http://www.ebi.ac.uk/~guy/exonerate/*

# Search for conserved protein domains

INTERPROSCAN http://www.ebi.ac.uk/interpro/




**conserved protein domains = signatures**

database of predictive protein "signatures" can be used for the classification and automatic annotation of proteins.

Interproscan classifies sequences at superfamily, family and subfamily levels, predicting the occurrence of functional domains and important sites.

Domain databases used by interproscan:

| | |
|---|---|
| Prosite patterns | GENE3D |
| Pfam | HAMAP |
| ProDom | PANTHER |
| Superfamily | PIRSF |
| TIGRFAMs | |

# database limits

- Proteins databases are almost derived from the automatic translation of nucleic sequences. For example, TrEMBL is the automatic translation of the nuclein EMBL (NR) database. (No biological evidence)

→ SWISSPROT, is another protein database that contains less sequences than TrEMBL, but sequences are manually curated by biologists.

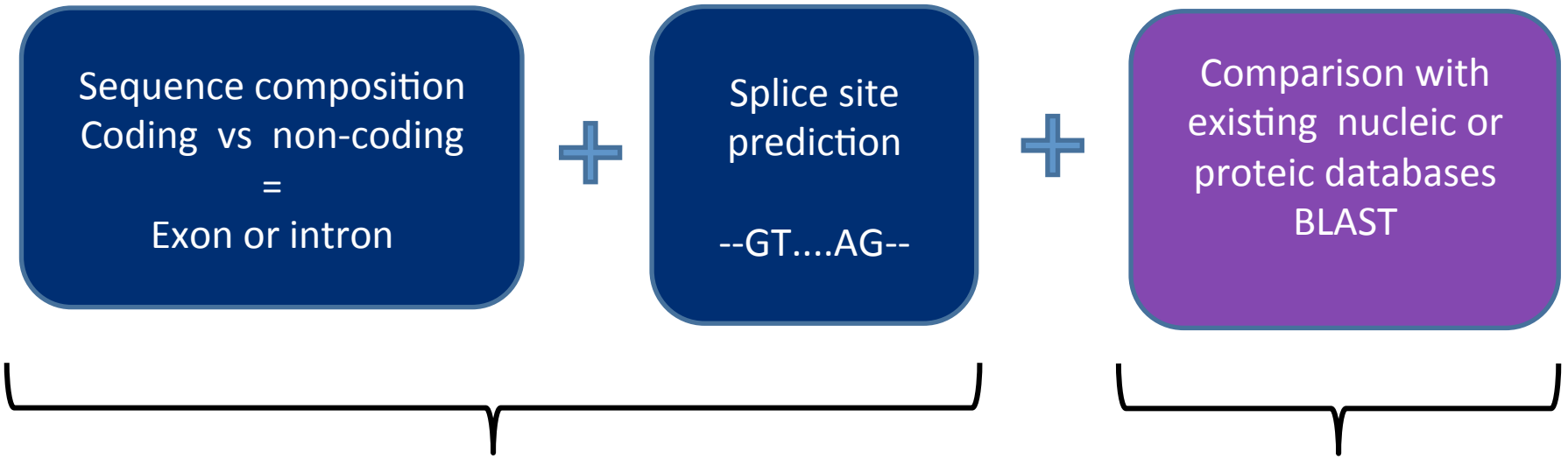
- ESTs sequences are often produced from single strand sequencing and contain mistakes.

→ESTs represent part of genes that are really expressed.

- Genome annotations are useful, but gene predictions are based on automatic annotations. Taking in account genome evolutions, only close relative species can be used to transfer the annotation.

→For sugarcane annotation, the sorghum sequence is available and represents a good model for comparison.

# Integrative method – The combiner




intrinsec methods

Ab initio - HMM
with training step

Extrinsec methods

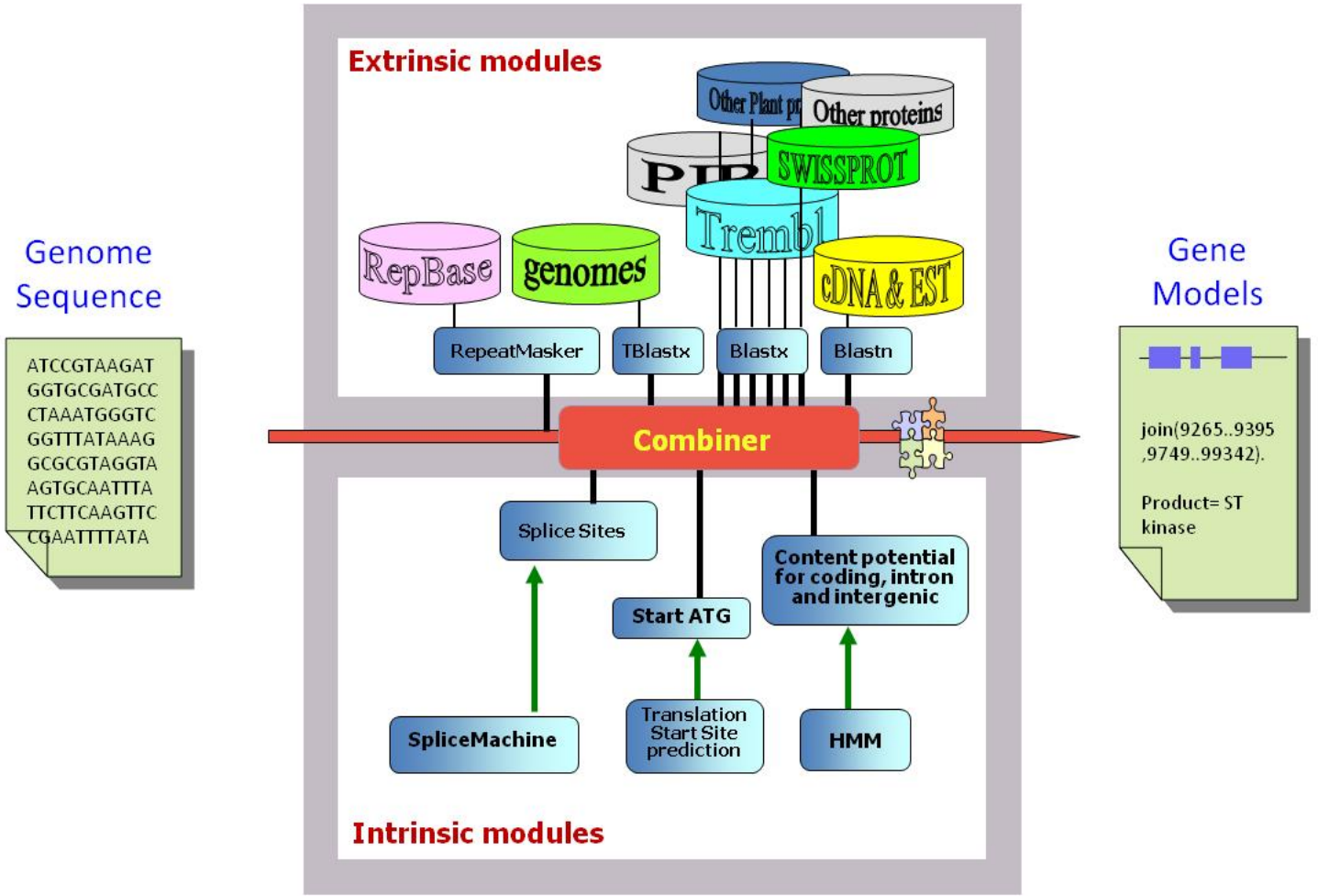
By similarity
= sequence comparisons

# Integrative method – The combiner

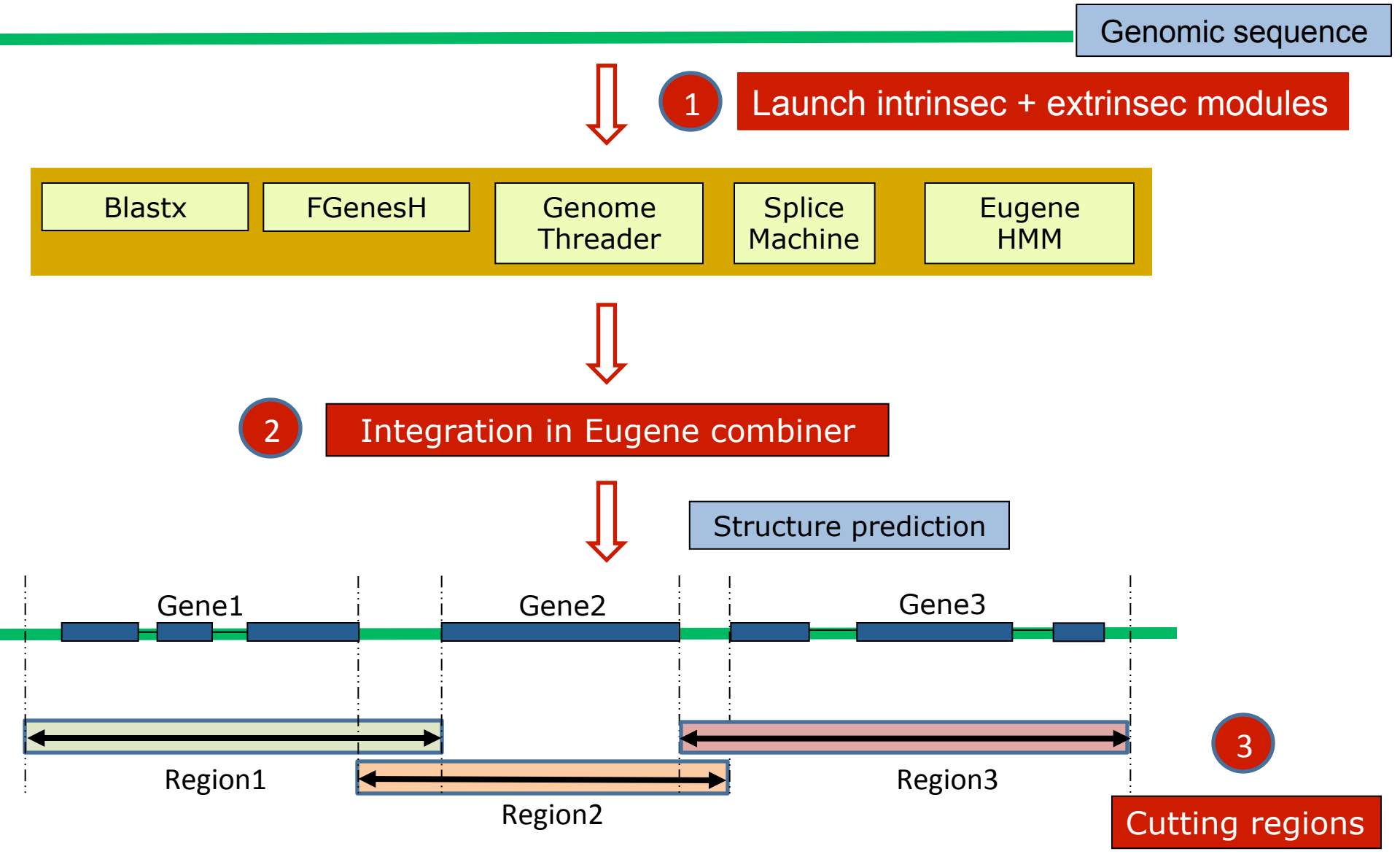
Integrative methods = ab-initio + comparative approaches

Predictions of ab-initio gene finders combined with the database similarities improve significantly the annotation.

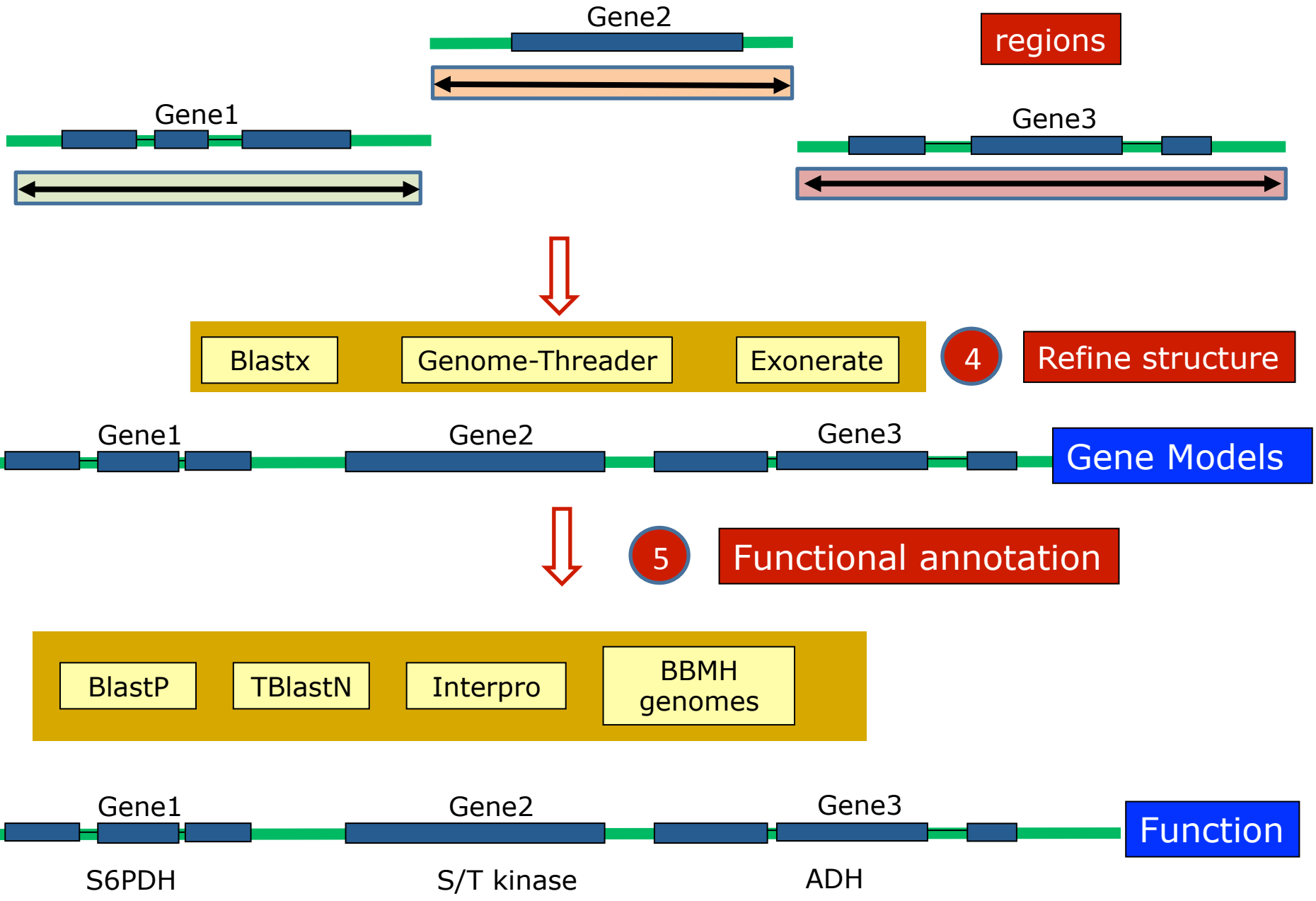
Intrinsic and extrinsic method complement each other.



# Gene annotation Pipeline (example)



# Annotation Pipeline (continue)



# Automatic annotation tool : Eugene (combiner)

http://eugene.toulouse.inra.fr/

## EuGène : A Gene Finding Software

P. BARDOU · M.J. CROS · S. FOISSAC · J. GOUZY · A. MOISAN · T. SCHIEX

Home
Plugins
Organisms
References
Documentation
Download
GForge
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Organisms

### Arabidopsis thaliana :

| *Arabidopsis thaliana* (L.) Heynh. mouseear cress | |
|---|---|
| **Group** | Dicot |
| **Family** | Brassicaceae |
| **Growth Habit** | Forb/herb |
| **Duration** | Annual |
| **Links** | |
| **USDA, NRCS. 2002. The PLANTS Database, Version 3.5.** | |
| **ARS Germplasm Resources Information Network (GRIN)** | |

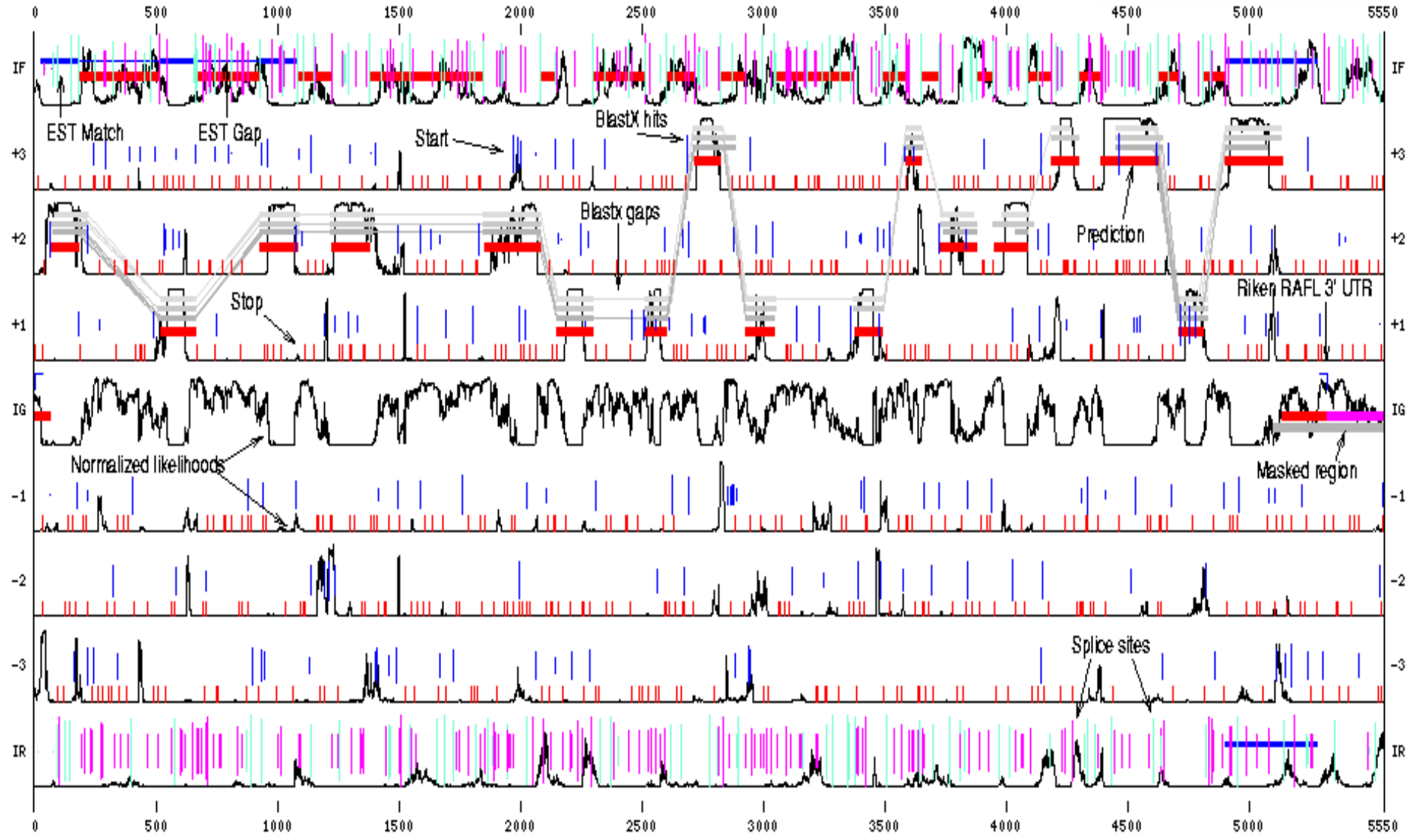
An evaluation of EuGène and several gene prediction programs was performed.

### Oryza sativa :

| *Oryza sativa* L. rice | |
|---|---|
| **Group** | Monocot |
| **Family** | Proceae |
| **Growth Habit** | Graminoid |
| **Duration** | Annual |
| **Links** | |
| **USDA, NRCS. 2002. The PLANTS Database, Version 3.5.** | |
| **ARS Germplasm Resources Information Network (GRIN)** | |

### Medicago truncatula :

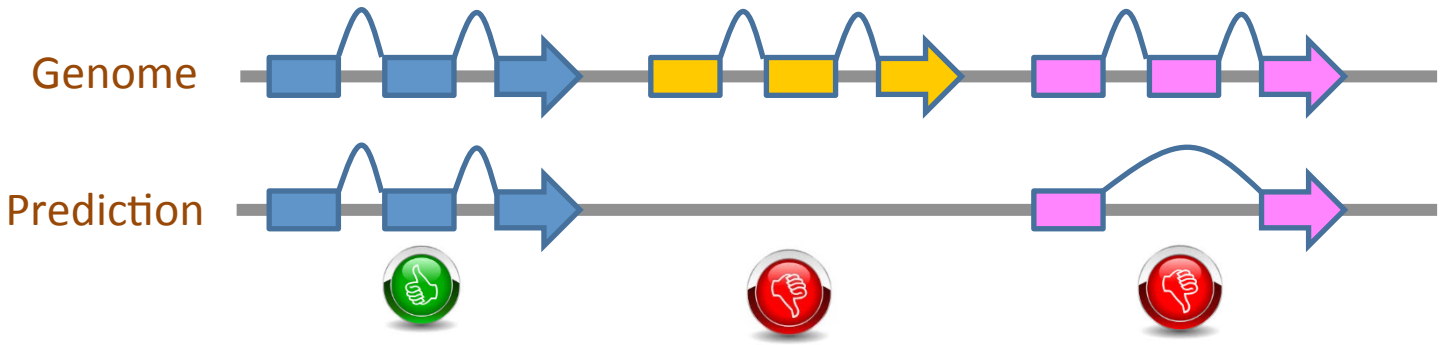
# Eugène output



# Automatic annotation

The automatic prediction is based on statistical models and on computational analyses

Some genes can be missed, over-predicted or can be partially detected (structure)




Manual curation of annotation is required when a gene of interest (or gene family) is targeted

Manual curation of annotations can be facilitated using dedicated tools allowing visual representation of genomic regions (artemis)

# Evaluation of the relevance of annotations

Based using a set of manually annotated genes (comparison of results automatic vs manual)
TP (true positives) = correctly predicted genes
FP (False positives) = predicted genes that are normally absents → over prediction
FN (False negatives) = genes that are missed by the automatic annotation → under prediction

Sensibility (Sn) = TP / (TP+FN) → number of correct predictions / number of real genes to find
Specificity (Sp) = TP / (TP+FP) → number of correct prediction among all predictions

Sn/Sp ratio to estimate efficiency of automatic predictions

| | Base level (coding/non-coding) | | Exon level | | Gene level | |
|---|---|---|---|---|---|---|
| **Gene finder** | **Sn N** | **Sp N** | **Sn E** | **Sp E** | **Sn G** | **Sp G** |
| GenScan+ | 83.2 | 98.2 | 69.6 | 78 | 25.8 | 29 |
| GenMarkHMM | 89.9 | 94.8 | 73.1 | 76.6 | 32.4 | 31.6 |
| FgenesH-At | 95.1 | 93 | 85.3 | 81.4 | 47 | 46.5 |
| FgenesH-Mt | 97.6 | 92.1 | 85.1 | 80.7 | 52.8 | 47.8 |
| EGN | 93.7 | 95 | 84.7 | 85.4 | 55.5 | 50.5 |
| EGN+FgenesH | 97.8 | 94.2 | 90 | 86.9 | 63.2 | 56.4 |
| EGN+FH+AA | 98.6 | 93.9 | 92.4 | 88 | 69.2 | 61.8 |
| EGN+FH+AA+ EST | 98.2 | 99.9 | 94.4 | 94.6 | 80.2 | 79.4 |

Integrative approach (combined)

# Transposable Elements (TEs)

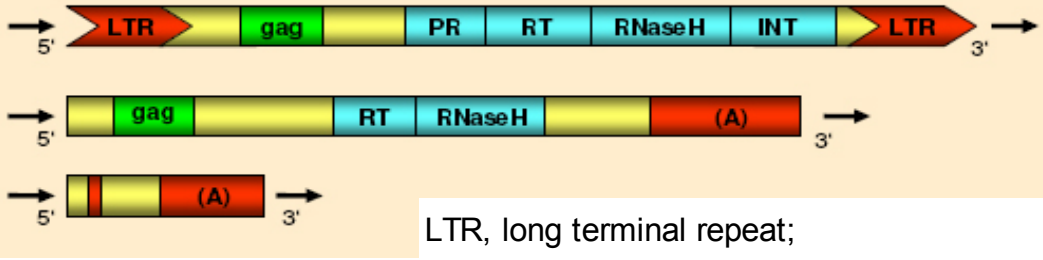
Genes represent only a little part of the genome. Some regions can be gene-rich but some other can contain a majority of repeated elements.

Retrotransposons CLASS I

LTR

Non-LTR (LINEs)

SINEs

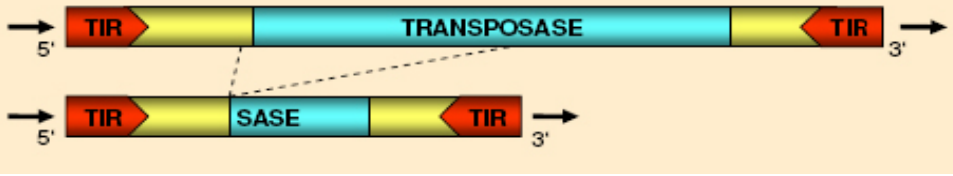



LTR, long terminal repeat;
LINE, long interspersed nuclear element;
SINE, short interspersed nuclear element;

DNA transposons CLASS II

TIR

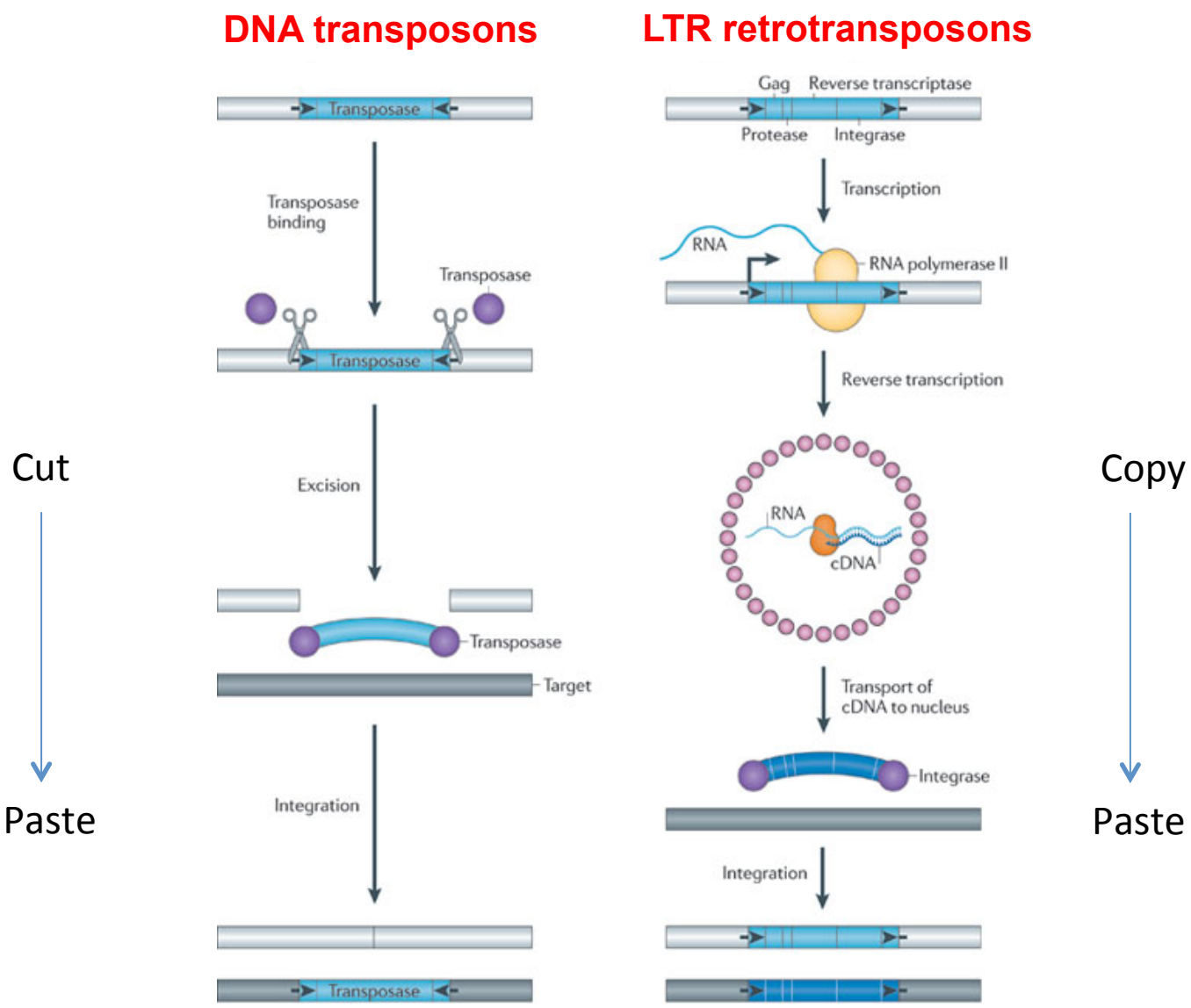
MITEs




Helitrons

TIR , terminal inverted repeat.
MITE, Miniature Inverted Transposable Element

# Dynamics of TEs

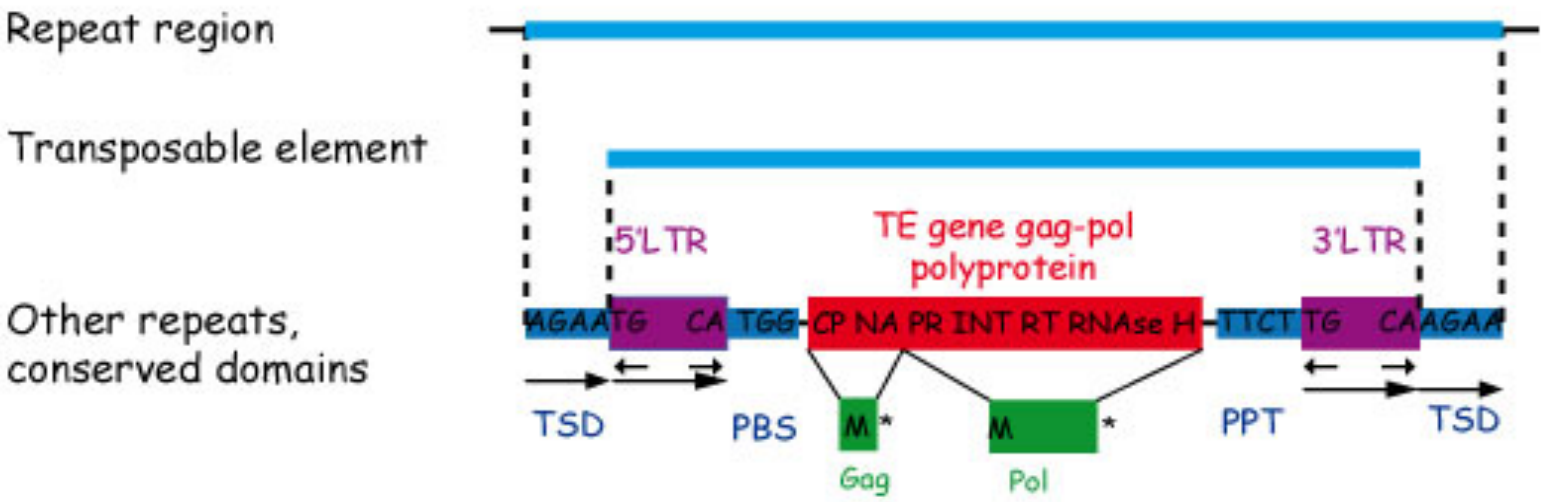



From “Dynamic interactions between transposable elements and their hosts” . Henry L. Levin & John V. Moran .Nature Reviews Genetics (2011) 12, 615-627

# LTR-Retrotransposons, Ty1 (*copia*) & Ty3 (*gypsy*)

In plants, the most frequent mobile elements are LTR retrotranposons

2 super families of LTR-retrotransposons : *Copia* and *Gypsy*



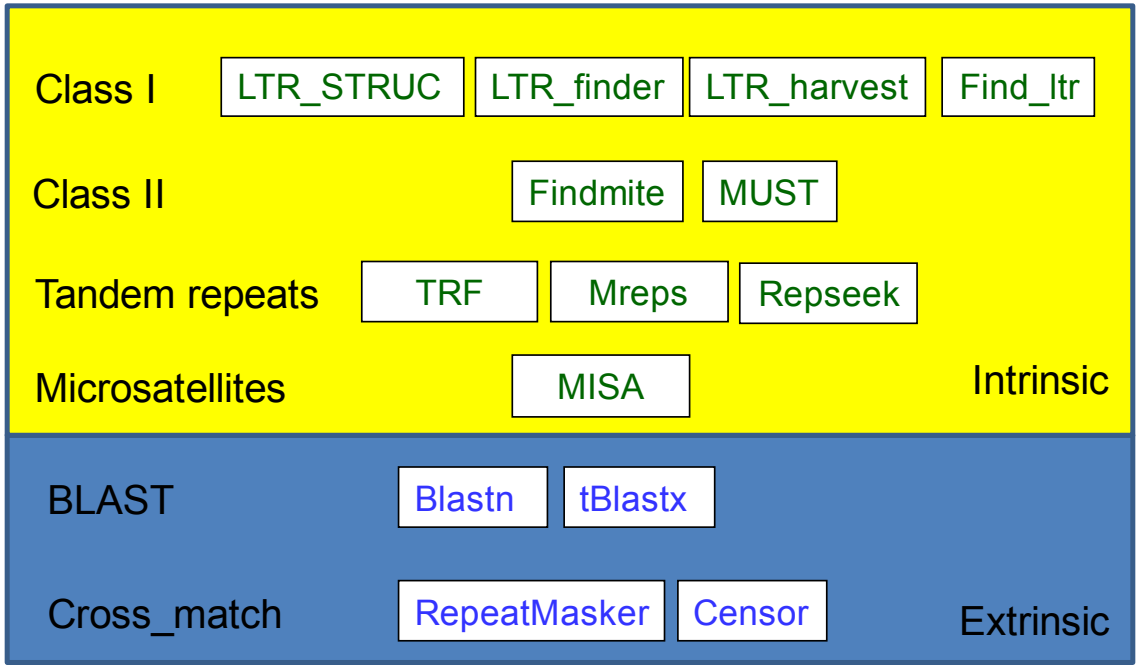

Example of LTR retrotransposon Ty1-copia

In Ty3-gypsy superfamily, the order of the protein domain differ: CP-NA-PR-RT-RNAseH-INT

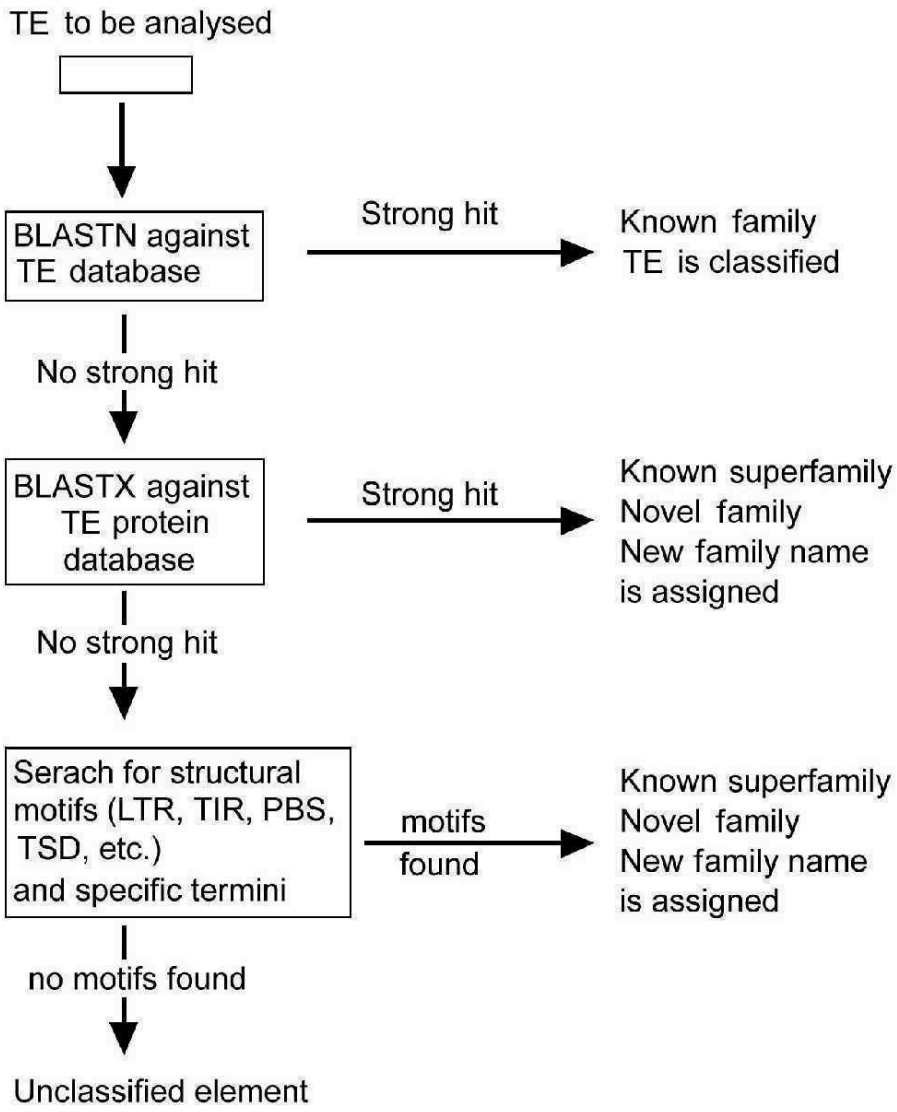
LTR, long terminal repeat; TSD, target site duplication; PBS, primer binding site;
PPT, polypurine tract (RR tract); CP, capsid-like protein; NA, nucleic acid binding moiety;
PR, protease; INT, integrase; RT, reverse transcriptase.

# Annotation of transposable elements : tools

Several ab-initio programs can be used to detect structures of Tes
Comparisons with databases can be used to classify the elements.



# Annotation of TE, general approach based on comparison



# Transposable Elements

Classification from Wicker et al (2007). A unified classification system for eukaryotic transposable elements. *Nat Rev Genet, 8, 973-982.*

| Class | Order | Superfamily | Family | Code / Label |
|---|---|---|---|---|
| Retrotransposon | LTR | Copia | opie | RLC |
| | | Gypsy | maggy | RLG |
| | | Unclassified | | RLX |
| | LINE | L1 | | RIL |
| | | Unclassified | | RIX |
| | SINE | Alu | | RSA |
| | | Unclassified | | RSX |
| DNA transposons | TIR | CACTA | | DTC |
| | | Mutator | | DTM |
| | MITE | Stowaway | | DTT |
| | | Tourist | | DTH |
| | Helitron | Helitron | | DHH |

Retrotransposon, LTR, Copia, opie = RLC_opie

Classification of TEs based on sequence similarities : the rule 80-80-80

# Refine the structure of TE  search for LTR and IR

DOT PLOT

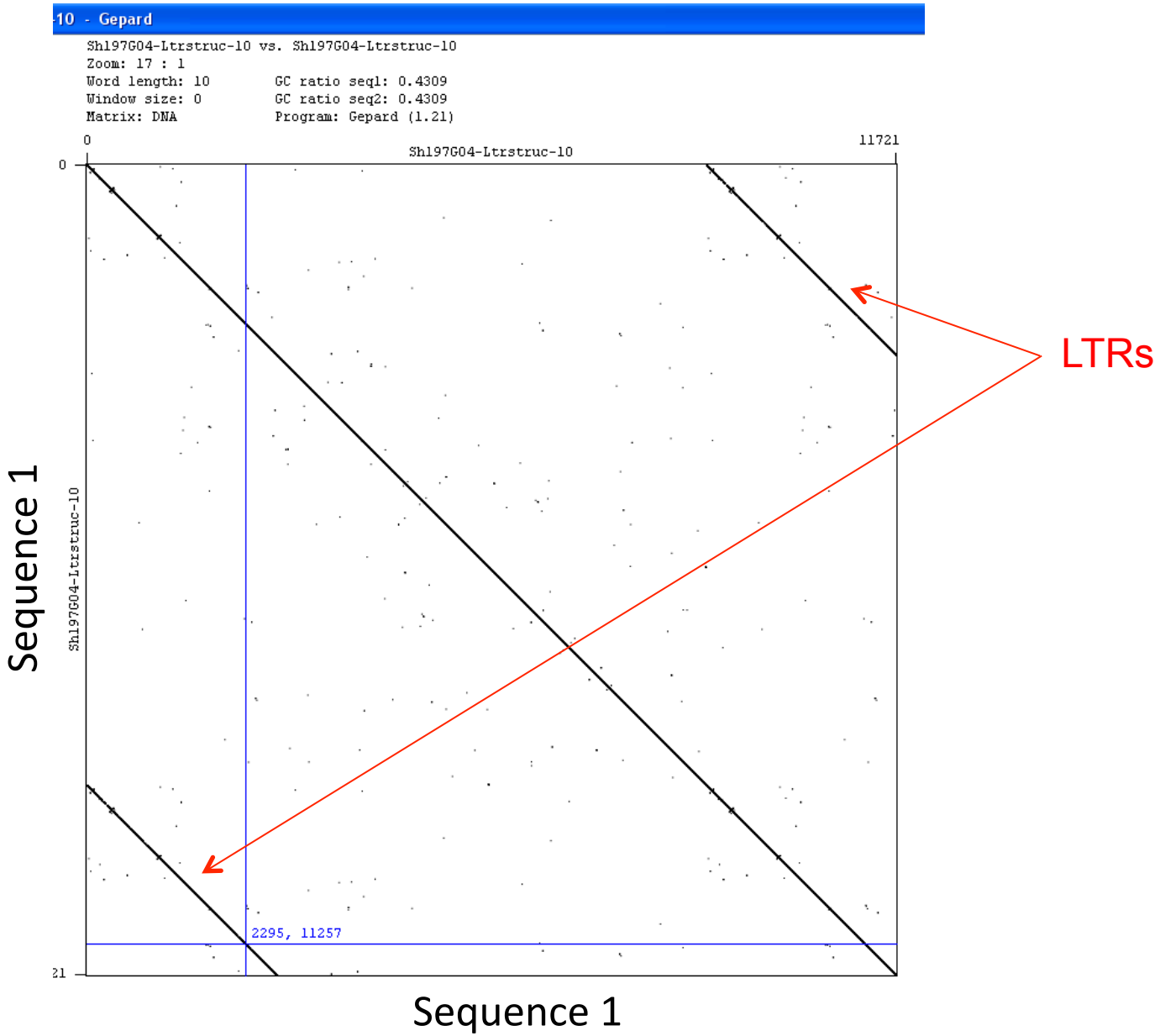
## Gepard

**Gepard** (German: "cheetah", Backronym for "GEnome PAir - Rapid Dotter") allows the calculation of dotplots even for large sequences like chromosomes or bacterial genomes.

**Reference:**
Krumsiek J, Arnold R, Rattei T. Gepard: A rapid and sensitive tool for creating dotplots on genome scale. Bioinformatics 2007; 23(8): 1026-8. PMID: 17309896

- **Use cases** +
- **Features** +
- **Screenshot** +
- **System requirements** +
- **Download** +
- **Bugs** +
- **Source code** +
- **Tutorial** +
- **Method** +
- **Memory issues / Vmatch support** +
- **Contact** +

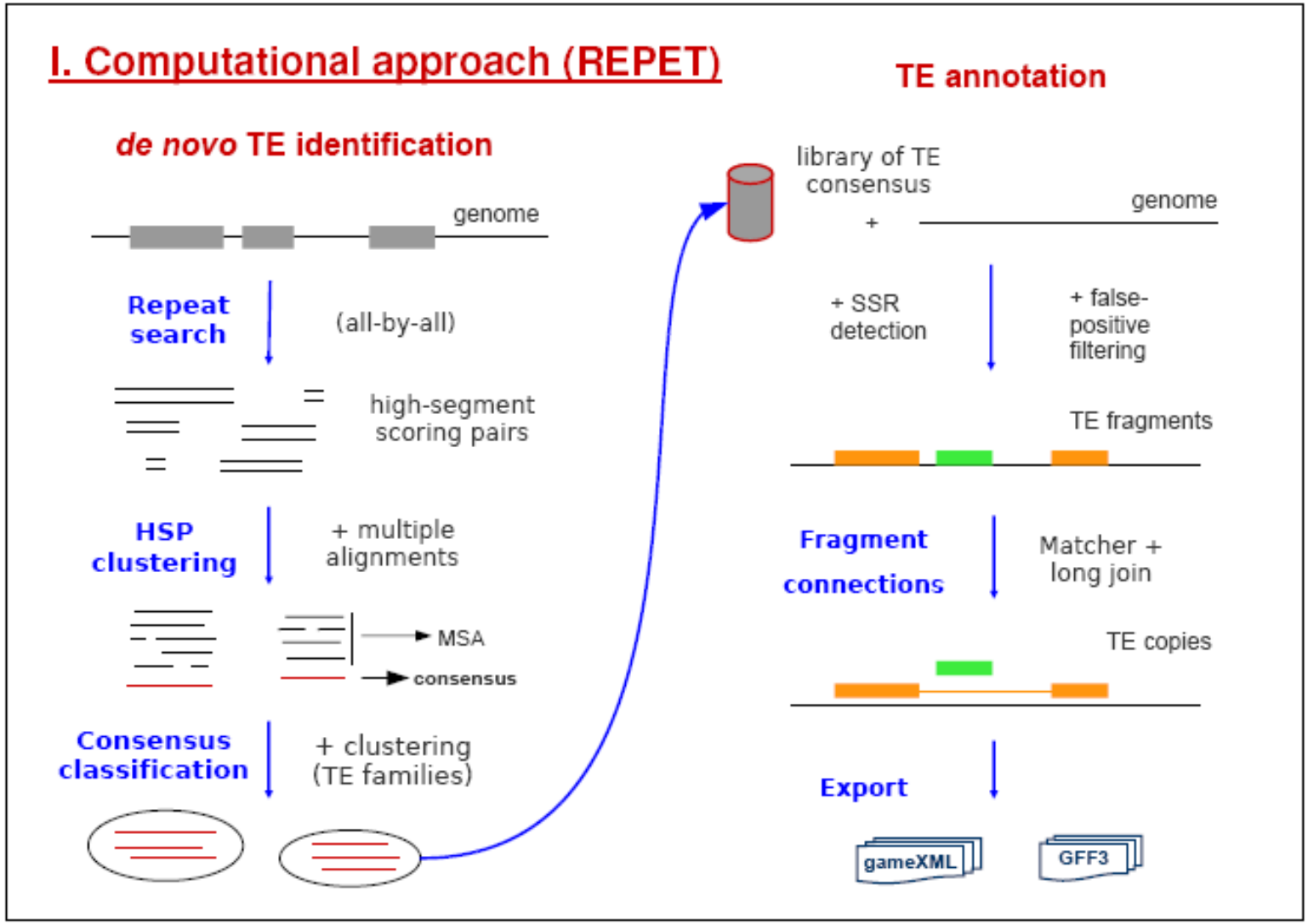
# Refine the structure of TE, e.g. LTR-retrotransposon




Guepard : http://www.helmholtz-muenchen.de/icb/gepard

# Pipeline for Classification, Screening and Mapping of TEs

Considering Transposable Element Diversification in De Novo Annotation Approaches
Flutre et al, 2011 PLOS one



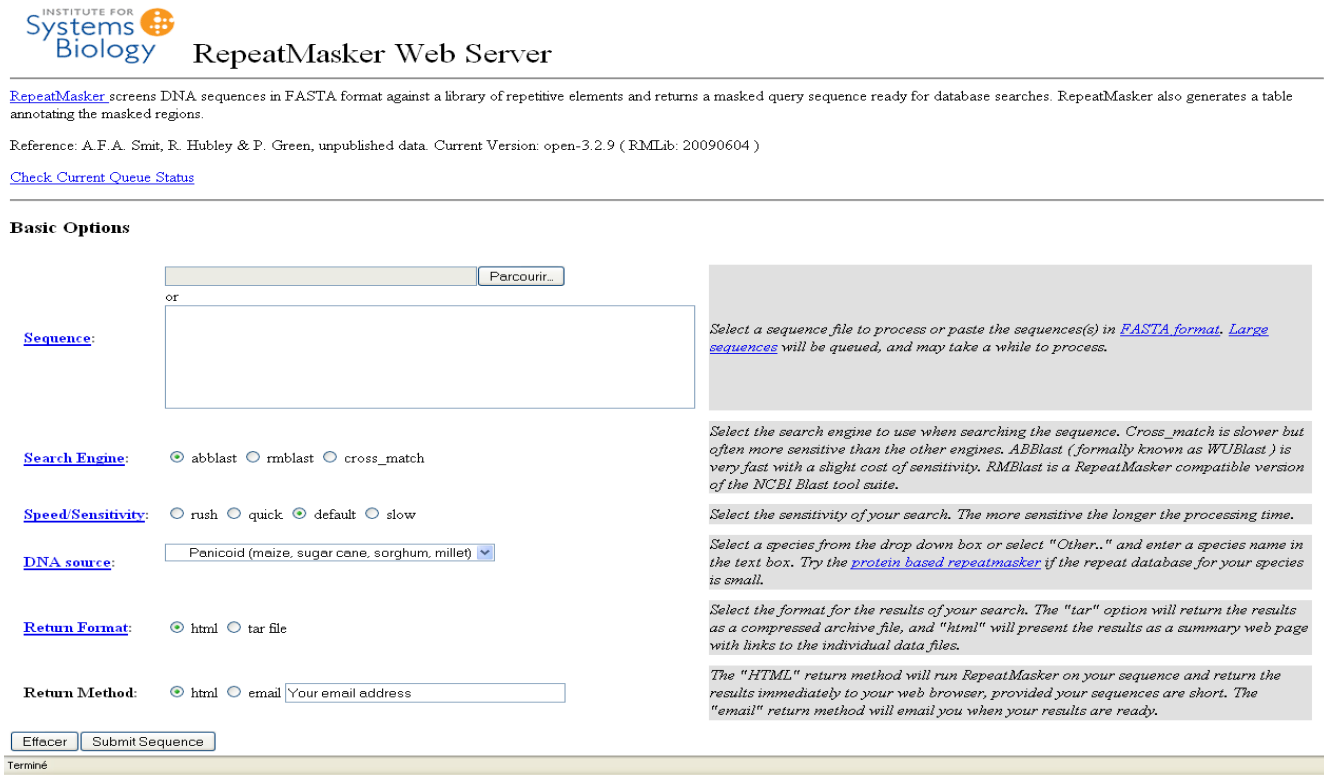
# Before gene annotation, mask repeat sequences

Gene modelling is generally done after filtering out repeats because this makes gene finding even more difficult :

TEs may look like protein encoding genes (transposase and remains)

TEs perturb the structure of gene models, e.g. by inserting in introns (long insertion, and gene model is cut in many parts)

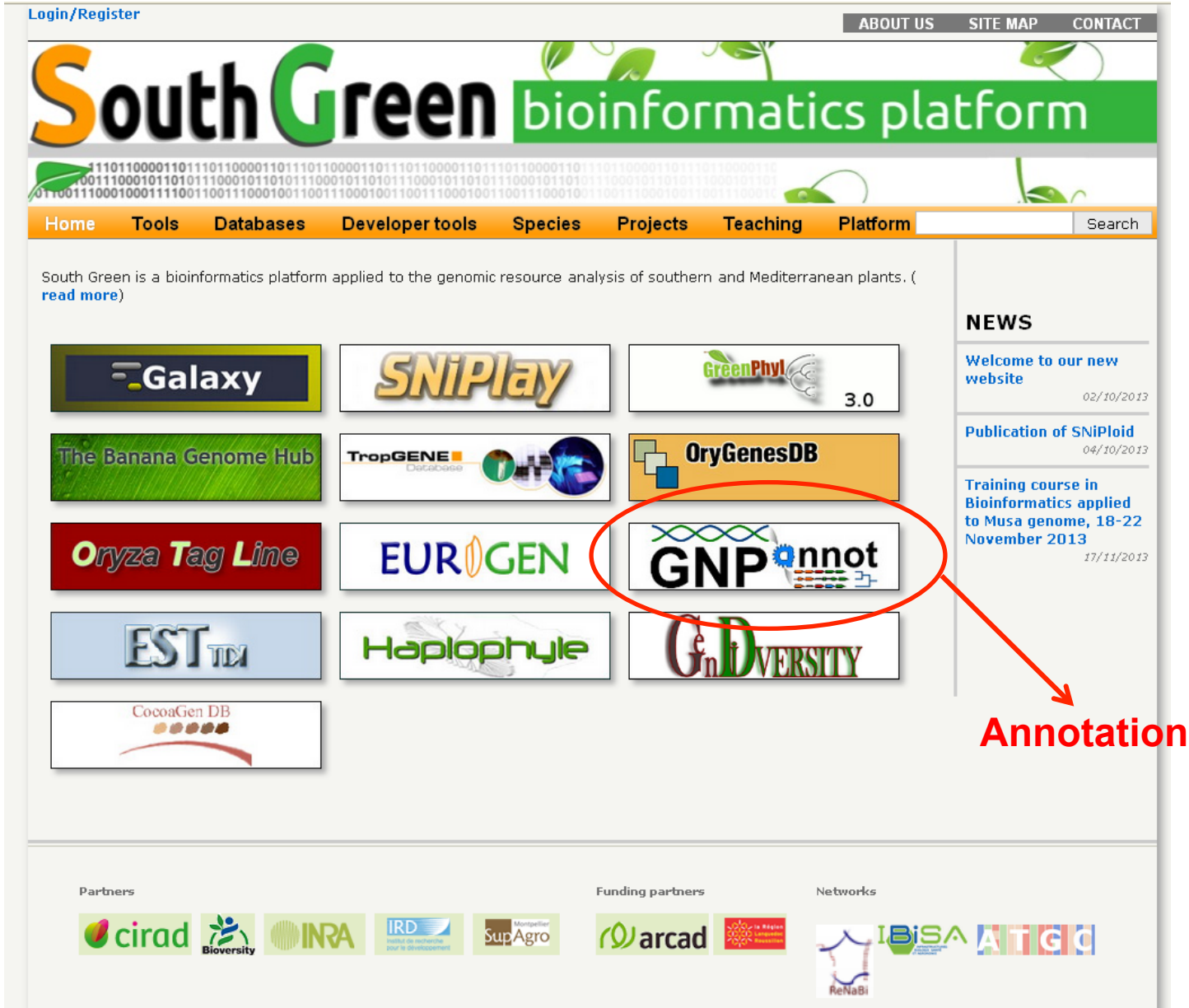
→Repeat masker is the tool the mostly used to mask repeats.




http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker

# Pipelines and database dedicated for annotations

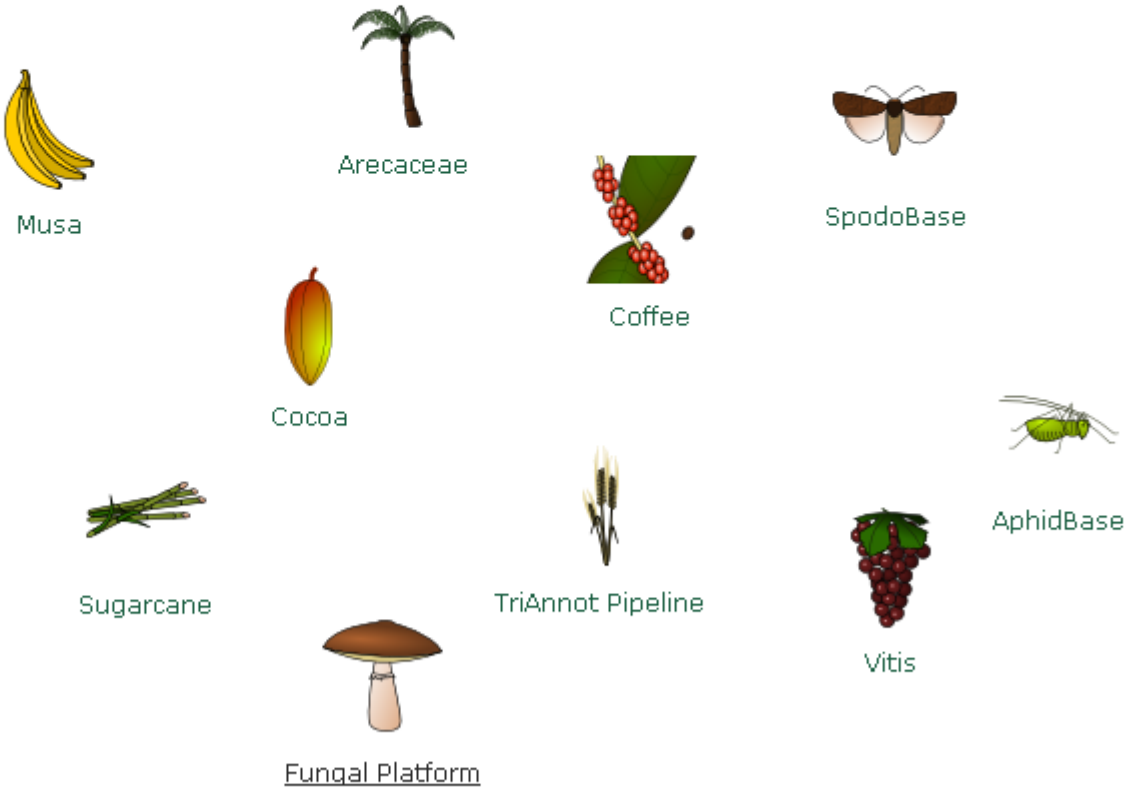
http://southgreen.cirad.fr/



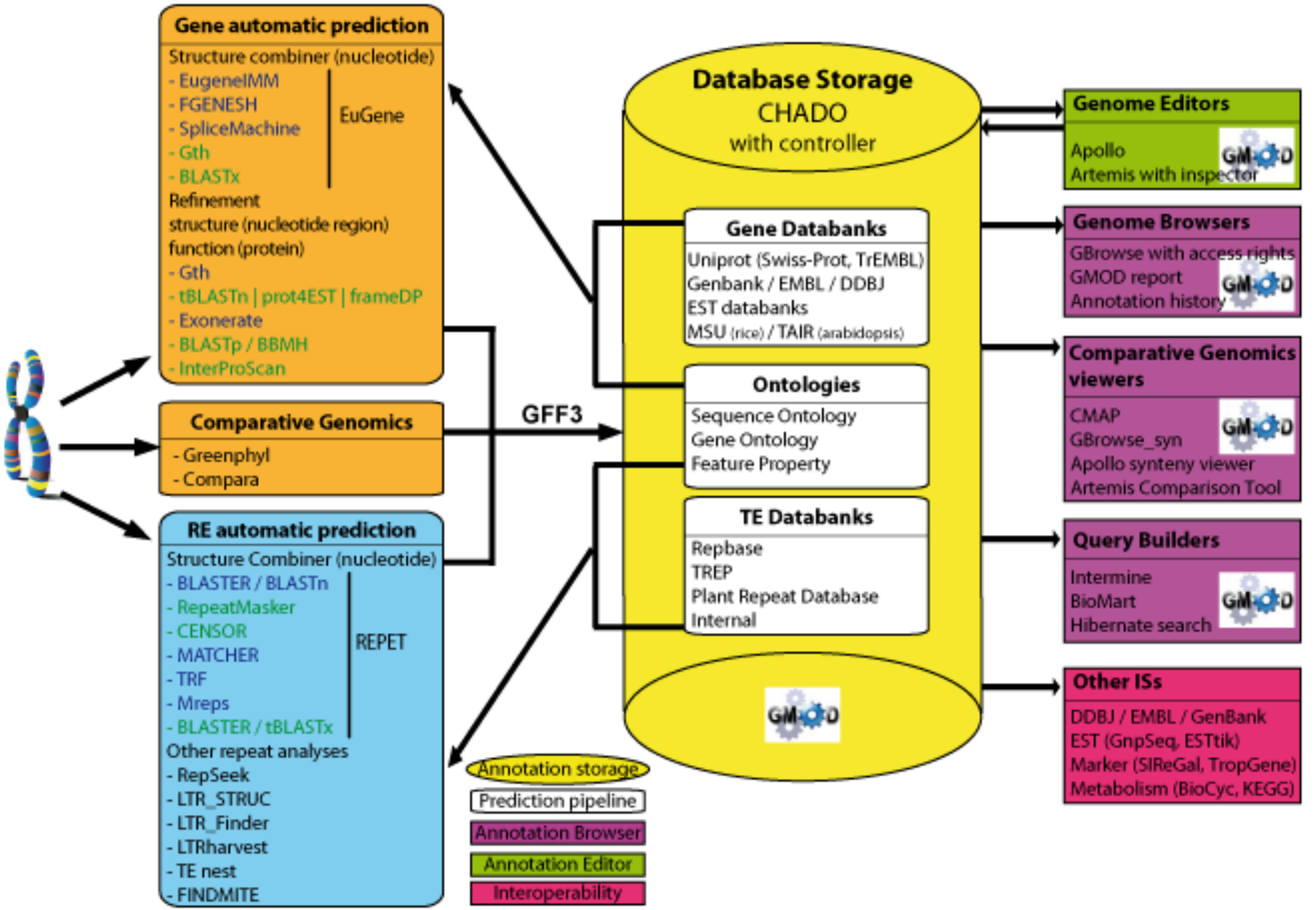
# Pipelines and database dedicated for annotations

GNPAnnot is a community system for structural and functional annotation dedicated to plants, insects and fungus genomes allowing both automatic predictions and manual curations of genomic objects.

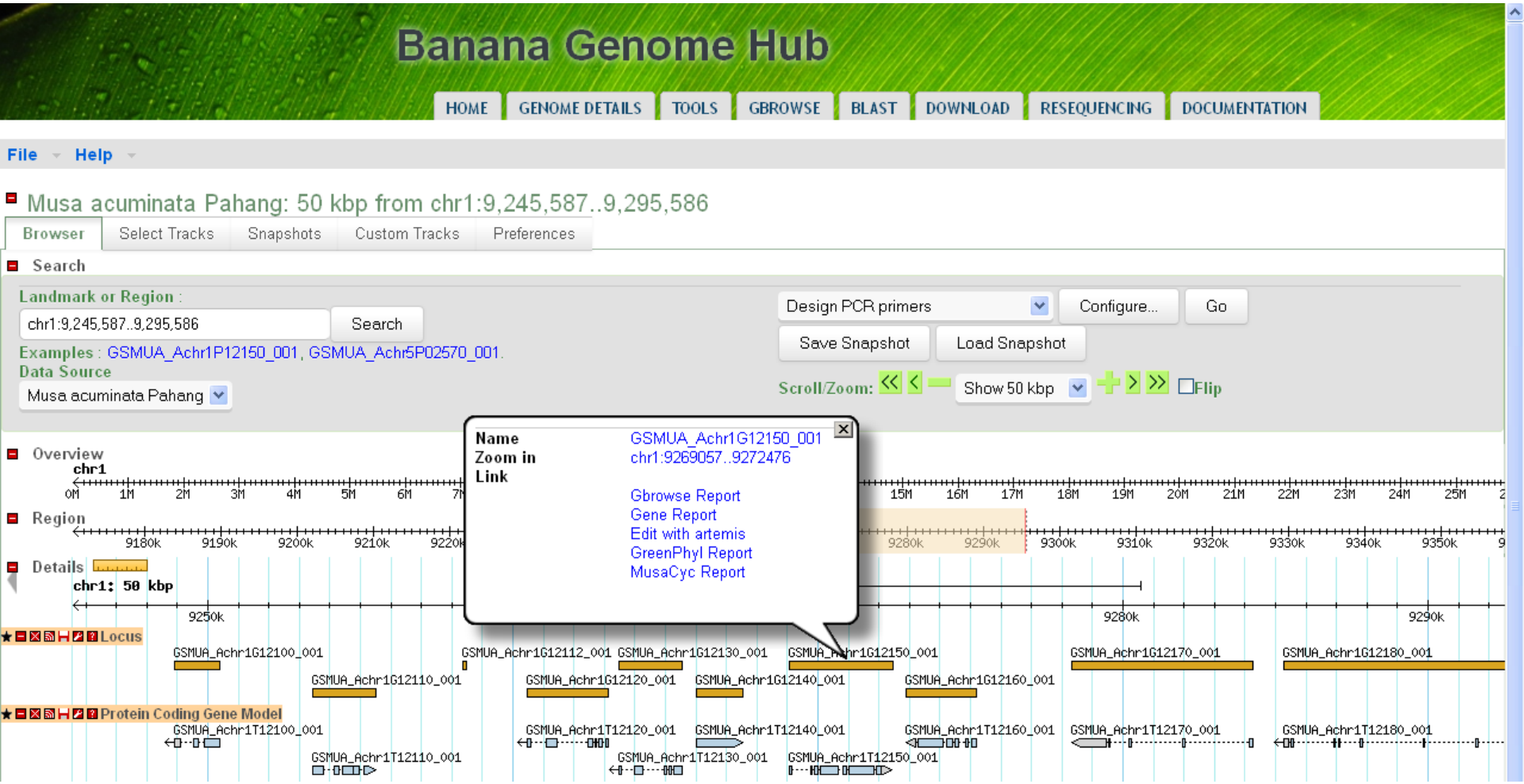
GNPAnnot



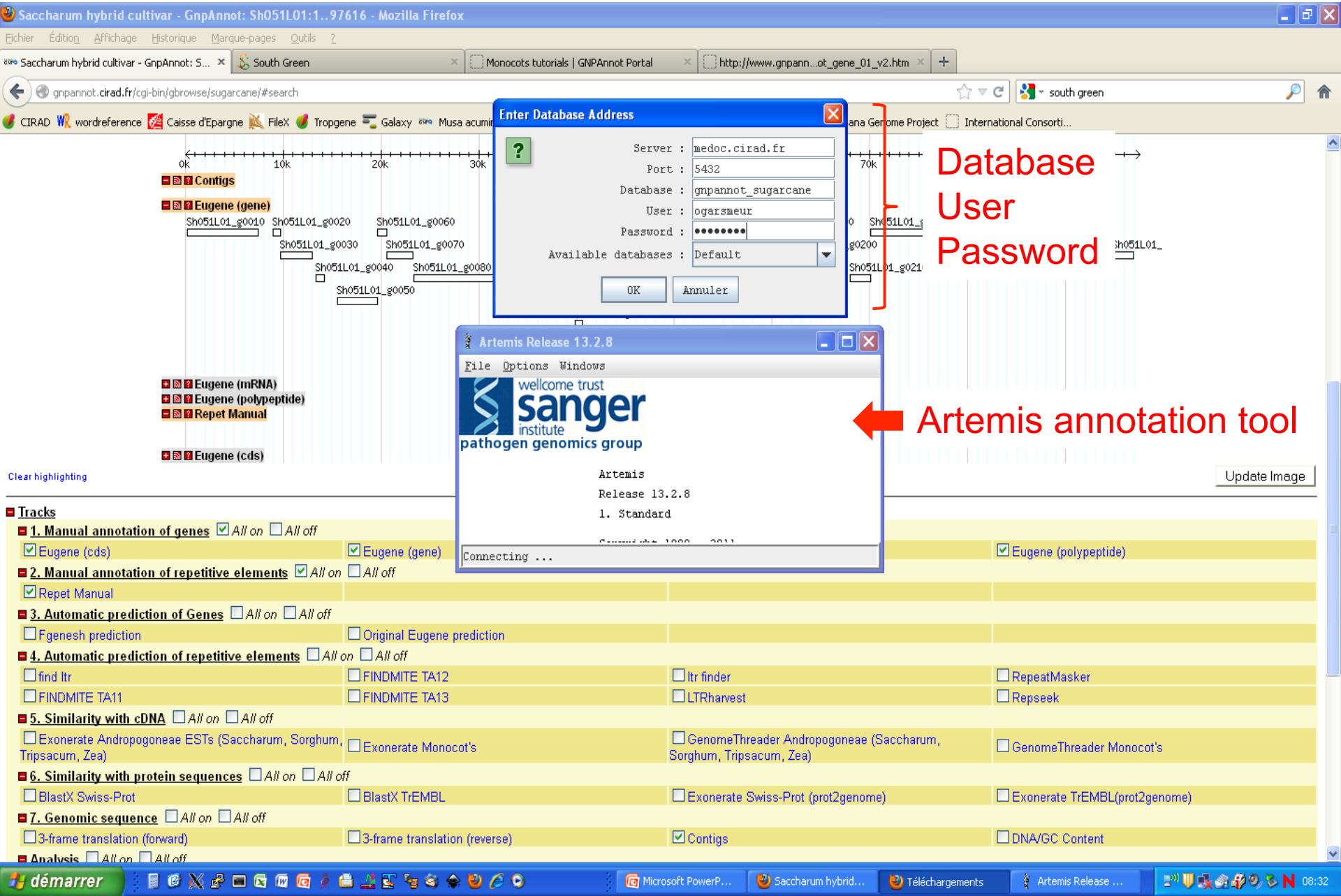
# GNPAnnot platform



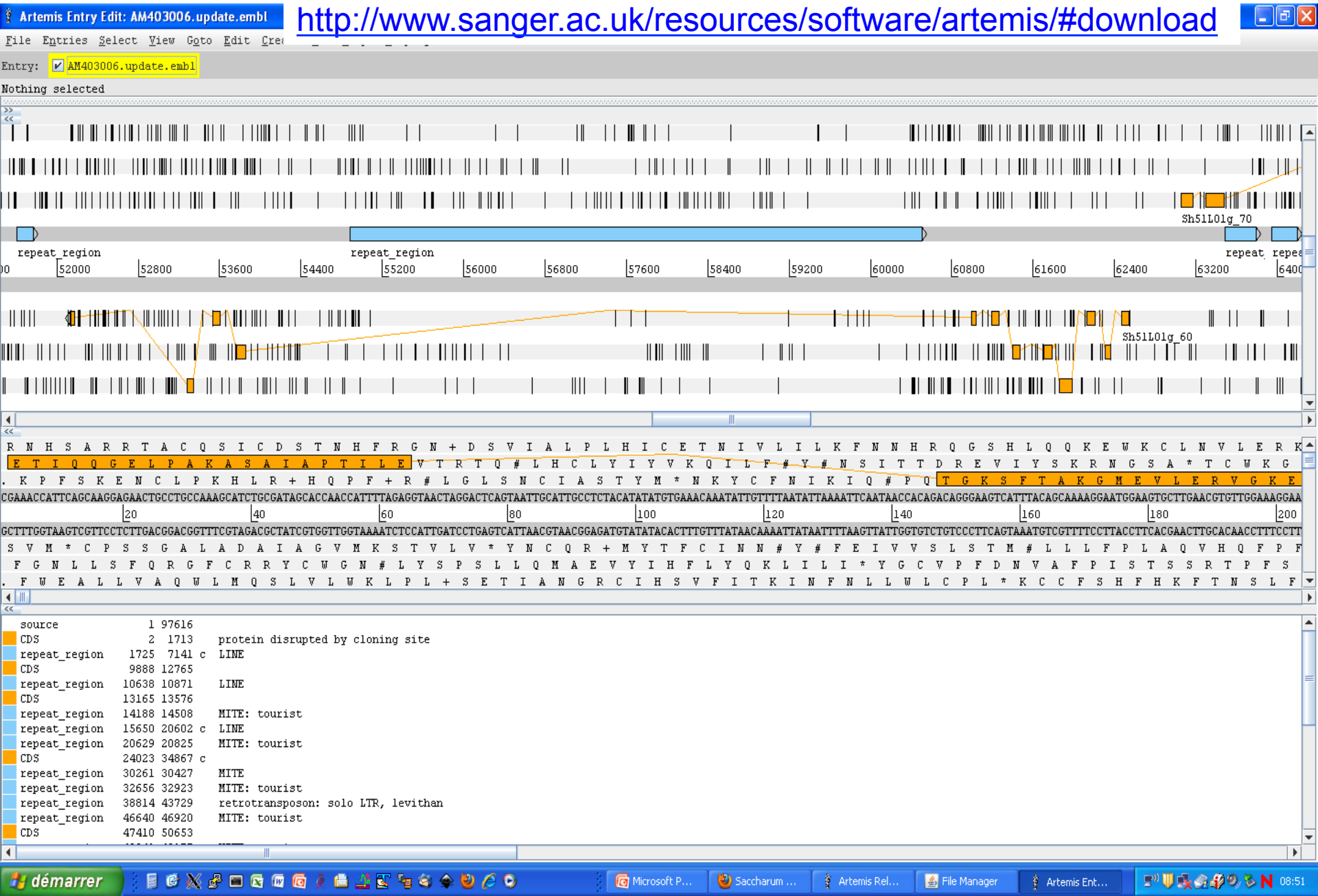
# Manual curation of annotations



# Gbrowse is connected to artemis through the chado database



# artemis : to see, to check, to correct, to validate annotations




http://www.sanger.ac.uk/resources/software/artemis/#download