Hands on session:
Basic promoter analysis

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A real life project

A project fully integrating in silico & in vitro methods

This application example was taken from a scientific project that had lasted for about five years and included experimental verification of every prediction made by bioinformatics analysis.

The results of the combined *in silico* and *in vitro* apporaches were published in two articles:

Benkhart E., Siedler M., Wedel A., Werner T., Ziegler-Heitbrock H.W.L. (2000) Role of Stat3 in Lipopolysaccharide-Induced IL-10 Gene Expression. J. Immunol. 165, 1612 - 1617.

Ziegler-Heitbrock H.W.L., Lötzerich M., Schaefer A., Werner T., Frankenberger M., Benkart E. (2003). IFN-a Induces Human IL-10 Gene by Recruiting Both IFN Regulatory Factor I and Stat3. J. Immunol. 171, 285 - 290.

Outline of tasks

What you will be doing during the next 2 hours

- General aim: Functional analysis of the IL-10 promoter
- Find and extract IL-10 promoters (***ElDorado***)
- Analyze IL-10 promoters for transcription factor binding sites (TFBS) (***MatInspector*** from ***GEMS*** package)
- Analyze literature for IL-10 Promoter (***BiblioSphere***)
- Analyze IL-10 promoters for TFBS frameworks (***FrameWorker*** from ***GEMS*** package)
- Database search with frameworks (***ModelInspector*** from ***GEMS*** package)

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Task 1

What you will be doing next

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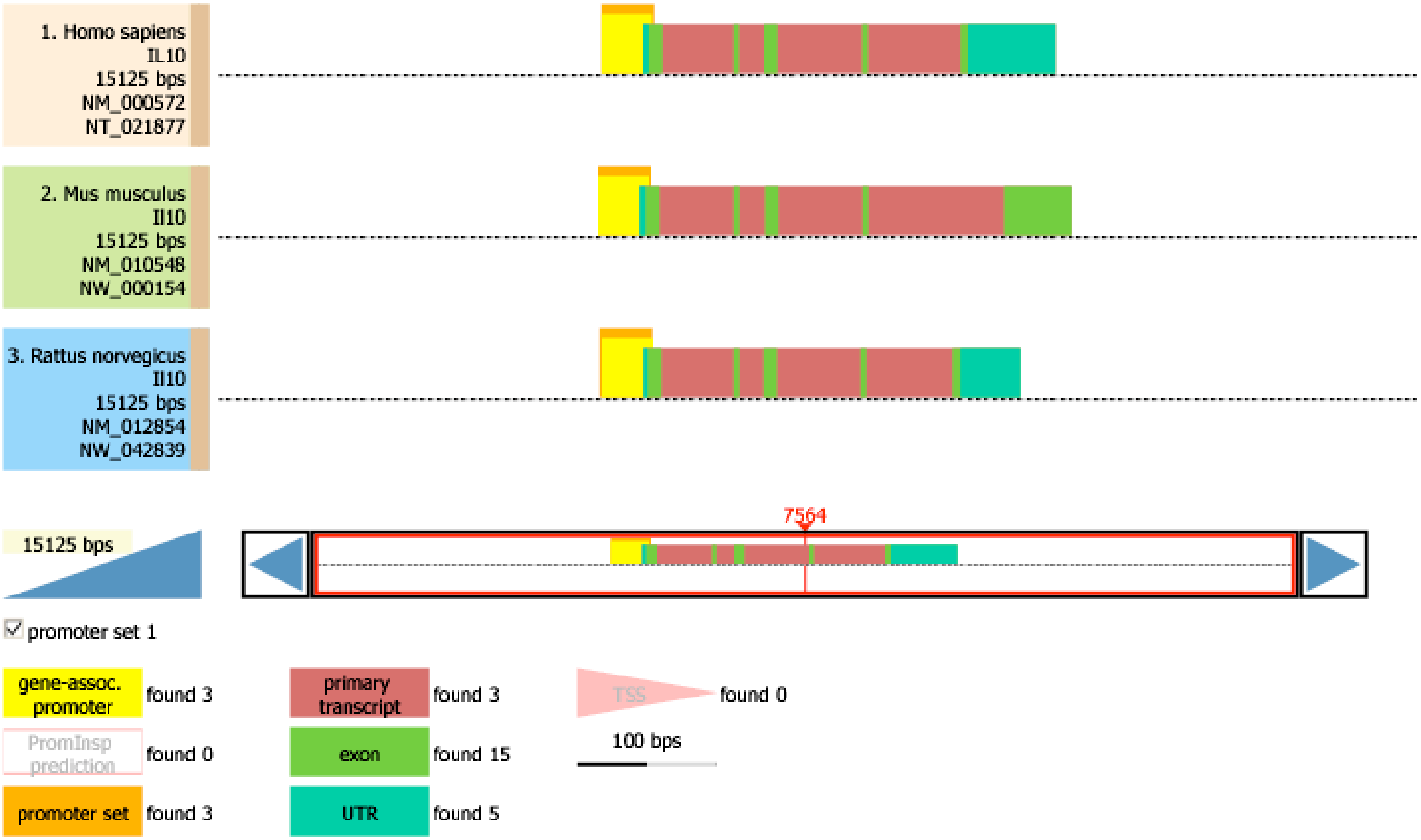
Genomatix ElDorado is a unique promoter source

ElDorado provides human, mouse, and rat IL-10 promoters



1. Homo sapiens
IL10
15125 bps
NM_000572
NT_021877

2. Mus musculus
Il10
15125 bps
NM_010548
NW_000154

3. Rattus norvegicus
Il10
15125 bps
NM_012854
NW_042839

☑ promoter set 1

gene-assoc. promoter	found 3	primary transcript	found 3	TSS	found 0
PromInsp prediction	found 0	exon	found 15		
promoter set	found 3	UTR	found 5		

Task 2

What you will be doing next

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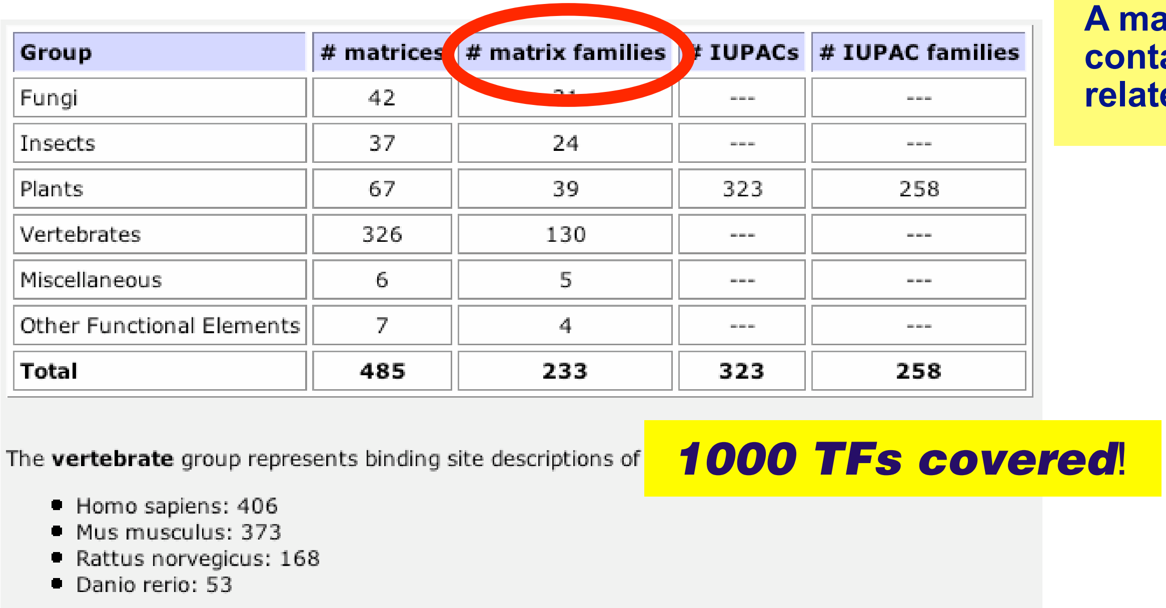
Genomatix IL-10 project initial in silico analysis

***MatInspector*: Finding TF binding sites by weight matrices**

- A IUPAC consensus sequence cannot tolerate mismatches
- A weight matrix can tolerate mismatches



Group	# matrices	# matrix families	# IUPACs	# IUPAC families
Fungi	42	[illegible]	---	---
Insects	37	24	---	---
Plants	67	39	323	258
Vertebrates	326	130	---	---
Miscellaneous	6	5	---	---
Other Functional Elements	7	4	---	---
Total	**485**	**233**	**323**	**258**

The **vertebrate** group represents binding site descriptions of

- Homo sapiens: 406
- Mus musculus: 373
- Rattus norvegicus: 168
- Danio rerio: 53

***1000 TFs covered*!**

A matrix family contains several related matrices

MatInspector can rapidly identify candidate binding sites and factors

IL-10 project TFBS analysis

MatInspector analysis of the human IL-10 promoter


132 matches found.

Human IL-10 promoter

```
TCCAGCCTCCATGGAATCCTGACTTCTTTTCCTTGTTATTTCAACTTCTTCCACCCCATCTTTTAAACTTTAGACTCCAG
CCACAGAAGCTTACAACTAAAAGAAACTCTAAGGCCAATTTAATCCAAGGTTTCATTCTATGTGCTGGAGATGGTGTACA
GTAGGGTGAGGAAACCAAATTCTCAGTTGGCACTGGTGTACCCTTGTACAGGTGATGTAACATCTCTGTGCCTCAGTTTG
CTCACTATAAAATAGAGACGGTAGGGGTCATGGTGAGCACTACCTGACTAGCATATAAGAAGCTTTCAGCAAGTGCAGAC
TACTCTTACCCACTTCCCCCAAGCACAGTTGGGGTGGGGGACAGCTGAAGAGGTGGAAACATGTGCCTGAGAATCCTAAT
GAAATCGGGGTAAAGGAGCCTGGAACACATCCTGTGACCCCGCCTGTCCTGTAGGAAGCCAGTCTCTGGAAAGTAAAATG
GAAGGGCTGCTTGGGAACTTTGAGGATATTTAGCCCACCCCCTCATTTTTACTTGGGGAAACTAAGGCCCAGAGACCTAA
GGTGACTGCCTAAGTTAGCAAGGAGAAGTCTTGGGTATTCATCCCAGGTTGGGGGGACCCAATTATTTCTCAATCCCATT
GTATTCTGGAATGGGCAATTTGTCCACGTCACTGTGACCTAGGAACACGCGAATGAGAACCCACAGCTGAGGGCCTCTGC
GCACAGAACAGCTGTTCTCCCAGGAAATCAACCTTTTTTTAATTGAGAAGCTAAAAAATTATTCTAAGAGAGGTAGCCCA
TCCTAAAAATAGCTGTAATGCAGAAGTTCATGTTCAACCAATCATTTTTGCTTACCATCCAAAAAATTGAAAACTAAGTTT
ATTAGAGAGGTTAGAGAAGGAGGAGCTCTAAGCAGAAAAATCCTGTGCCGGGAAACCTTGATTGTGGCTTTTTAATGCAA
TGAAGAGGCCTCCCTGAGCTTACAATATAAAAGGGGGACAGAGAGGTGAAGGTCTACACATCAGGGGCTTGCTCTTGCAA
AACCAAACCACAAGACAGACTTGCAAAAGAAGGC
```

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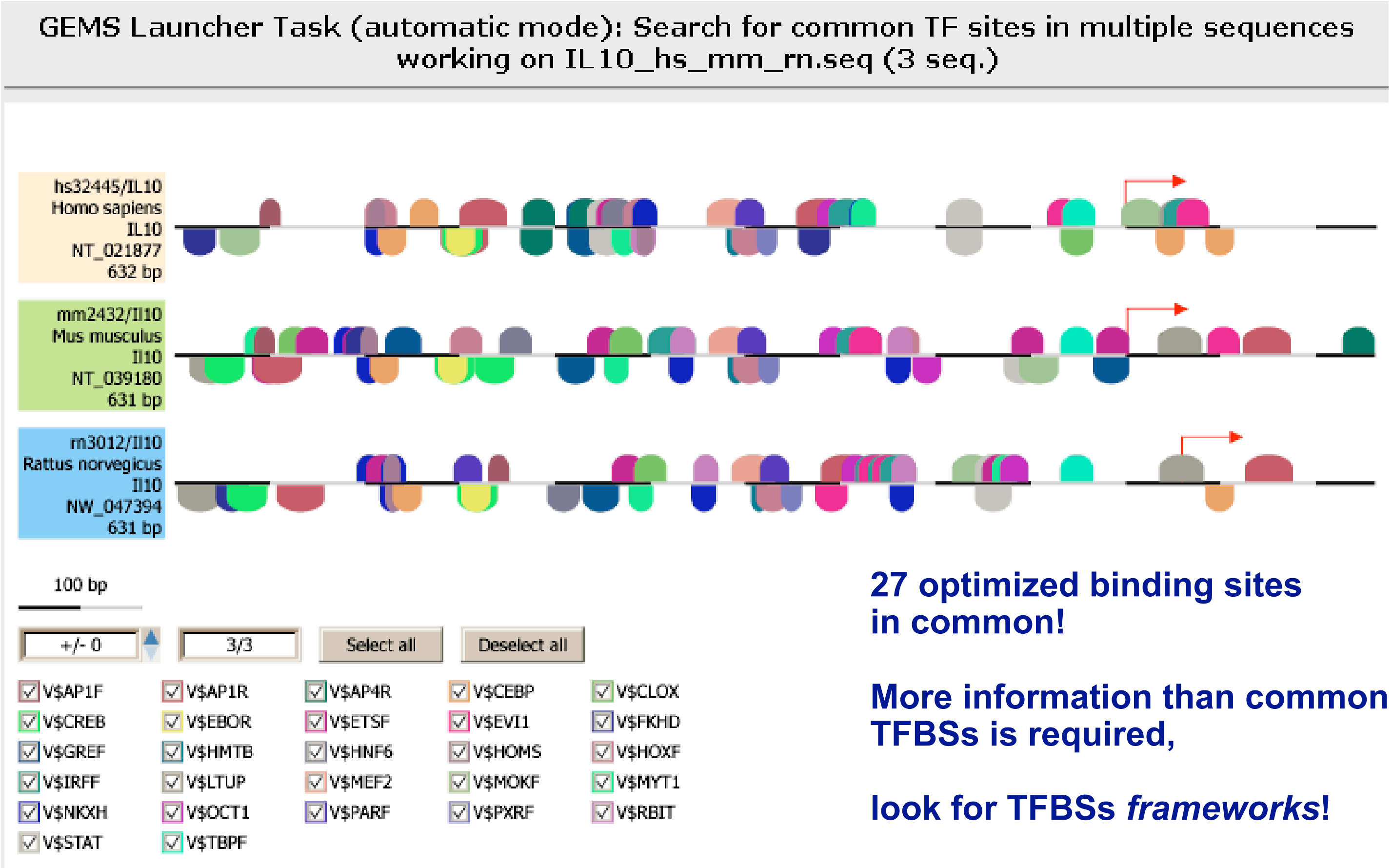
Beyond simple TFBS analysis

TFBSs shared between human mouse and rat promoter



GEMS Launcher Task (automatic mode): Search for common TF sites in multiple sequences
working on IL10_hs_mm_rn.seq (3 seq.)

☑ V$AP1F ☑ V$AP1R ☑ V$AP4R ☑ V$CEBP ☑ V$CLOX
☑ V$CREB ☑ V$EBOR ☑ V$ETSF ☑ V$EVI1 ☑ V$FKHD
☑ V$GREF ☑ V$HMTB ☑ V$HNF6 ☑ V$HOMS ☑ V$HOXF
☑ V$IRFF ☑ V$LTUP ☑ V$MEF2 ☑ V$MOKF ☑ V$MYT1
☑ V$NKXH ☑ V$OCT1 ☑ V$PARF ☑ V$PXRF ☑ V$RBIT
☑ V$STAT ☑ V$TBPF

27 optimized binding sites in common!

More information than common TFBSs is required,

look for TFBSs *frameworks*!

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Task 3

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IL-10 promoter frameworks

Exploit knowledge about the IL-10 promoters

- IL-10 is very well covered in the literature (more than 1000 papers!)
- At this point we are only interested in TFs linked to IL-10
- ***BiblioSphere*** automatically analyzes the whole PubMed for TF links
- Get candidates from the IL-10 ***BiblioSphere***!
- Start with the TFs with BSs in the IL-10 promoter and cocited with IL-10

Focus the framework analysis on likely candidate TFBSs already known

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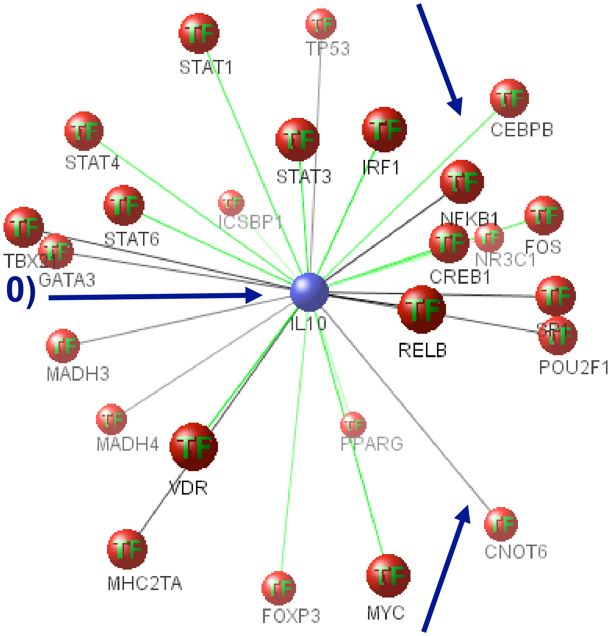
IL-10 promoter frameworks

Transcriptional context of IL-10 identified by ***BiblioSphere***

- IL-10 is cocited with 1209 genes
- 96 are transcription factors
- ***BiblioSphere*** determined TFBS for 10 TFs in the IL-10 promoter
  - **AP1**
  - **CEBP**
  - **CREB**
  - **EBOX**
  - **FKHD**
  - **GRE**
  - **IRF**
  - **PERO**
  - **RXRF**
  - **STAT**

Green connection:
Binding site confirmed
In promoter sequence

Reference gene (IL-10)



Gray connection:
Binding site not found
In promoter sequence

Parameters (default)
TFs, cocited on sentence level
minimum 2 cocitations

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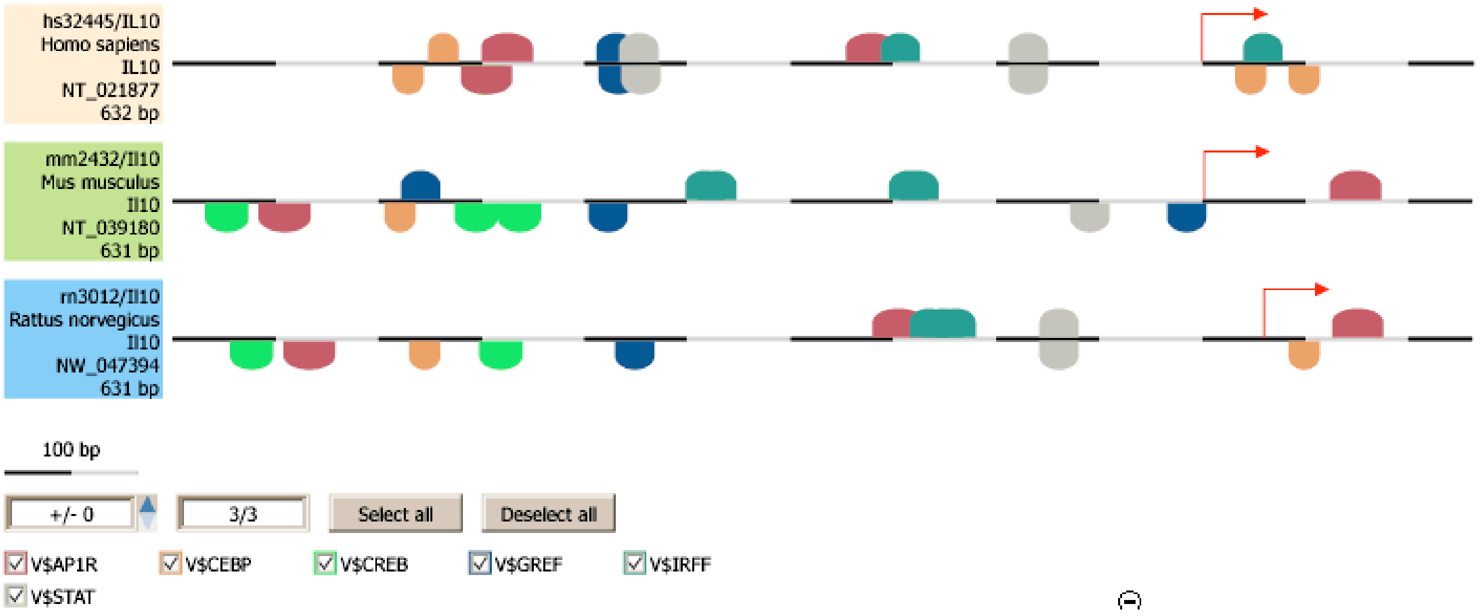
IL-10 promoter frameworks

Common TFs of human and mouse IL-10 promoter

- Only the BS for the 10 TFs identified by ***BiblioSphere*** are shown



+/- 0 | 3/3

☑ V$AP1R ☑ V$CEBP ☑ V$CREB ☑ V$GREF ☑ V$IRFF
☑ V$STAT

Frameworks are not obvious even with only 6 TFBSs to be considered!

Task 4

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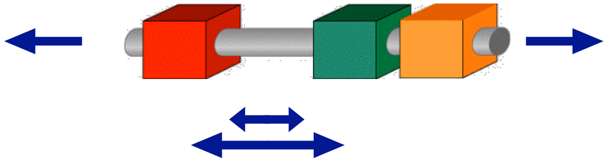
FrameWorks and Modules

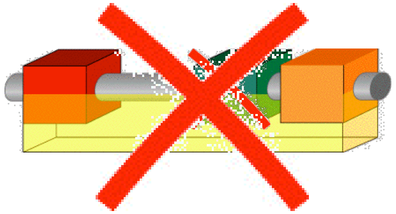
Describing promoter/enhancer TFBSs organization

- A *framework* describes a conserved set of transcription elements



- The distances of TFBSs within a *framework* are variable as is the position of the whole framework within different sequences

- A *module* describes experimentally verified mutually dependent functional transcription elements



Delete a single site - lose function of all

Modules definitely have a biological function, frameworks may have a function

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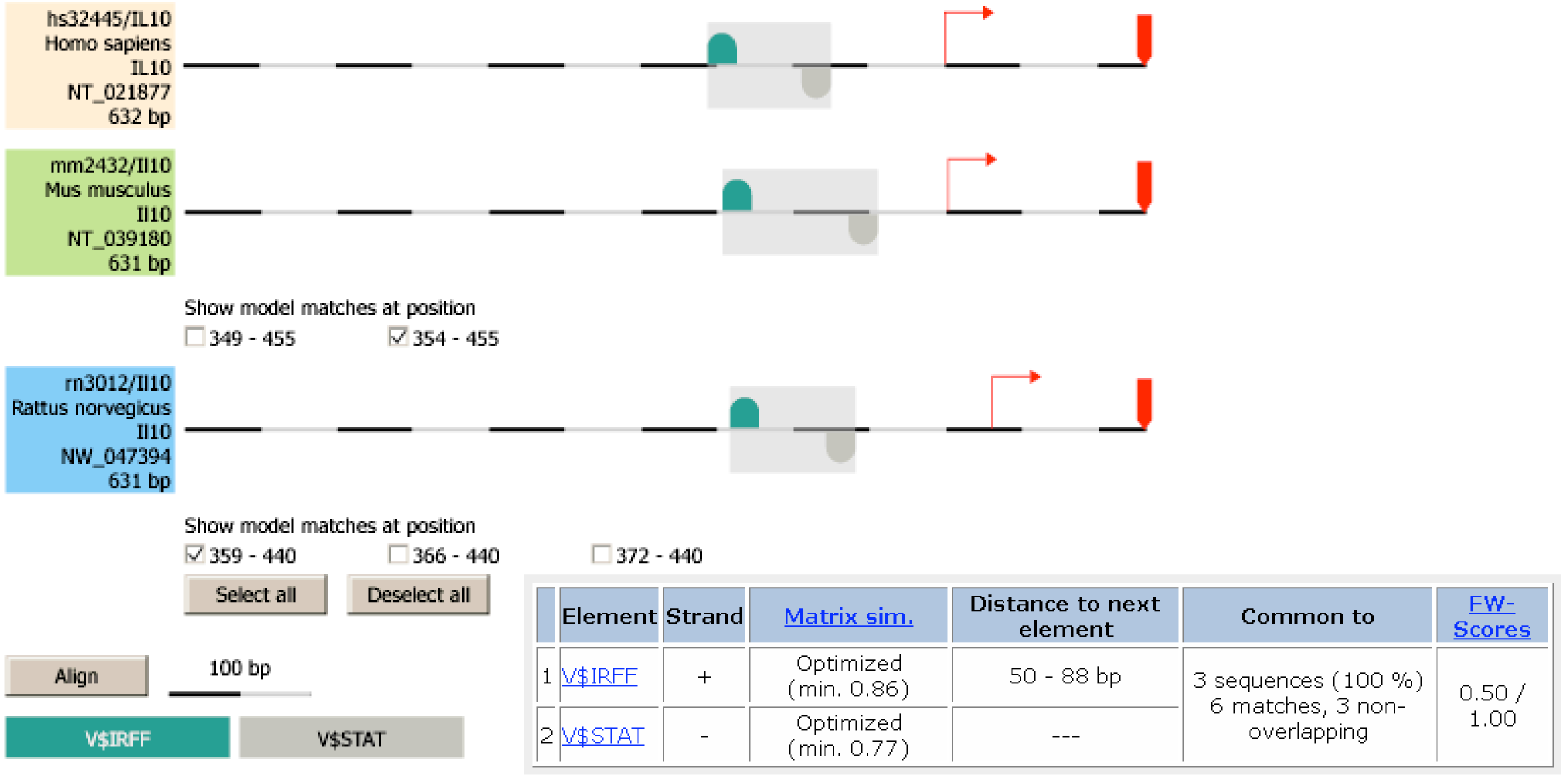
IL-10 promoter frameworks

FrameWorker identified a single framework including the functional STAT binding site



Show model matches at position
☐ 349 - 455 ☑ 354 - 455

Show model matches at position
☑ 359 - 440 ☐ 366 - 440 ☐ 372 - 440

	Element	Strand	Matrix sim.	Distance to next element	Common to	FW-Scores
1	V$IRFF	+	Optimized (min. 0.86)	50 - 88 bp	3 sequences (100 %) 6 matches, 3 non-overlapping	0.50 / 1.00
2	V$STAT	-	Optimized (min. 0.77)	---		

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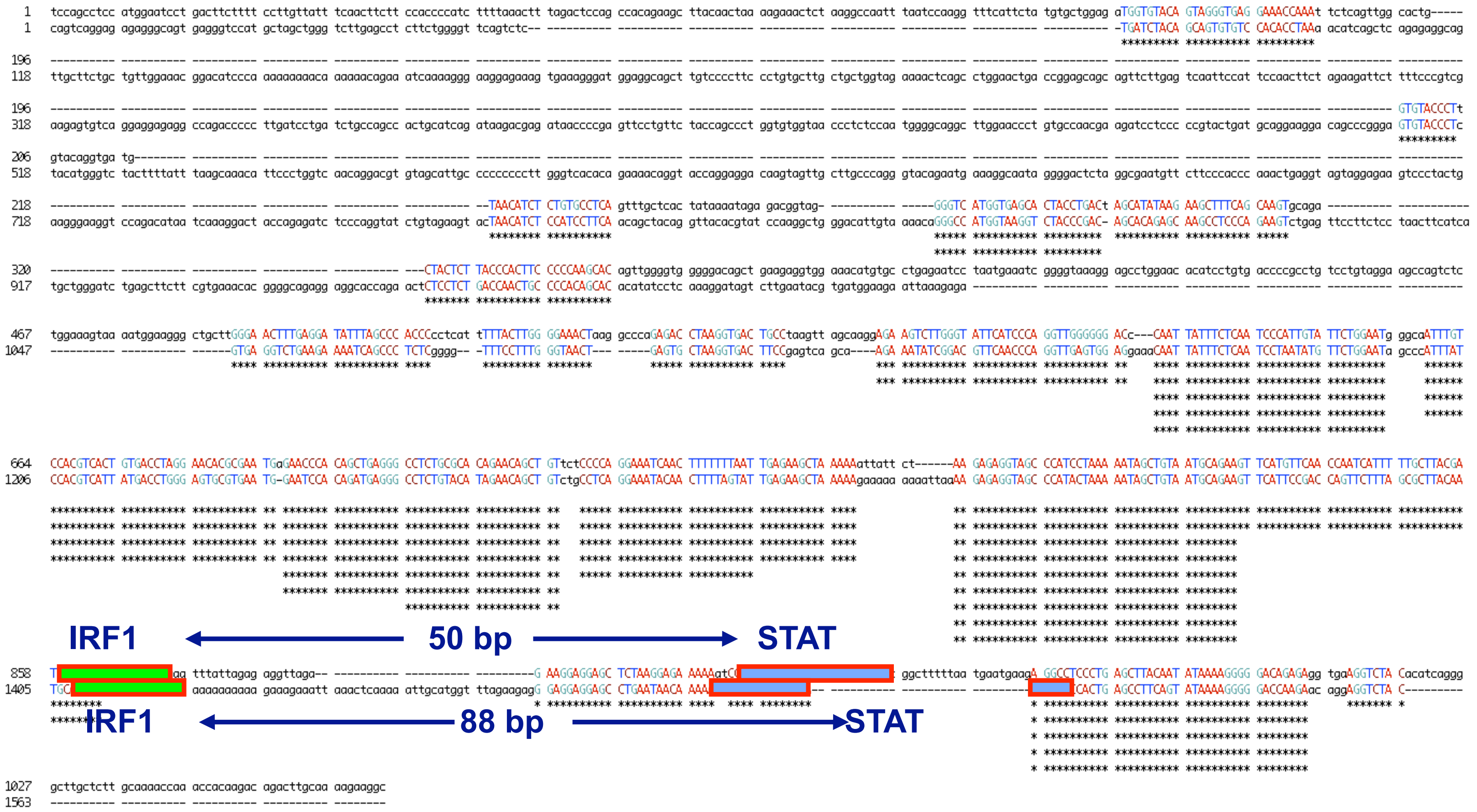
IL-10 promoter frameworks

The IRF1-STAT TFBS pair is conserved, the sequence is not!



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   1  tccagcctcc atggaatcct gacttctttt ccttgttatt tcaacttctt ccaccccatc ttttaaactt tagactccag ccacagaagc ttacaactaa aagaaactct aaggccaatt taatccaagg tttcattcta tgtgctggag aTGGTGTACA GTAGGGTGAG GAAACCAAAt tctcagttgg cactg-----
   1  cagtcaggag agagggcagt gagggtccat gctagctggg tcttgagcct cttctggggt tcagtctc-- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -TGATCTACA GCAGTGTGTC CACACCTAAa acatcagctc agagaggcag
                                                                                                                                                                                    ********* ********** *********

 196  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
 118  ttgcttctgc tgttggaaac ggacatccca aaaaaaaaca aaaaacagaa atcaaaaggg aaggagaaag tgaaagggat ggaggcagct tgtccccttc cctgtgcttg ctgctggtag aaaactcagc ctggaactga ccggagcagc agttcttgag tcaattccat tccaacttct agaagattct tttcccgtcg

 196  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- GTGTACCCTt
 318  aagagtgtca ggaggagagg ccagaccccc ttgatcctga tctgccagcc actgcatcag ataagacgag ataaccccga gttcctgttc taccagccct ggtgtggtaa ccctctccaa tggggcaggc ttggaaccct gtgccaacga agatcctccc ccgtactgat gcaggaagga cagcccggga GTGTACCCTc
                                                                                                                                                                                                                       *********

 206  gtacaggtga tg-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
 518  tacatgggtc tacttttatt taagcaaaca ttccctggtc aacaggacgt gtagcattgc cccccccctt gggtcacaca gaaaacaggt accaggagga caagtagttg cttgcccagg gtacagaatg aaaggcaata ggggactcta ggcgaatgtt cttcccaccc aaactgaggt agtaggagaa gtccctactg

 218  ---------- ---------- ---------- ---------- ---------- ---------- --TAACATCT CTGTGCCTCA gtttgctcac tataaataga gacggtag- ---------- -----GGGTC ATGGTGAGCA CTACCTGACt AGCATATAAG AAGCTTTCAG CAAGTgcaga ---------- ----------
 718  aagggaaggt ccagacataa tcaaaggact accagagatc tcccaggtat ctgtagaagt acTAACATCT CCATCCTTCA acagctacag gttacacgtat ccaaggctg ggacattgta aaacaGGGCC ATGGTAAGGT CTACCCGAC- AGCACAGAGC AAGCCTCCCA GAAGTctgag ttccttctcc taacttcatca
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                                                                                                                                               ***** ********** *********

 320  ---------- ---------- ---------- ---------- ---------- ---CTACTCT TACCCACTTC CCCCAAGCAC agttggggtg ggggacagct gaagaggtgg aaacatgtgc ctgagaatcc taatgaaatc ggggtaaagg agcctggaac acatcctgtg accccgcctg tcctgtagga agccagtctc
 917  tgctgggatc tgagcttctt cgtgaaacac ggggcagagg aggcaccaga actCTCCTCT GACCAACTGC CCCACAGCAC acatatcctc aaaggatagt cttgaatacg tgatggaaga attaaagaga ---------- ---------- ---------- ---------- ---------- ---------- ----------
                                                                ******* ********** **********

 467  tggaaagtaa aatggaaggg ctgcttGGGA ACTTTGAGGA TATTTAGCCC ACCCcctcat tTTTACTTGG GGAAACTaag gcccaGAGAC CTAAGGTGAC TGCCtaagtt agcaaggAGA AGTCTTGGGT ATTCATCCCA GGTTGGGGGG ACc---CAAT TATTTCTCAA TCCCATTGTA TTCTGGAATg ggcaATTTGT
1047  ---------- ---------- ------GTGA GGTCTGAAGA AAATCAGCCC TCTCgggg-- -TTTCCTTTG GGTAACT--- -----GAGTG CTAAGGTGAC TTCCgagtca gca----AGA AATATCGGAC GTTCAACCCA GGTTGAGTGG AGgaaaCAAT TATTTCTCAA TCCTAATATG TTCTGGAATa gcccATTTAT
                                  **** ********** ********** ****        ********* *******         ***** ********** ****              *** ********** ********** ********** **    **** ********** ********** *********      ******
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 664  CCACGTCACT GTGACCTAGG AACACGCGAA TGaGAACCCA CAGCTGAGGG CCTCTGCGCA CAGAACAGCT GTtctCCCCA GGAAATCAAC TTTTTTTAAT TGAGAAGCTA AAAAattatt ct------AA GAGAGGTAGC CCATCCTAAA AATAGCTGTA ATGCAGAAGT TCATGTTCAA CCAATCATTT TTGCTTACGA
1206  CCACGTCATT ATGACCTGGG AGTGCGTGAA TG-GAATCCA CAGATGAGGG CCTCTGTACA TAGAACAGCT GTctgCCTCA GGAAATACAA CTTTTAGTAT TGAGAAGCTA AAAAgaaaaa aaaattaaAA GAGAGGTAGC CCATACTAAA AATAGCTGTA ATGCAGAAGT TCATTCCGAC CAGTTCTTTA GCGCTTACAA

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 858  T                  ag tttattagag aggttaga-- ---------- ---------- ---------G AAGGAGGAGC TCTAAGGAGA AAAAatC                       ggctttttaa tgaatgaagA GGCCTCCCTG AGCTTACAAT ATAAAAGGGG GACAGAGAgg tgaAGGTCTA Cacatcaggg
1405  TGC                   aaaaaaaaaa gaaagaaatt aaactcaaaa attgcatggt ttagaagagG GAGGAGGAGC CTGAATAACA AAA                  -- ---------- ---------- --------       CACTG AGCCTTCAGT ATAAAAGGGG GACCAAGAac aggAGGTCTA C---------
      ********                                                                   * ********** ********** ****  **** ********                                  * ********** ********** ********** ********      ******* *
      *******                                                                                                                                                 * ********** ********** ********** ********
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1027  gcttgctctt gcaaaaccaa accacaagac agacttgcaa aagaaggc
1563  ---------- ---------- ---------- ---------- --------
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Task 5

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IL-10 promoter frameworks

Database search with the IL-10 IRF-STAT framework

- 221 matches located in the Eukaryotic Promoter Database (EPD, 4.6%)
- Among those were:

- IFNA13
- IFNA6
- IFNA5
- IFNA7

- IFNA16
- IL-2
- IL1B
- IL2RG

- MHCI
- HLA-D
- HLA-E

- and several other Interferon target genes

- All together 11 of 221 matches were directly IFN/IRF/STAT related

The IL-10 IRF-STAT module identified other potential IFN-alpha targets

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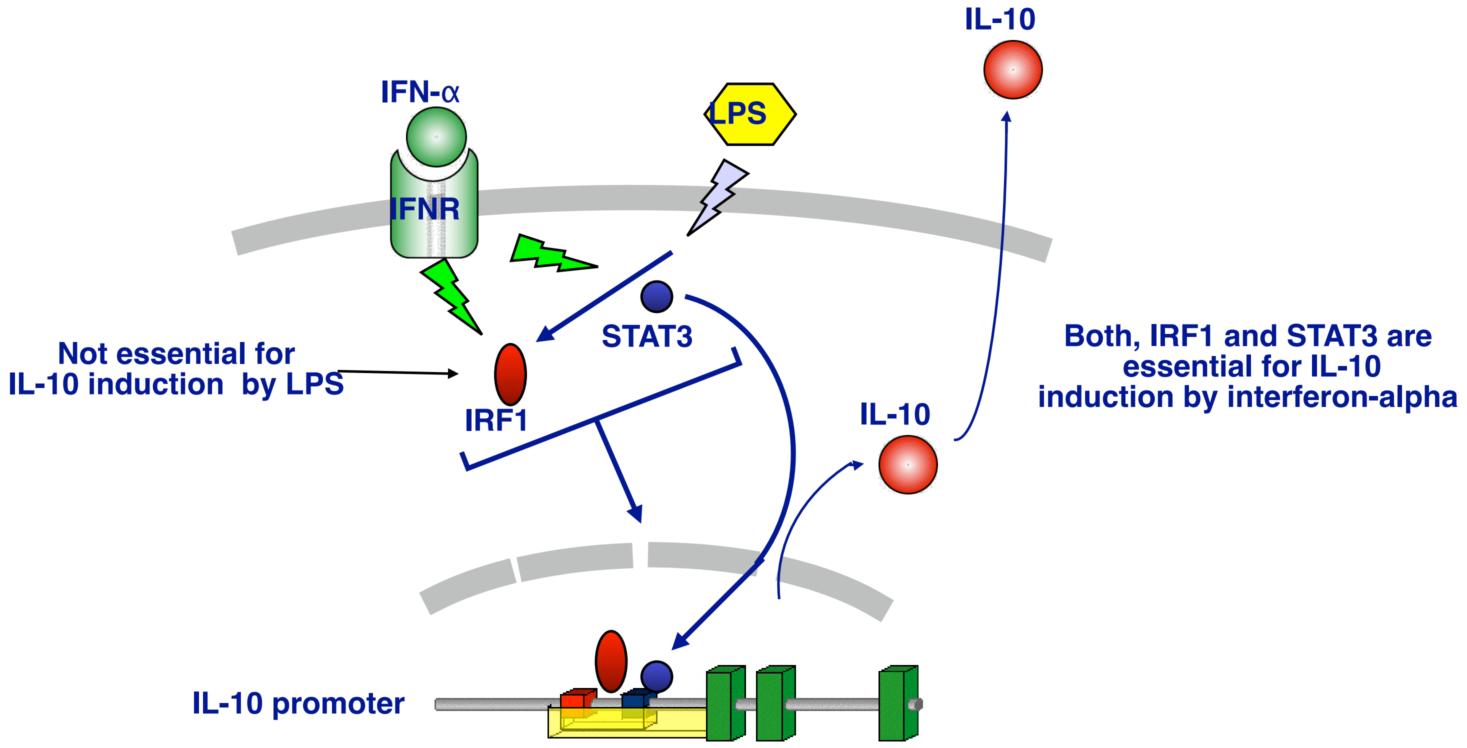
IL-10 promoter summary

The IL-10 promoter as signal processor



Not essential for
IL-10 induction by LPS

Both, IRF1 and STAT3 are
essential for IL-10
induction by interferon-alpha

Molecular basis for two pathways converging on the IL-10 promoter elucidated

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Acknowledgements

The IL-10 promoter analysis was an international cooperation

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Marion Frankenberger
Löms Ziegler-Heitbrock

GSF-National Research Center for
Environment and Health
Gauting, Germany

Annette Schäfer

...and *Genomatix*

...now it is your turn!