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U599 Cancer Institute of Marseille

INSTITUT
CENTRE RÉGIONAL DE LUTTE CONTRE LE CANCER
PAOLI-CALMETTES
PROVENCE-ALPES-CÔTE D'AZUR

UNIVERSITÉ DE LA MEDITERRANÉE AIX-MARSEILLE II

Analysis of Gene Regulatory Regions using Comparative Genomics: From the wet lab to computer and back

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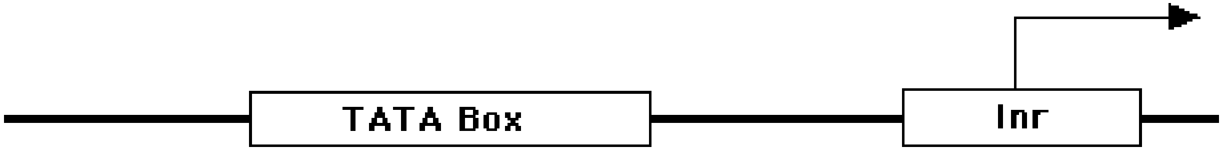
et d 'Immunologie de Marseille

INSERM Workshops 2004 n°152:
Comparative genomics of vertebrate: concepts and bioinformatic tools
May 27-28, 2004
La Londe – Les Maures, France

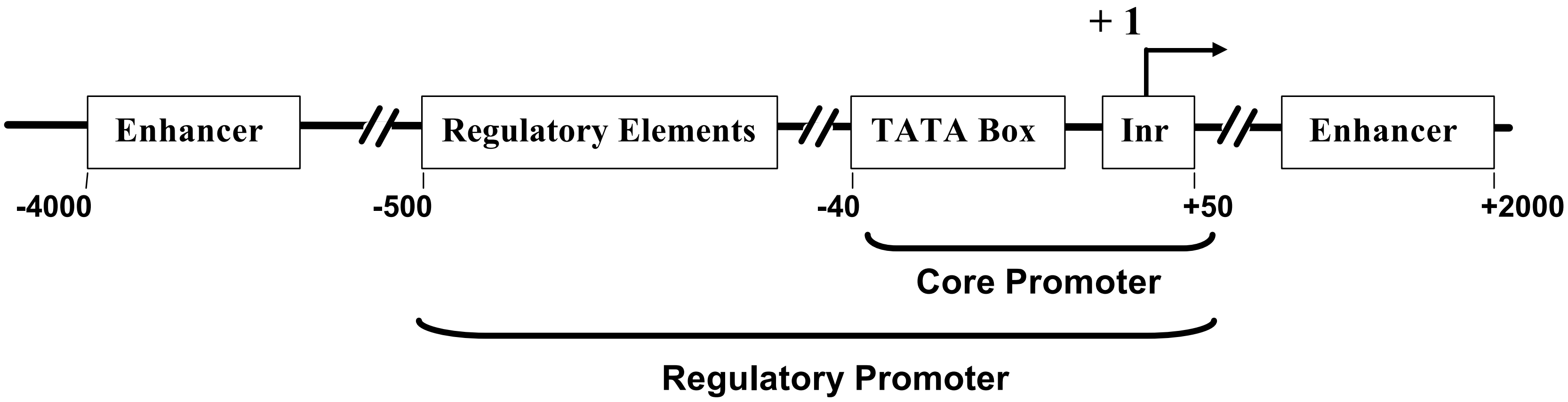
CORE PROMOTER



TATA Box: TATAAAA (about 25 base pairs upstream of the start point)

Initiator (Inr): PyPyCAPyPyPyPyPy

MODEL OF TYPICAL GENE PROMOTER AND REGULATORY REGIONS



Eukaryotic Promoter Classes

Pol I	< 1%	3/4 rRNAs (28S, 18S, 5.8S)
Pol II with TATA-box	> 70%	mRNAs
Pol II without TATA-box	~ 20%	
Pol III internal	~ 5%	Small RNAs tRNAs 5S RNAs
Pol III upstream with TATA-box	< 1%	
Pol III upstream without TATA-box	< 1%	

Components of Eukaryotic Promoters and Regulatory Regions

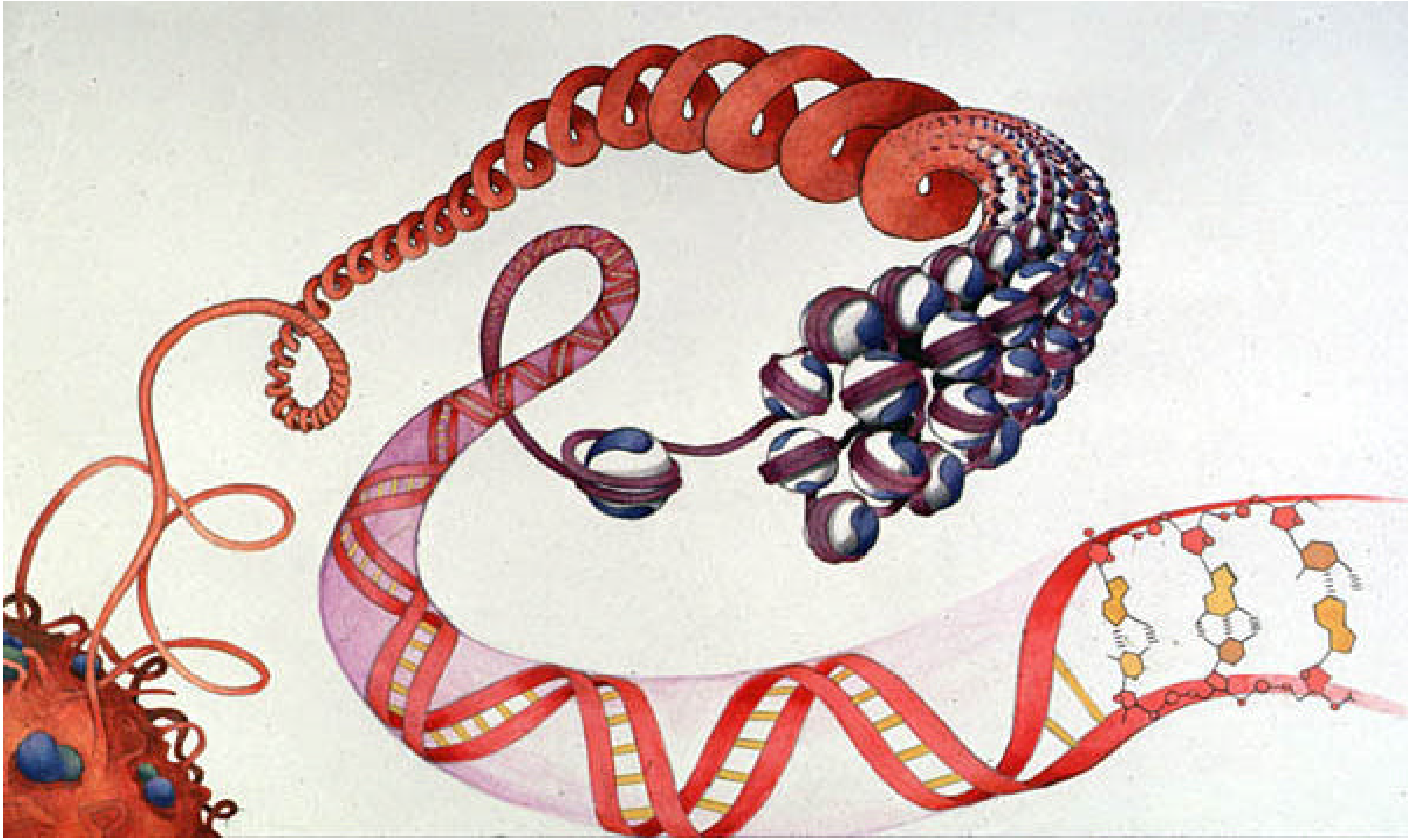
• Site selector elements	TATA-box, Initiator
• Common upstream elements	CCAAT-box, GC-box
• Regulatory elements	HSE, SRE, GRE, etc.

- Enhancers / Silencers
- Locus control regions (LCRs)
- Scaffold / Matrix attachment sites (SARs / MARs)

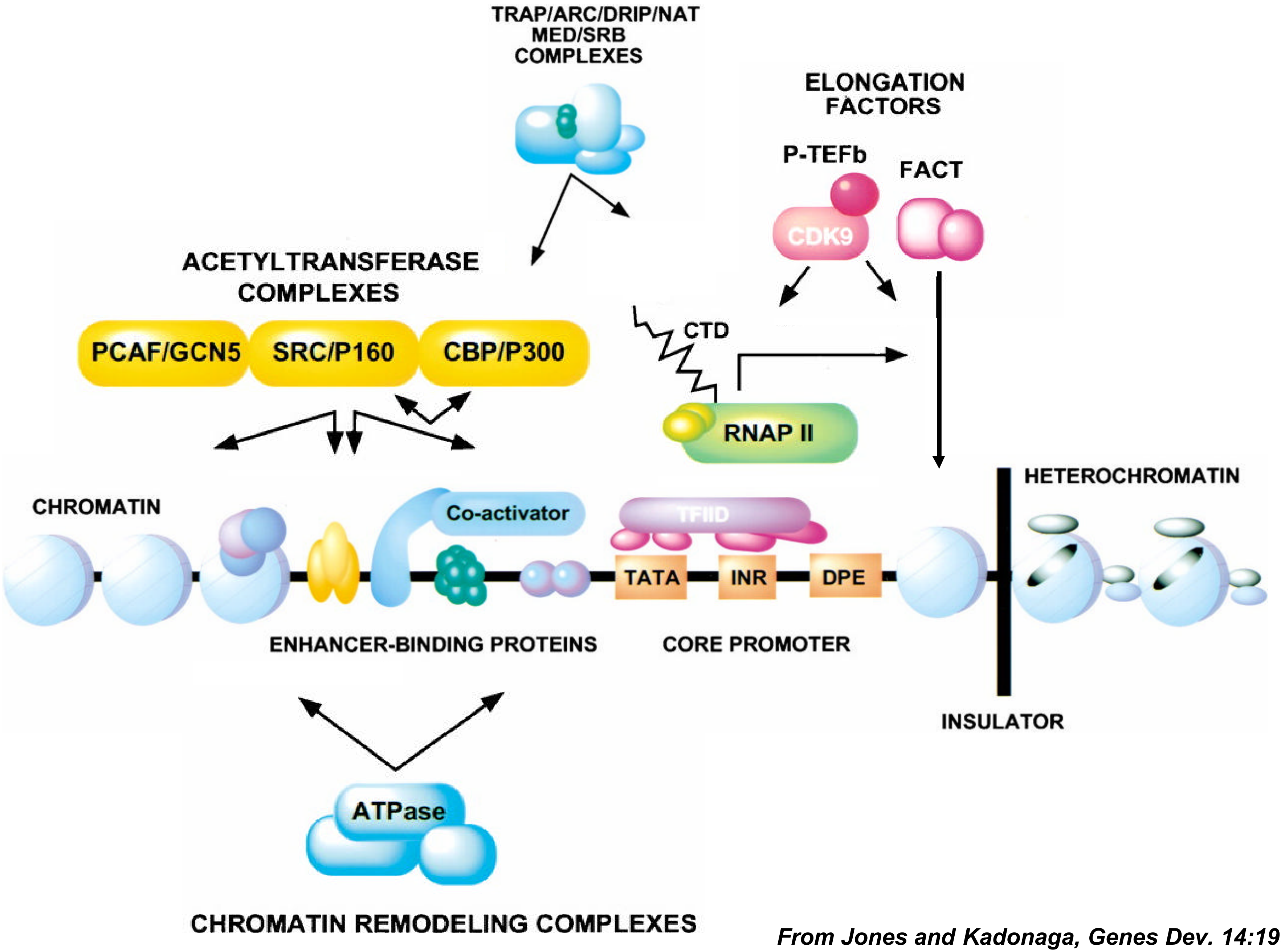
- CpG islands

Promoter Regulatory Elements: Features and Facts

- Degenerate sequence motifs
- Length: 6 to 20 bp
- Low complexity (8-12 bits)
- Binding sites of transcription factors
- Excess of binding sites over binding proteins in the nucleus
- Most in vitro binding sites not functional in vivo
- Some in vivo binding sites also not functional
- Regulatory potentials depends on cooperative effects between multiple elements



From G. Almouzni, Institut Curie, Paris



From Jones and Kadonaga, Genes Dev. 14:1992-1996, 2000.

TOOLS FOR THE IDENTIFICATION OF REGULATORY SEQUENCES AND THEIR COGNATE TRANSCRIPTION FACTORS

1. Identification and characterization of regulatory sequences

- Gene reporter assays: transient or stable transfection (CAT, Luciférase, SEAP, GFP, b-gal, etc.)
- *In vitro* transcription assay
- Sequencing, database mining (web: TESS, Euk. Pr. Database, TRANSFAC, MATINSPECTOR, TFSEARCH, etc.)
- Animal or cellular models: enhancer trap, enhancer knock-in, minichromosomes, etc.

2. Identification and characterization of specific transcription factors (TFs)

- EMSA and sequels: UV-crosslinking, pull-down assay using biotynilated oligonucleotide
- Footprint detection:
  - * *in vitro :* nucleases (DNAse I hypersensitivity, S1 nuclease, Mmase) or chemical compounds
  - * *in vivo* : genomic footprinting, Chromatin ImmunoPrecipitation (ChIP) and sequels

3. TFs physical and functional interactions

- Transfection and biochemistry
- ChIP-on-chip

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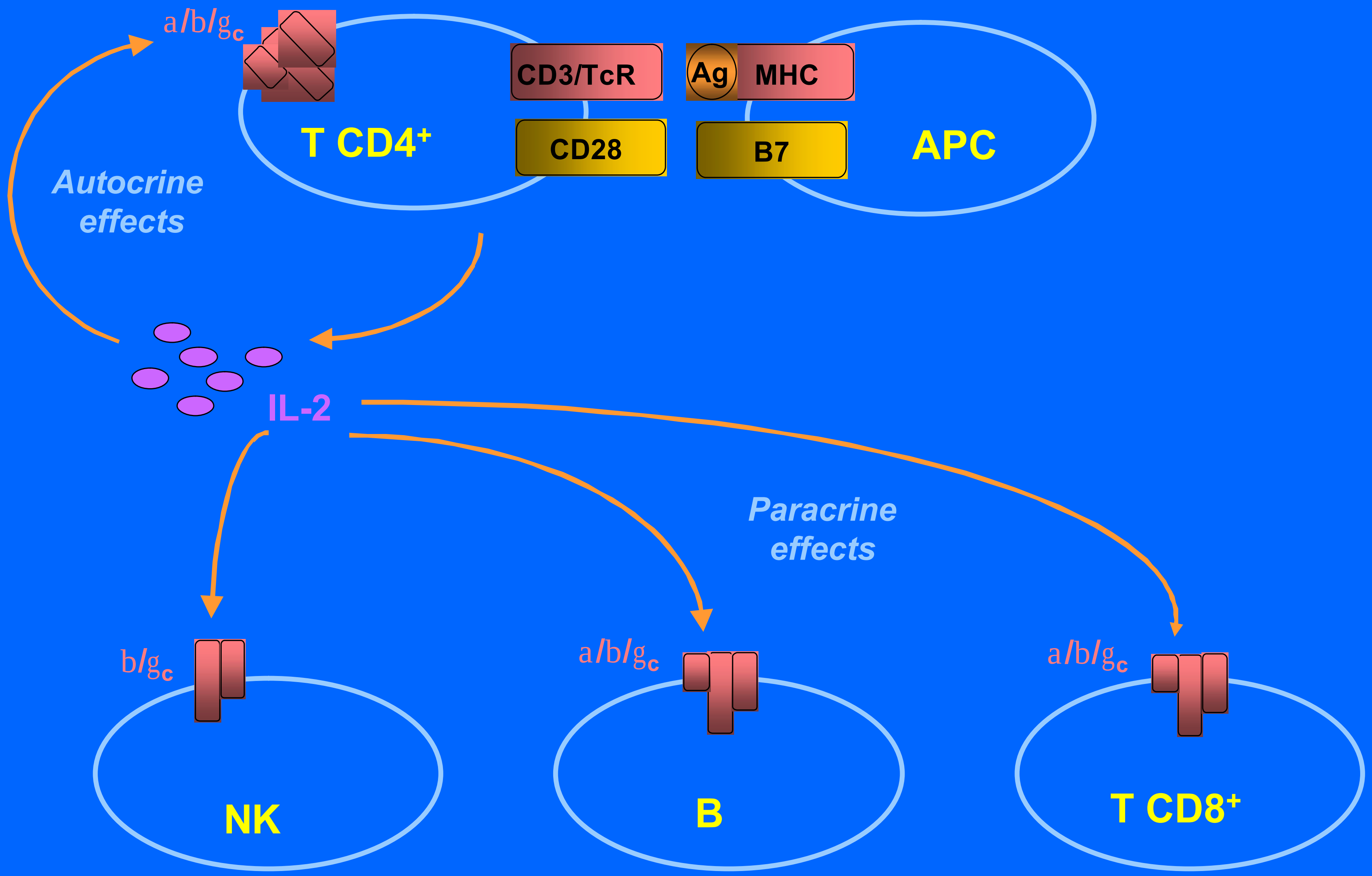
Control of CD25/IL-2Ra gene transcription

icim
Institut de Cancérologie
et d 'Immunologie de Marseille

Primary Activation



ROLE OF THE IL-2Ra CHAIN

- SOLE IL-2 SPECIFIC CHAIN

- *DE NOVO* EXPRESSION CREATES AN HIGH AFFINITY IL-2 RECEPTOR (Kd=10^{-11}) IN ASSOCIATION WITH THE INTERMEDIATE AFFINITY RECEPTOR b/g$_c$ (Kd=10^{-9}) SHARED WITH IL-4R, -7R, -9R AND -15R

- IL-2 $^{-/-}$ MICE ~ IL-2Ra $^{-/-}$ MICE

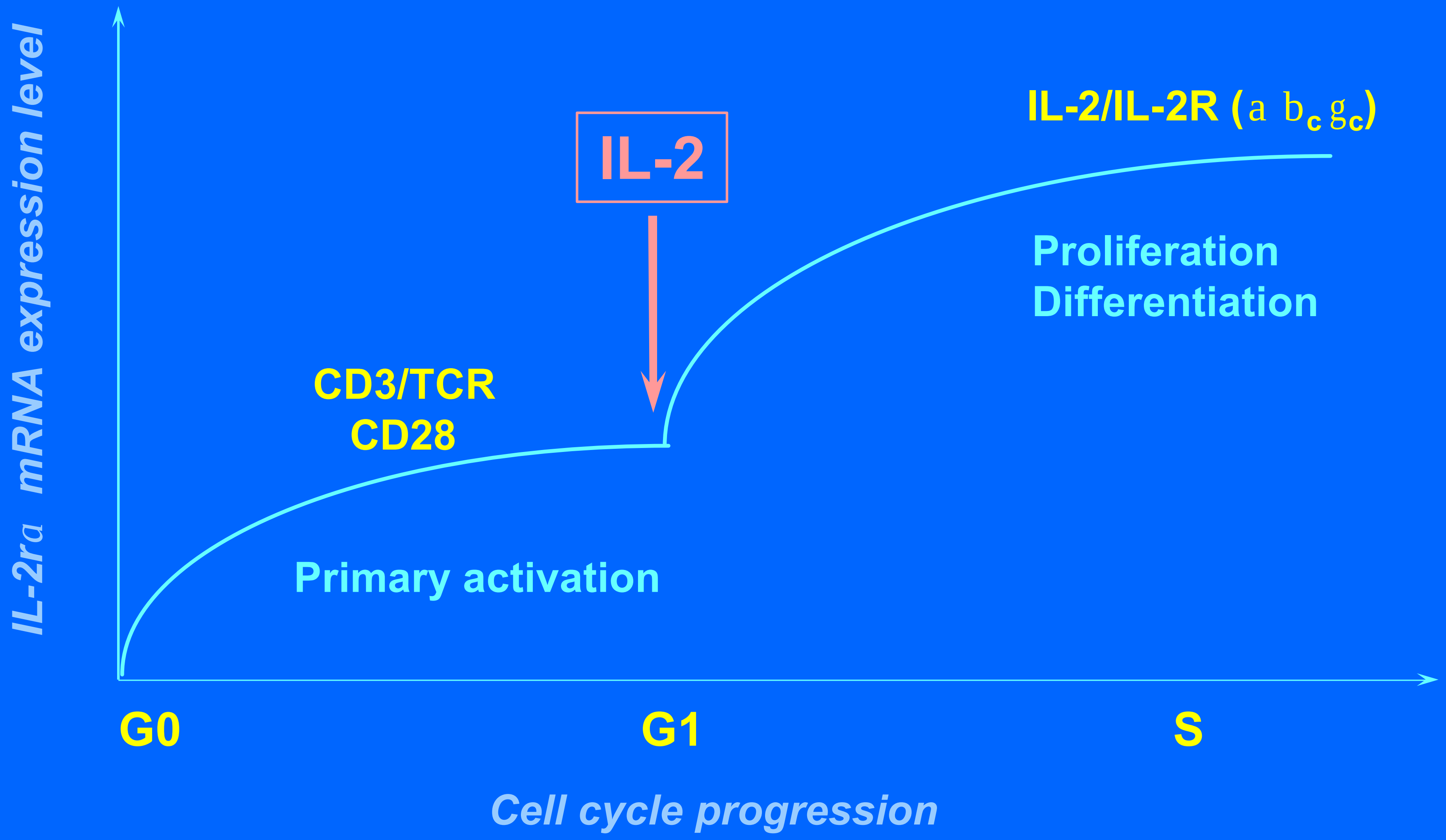
THE HIGH AFFINITY IL-2 RECEPTOR IS ESSENTIAL TO TRIGGER AND SUSTAIN AN EFFICIENT RESPONSE TO THE LIMITED AMOUNT OF SECRETED IL-2 AVAILABLE IN PHYSIOLOGICAL CONDITIONS

IL-2Ra CHAIN EXPRESSION IS CONTROLLED A TWO MAIN LEVELS:

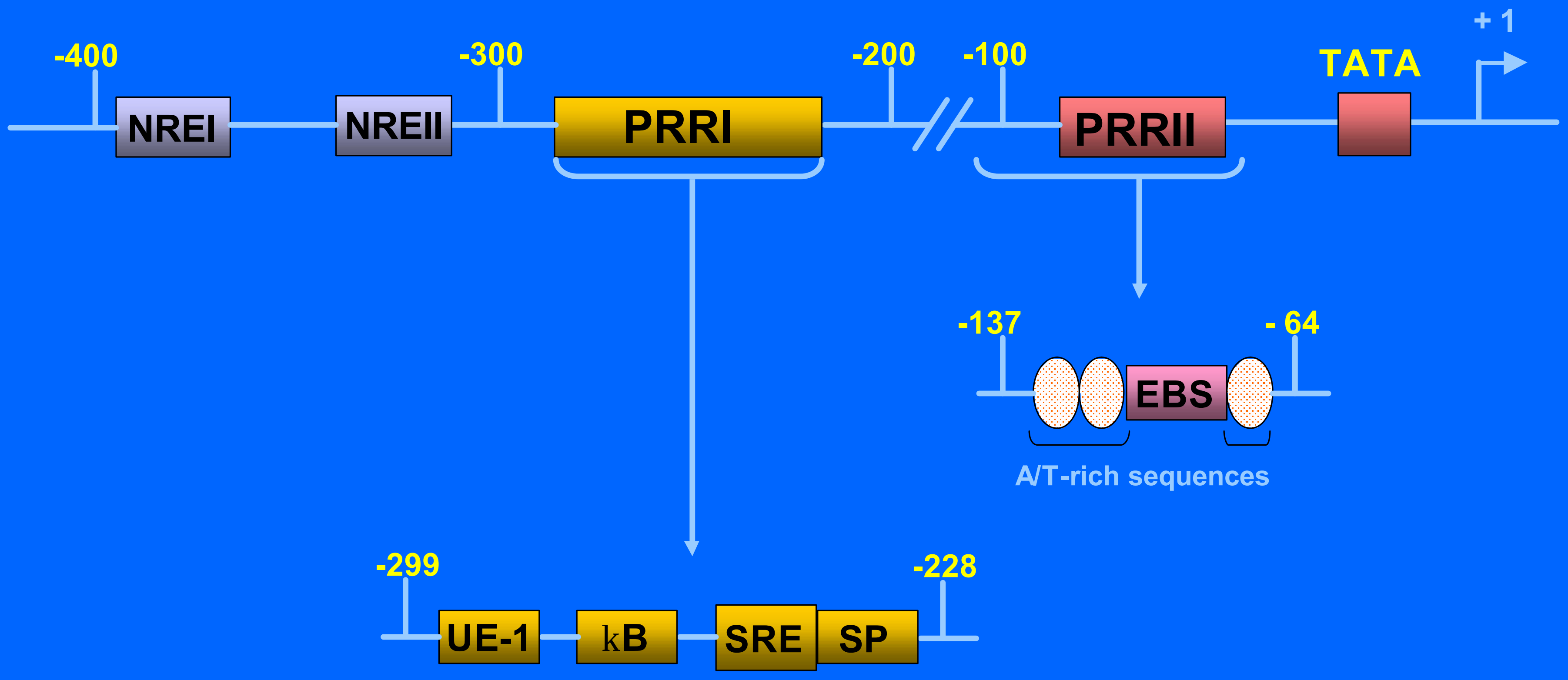
- TRANSCRIPTIONAL

- POST-TRANSCRIPTIONAL
  (mRNA stabilization)

CD25/IL-2Ra GENE TRANSCRIPTION DURING T CELL ACTIVATION

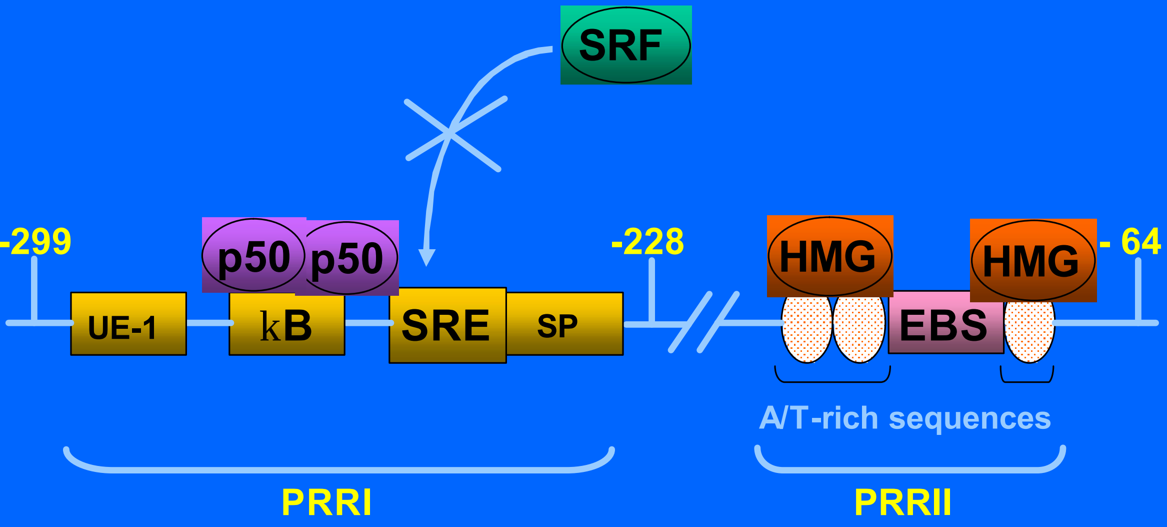


PROXIMAL REGULATORY REGIONS OF THE HUMAN CD25/IL-2Ra GENE



PRR: POSITIVE REGULATORY REGION

RESTING T LYMPHOCYTE



PRRI : Inactive NF-kB p50/p50 homodimers binding

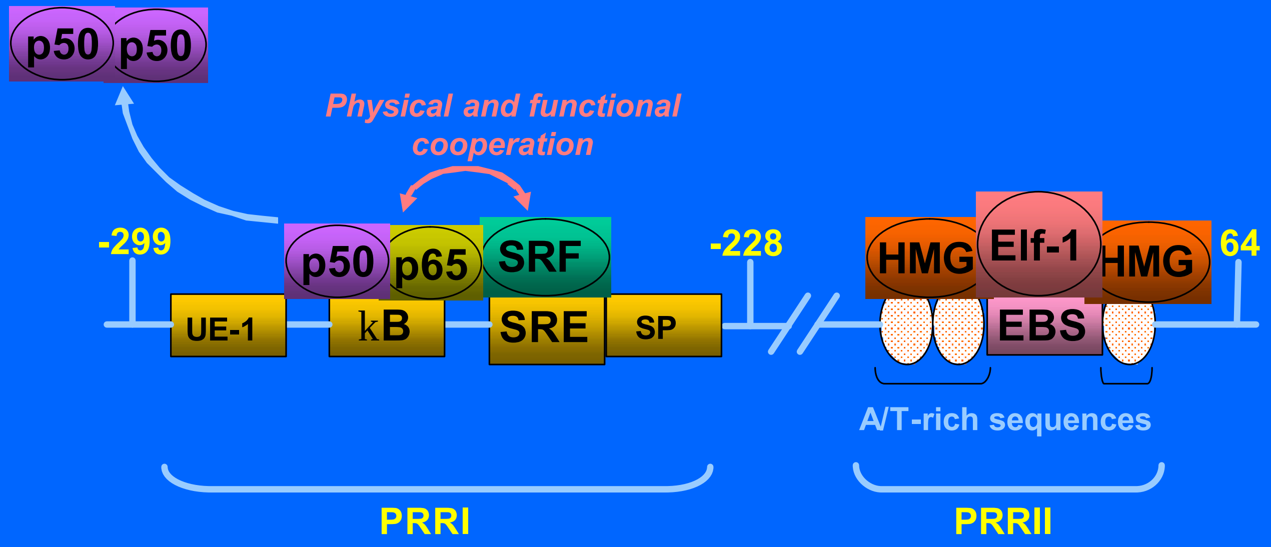
PRRII : HMGI(Y) constitutive binding

Algarte, M., Lecine, P., Costello, R., Plet, A., Olive, D. and Imbert, J. In vivo regulation of interleukin-2 receptor alpha chain gene transcription by the coordinated binding of constitutive and inducible factors in human primary T-cells. EMBO J. 14: 5060-5072; 1995.

Primary activation

↓

ACTIVATED T LYMPHOCYTE



PRRI
(induction)

- Active NF-kB p50/p65 or p65/c-Rel heterodimer binding
- SRF binding
- Physical and functional intercation SRF / NF-kB

Ballard et al., Science, 1988; Toledano et al., PNAS, 1990; Costello et al., CGD, 1993; Algarte et al., EMBO J, 1995; John et al., MCB, 1995

PRRII
(T cell specificty)

- Elf-1 binding

John et al., MCB, 1995

Homo Sapiens versus Mus Musculus PRRI/II conservation

Local identities (aligment n°6)

```
8724-8798 <--> 11629-11703 83% (75 nt)
8800-8886 <--> 11704-11790 76% (87 nt)
8887-8911 <--> 11792-11816 96% (25 nt)
8913-8946 <--> 11817-11850 97% (34 nt)
8954-8979 <--> 11851-11876 100% (26 nt)
8982-8999 <--> 11877-11894 83% (18 nt)
9003-9006 <--> 11895-11898 100% (4 nt)
9007-9015 <--> 11901-11909 78% (9 nt)
9017-9079 <--> 11910-11972 70% (63 nt)
9082-9128 <--> 11973-12019 51% (47 nt)
9129-9153 <--> 12021-12045 64% (25 nt)
9160-9202 <--> 12046-12088 58% (43 nt)
9203-9292 <--> 12090-12179 73% (90 nt)
9293-9313 <--> 12181-12201 62% (21 nt)
```

```
Seq 1 = ">HS_IL-2Ra_9.3KB genomic fragment from ENSG00000134460 5'->3'"
Seq 2 = ">MmIL-2Ra_12.5kb genomic fragment from phage Ch4C19 5'->3'"

         Local Alignment Number 6
         Similarity Score:  24894
         Match Percentage:  71 %
         Number of Matches:  425
         Number of Mismatches:  142
         Total Length of Gaps:  29
         Begins at (8724,11629) and Ends at (9313,12201)
    0     .    :    .    :    .    :    .    :    .    :
 8724 GAGGACTCAGCTTATGAAGTGCTGGGTGAGACCACTGCCAAGAAGTGCTT
      ||||||||||:|||::||:: ||::|||||||||||||||||||||||||
11629 GAGGACTCAGTTTACAAAACCCTAAGTGAGACCACTGCCAAGAAGTGCTT
   50     .    :    .    :  kB site   :    .  SRE    .SP1/
 8774 GCTCACCCTACCTTCAACGGCAGGGGAATCTCCCTCTCCTTTTATGGGCG
      ||||||||: ||| | :||||||||-||||:||||:||||| ||::|||:
11679 GCTCACCCCTCCTGCCGCGGCAGGG AATCCCCCTTTCCTTGTACAGGCA
  100 GC-box   :    .    :    .    :    .    :    .    :
 8824 TAGCTGAAGAAAGGATTCATAAATGAAGTTCAATCCTTCTCATCAACCCC
       |:|  ||:||||||:||||||:|||||::::||||||||||:||| | |
11728 AAACACAAAAAAGGACTCATAAGTGAAGCCTGATCCTTCTCACCAAACAC
  150     .    :    .    :    .    :    .    :    .    :
 8874 AGCCCACACCTCC AGCAATTGAACTTGAAAAAAAAAACCTGGTTTGAAA
       ||||||||||||-||:||||||||||||||||||||||-||||||||||
11778 TGCCCACACCTCCTAGTAATTGAACTTGAAAAAAAAAAC TGGTTTGAAA
  200     .    : HMGI(Y)        .HMGI(Y)   .    :    .EBS/:
 8923 AATTACCGCAAACTATATTGTCATCaaaaaaaaaaaaaaaaaaaaaCACT
      |||||||||||||:||||||||||-------|||||||||||||||||||
11827 AATTACCGCAAACCATATTGTCAT       AAAAAAAAAAAAAAACACT
  250 Elf-1 HMGI(Y) .    :    .    :    .    :    .    :
 8973 TCCTATATTTGAGATGAGAGAAGAGAGTGCTAGG  CAGTTTCCTGGCTG
      |||||||--|||||| | |||| ||||---||||--||: |||||-||||
11870 TCCTATA  TGAGATCACAGAACAGAG   TAGGCACAAGTTCCT GCTG
  300     .    :    .TATA box .    :    .    :    .+1  :
 9021 AACACGCCAGCCCAATACTTAAAGAGAGCAACTCCTGACTCCGATAGAGA
      |:|| ::|||||:|||:|||||| |||:|||||||||:|| : || || |
11914 AGCAGATCAGCCTAATGCTTAAATAGAACAACTCCTGGCTGTCATTGACA
  350     .    :    .    :    .    :    .    :    .    :
 9071 CTGGATGGACCCACAAGGGTGACAGCCCAGGCGGACCGATCTTCCCATCC
      :||  |::|-- :| |:|:|||||| |:::| || |:|| |::::  ||:
11964 TTGTCTAAA  AGCCAAGATGACAGACTGAGAGGCCTGAGCCCTTGTTCT
  400     .    :    .    :    .    :    .    :    .    :
 9121 CACATCCT CCGGCGCGATGCCAAAAAGAGGCTGACGGCAACTGGGCCTT
       :|||:||-||:| : ||||| : ||||:||:||------||: ::: | 
12012 GGCATTCTCCCAGGAAGATGCAGTAAAGGGGTTG      ACCCAATATA
  450     .    :    .    :    .    :    .    :    .    :
 9170 CTGCAGAGAAAGACCTCCGCTTCACTGCCCCGG CTGGTCCCAAGGGTCA
      ||||||||||   | |||: ||| || |:||: -|||:||||| | |:||
12056 CTGCAGAGAATTTCATCCAGTTCCCTCCTCCATCCTGATCCCATGTGCCA
  500     .    :    .    :    .    :    .    :    .    :
 9219 GGAAGATGGATTCATACCTGCTGATGTGGGGACTGCTCACGTTCATCATG
      |||||||||| :||::|:||||||||| |||::| ||| |:|| |:|||:
12106 GGAAGATGGAGCCACGCTTGCTGATGTTGGGGTTTCTCTCATTAACCATA
  550     .    :    .    :    .    :    .    :    .
 9269 GTGCCTGGCTGCCAGGCAGGTAAG GGCCTGTGGGTGCCCCCGGAA
      ||:||::|:||:|:||||||||||-|:|| | :: |||||: ||||
12156 GTACCCAGTTGTCGGGCAGGTAAGAGACCAGGAACTGCCCTGGGAA
```

PRRI
[-276,-244]

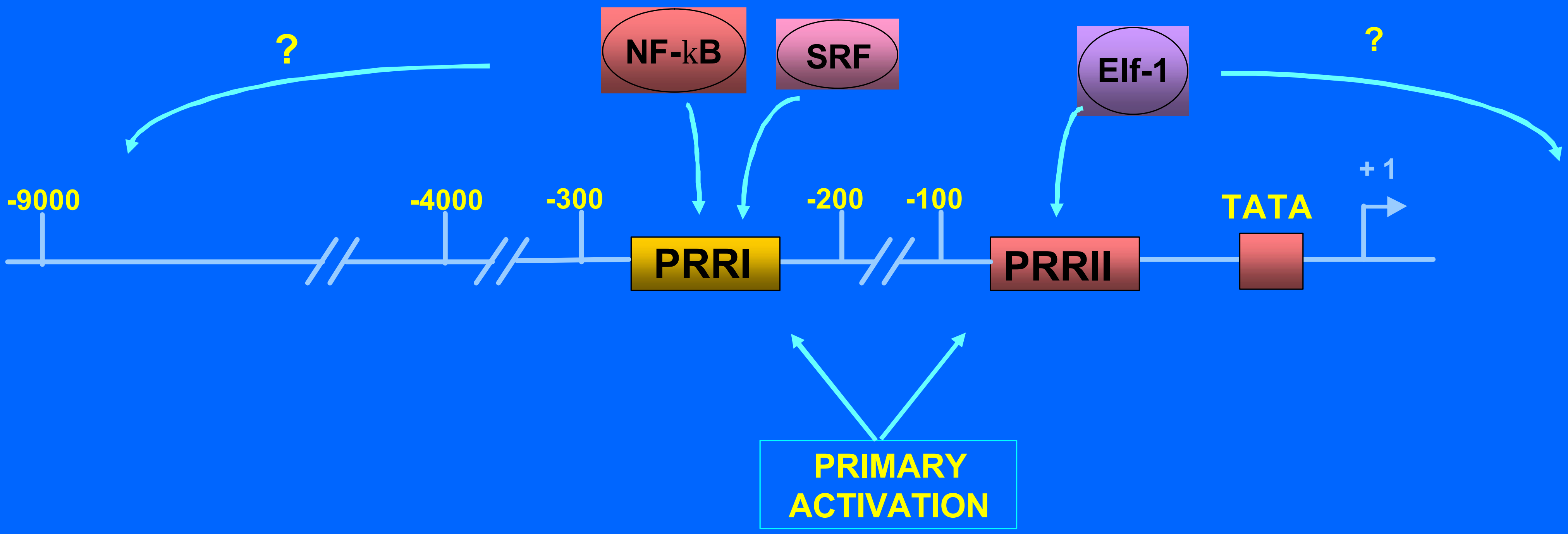
PRRII
[-137,-64]

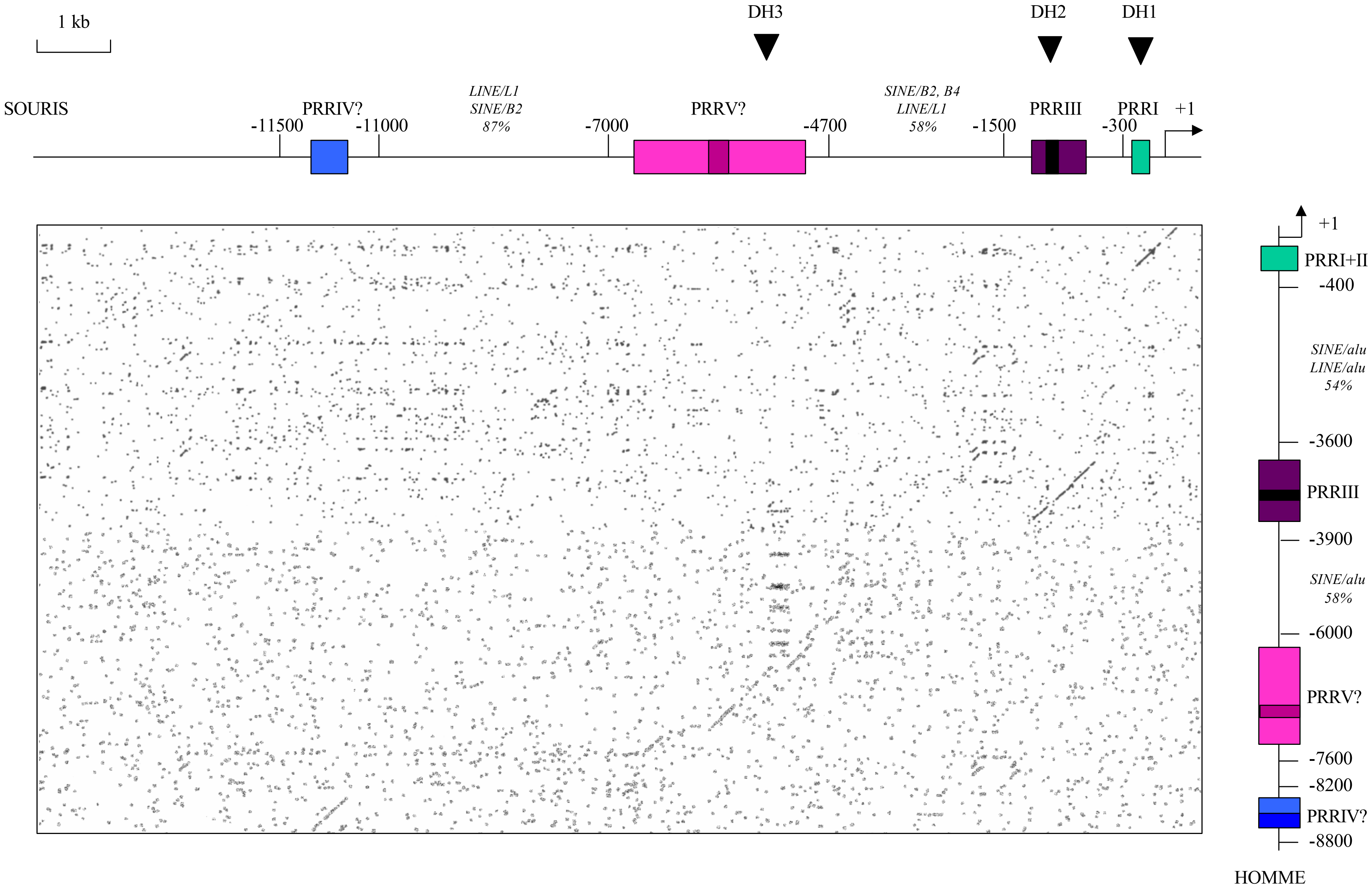
HOWEVER NEITHER PRRI NOR PRRII, ALONE OR IN ASSOCIATION, ARE SUFFICIENT

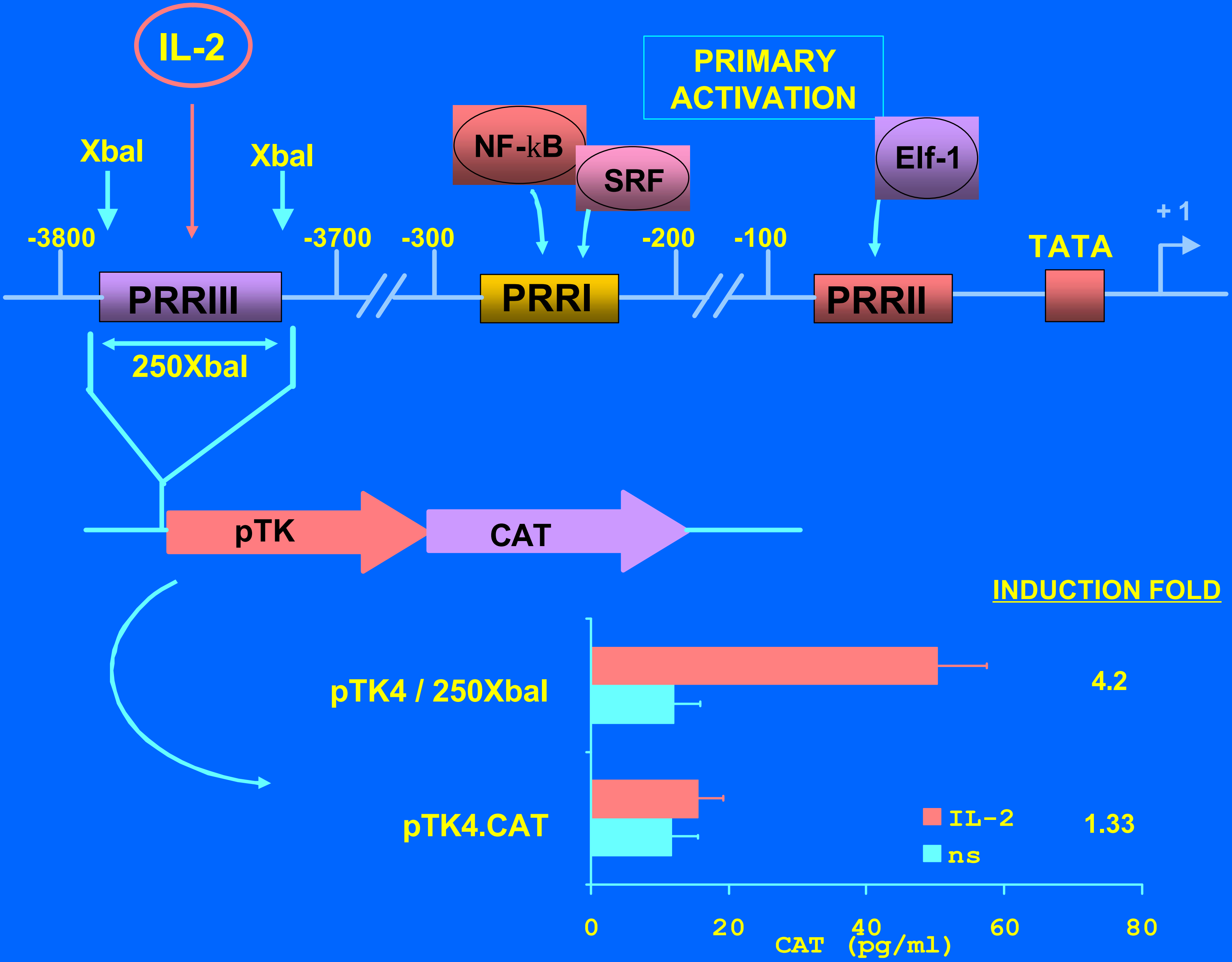
TO ELICIT AN EFFICIENT TRANSCRIPTION

IN GENE REPORTER ASSAYS USING VARIOUS T CELL MODELS

SEARCH FOR IL-2 AND/OR CD28 RESPONSIVE REGULATORY REGIONS WITHIN CD25/IL-2Rα GENE LOCUS




1 kb
DH3
DH2
DH1
SOURIS
PRRIV?
LINE/L1
SINE/B2
87%
PRRV?
SINE/B2, B4
LINE/L1
58%
PRRIII
PRRI
+1
-11500
-11000
-7000
-4700
-1500
-300
+1
PRRI+II
-400
SINE/alu
LINE/alu
54%
-3600
PRRIII
-3900
SINE/alu
58%
-6000
PRRV?
-7600
-8200
PRRIV?
-8800
HOMME

IL-2
PRIMARY
ACTIVATION
XbaI
XbaI
NF-kB
SRF
Elf-1
+ 1
-3800
-3700
-300
-200
-100
TATA
PRRIII
PRRI
PRRII
250XbaI
pTK
CAT
INDUCTION FOLD
pTK4 / 250XbaI
4.2
pTK4.CAT
1.33
IL-2
ns
0
20
40
60
80
CAT (pg/ml)

PRRIII

Homo Sapiens

GASd/EBSd   GASp/GATA   EBSp

```
- 3772                                                            - 3718
        TTTCTTCTAGGAAGTACCAAACATTTCTGATAATAGAATTGAGCAATTTCCTGAT
        IIIIIIII--IIIIIIIII-IIIIIIIIIIIII-III-IIIIIIII-IIIIIIII
        TTTCTTCTGAGAAGTACCAGACATTTCTGATAAGAGAGTTGAGCAACTTCCTGAT
- 1369                                                            - 1315
```

Site I   Site II   Site III

Mus Musculus

IL-2rE

In Vivo Footprinting

- 1) Methylation of guanines (major groove) and to a lesser extent of adenines (minor groove) by DMS on living cells

  The level of methylation is affected by protein binding to DNA

- 2) Genomic DNA extraction

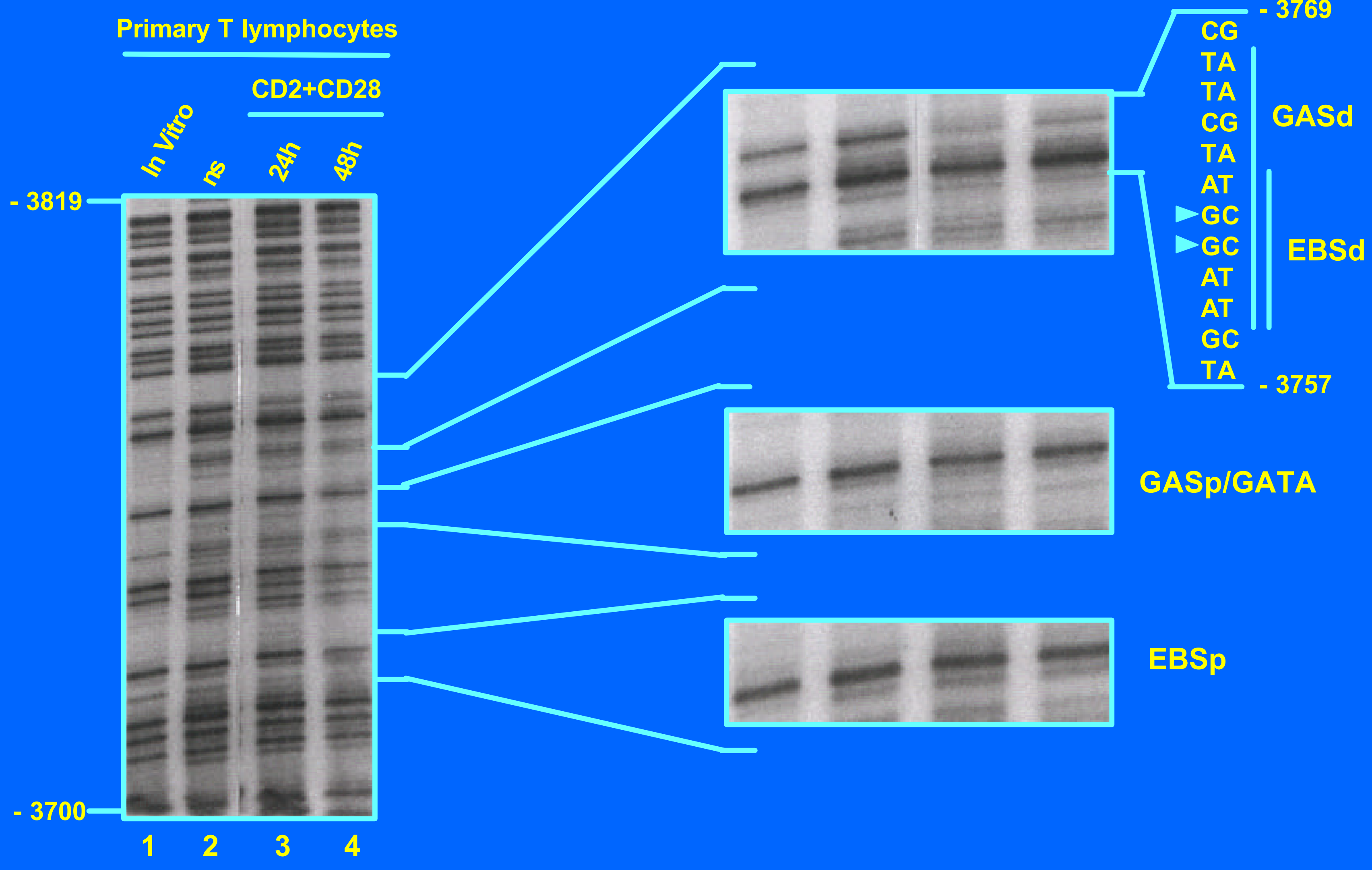
- 3) Cleavage of methylated residues by piperidine

- 4) LMP-PCR amplification of the region to be analyzed

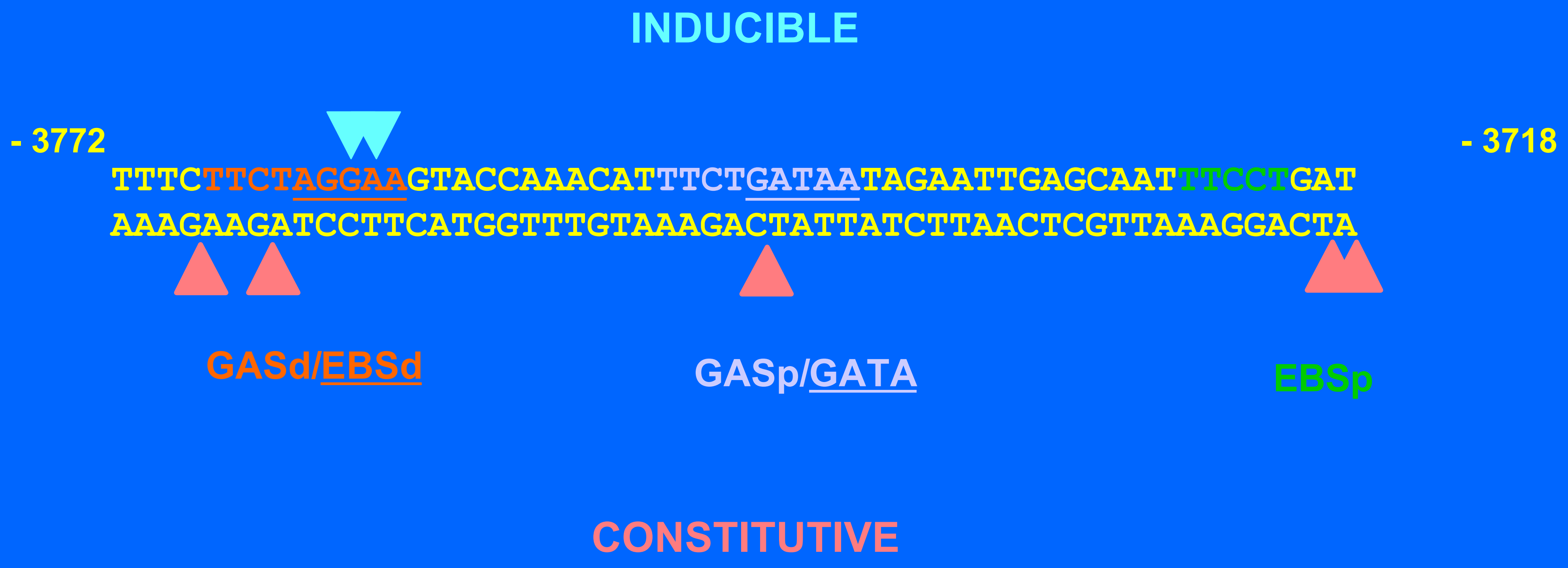
  Last amplification cycles performed with a ^{32}P-labeled primer

- 5) Analysis of the PCR products on sequencing gel

In vivo modification of the GASd/EBSd motif occupancy in response to CD2+CD28 costimulation in purified human primary T cells



The GASd/EBSd motif is the only putative regulatory element within PRRIII modified *in vivo* in response to an IL-2-dependent induction in human T lymphocytes



INDUCIBLE

- 3772

- 3718

TTTCTTCTAGGAAGTACCAAACATTTCTGATAATAGAATTGAGCAATTTCCTGAT

AAAGAAGATCCTTCATGGTTTGTAAAGACTATTATCTTAACTCGTTAAAGGACTA

GASd/EBSd

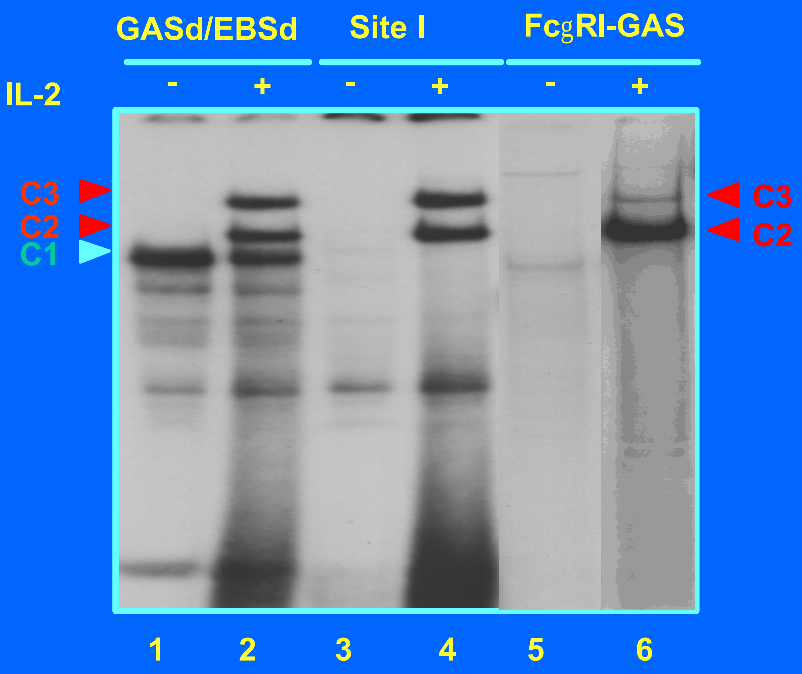
GASp/GATA

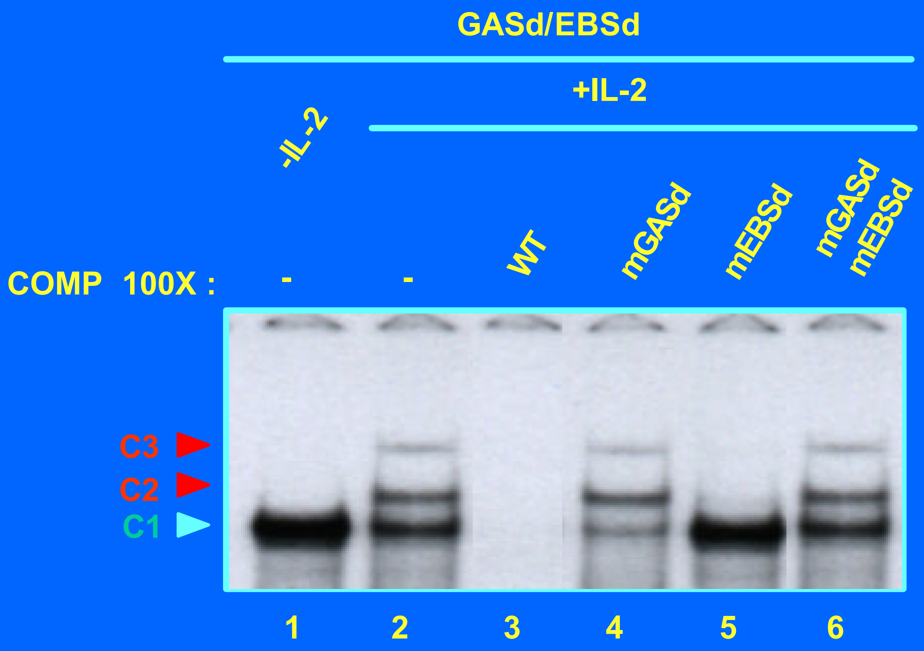
EBSp

CONSTITUTIVE

Lecine, P., Algarte, M., Rameil, P., Beadling, C., Bucher, P., Nabholz, M. and Imbert, J. Elf-1 and Stat5 bind to critical element in a new enhancer of the human interleukin-2 receptor alpha gene. Mol.Cell.Biol. 16: 6829-6840; 1996.

Inducibles complex C2 and C3 are GAS-specific
Constitutive complex C1 is EBS-specific





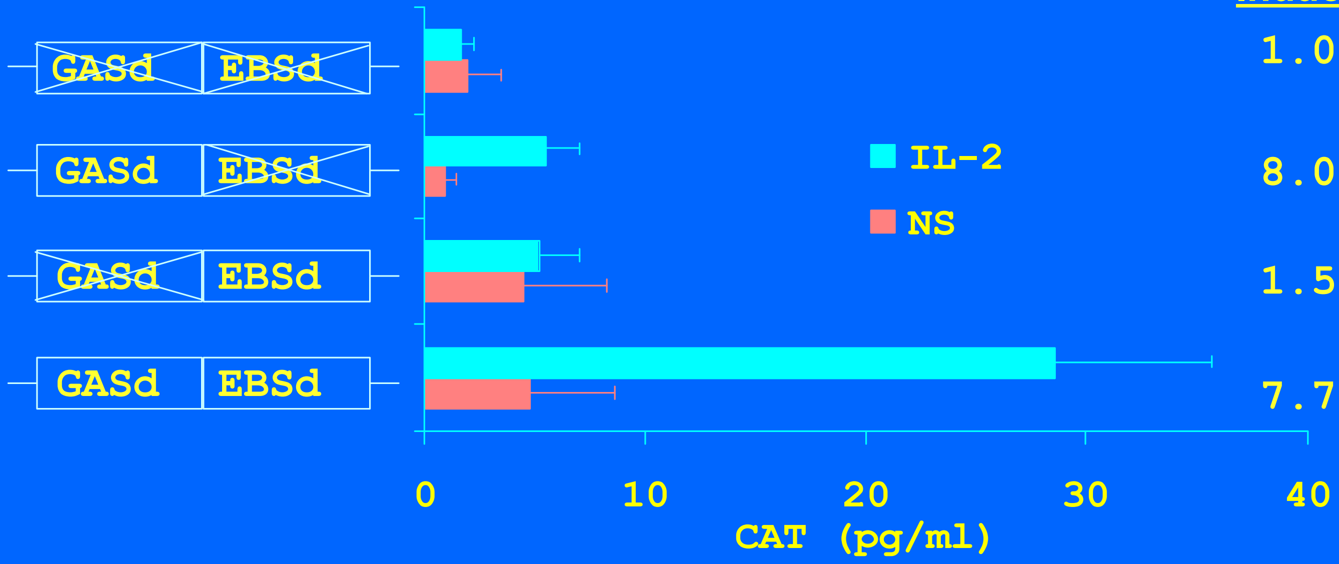
CD25/IL-2Ra GASd/EBSd EMSA probe:

GASd

```
TTTCTTCTAGGAAGTACC
AAAGAAGATCCTTCATGG
```

EBSd

Disruption of the GASd motif by site-directed mutagenesis fully abolish IL-2 response



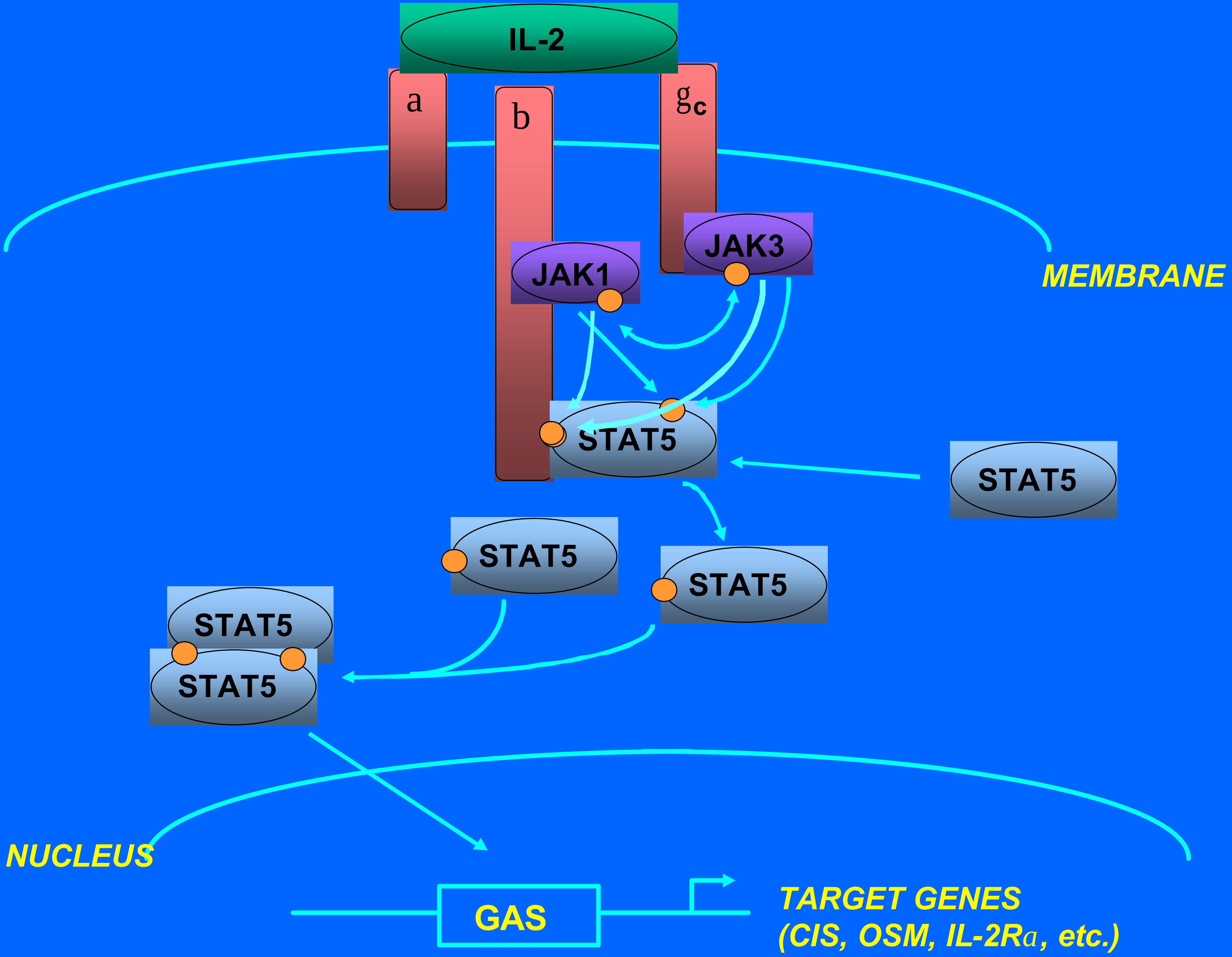
Induction Fold

1.0 +/- 0.1

8.0 +/- 3.7

1.5 +/- 0.1

7.7 +/- 2.4



IL-2

a

b

g_c

MEMBRANE

JAK1

JAK3

STAT5

STAT5

STAT5

STAT5

STAT5

STAT5

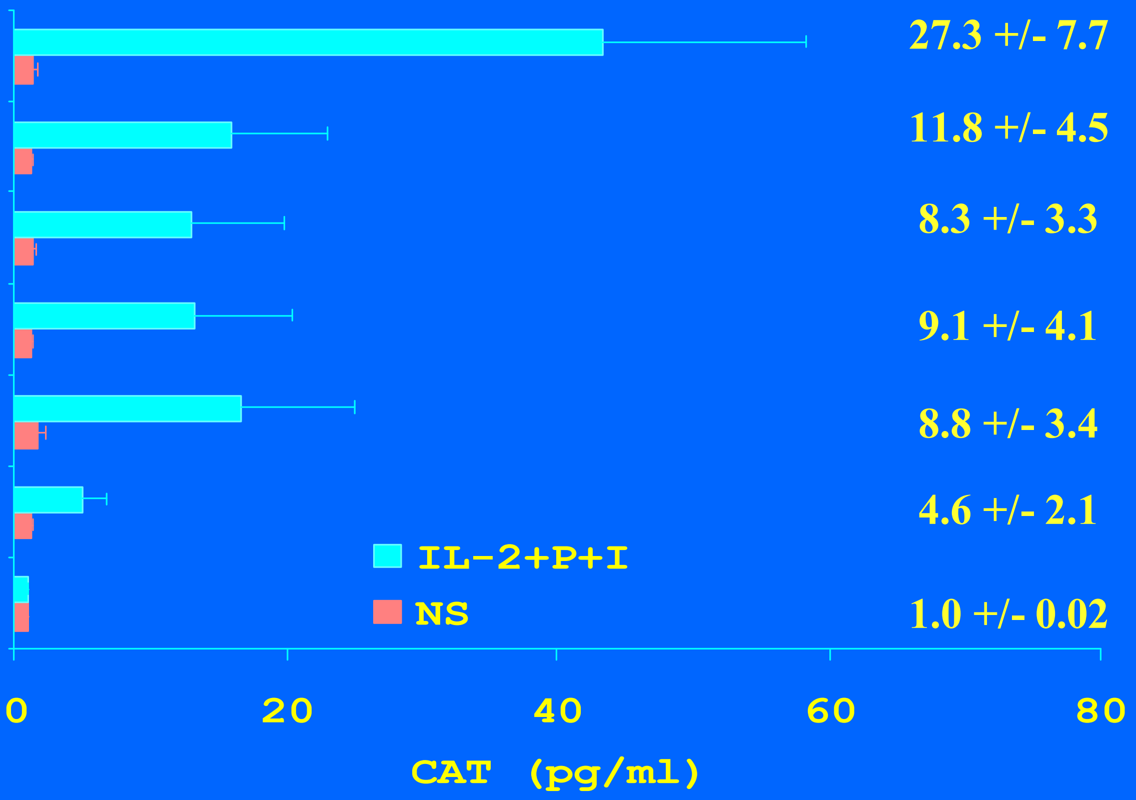
NUCLEUS

GAS

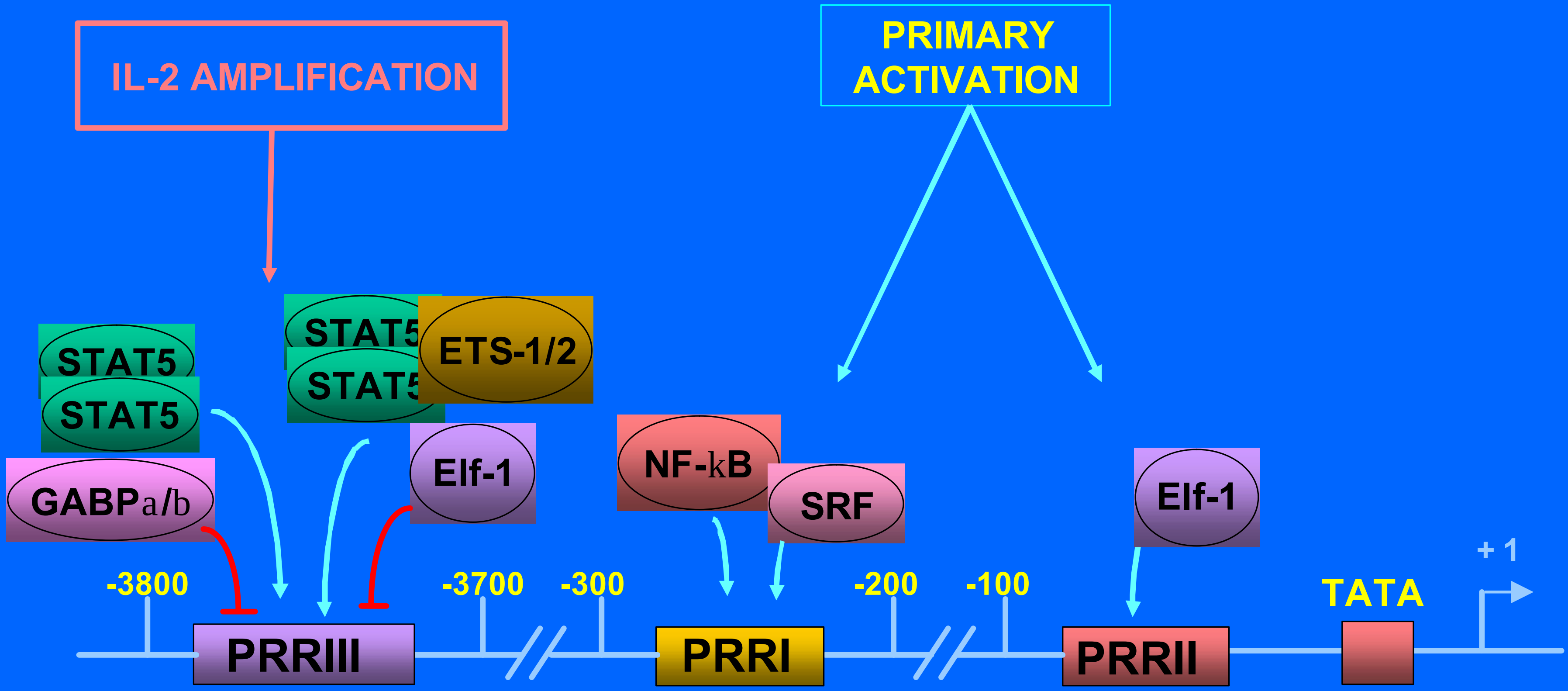
TARGET GENES
(CIS, OSM, IL-2Ra, etc.)

Stat5b, Ets-1 and Ets-2 cooperate functionally in response to IL-2 +PMA+Ionomycin

REPORTER	EFFECTOR Stat5b	EFFECTOR Ets-1	EFFECTOR Ets-2	INDUCTION FOLD
GASd EBSd	+	+	+	27.3 +/- 7.7
GASd EBSd	-	+	+	11.8 +/- 4.5
GASd EBSd	-	-	+	8.3 +/- 3.3
GASd EBSd	-	+	-	9.1 +/- 4.1
GASd EBSd	+	-	-	8.8 +/- 3.4
GASd EBSd	-	-	-	4.6 +/- 2.1
pTK4.CAT	-	-	-	1.0 +/- 0.02



Rameil, P., Lecine, P., Ghysdael, J., Gouilleux, F., Kahn-Perles, B. and Imbert, J. IL-2 and long-term T cell activation induce physical and functional interactions between STAT5 and ETS transcription factors in human T cells. Oncogene 19: 2086-2097; 2000.



IL-2 AMPLIFICATION

PRIMARY ACTIVATION

STAT5

STAT5

GABPa/b

STAT5

STAT5

ETS-1/2

Elf-1

NF-kB

SRF

Elf-1

-3800

-3700

-300

-200

-100

TATA

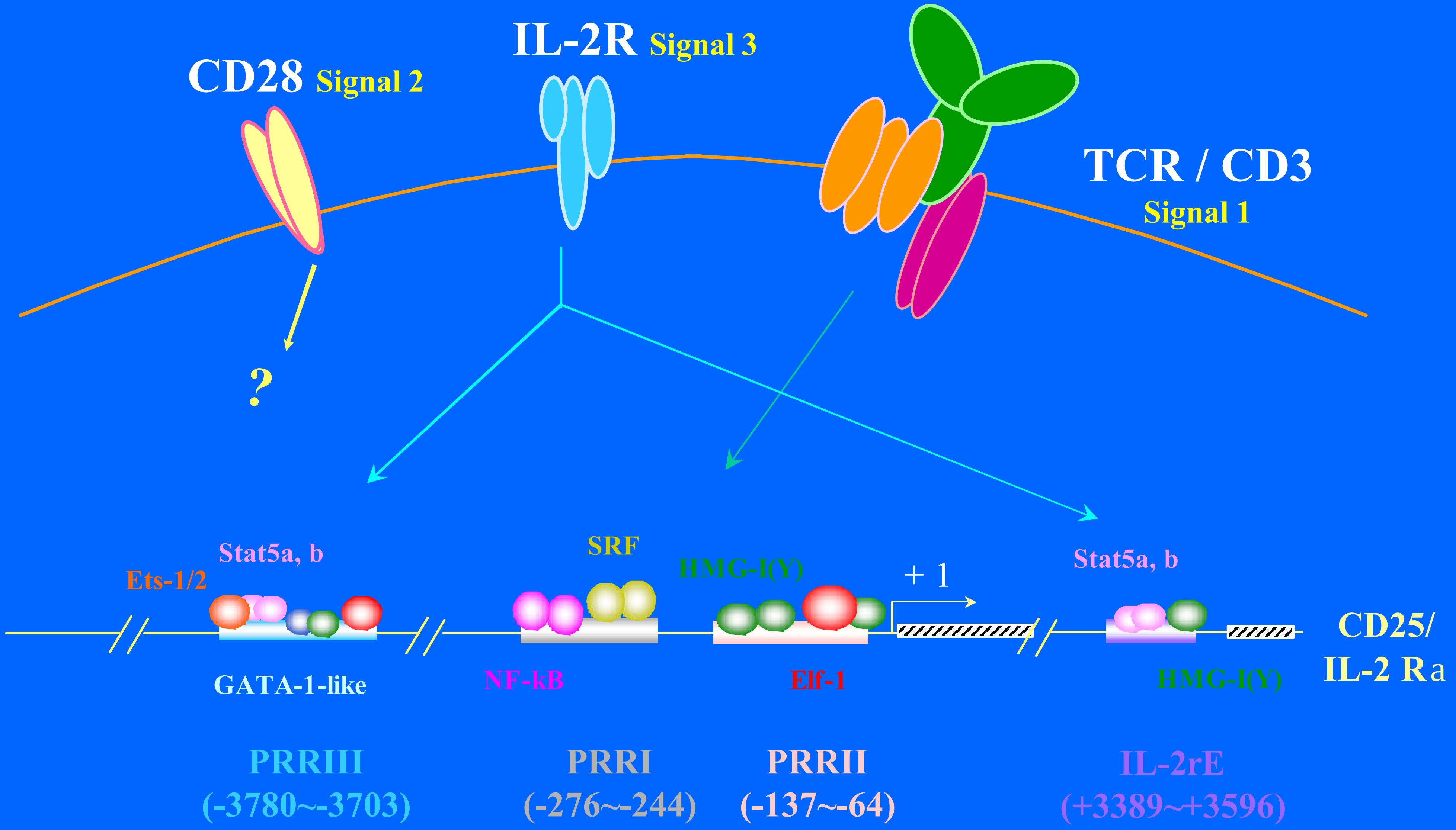
+ 1

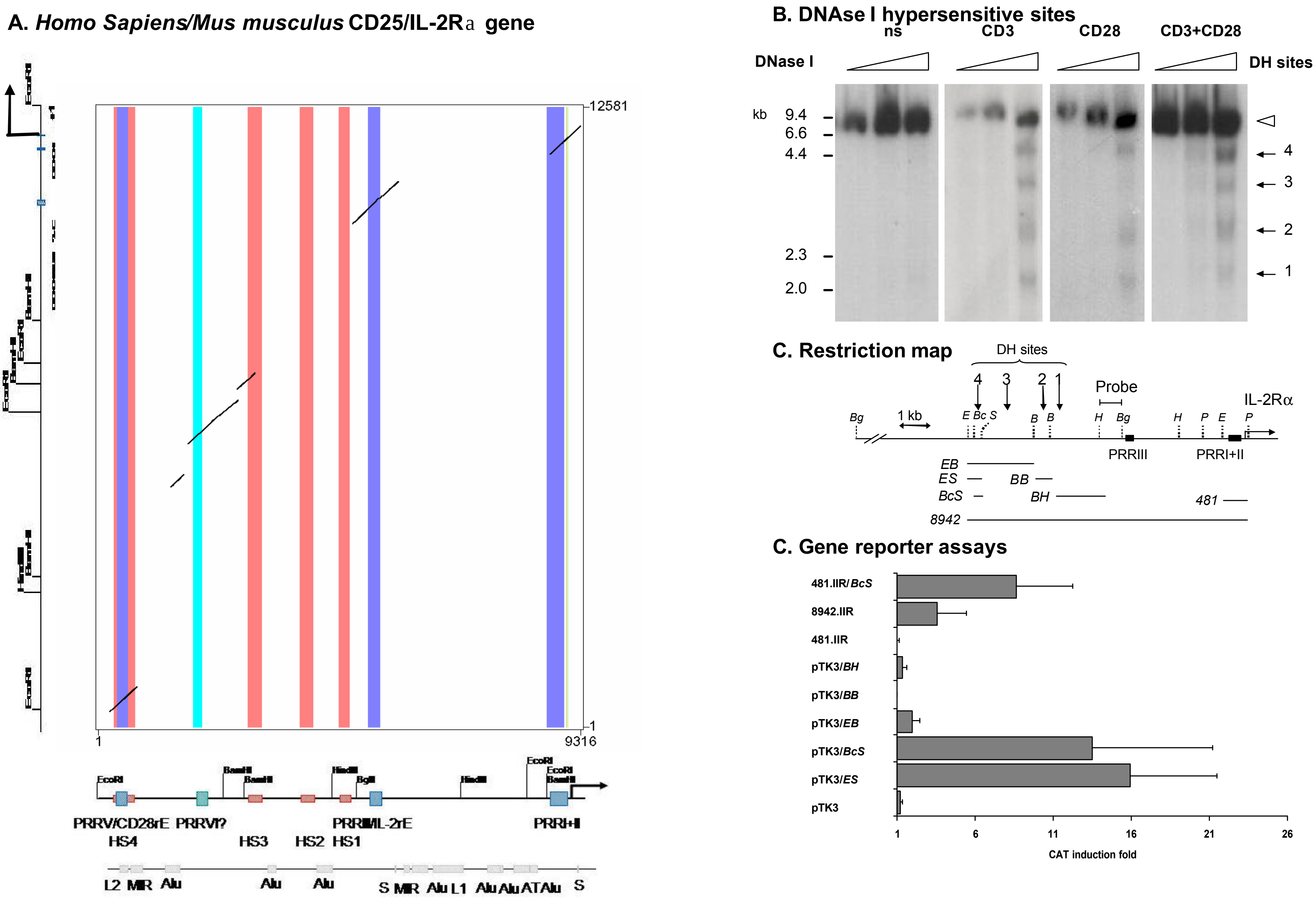
PRRIII

PRRI

PRRII

Regulatory elements and their cognate transcription factors in the human CD25/IL-2Ra gene





A. *Homo Sapiens/Mus musculus* CD25/IL-2Rα gene

B. DNAse I hypersensitive sites

C. Restriction map

C. Gene reporter assays

A Well-Conserved but not Functional Candidate for a CD28 Responsive Enhancer

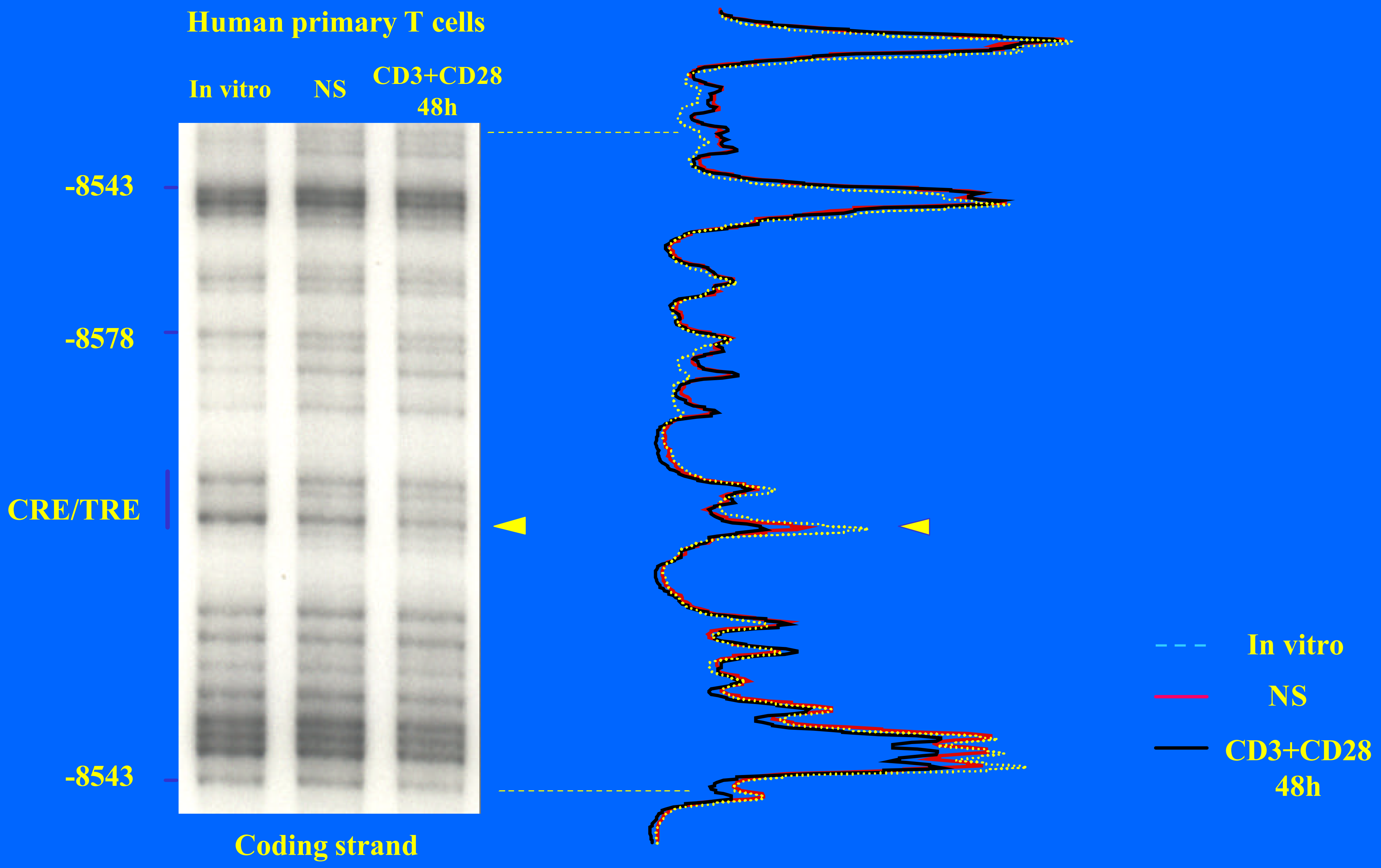
Comparison of two nucleotidic sequences:
Sequence 1 : cons4HS
Sequence 2 : cons4mus

```
 resetting to DNA matrix
 LALIGN finds the best local alignments between two sequences
 version 2.0u4 Feb. 1996
Please cite:
 X. Huang and W. Miller (1991) Adv. Appl. Math. 12:373-381

 Comparison of:
(A) cons4HS
(B) cons4mus
 using matrix file: DNA, gap penalties: -16/-4

  79.6% identity in 201 nt overlap; score:  612

                       NF-ATp
               10        20        30        40        50        60
cons4H CATAGTGGATTTTGGTTTTCCACGGGACCCCTGTGCCCTTGTCTAGTAGAATCTGGTGGA
       ::::::::::: ::::::::::: ::::::         ::::::::: :: :: :::::
cons4m CATAGTGGATTCTGGTTTTCCACAGGACCC---------TGTCTAGTAAAACCTAGTGGA
               10        20        30                 40        50
                    NF-kB/CD28RC  CREB         Ets
               70        80        90       100       110       120
cons4H AATTACAAACTGCAGAAATTCAACTCAGTGCCGCAATAACAGGATGCACCTGTAGATTTC
       :::::::: ::: ::::::::: :   ::::: :::::::::::::::::::::::::::
cons4m AATTACAAGCTG-AGAAATTCAGCCTTGTGCCACAATAACAGGATGCACCTGTAGATTTC
              60         70        80        90       100       110
       GAS
              130       140       150       160       170       180
cons4H GTAGAATTAGCAGCAGCATTCTTTCAATACCAGTTTGAGAGAAATAACCCTGTTTGCATA
         ::::::::: :: :  :::: : ::  ::: ::::::::::: :: ::::::::  : 
cons4m ACAGAATTAGCTGCTGTCTTCTCTTAACGCCAATTTGAGAGAAAGAAGCCTGTTTGTCTG
              120       130       140       150       160       170

              190       200
cons4H GTGCCAACTGGGGCAGAATCT
        ::::::   :::::::::::
cons4m CTGCCAAACAGGGCAGAATCT
              180       190
```

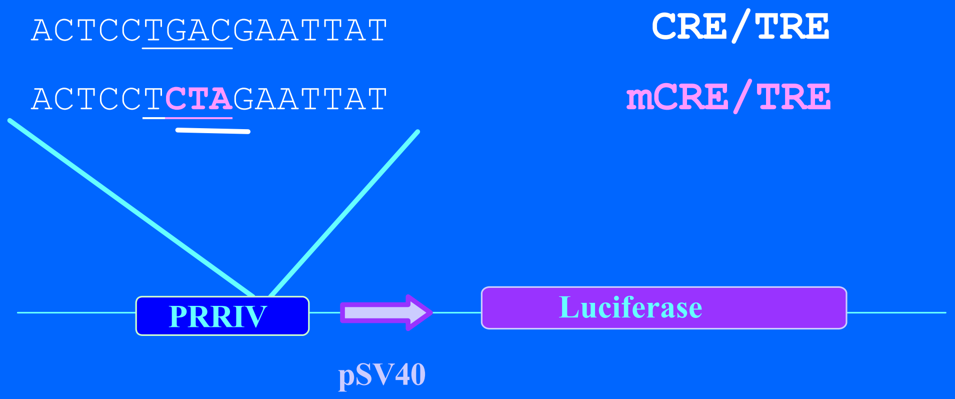
The Functional PRRV/CD28rE within Human CD25/IL-2Ra Gene

```
         Bcl-I    .         .         .         .         .
-8688    TGATCAGCCGTGTCTCCAGAGAGCTACAAGGCAGTTTTCAATTGGTAAAT
         ACTAGTCGGCACAGAGGTCTCTCGATGTTCCGTCAAAAGTTAACCATTTA
                                            NFAT

                  .         .         .         .         .
-8638    GCCCTGAGAGTGATGGGCTTGTGGCATGTGTAAGGGTTAGACAGACCTGG
         CGGGACTCTCACTACCCGAACACCGTACACATTCCCAATCTGTCTGGACC
                  .TRE      .CRE/TRE  .         .         .
-8588    GACTAGACATGACACCACTCCTGACGAATTATGTGAGTGTGGGTGTTTCA
         CTGATCTGTACTGTGGTGAGGACTGCTTAATACACTCACACCCACAAAGT
                   TCF 11      TCF 11
                                             NFAT         
                  .         .         .      NFAT         .
-8538    CAACCACAATGAGATGCAATGCCTGCACTTGTAACATGGAAATAGTGATG
         GTTGGTGTTACTCTACGTTACGGACGTGAACATTGTACCTTTATCACTAC
                  TCF 11

         SphI
-8488    GCATGC
         CGTACG
```

In vivo LM-PCR characterized the regulatory elements in PRRIV/IL-2Ra

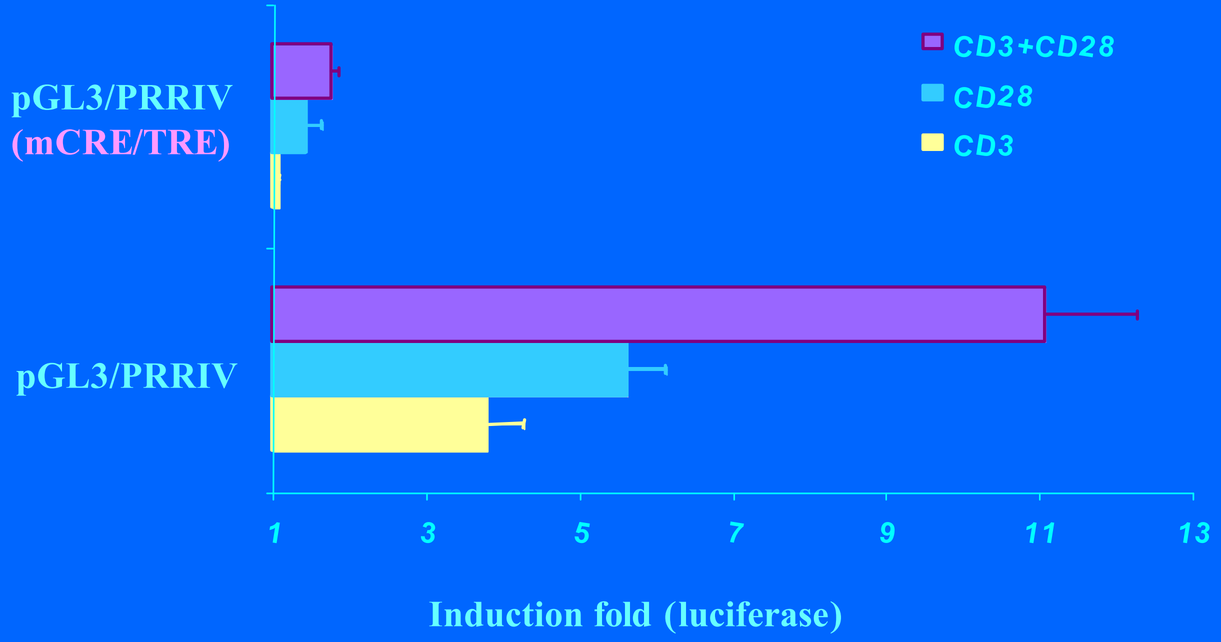


The CRE/TRE within PRRIV is essential for the response of PRRIV to TCR-CD3 and CD28 signals



ACTCCTGACGAATTAT CRE/TRE

ACTCCTCTAGAATTAT mCRE/TRE



The crucial CRE/TRE motif within CD25/IL-2Ra PRRV/CD28rE is contained in a SINE/MIR repeat and is not conserved between *Homo Sapiens* and *Mus Musculus*

```
Local identities (aligment n°1)

211-245 <--> 312-346 74% (35 nt)
261-312 <--> 347-398 62% (52 nt)
313-322 <--> 400-409 70% (10 nt)
323-371 <--> 414-462 61% (49 nt)
374-388 <--> 463-477 60% (15 nt)
389-444 <--> 480-535 75% (56 nt)
447-482 <--> 536-571 53% (36 nt)
491-542 <--> 572-623 67% (52 nt)
544-602 <--> 624-682 61% (59 nt)
603-664 <--> 684-745 66% (62 nt)
666-686 <--> 746-766 57% (21 nt)
690-709 <--> 767-786 85% (20 nt)
712-744 <--> 787-819 58% (33 nt)
```

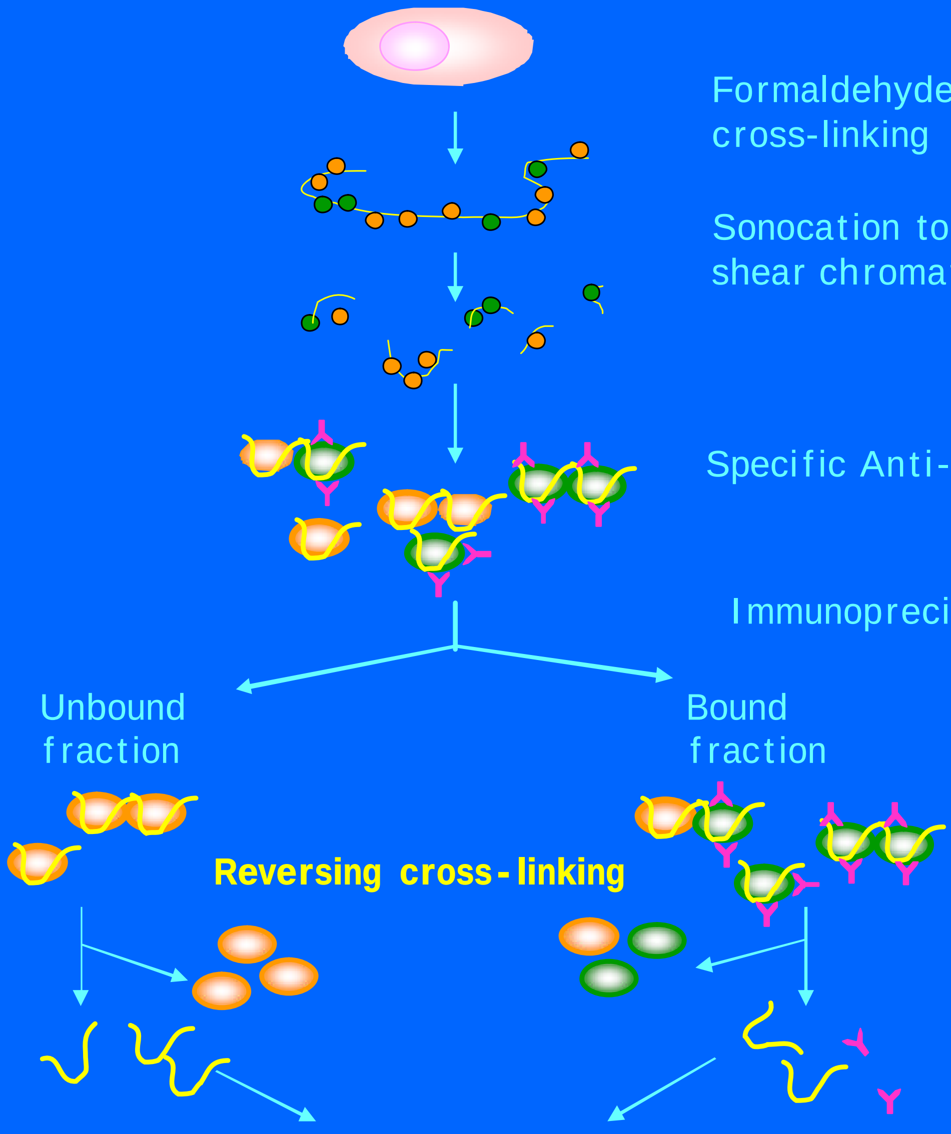
```
Seq 1 = ">HS_IL-2Ra_9.3KB genomic fragment from ENSG00000134460 5'->3'"
Seq 2 = ">MmIL-2Ra_12.5kb genomic fragment from phage Ch4Cl9 5'->3'"

        Local Alignment Number 1
        Similarity Score:  12780
        Match Percentage:  59 %
        Number of Matches:  325
        Number of Mismatches:  175
        Total Length of Gaps:  42
        Begins at (211,312) and Ends at (744,819)

   0    .    :    .    :    .    :    .    :    .    :
 211 CTCTAAAAAGGTTCTGCATACAGtcattcattcaatgcttaacgactgag
     ||:| ||: |||||| ||:| |:||||| ||||||---------------
 312 CTTTTAAGTGGTTCTCCACAGAATCATTAATTCAA

  50    .    :    .    :    .    :    .    :    .    :
 261 cattaattccatgctaagtactgaactcagcactaggaataagaaggcga
     ||   ||: ::|||||:|| ||: |||||| |||::|| |||:| ||::|
 347 CAGGTATCATGTGCTAGGTCCTATACTCAGAACTGAGACTAAAATGGTAA

 100    .    :    .    :    .    :    .    :    .    :
 311 cc tagaggcata    tcctctctctaaagatgcatagagcctcattgg
     | -||||:| | |----|:||:|:||||||:|::||:||||| : ::|||
 397 CAATAGAAGAAGACAATTTCTTTTTCTAAAAACACACAGAGCACAGCTGG

 150   BclI  :    .    :    .    :    .    :    .    :
 356 aatgatcagccgtgtctccagagagctacaagg  cagTTTTCAATTGGT
     |||  |::| |:||| --||||  | |: |:||--||||||| |:|||||
 447 AATTCTTGGGCATGTA  CAGATTGATGGAGGGTACAGTTTTAAGTTGGT

 200    .    :    .    :    .    :    .    :    .    :
 404 AAATGCCCTGAGAGTGATGGGCTTGTGGCATGTGTAAgggttagacagac
     |||||:|:||||||: |||:  | ||:|| ||| |:|||||--||::: :
 495 AAATGTCTTGAGAGCTATGACATGGTAGCCTGTTTGAGGGT  GATGATT

 250    .    :    .    :  CRE/TRE:    .    :    .    :
 454 ctgggacctagacatgacaccactcctgacgaattatgtgagtgtgggtg
      |:|:  |||  | | |||:| ||||||:---------||||| |||||::
 543 GTAGACACTACTCCTCACATCTCTCCTGG        GTGAGAGTGGGCA

 300    .    :    .    :    .    :    .    :    .    :
 504 tttcacaaccacaatgagatgcaatgcctgcacttgtaacatggaaatag
     |||||:||::|||:|:|:||::||||:|| ||:: |:||-|||||||| |
 585 TTTCATAATTACAGTAAAATATAATGTCTTCATCAGCAA ATGGAAATCG

 350    .SphI    .    :    .    :    .    :    .    :
 554 tgatggcatgccggccccgccagattgctgtgagaagtcagcggcagag
     |||:::::||:|:||:  :|  |:||||||||| |||:||||::: | |:-
 634 TGACAATGTGTCAGCTAGACATGGTTGCTGTGTGAAATCAGTAAGATAAC

 400    .    :    .    :    .    :    .    :    .    :
 603 acatgcaacattctcagcacagtgcttgccatgtagtaagggcctagtca
     ||||  || || : ::|||||| ||:|||:|||||:|||: :::|| |||
 684 ACATTAAAAATGTATGGCACAGAGCCTGCTATGTAATAAACATTTATTCA

 450    .    :    .    :    .    :    .    :    .    :
 653 gtgctagTGATTCCTTTCAATATTCCTAAGATGCAGATAAGGGAACAGCC
     :|| |||||:||- |||| :||:|   :| ||||---||||| ||||||:
 734 ATGGTAGTGGTT ATTTCCGTACTAGAGATATGC   TAAGGCAACAGCT

 500    .    :    .    :    .    :    .    :
 703 CAGAGGAGGGGGAGCACTTCCAGAGGGAGGGATGCGGTGAGA
     |||||:|--|:||::||:||:| | :: ||||| :|:|||:|
 780 CAGAGAA  GAGAATACCTCTACATAATGGGATCTGATGAAA
```

HS4 (-8.5Kb)
CD28rE (PRRV)
[-8689,-8484]

BclI-SphI fgt

underlined:
SINE/MIR repeat

Chromatin immunoprecipitation (ChIP)



Formaldehyde cross-linking

Sonocation to shear chromatin

Specific Anti-bodies

Immunoprecipitation

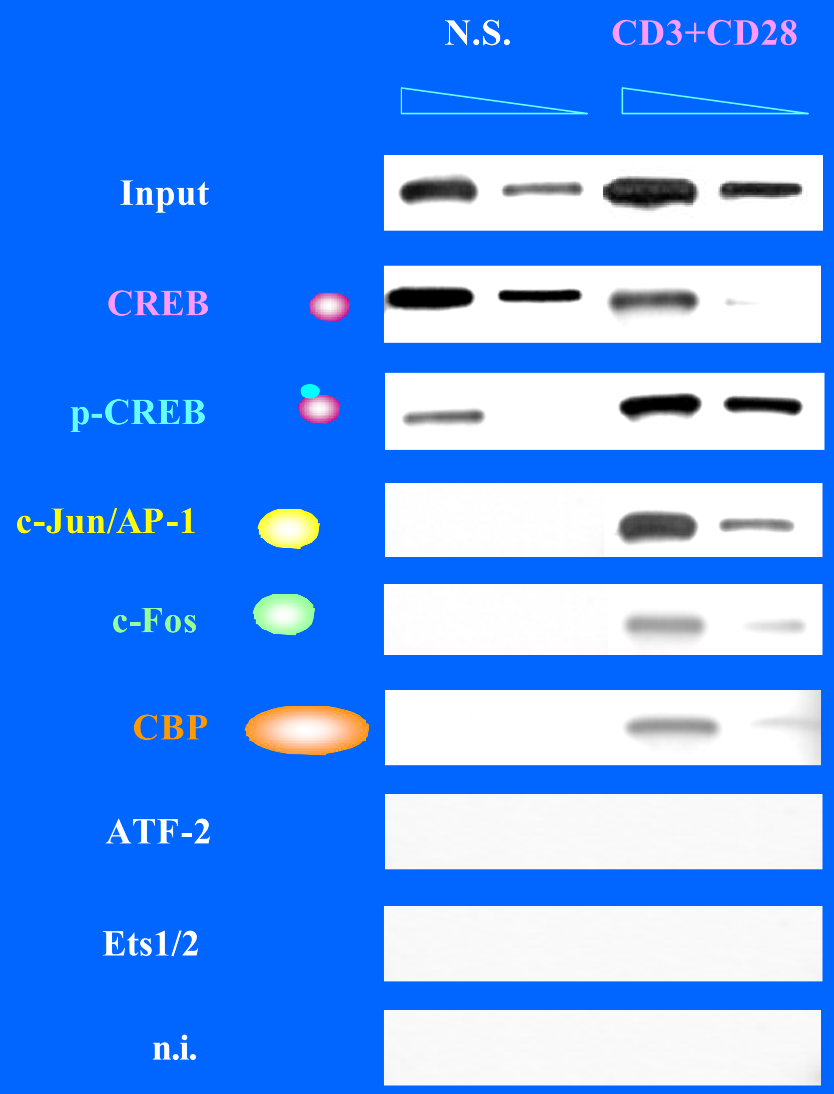
Unbound fraction

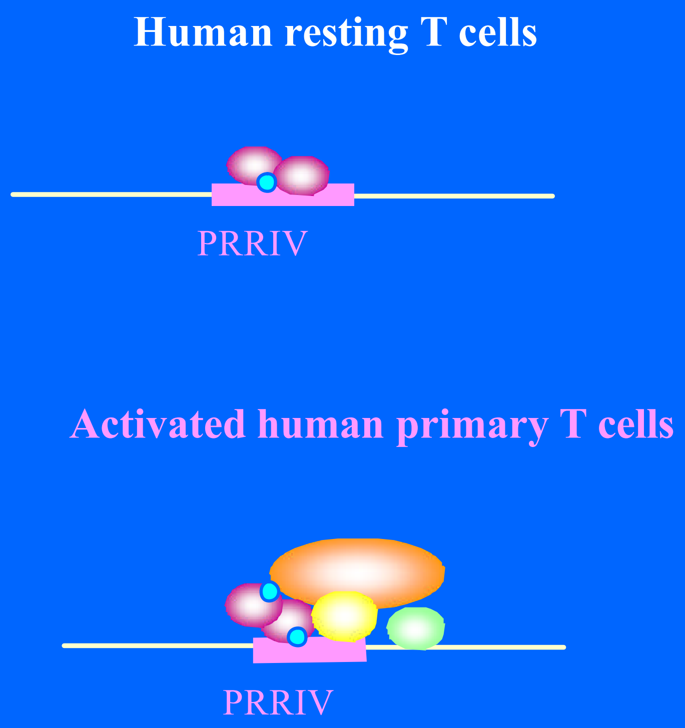
Bound fraction

Reversing cross-linking

Semi-quantitative PCR Assays

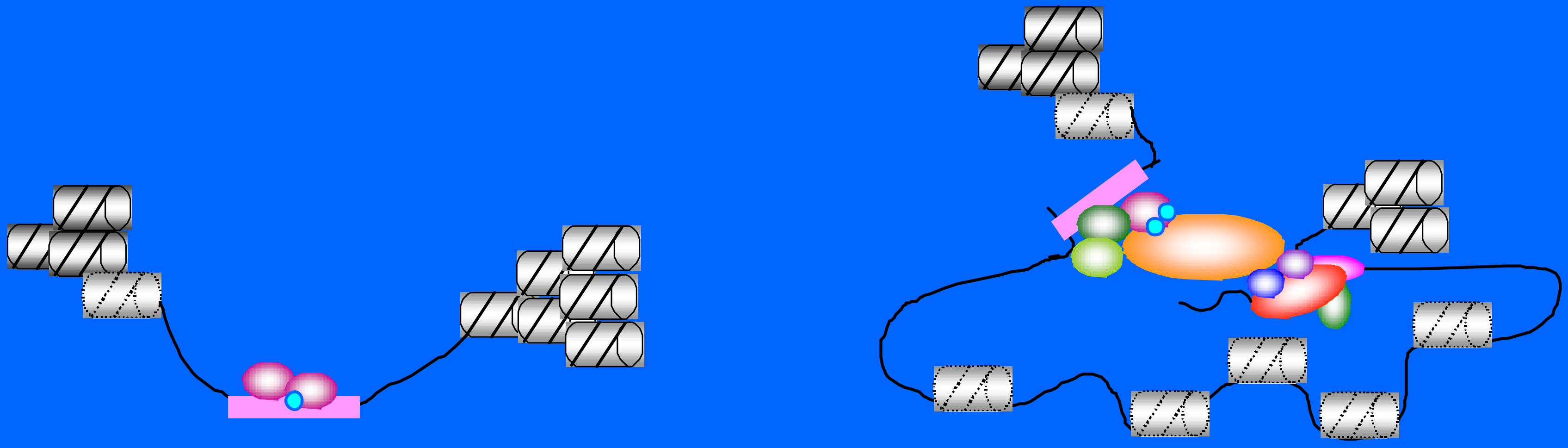
Specific recruitment of CREB, CBP, c-Jun/AP-1, and c-Fos/AP-1 to PRRIV in vivo





Resting human primary T cells

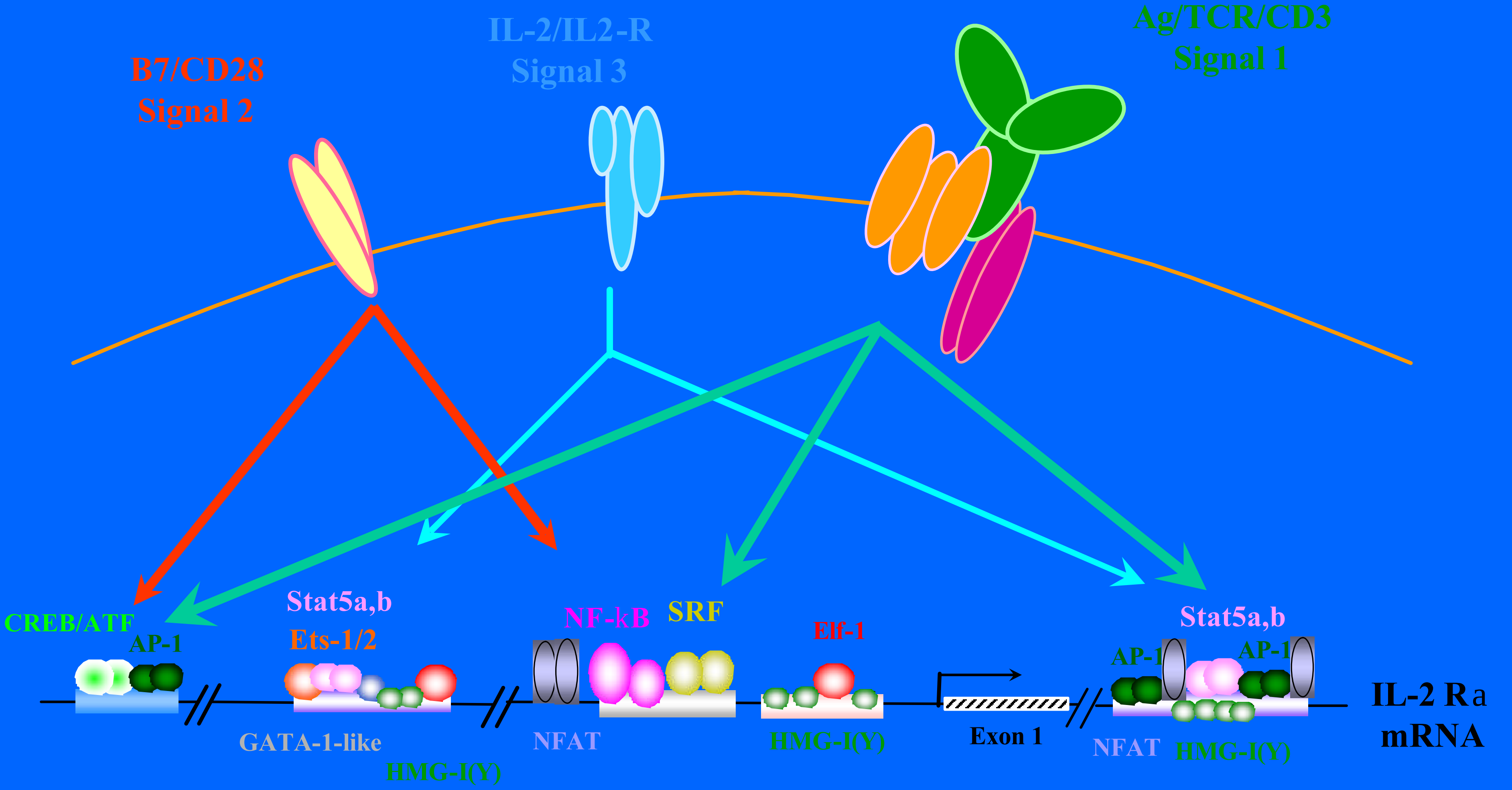
Activated human primary T cells



IL-2Ra gene transcription : Off On

▨	Nucleosome	▬	CRE/TRE	●	c-Jun/AP-1
▒	Remodelled nucleosome	●	CREB	⬭	CBP/p300
●	RNA polymerase II complex	●	Phosphorylated Ser^{133} of CREB	●	c-Fos/AP-1

Yeh, J.H., Lecine, P., Nunes, J.A., Spicuglia, S., Ferrier, P., Olive, D. and Imbert, J. Novel CD28-responsive enhancer activated by CREB/ATF and AP-1 families in the human IL-2Ralpha locus. Mol.Cell.Biol., 21: 4515-4527; 2001.



B7/CD28
Signal 2

IL-2/IL2-R
Signal 3

Ag/TCR/CD3
Signal 1

CREB/ATF
AP-1

Stat5a,b
Ets-1/2
GATA-1-like
HMG-I(Y)

NF-kB
SRF
NFAT

Elf-1
HMG-I(Y)

Exon 1

Stat5a,b
AP-1
AP-1
NFAT
HMG-I(Y)

IL-2 Ra
mRNA

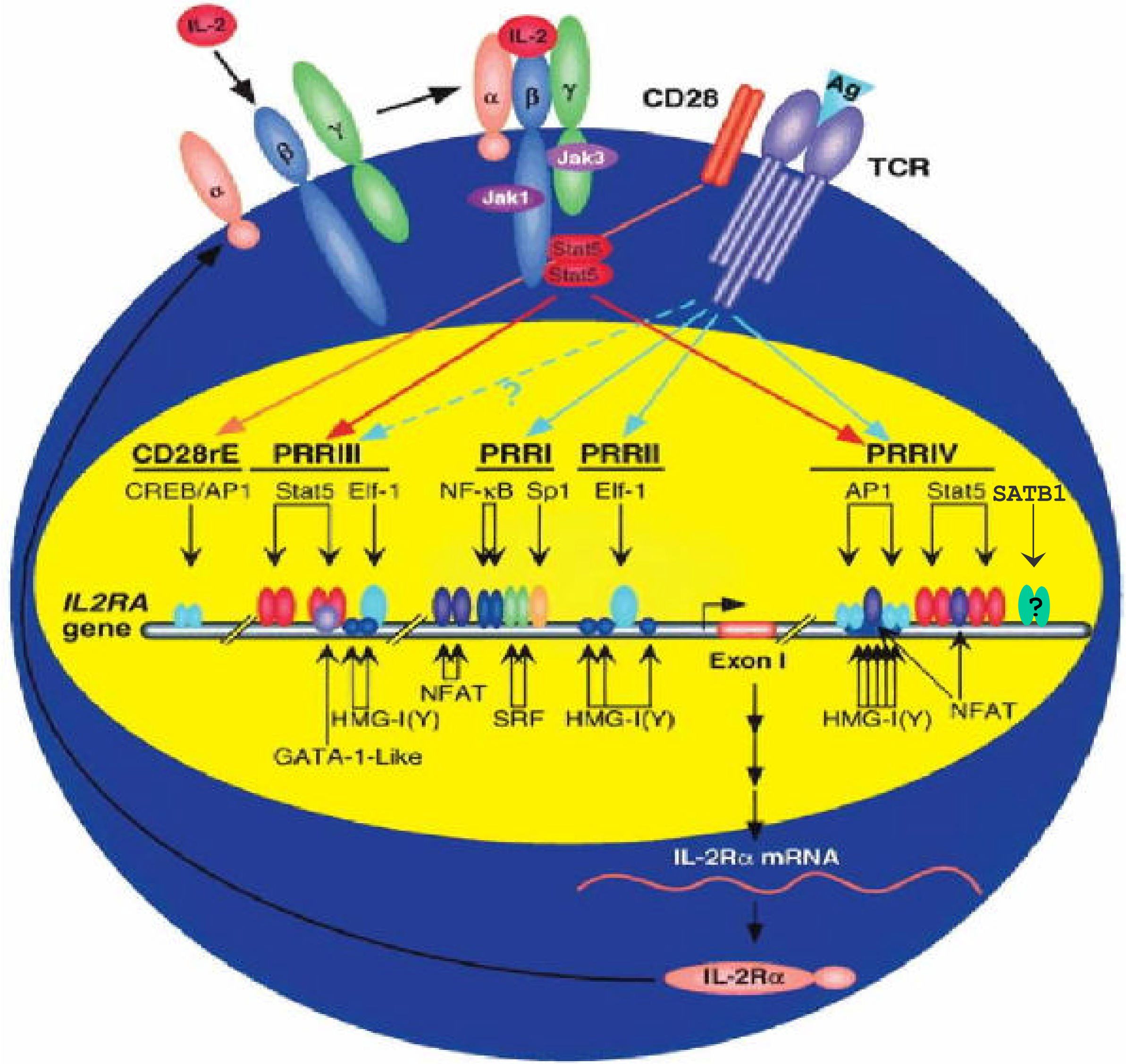
PRRV/CD28rE
(-8689~-8483)

PRRIII/IL-2rE
(-3780~-3703)

PRRI
(-276~-244)

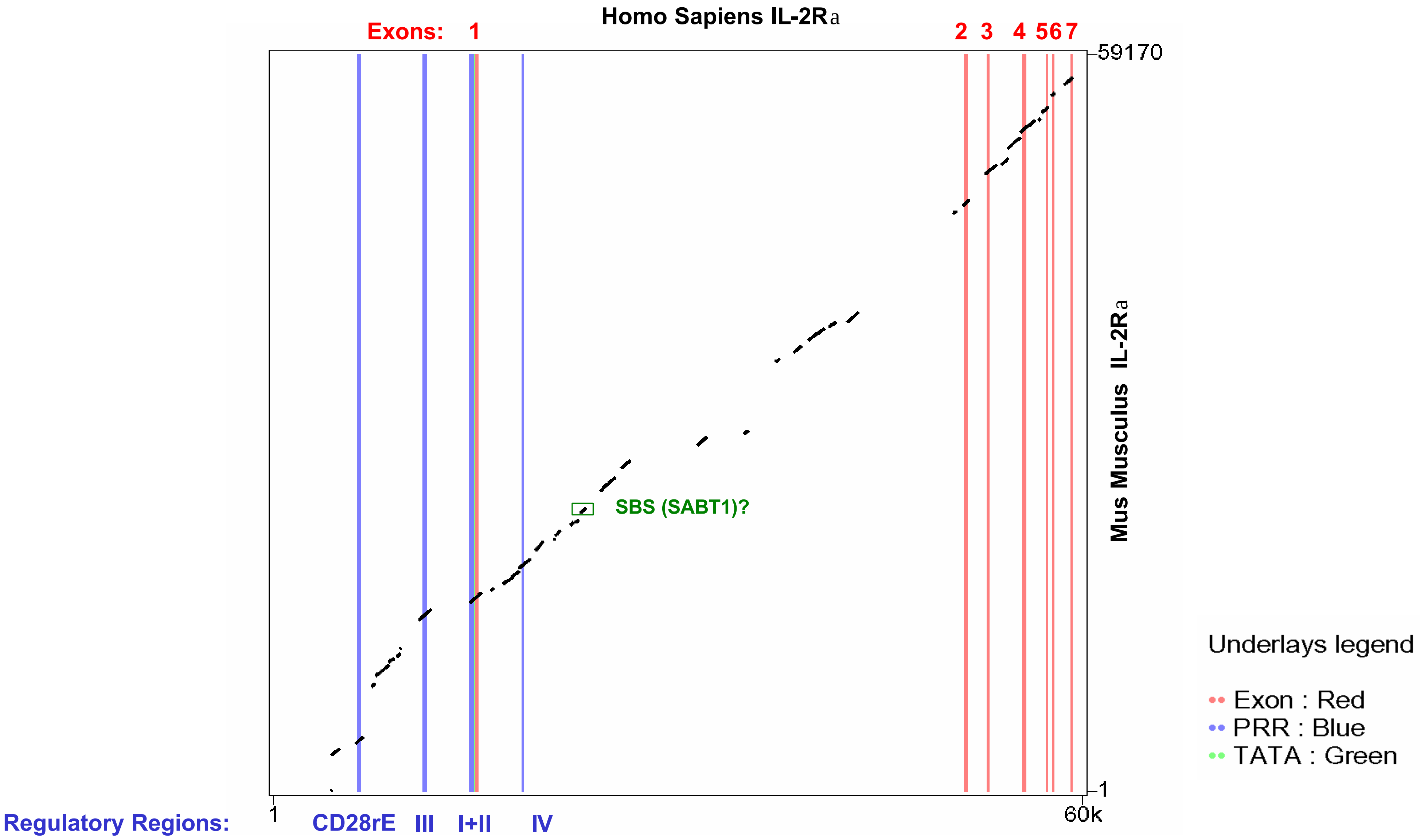
PRRII
(-137~-64)

PRRIV/IL-2rE
(+3389~+3596)



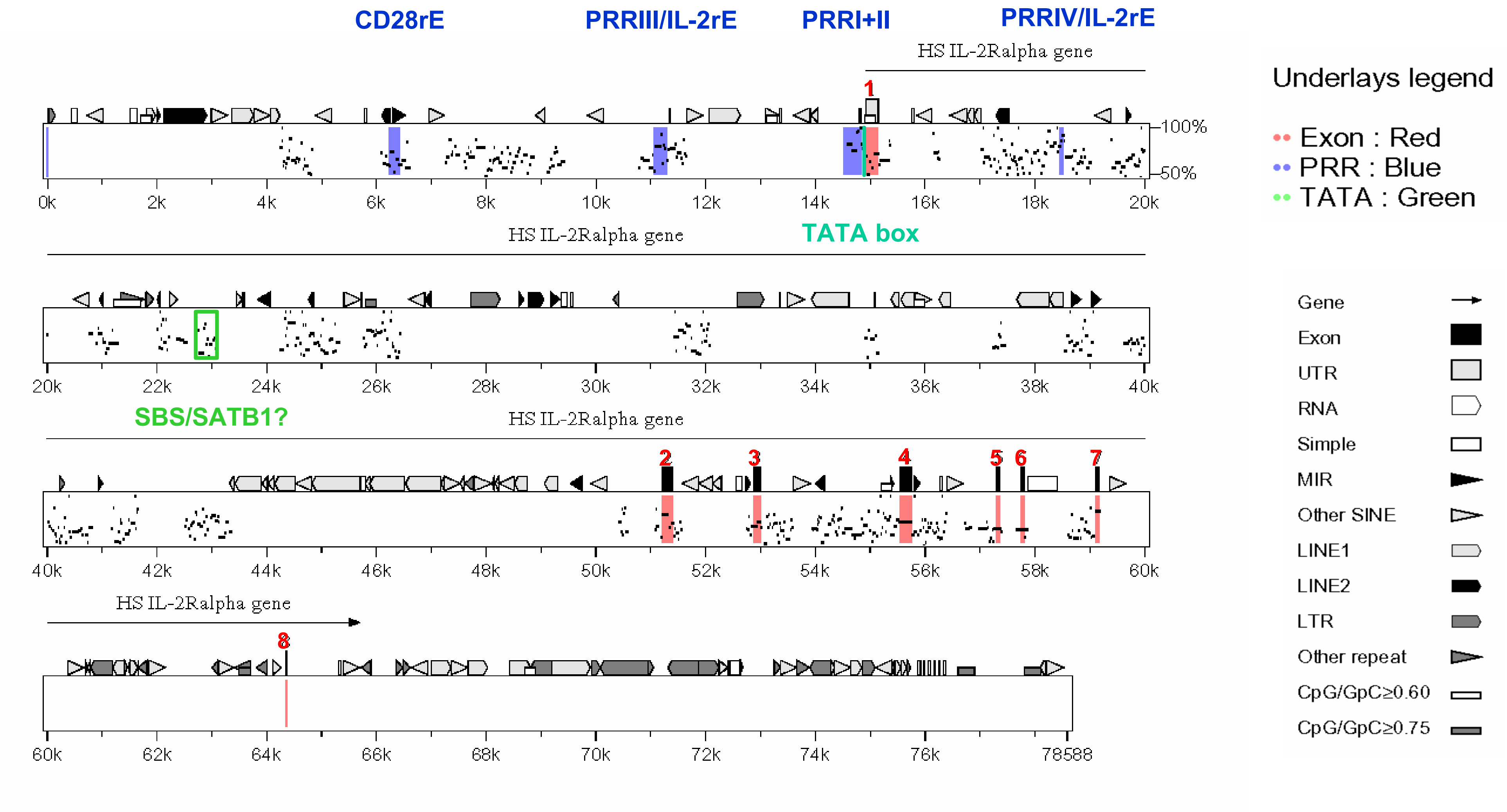
From Kim et al., EMBO J., 21:3051-3059, 2002.

Homo Sapiens/Mus Musculus IL-2Ra locus dotplot comparison



Schwartz, S., Zhang, Z., Frazer, K.A., Smit, A., Riemer, C., Bouck, J., Gibbs, R., Hardison, R., and Miller, W. (2000). PipMaker: A Web Server for Aligning Two Genomic DNA Sequences. Genome Res. 10, 577-586.

Percent Identity Plot (PIP) of Homo Sapiens and Mus Musculus IL-2Rα locus



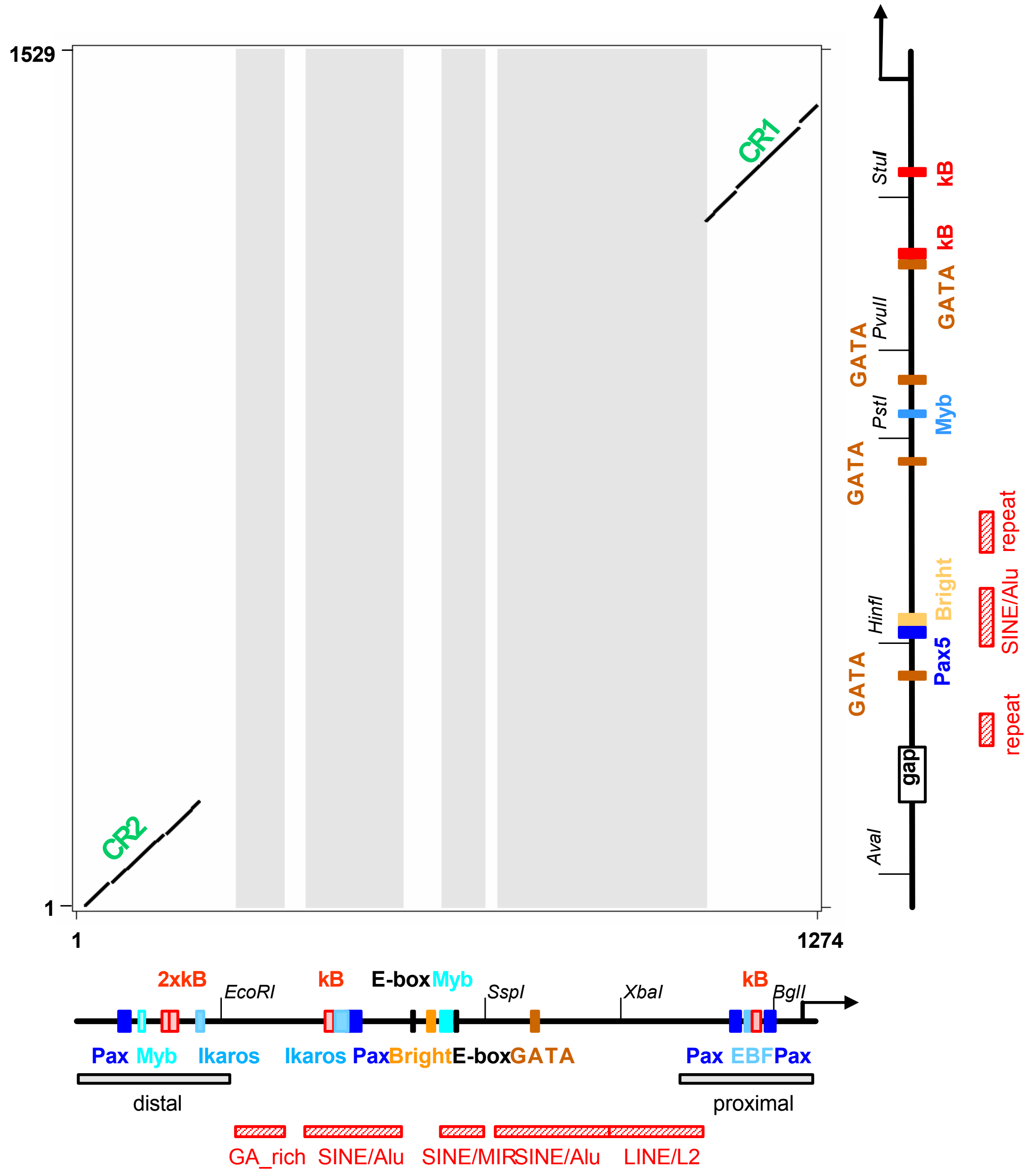
Schwartz, S., Zhang, Z., Frazer, K.A., Smit, A., Riemer, C., Bouck, J., Gibbs, R., Hardison, R., and Miller, W. (2000). PipMaker: A Web Server for Aligning Two Genomic DNA Sequences. Genome Res. 10, 577-586.

U119 Experimental Oncology

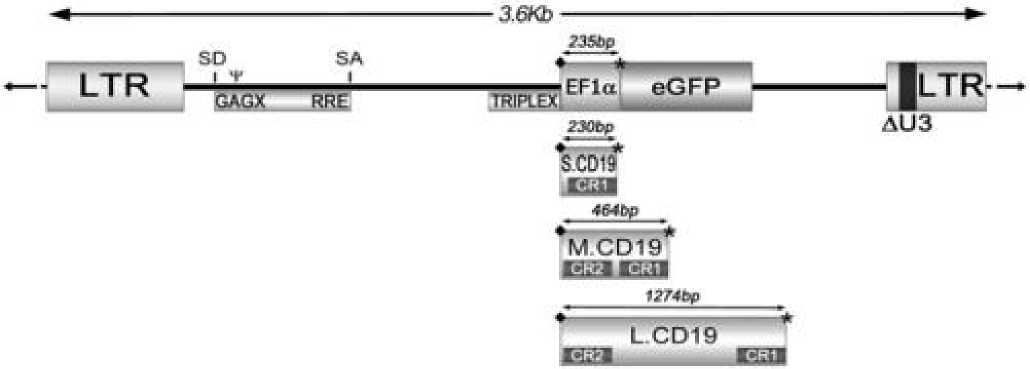
Selection of CD19 B-cell specific regulatory sequence and design of CD19-GFP lentiviral vector

Moreau, T., F. Bardin, J. Imbert, C. Chabannon, and C. Tonnelle. 2004. Restriction of transgene expression to the B-lymphoid progeny of human lentivirally transduced CD34+ cells. Mol.Ther,. 2004, in press.

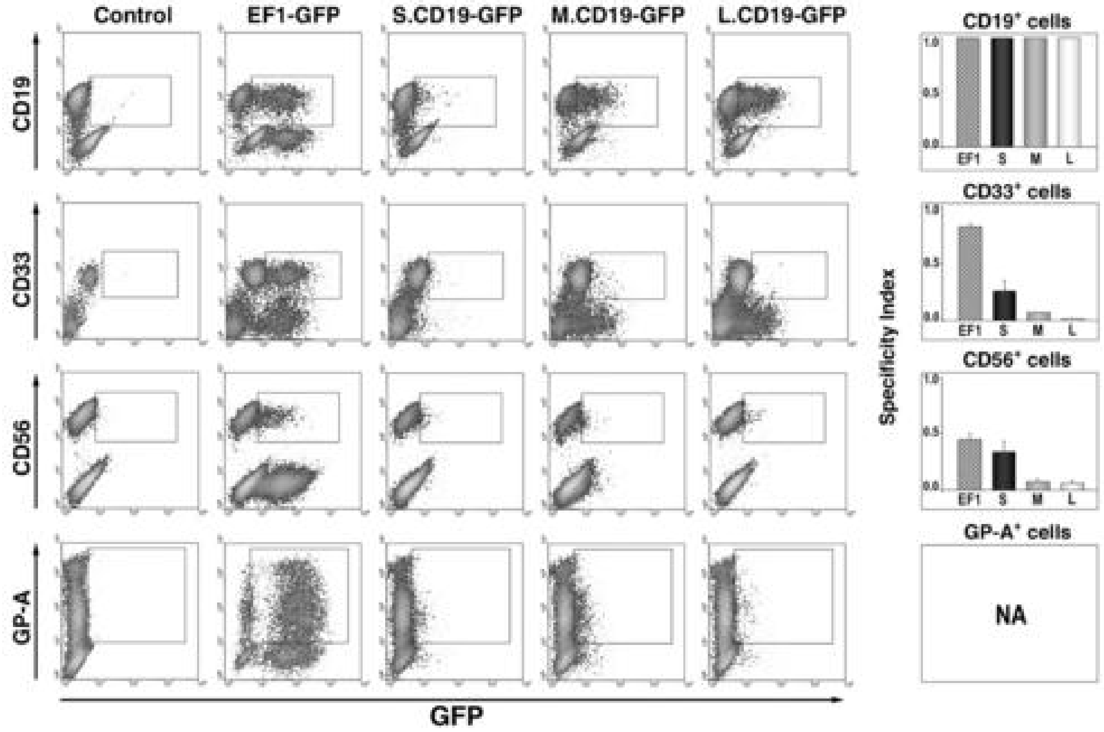
Computational analysis of homologies between Human and Mouse CD19 gene 5’ regions



A. Recombinant CD19-GFP lentiviral vectors



B. GFP expression in the progeny of transduced CD34$^+$ progenitor cells differentiated in vitro



From Moreau et al., Mol.Ther, 2004.

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