

Gene Transfer and Insertional Mutagenesis



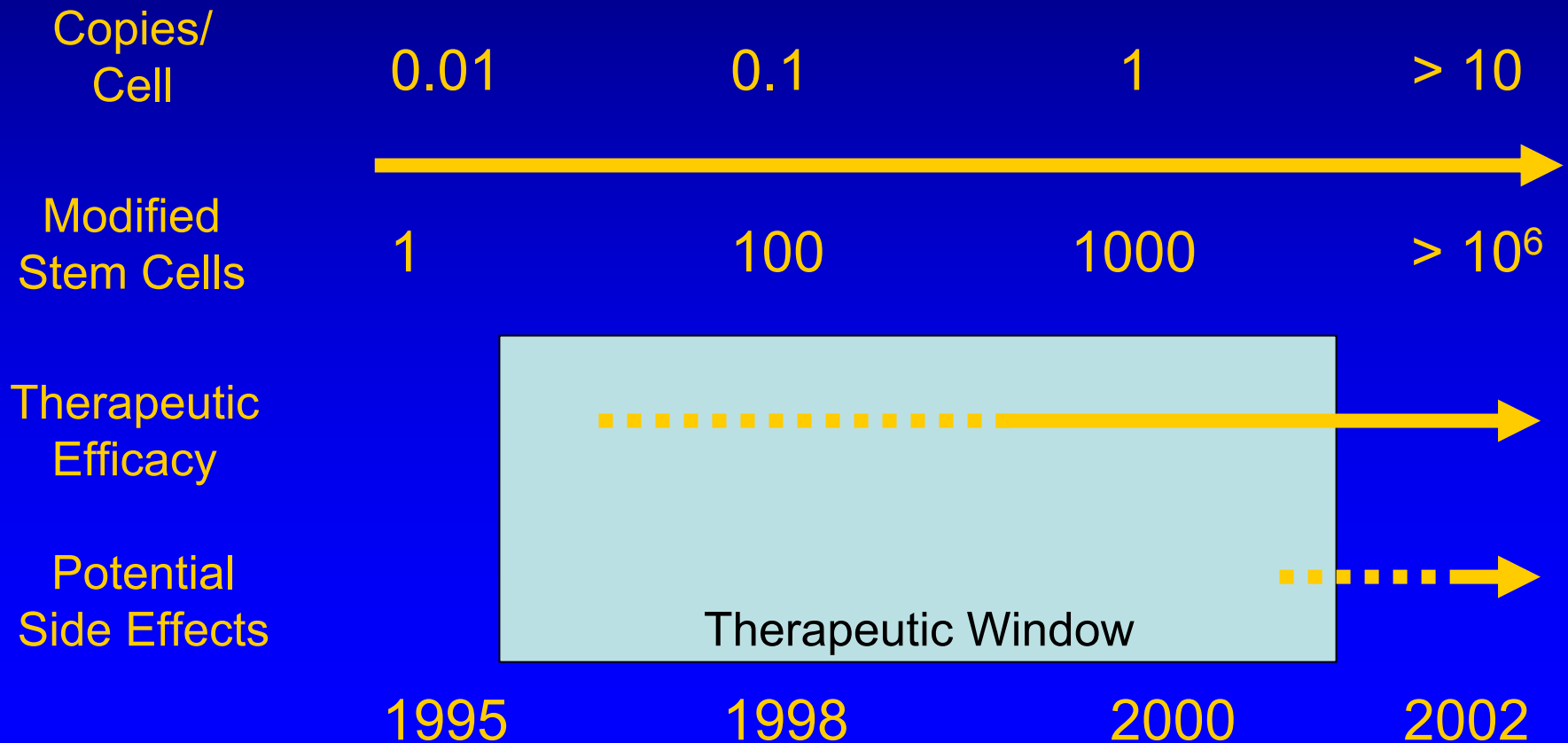
Molecular and Gene Therapy Program
Division of Experimental Hematology
Children's Hospital Research Foundation
Cincinnati



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und Poliklinik, Abt. Innere Medizin I

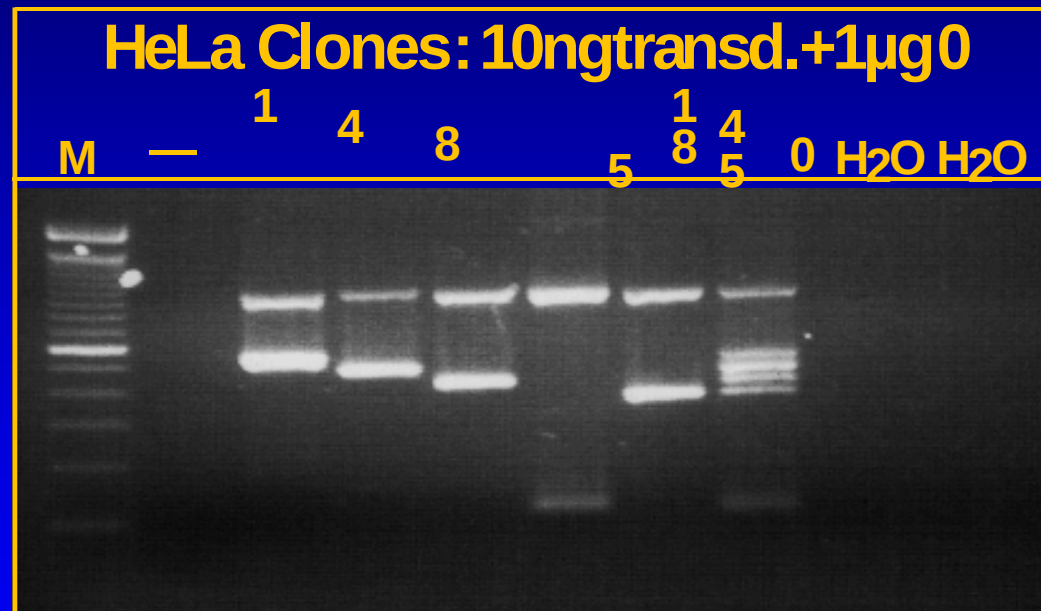
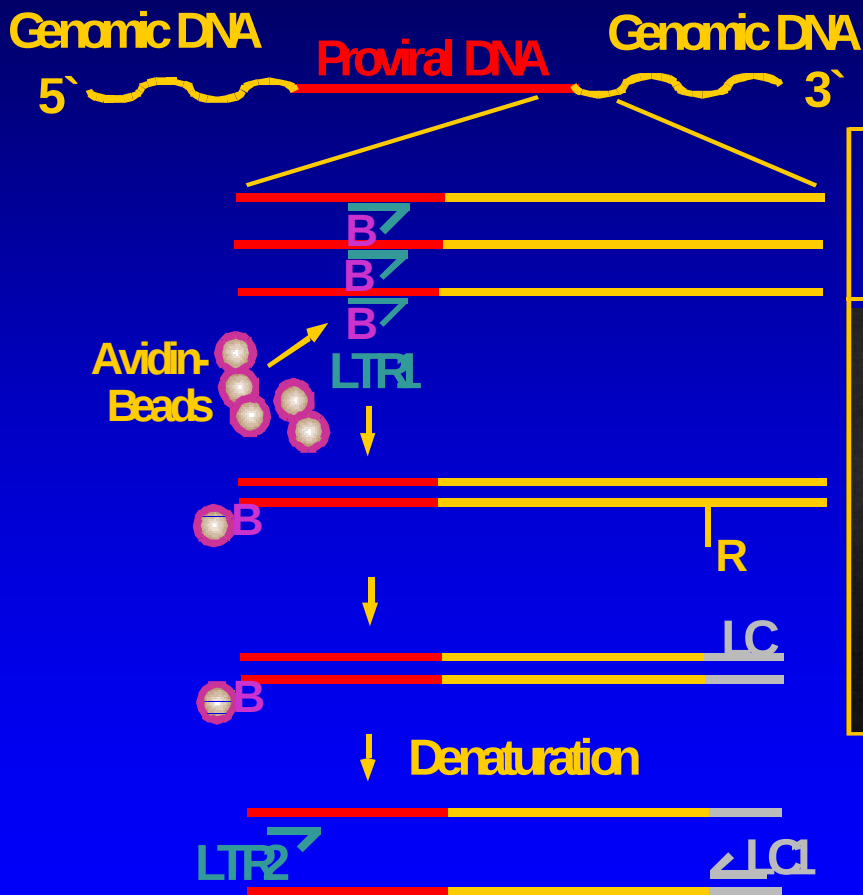
Risk/Benefit Assessment of Clinical Applications For Insertional Vector Systems As Pharmaceutical Agents



Insertion Site Analysis: Dose Finding in Gene Therapy

- Stem Cell Clonality
- Insertion Site Distribution Analysis
- Cell Type Dependence of Insertion
- Genomic Side Effects of Insertion
- Active Modification of Insertion Behavior

Integration Site Analysis by LAM-PCR



Schmidt *et al.*, Hum Gen Ther 2001

Schmidt *et al.*, Blood 2002

Ailles *et al.*, Hum Gen Ther 2002

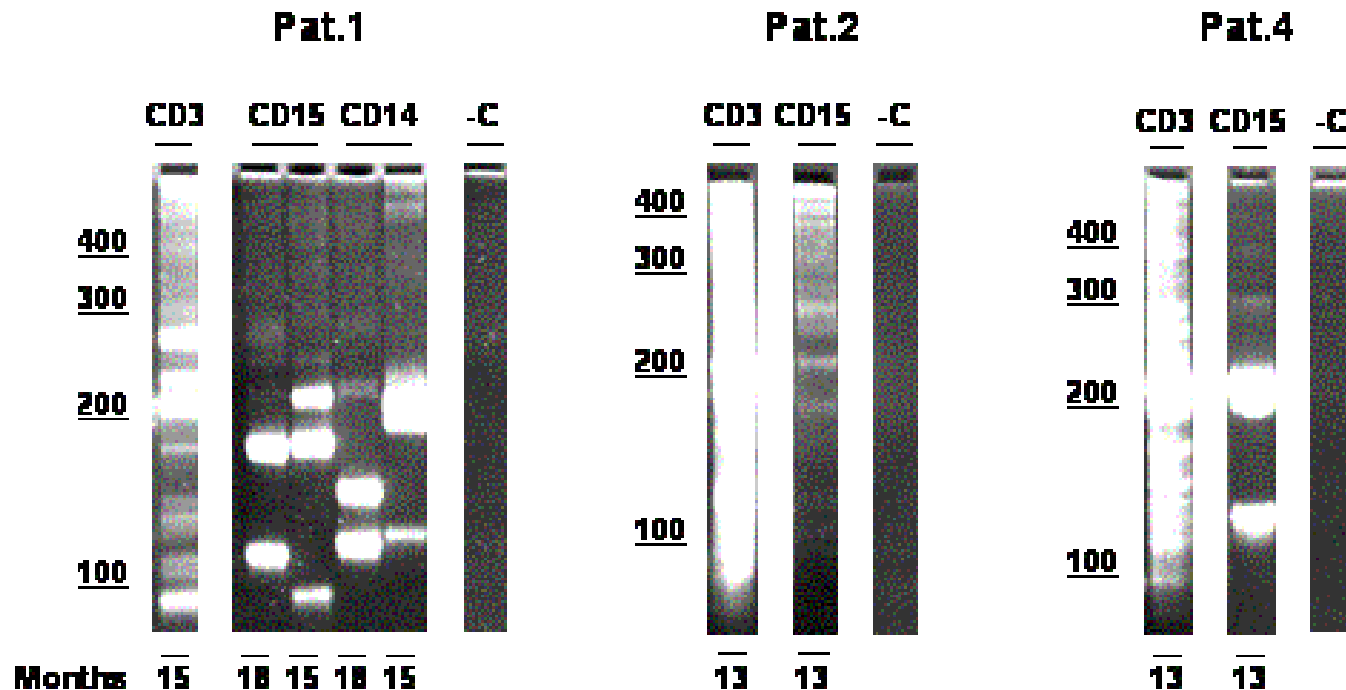
Woods *et al.*, Blood 2003

United States Patent No. 06514706 B1

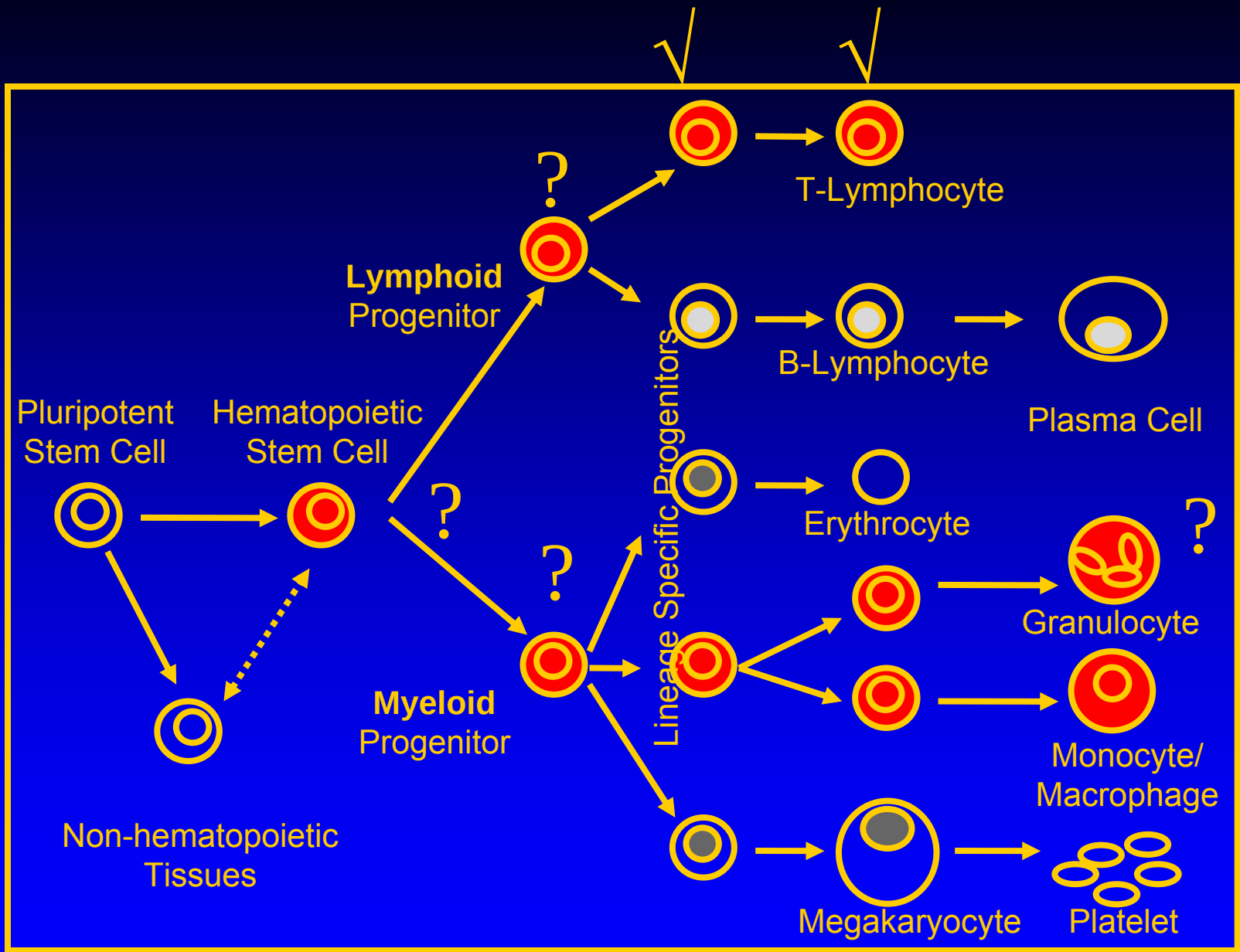
SCID-X1 Gene Therapy

(A. Fischer *et al.*, A. Thrasher *et al.*)

In vivo Clonality Analysis

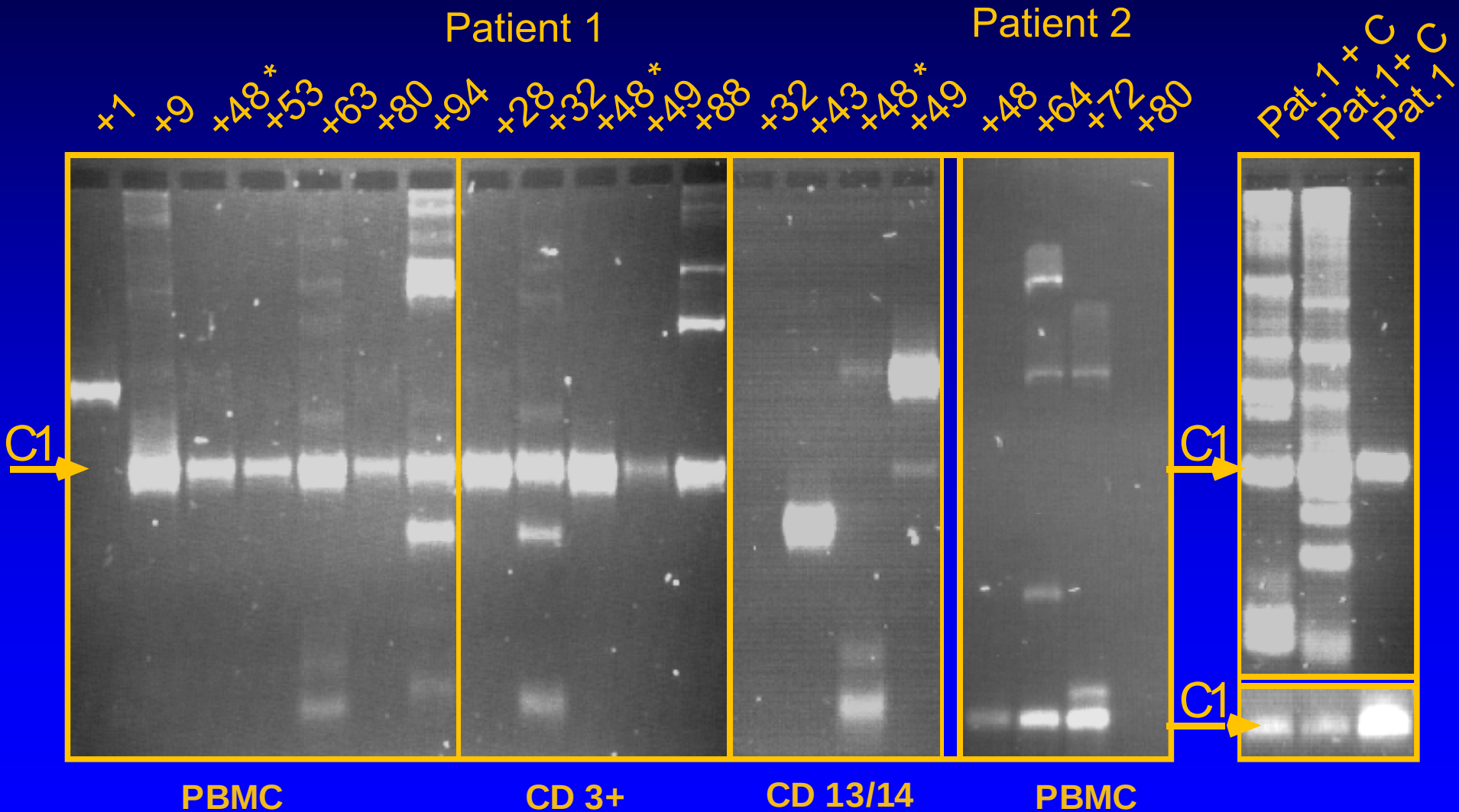


Gaspar *et al.*, *Lancet in press*, Schmidt M *et al.*, *Blood*, in press



ADA Gene Therapy

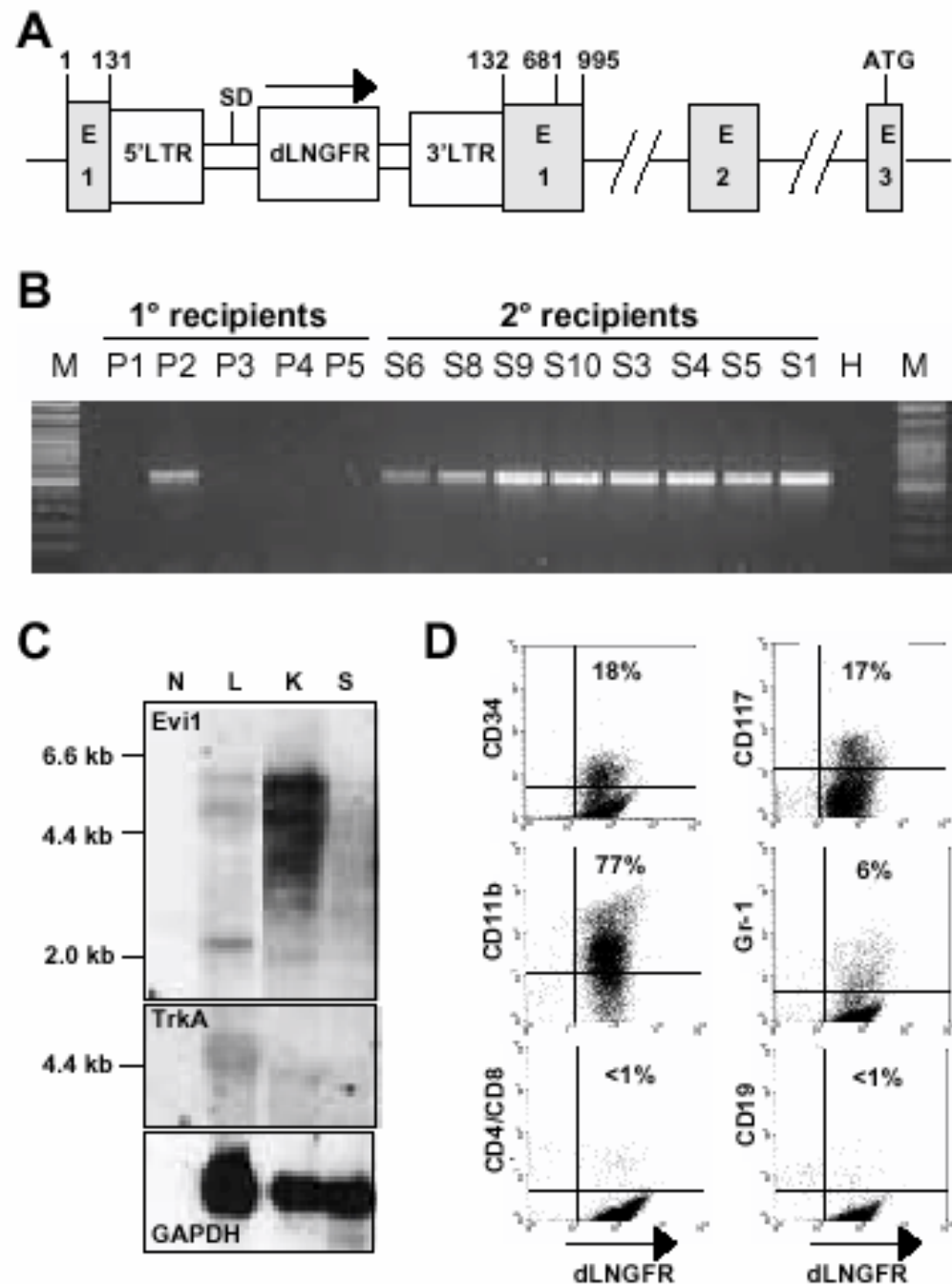
In Vivo Clonality Analysis



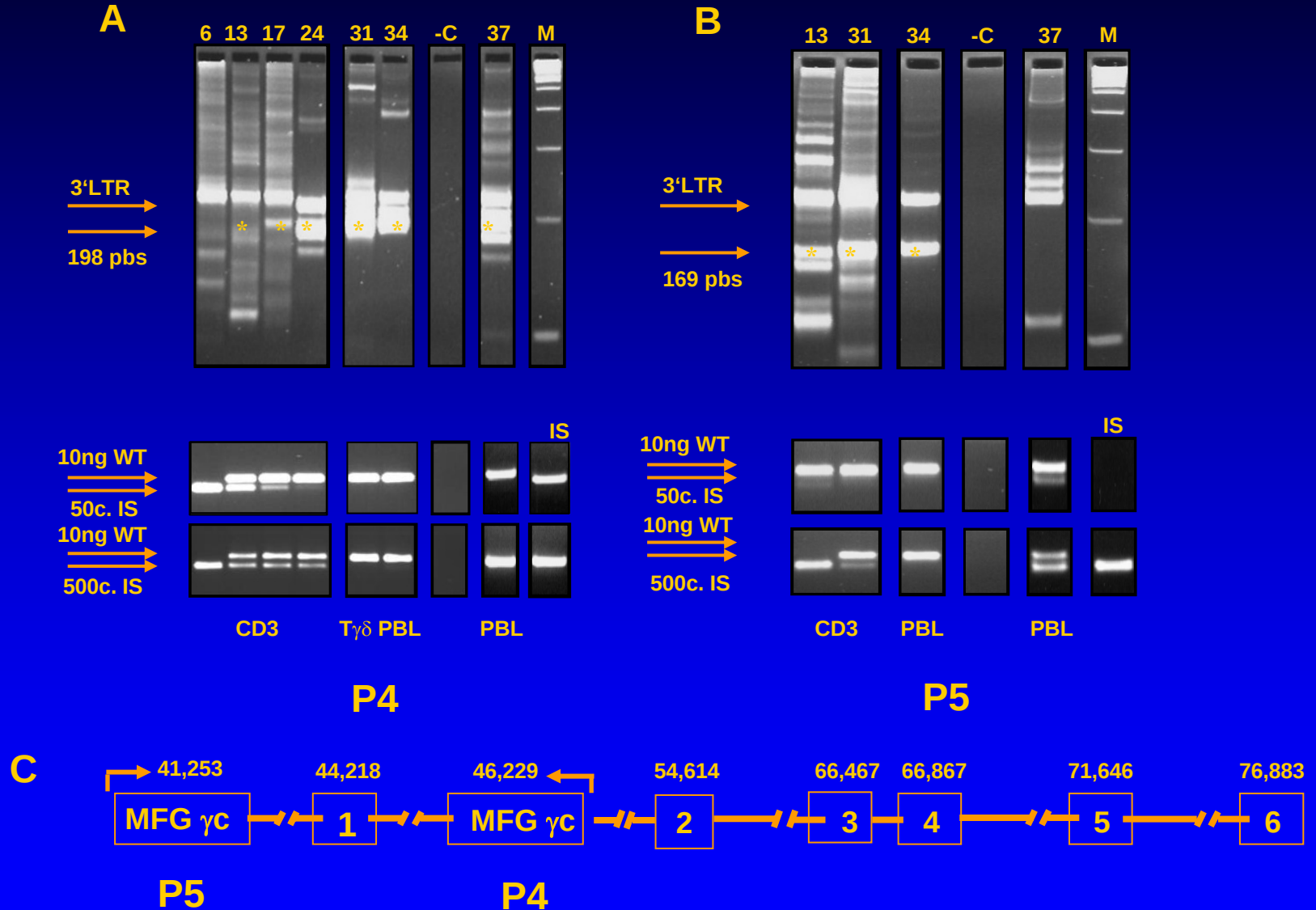
Schmidt M *et al.*, Nat Med 2003

The unmodified retroviral Vector LNGFR Fused into Evi-1 Exon 1, Inducing Constitutive Expression of the Proleukemic Transcription Factor

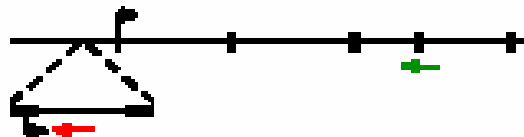
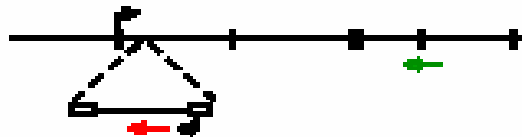
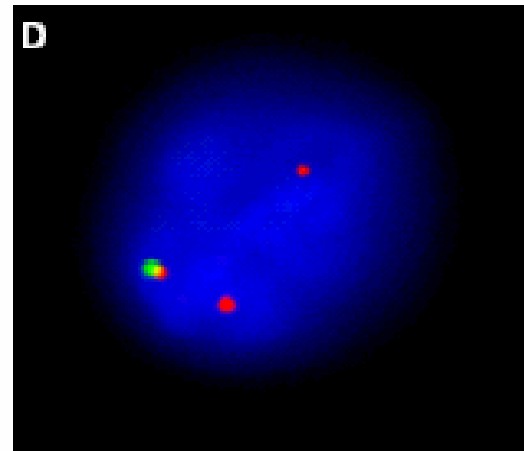
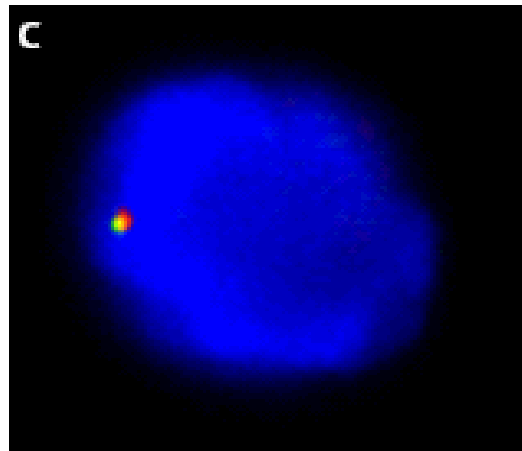
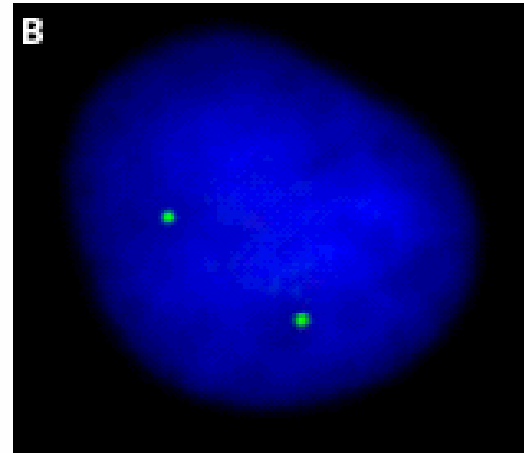
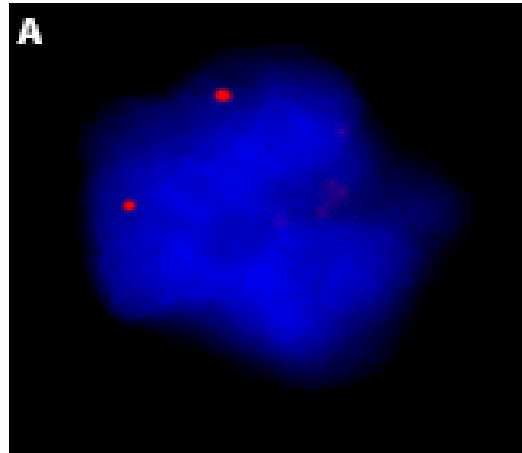
Li et. al, Science 2002



Development of Monoclonality in Human Hematopoiesis



Co-Transcription by RNA FISH



LAM High Throughput Data Pipeline

KALLE PROJECT BIOINFORMATICS - Microsoft Internet Explorer

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Address http://neo-c.chmcc.org:8080/~mkeddach/kalle/

KALLE PROJECT BIOINFORMATICS
-- Upload Zipped Sequence Files --

This interface takes .seq files from a ZIP archive and tries to find predefined motifs in the sequence (5' VECTOR, 3' VECTOR, LINKER, LTR and sequences to the read sequence, once a partial alignment of the specified word size is made. The entire motif is assumed to be present in the read is strictly limited to a subsequence of word size in length.

5' VECTOR:	ACCGAGCTCGGATCCCTAGTAACGGCCGCCAGT	word size:	12
3' VECTOR:	AAGGGCGAATTCTGCAGATATCCATCACACTGG	word size:	16
LTR:	TTCCATGCCTTGCAAAATGGCGTTACTTAAGCT	word size:	12
LINKER:	CCTAACTGCTGTGCCACTGAATTCAGATC	word size:	12
CONTROL:	CTTTCATTCCCCCTTTTCTGGAGACTAAATA	word size:	12

BLAST sequences equal or longer than bp in length.

To upload your zip file:

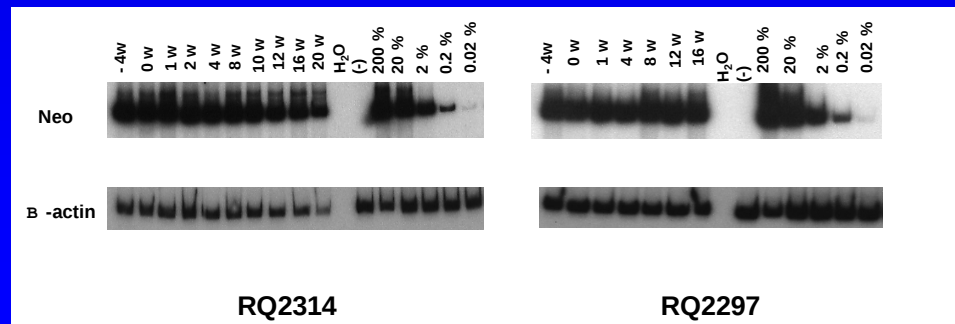
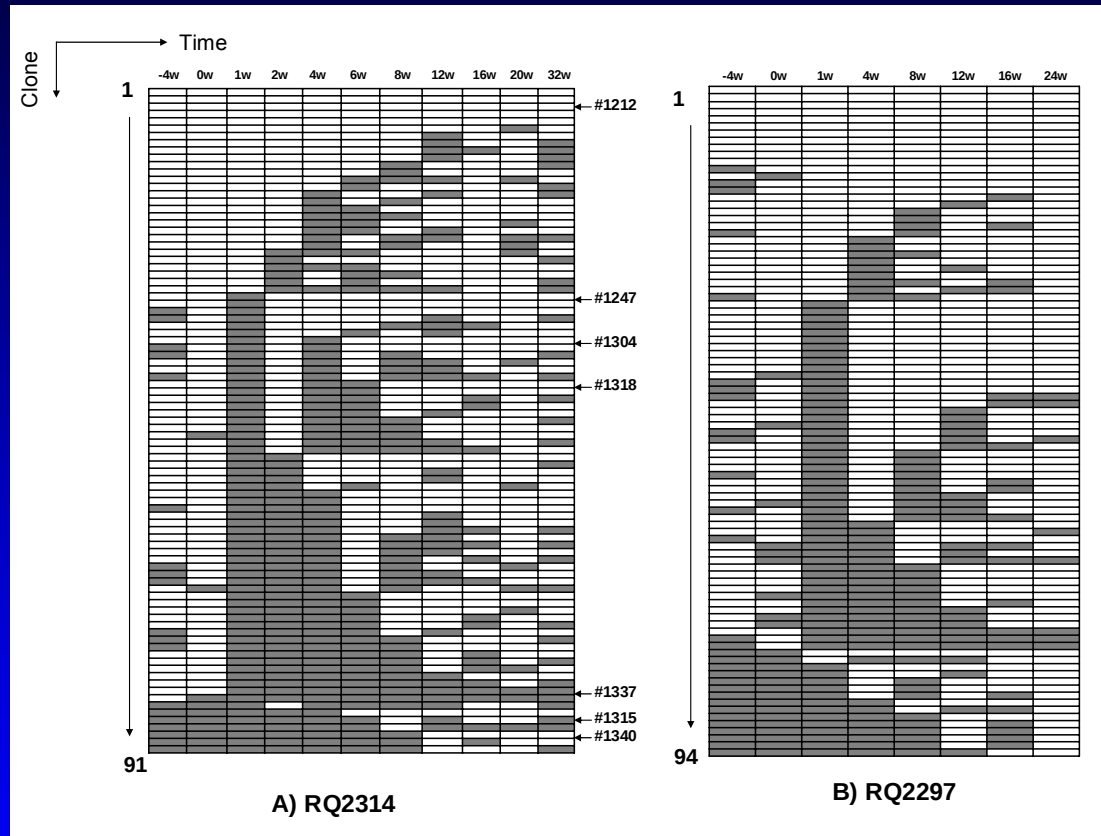
- Make sure the zip archive only contains .seq files in PC or UNIX text format
- Click BROWSE and select the file on your hard drive.
- Click PROCEED to let the system check the file. You will be taken to another page.

ZIP FILE:

Previous Uploads:

- seqfiles.zip
- Br.pt5-p1-b10+M13R.seq
- Br.pt5-p2-h11+M13R.seq
- Br.pt5-p2-a12+M13R.seq
- Br.pt5-p2-b12+M13R.seq
- Br.pt5-p3-d12+M13R.seq
- Br.pt2-p1-a12+M13R.seq
- Br.pt5-p1-b12+M13R.seq
- Br.pt5-p2-a11+M13R.seq
- Br.pt5-p1-a12+M13R.seq
- Br.pt5-p1-e12+M13R.seq
- Br.pt2-p1-a09+M13R.seq
- Br.pt5-p1-c12+M13R.seq
- Br.pt5-p3-a12+M13R.seq
- Br.pt5-p1-d12+M13R.seq
- Br.pt2-p1-d12+M13R.seq
- Br.pt5-p2-e12+M13R.seq
- Br.pt5-p1-e11+M13R.seq
- Br.pt2-p1-b11+M13R.seq
- Br.pt5-p2-b11+M13R.seq
- Br.pt2-p1-b12+M13R.seq
- Br.pt5-p1-h12+M13R.seq
- Br.pt5-p2-c12+M13R.seq
- Br.pt2-p1-e12+M13R.seq
- Br.pt2-p1-a11+M13R.seq

Busulfan Conditioning Impact On Stem Cell Clone Numbers

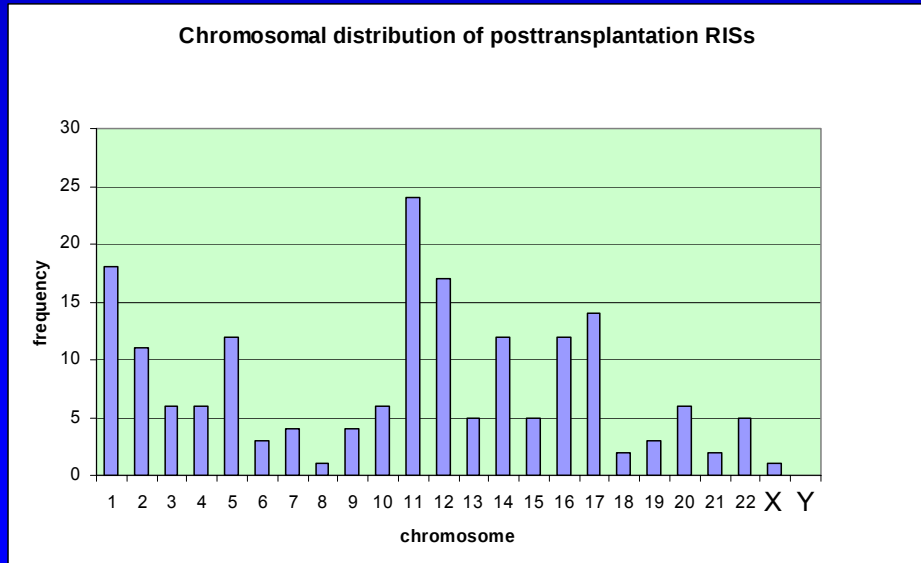
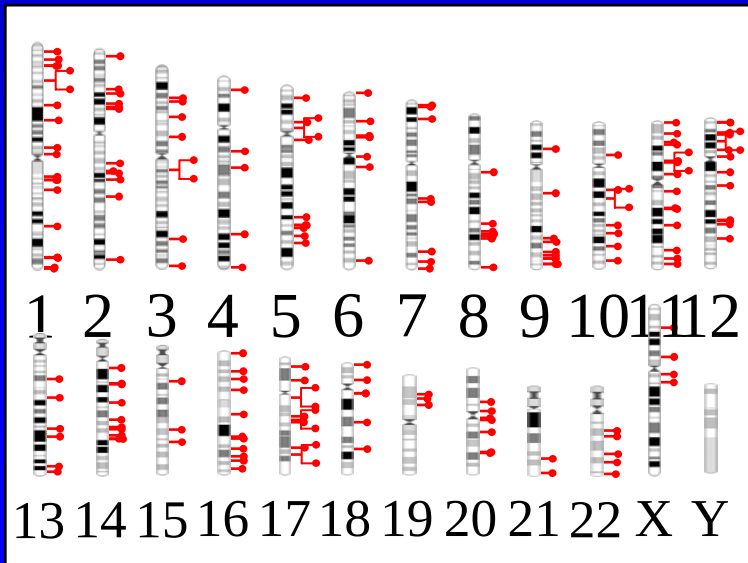
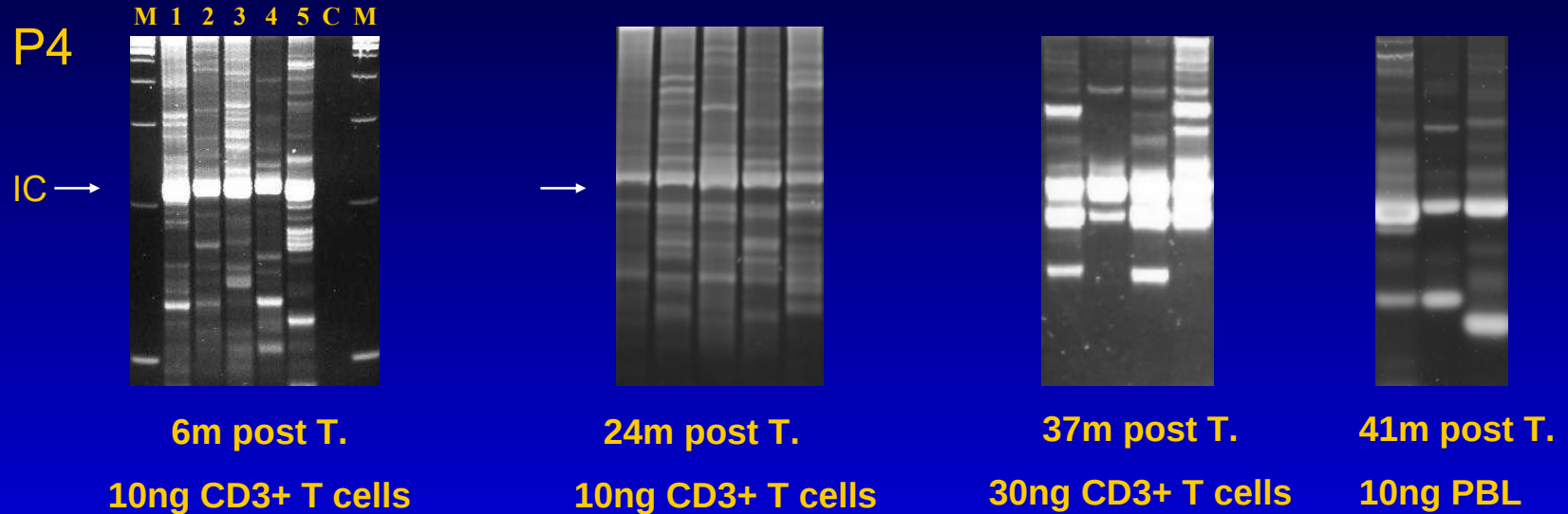


G-CSF:
Kuramoto *et al.*,
Blood 2004;
Bu:
Kuramoto *et al.*,
Blood 2004

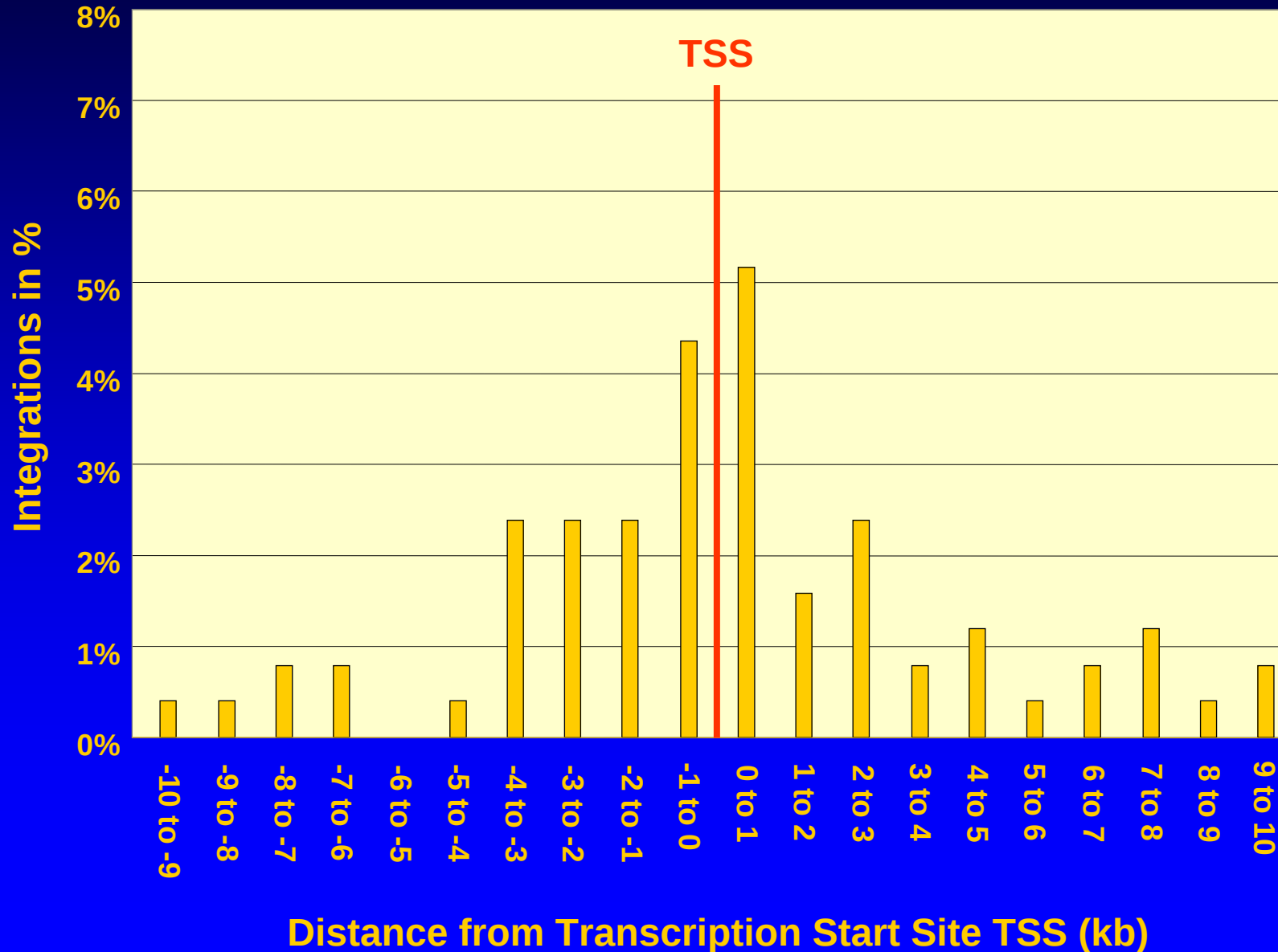
Analysis of Retroviral Integration Sites (IS)

	Post-Transplantation	
	Absolute	Percentage
Total number of integration sites (IS)	344	
Exactly mappable IS	252	100%
IS in RefSeq genes	99	39%
IS in RefSeq genes including the region 10 kb upstream and downstream of the gene	148	59%
IS more than 10 kb away from RefSeq genes	104	41%
IS within +/- 5 kb around transcription start site	58	23%

LAM Characterization of Post-Transplantation Hematopoiesis



Insertions around Transcription Start Sites



Gene Ontology Analysis (NCBI)

Molecular Function

Level	Category	List Hits	Fisher Exact
2	Kinase activity	14	0.000534
2	Transferase activity	19	0.00173
3	Protein kinase activity	10	0.00403
4	Phosphotransferase Activity	12	0.00134
4	Protein-tyrosine kinase Activity	5	0.00632

Total number of RefSeq genes in the list: 136

Insertional Activation – Relevant Parameters to Look for In Vivo

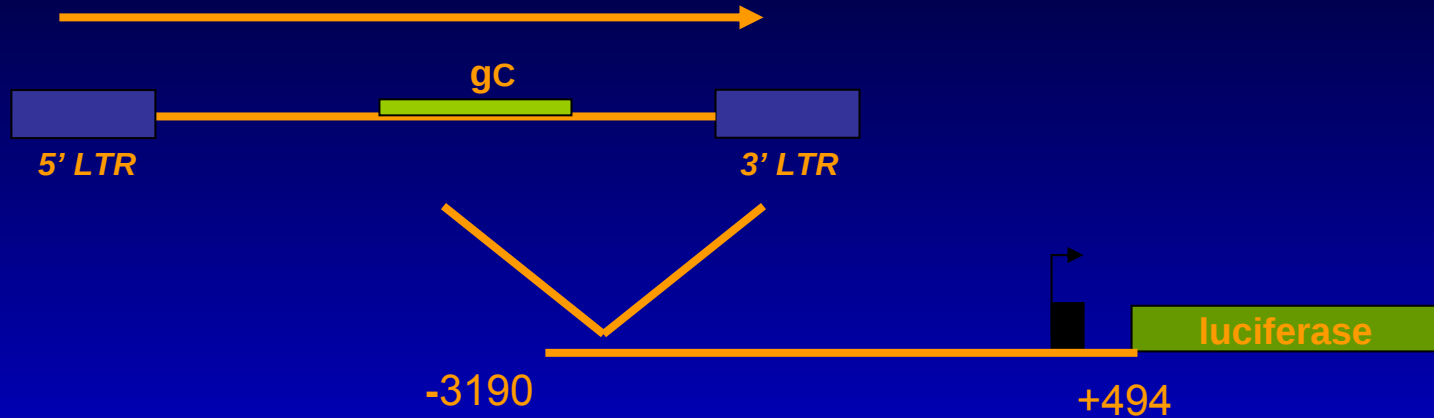
- Insertion Correlation to Expression Pattern
- Regulatory Insertions Should Prevail over Disruptive Insertions
- Increase of Contribution over Time

Vector-induced LMO2 activation

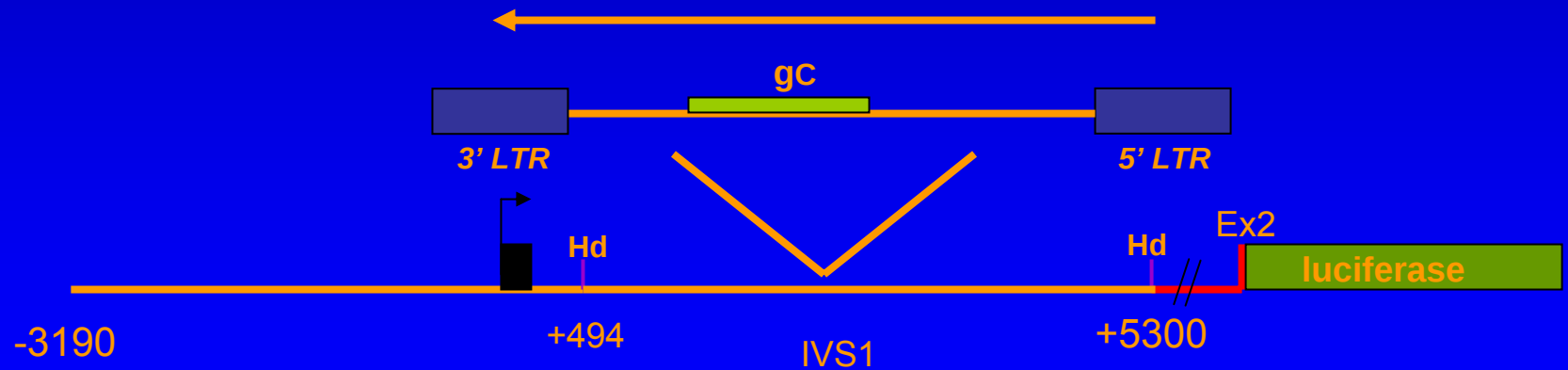
- Our data suggest:
 - Enhancer effect by the 3'LTR.
 - Promoter competition for the 5'LTR enhancer
- Position within the *LMO2* locus may affect activation.
- Negative regulatory elements upstream of the *LMO2* promoter need to be overcome.
(Precedence: Differential interactions between promoters of adult and fetal b-globin locus)
Townes and Behringer (1990), Crossley and Orkin (1993)
- Hypothesis: A “competent” transcriptional complex at the 5'LTR may be stabilized by components of the RNA processing machinery.

LMO2 Promoter Activation Test Constructs

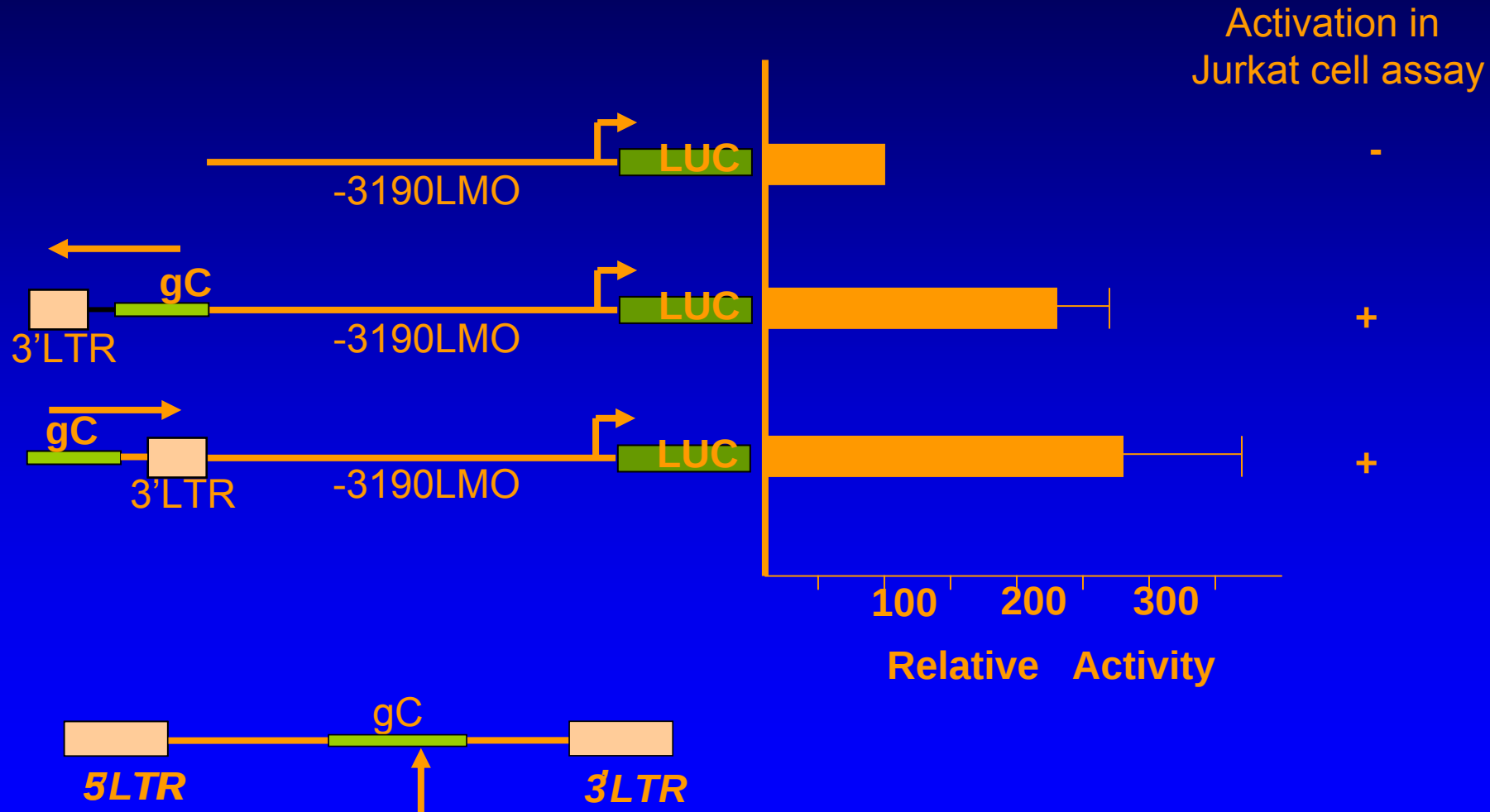
Patient 5



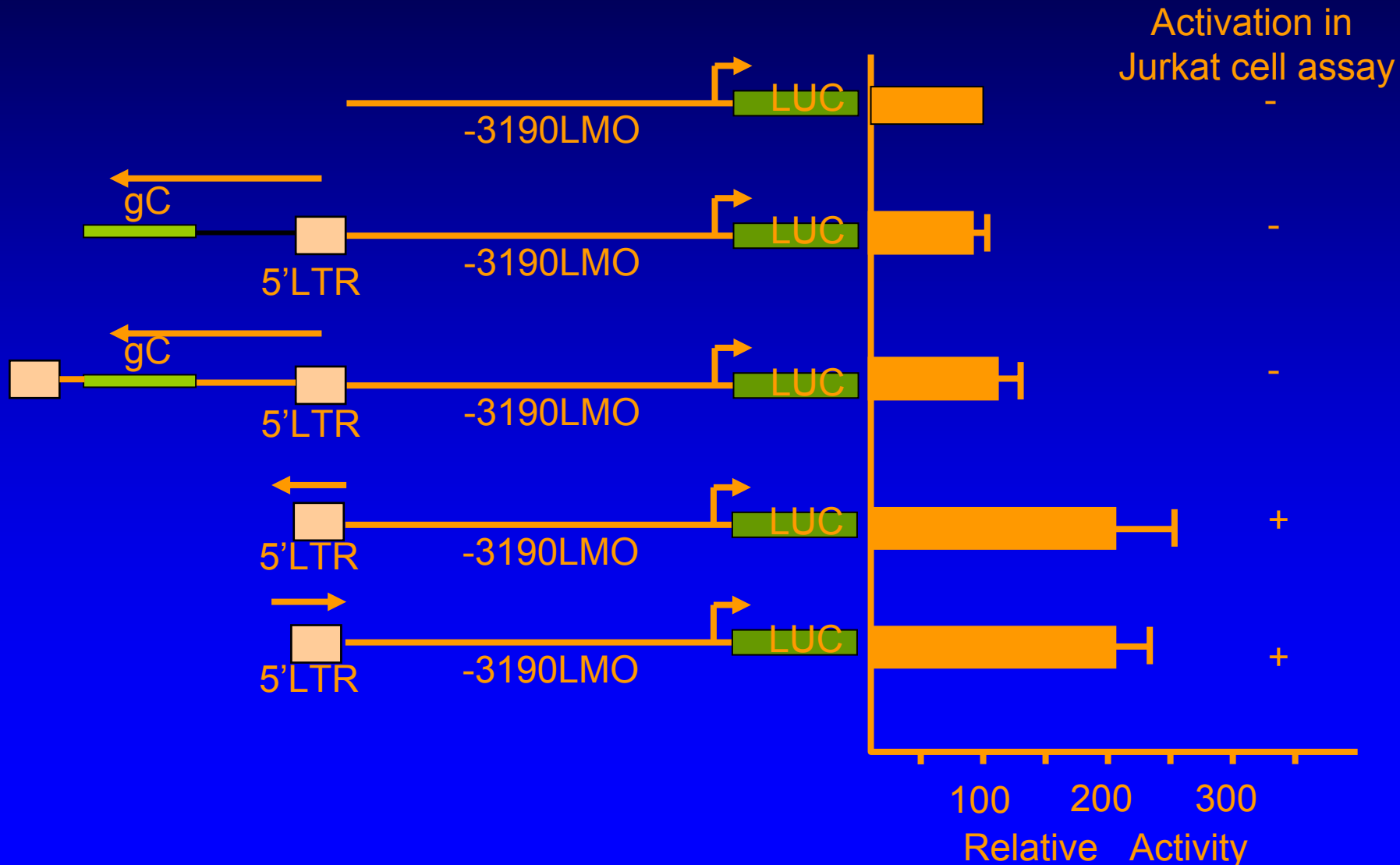
Patient 4



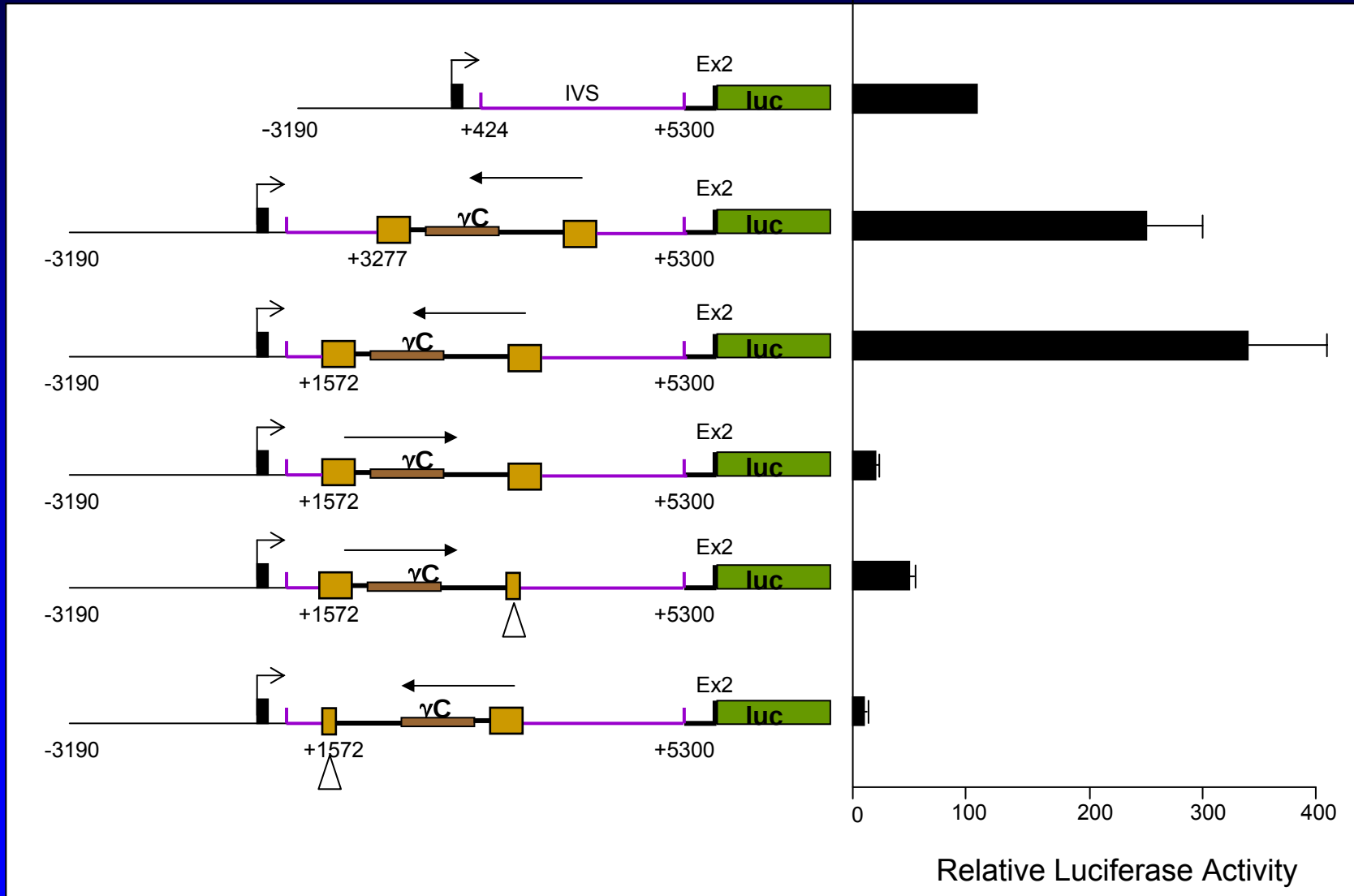
3'LTR Activates the LMO2 Promoter



Activation of the LMO2 promoter by the 5'LTR appears to be modulated by vector elements



SIN Vector Design Prevents LMO2 Promoter Activation



Conclusions

- Preferred integration of MLV-based vectors within or close to genes:

59% within or close to genes,

39% within the gene itself,

~50% of these within in the first 20% of the gene

- Insertion peaks around the start of transcription in a narrow segment (+/- 4 kb)
- Clustering in Common Insertion Sites
- Differences before and after engraftment
- Differences between trials

Insertion Site Analysis

Advancing to the Next Level

- Stem Cell Clonality – Novel Data Quality
- Insertion Site Distribution Analysis
 - Cell Type Dependence of Insertion
 - Gene Expression Dependence of Insertion
 - Genomic Side Effects of Insertion
- Active Modification of Insertion Behavior

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