

Post-transcriptional deregulation of gene expression caused by retroviral integration in the human genome

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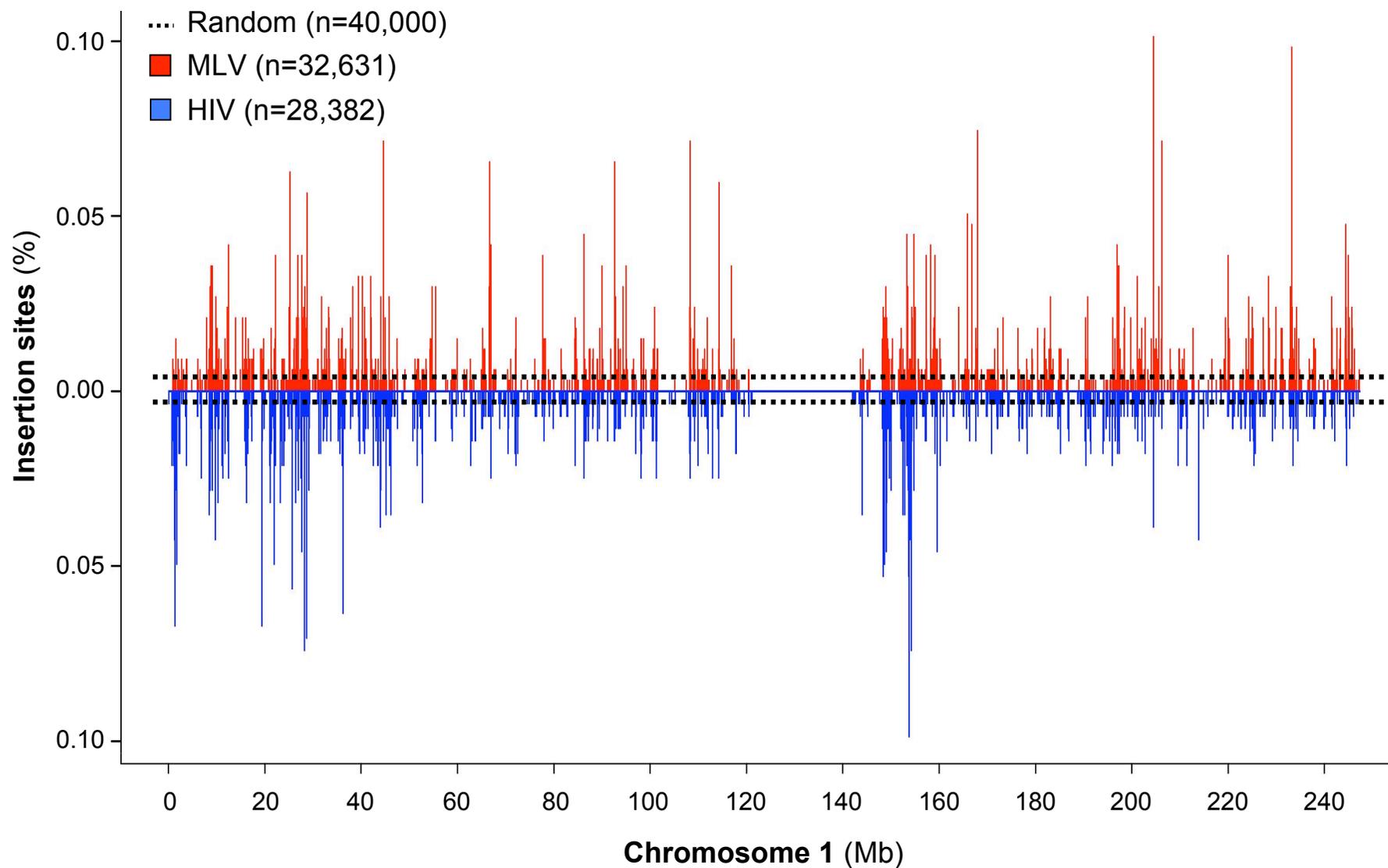
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and

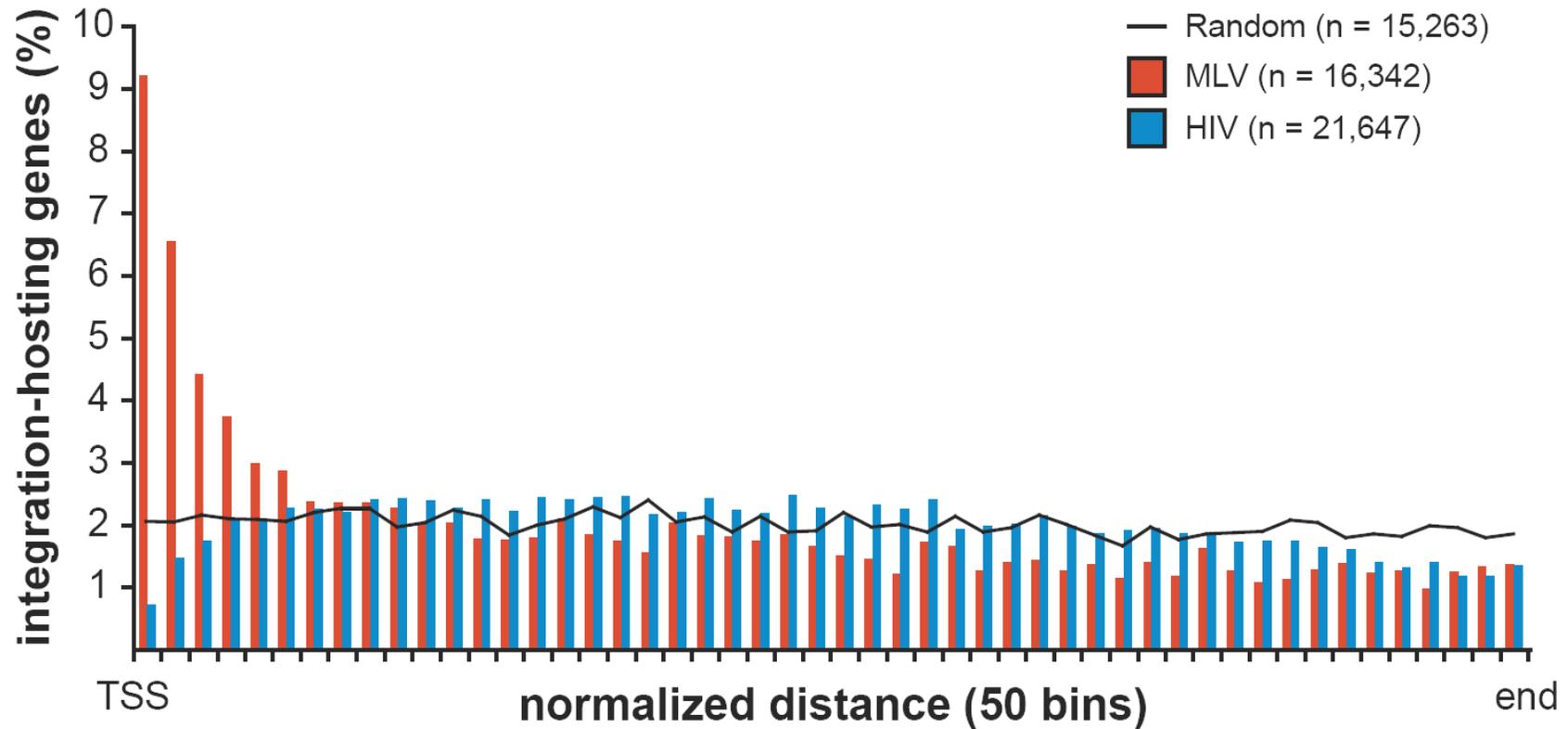
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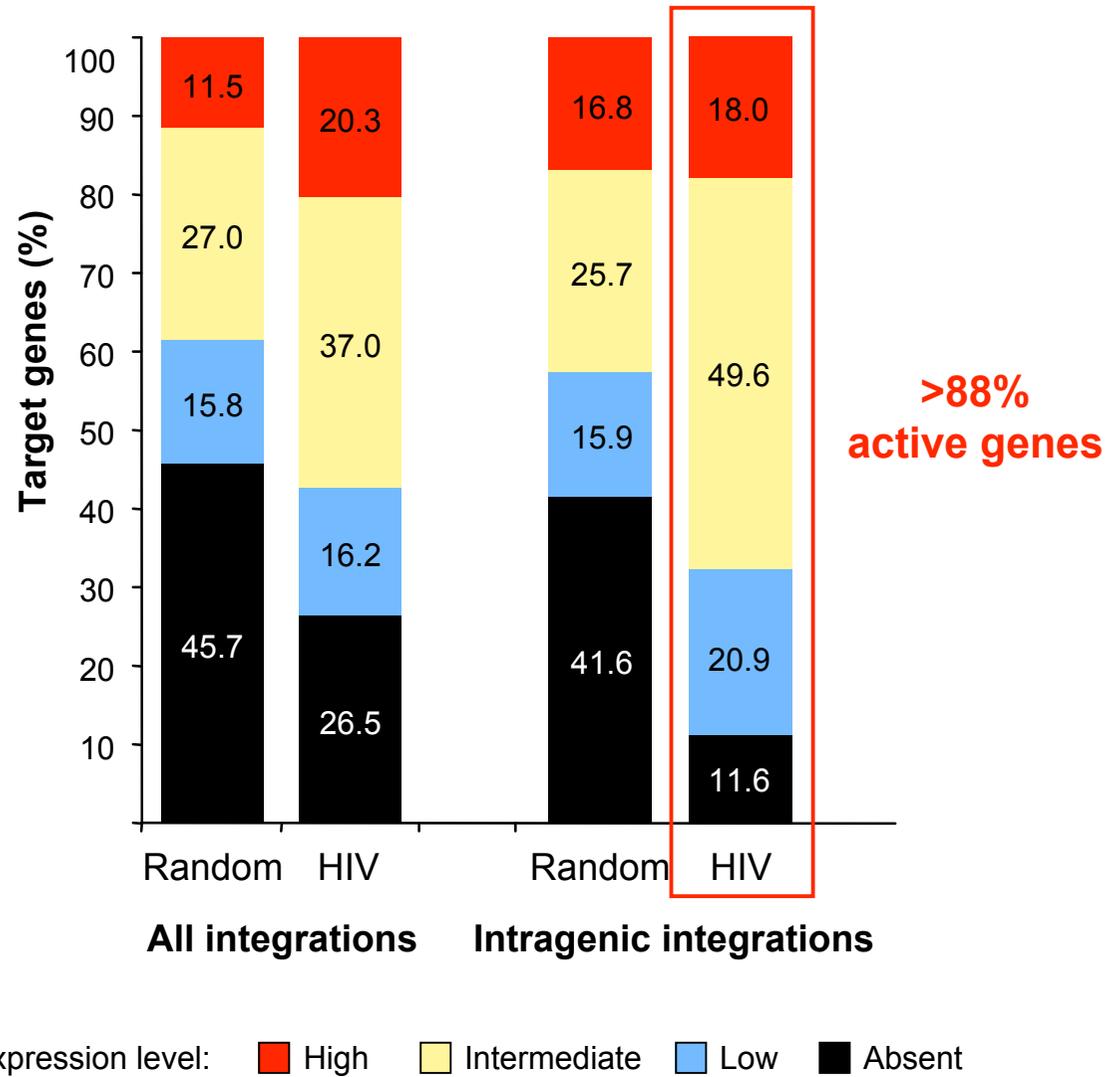
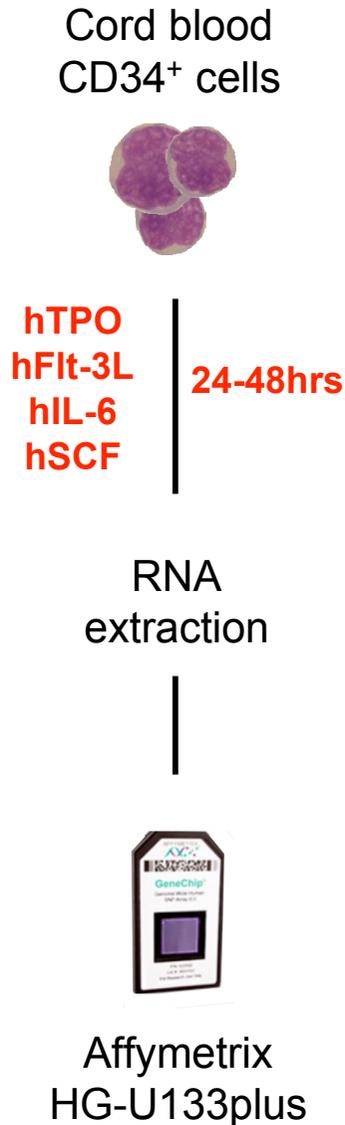
Lentiviral vector integrations are clustered (CD34⁺ HPCs)



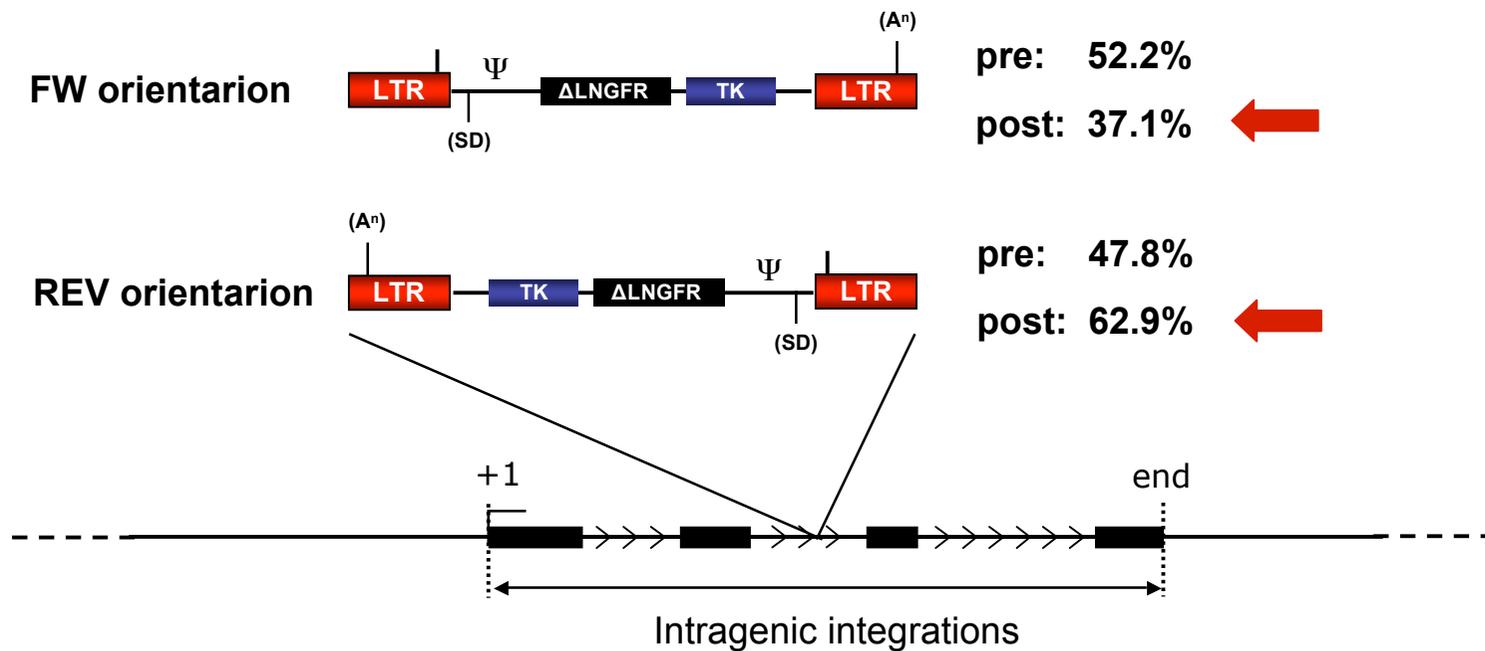
Lentiviral vector integrations target gene bodies



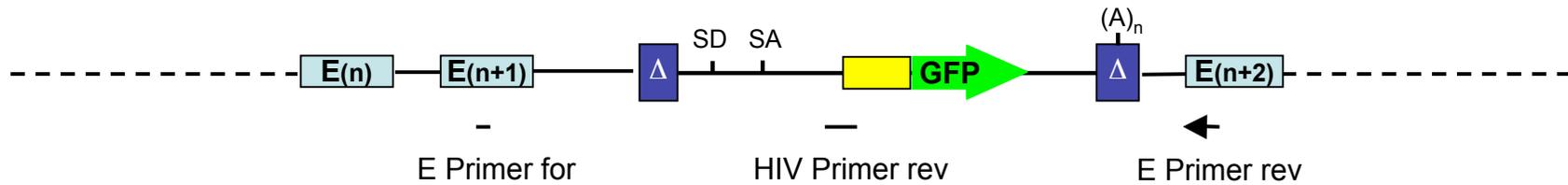
Lentiviral integrations target expressed gene bodies



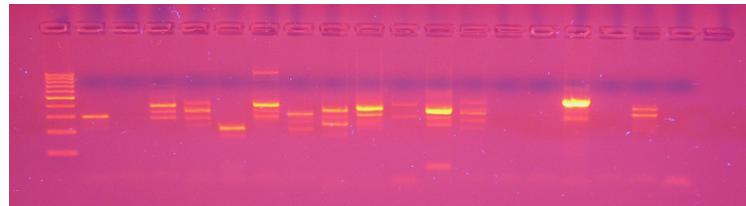
Forward-oriented, intragenic retroviral integrations are counterselected in T cells *in vivo*



Identification of aberrant splicing products

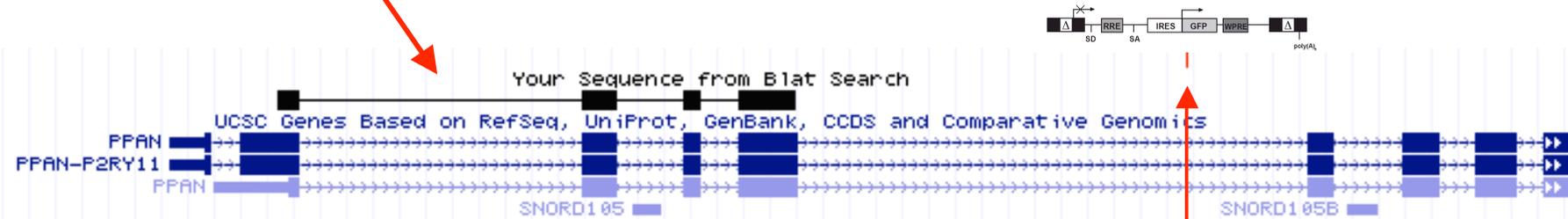


RT-PCR



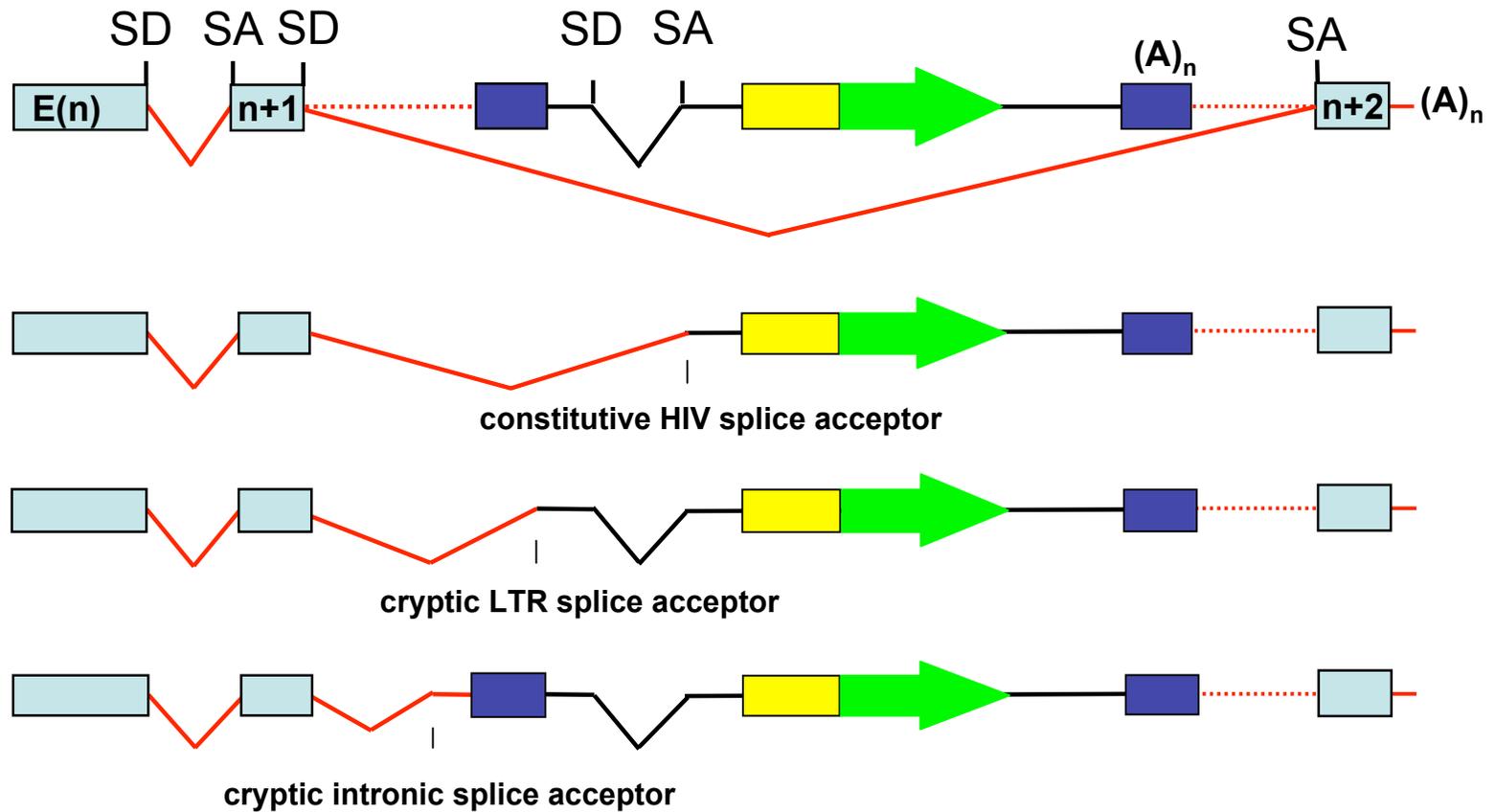
sequencing and mapping

alternatively spliced transcript



integration site

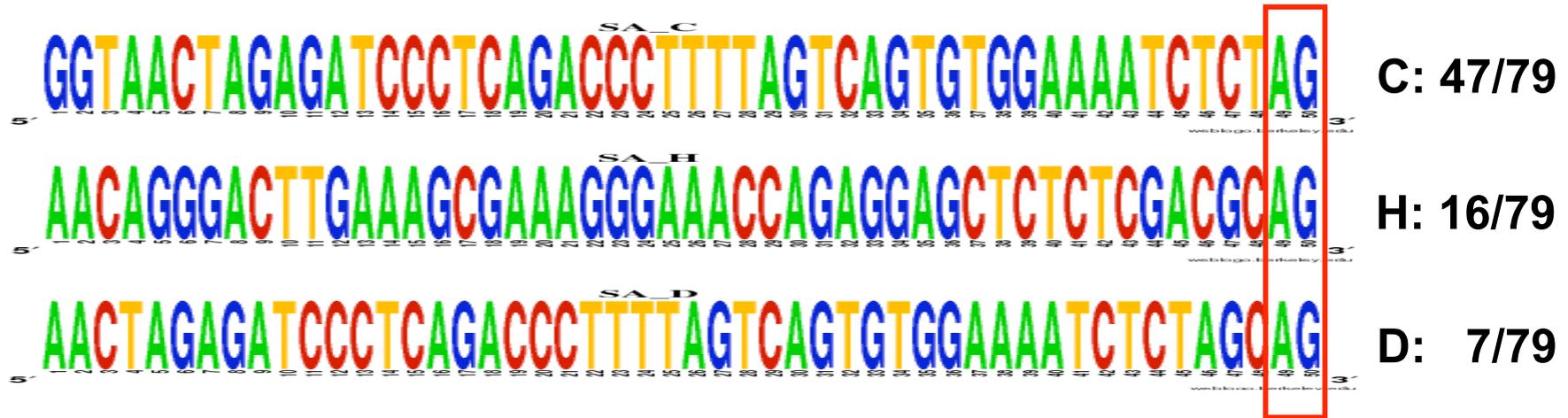
Three families of alternatively spliced transcripts in forward-oriented lentiviral vectors



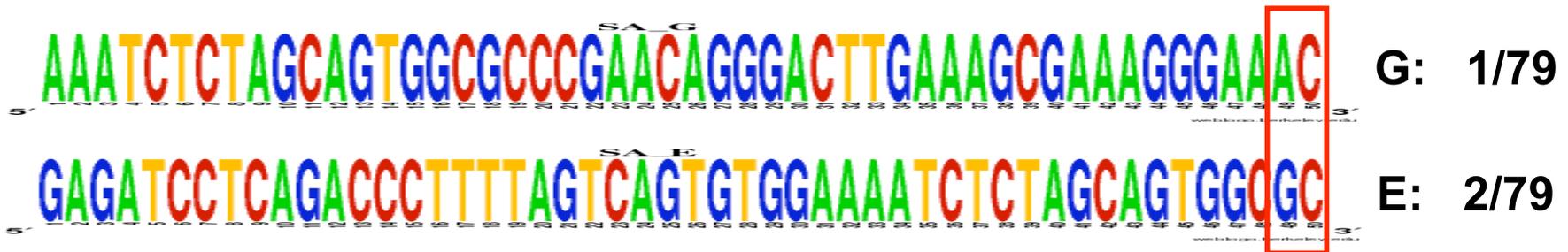
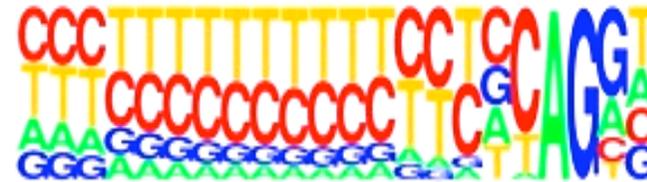
Cryptic splice sites in forward orientation

No Data Shown
Refer to Webcast

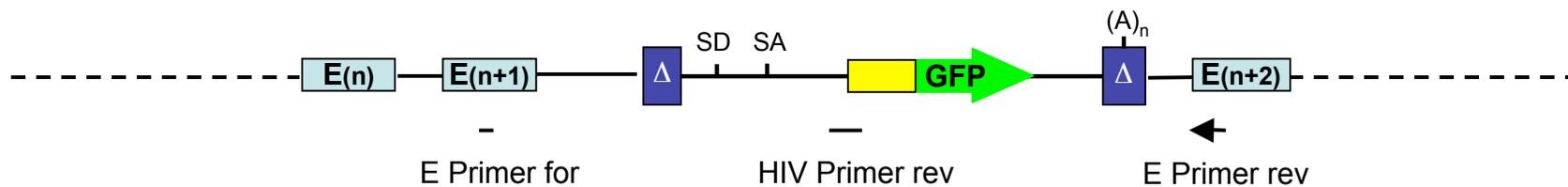
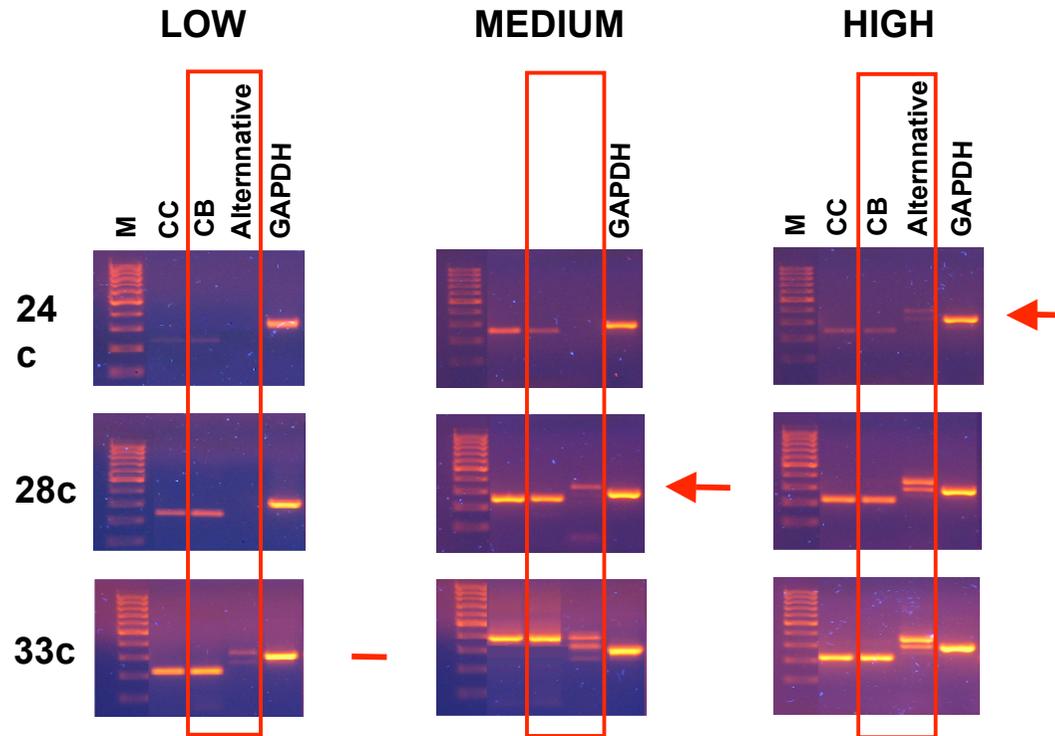
Cryptic acceptor splice site usage



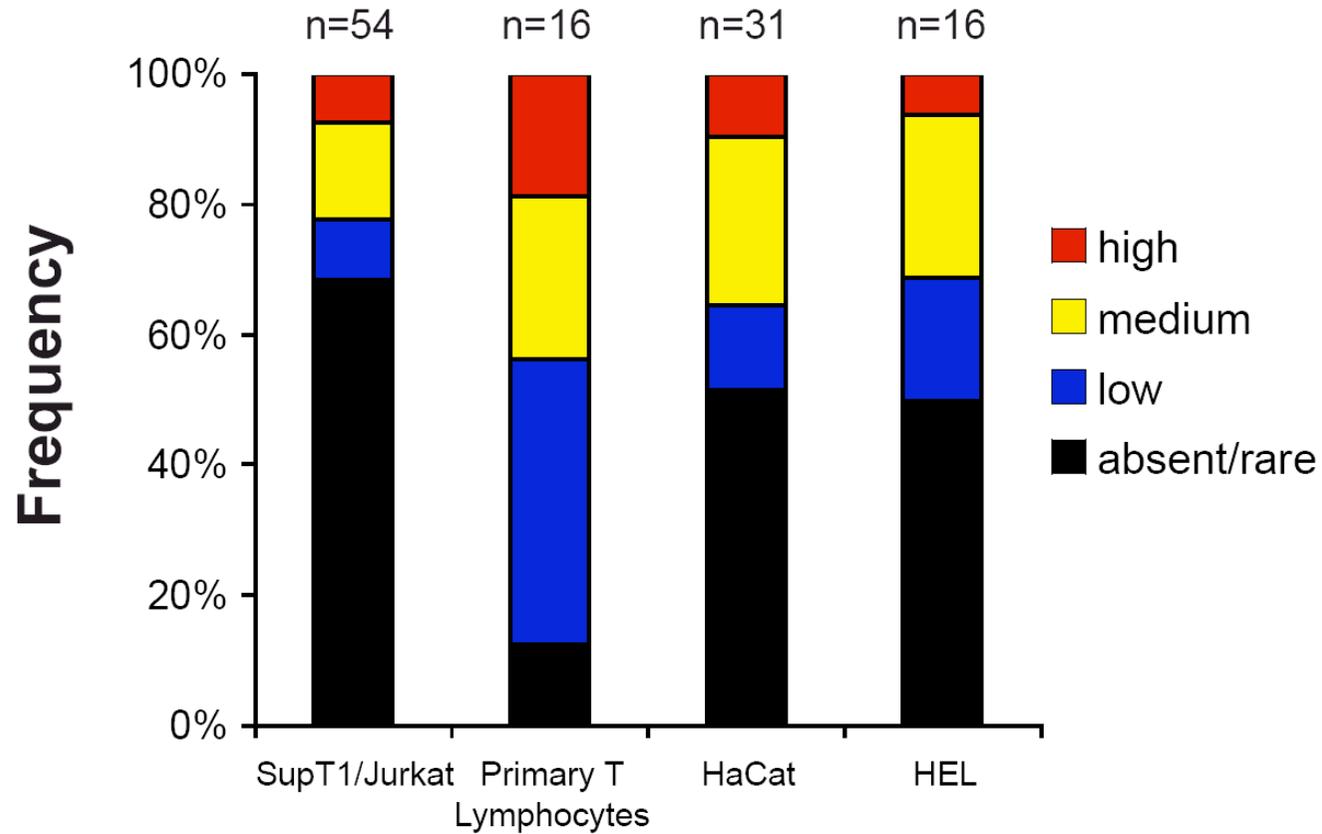
Human splice acceptor consensus sequence



Alternatively spliced transcripts can be abundant



Aberrantly spliced transcripts are frequent



Conclusions

- ❑ Integration of lentiviral vectors within transcribed regions causes abnormal splicing in a variable but significant percentage of targeted genes in all tested cell types
- ❑ Abnormal splicing is due to the usage of constitutive and cryptic splice signals located on both strands of the integrated provirus
- ❑ The proportion of aberrant, alternatively spliced transcripts is on average low compared to constitutively spliced transcripts
- ❑ The relative usage of cryptic splice sites is proportional to their homology to the mammalian splice consensus sequences
- ❑ The “strength” of constitutive splice signals in the targeted gene does not predict the extent of vector-induced alternative splicing
- ❑ Systematic analysis of alternatively spliced transcripts can be used to “recode” vector backbones and reduce their potential genotoxicity

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