Analysis of integration site distributions and clonal abundances for gene therapy correction of cystinosis (Groups 29-30)

John K. Everett, Ph.D. and Frederic Bushman, Ph.D.

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Contents

Summary of results

The goal of this analysis is to investigate the integration profile of a gene therapy vector for the correction of cystinosis in mouse subjects and assess potential clonal expansions. The list of mouse oncogenes was compiled from the retroviral tagged cancer gene database (RTCGD)[1] using an inclusion threshold of three or more incidents where the mouse oncogene list comprises 2.5% of all mouse genes. The frequency of integration near oncogenes was generally less than that of mice in a previously published β-thalassemia mouse trial from which no adverse events have been reported [2]. The code base for this analysis is available online (link).

Mouse samples studied

Integration sites were detected in 24 samples from mouse subjects (Tables 1 & S1).

Table 1. Overview of data collection.

Organism	Number of samples	Number of reads	Number of inferred cells	Number of integration sites
mouse	24	11,359,604	211,753	7,385

Subject reports

Subject specific reports for all subjects are available via an online archive (link).

UCSC browser exploration

UCSC browser sessions pre-loaded with the integration sites identified in this analysis are available via this (link). Integration sites are shown as blue (positive orientation integration) and red (reverse orientation integration) tick marks. For each integration site, the intensity of the color reflects the maximum abundance observed.

Description of analysis techniques

We investigate effects of integration on cell growth using the following criteria: Integration Frequency is the frequency at which unique integration sites are observed in or near a given gene. Clonal Abundance is determined by quantifying the number of sites of linker ligation associated with each unique integration site. This samples the number of DNA chains at the start of the experiment allowing clonal expansion to be quantified[4].

Relative clonal Abundance is determined per sample and is the percentage of identified cells attributed to a given clone. Integration sites and the clones harboring them are sampled from a larger population. It would be rare for all integration sites in a sample to be represented in the sequence data.

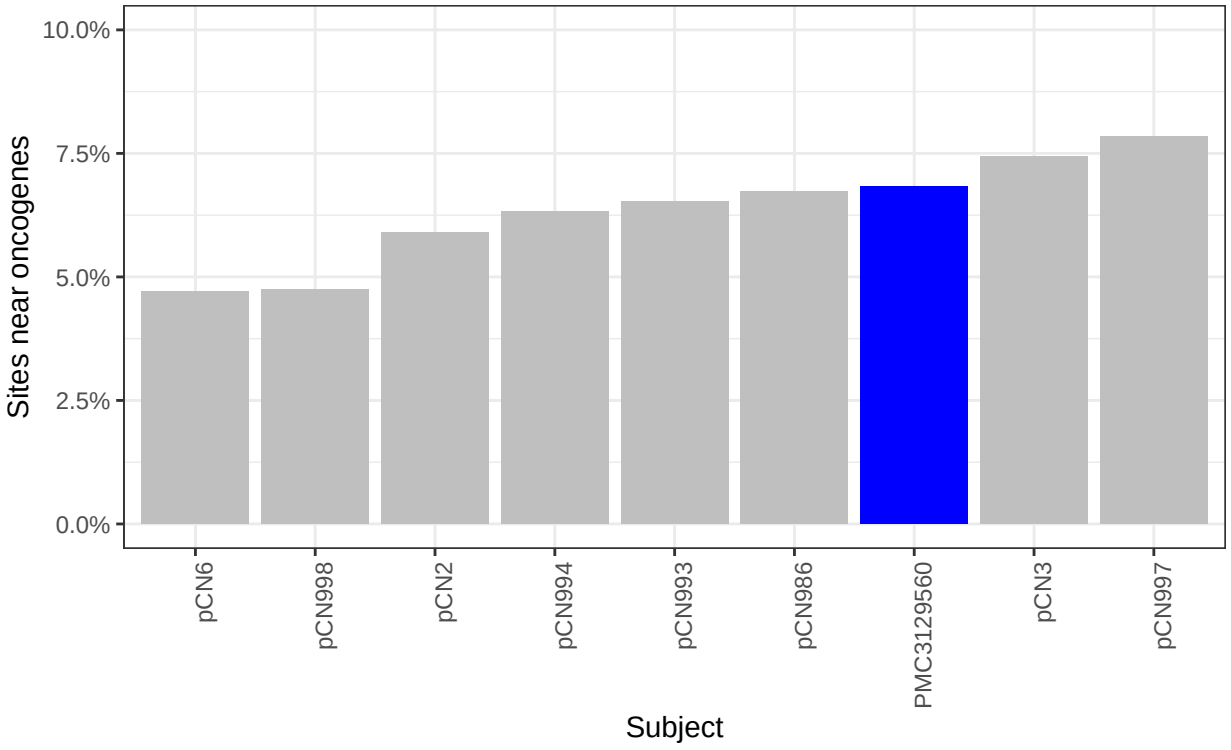
For this analysis, four technical replicates of each delivered sample were prepared, sequenced and analyzed with the INSPIIRED integration site analysis pipeline (v1.2)[4].

Comparisons to previous trials

Integration events near oncogenes in mouse subjects

In order to determine if the experimental vector has a higher propensity of integrating near suspected oncogene in mice than previously employed vectors, the frequency of integration near oncogenes was compared to a previously published mouse trial[2] which used a comparable lentiviral vector to correct β-thalassemia. The frequency of integration events near onco genes in *all transfected mice* was less than the mean frequency of integration events near oncogenes in the published trial (Figure 1 [experimental subjects: gray, previous β-thalassemia trial: blue]).

Figure 1. Comparison of frequencies of integration events near oncogenes.



Relative abundances of mouse subject samples

The sample relative abundance plots below (Figure 2) show the most abundant 25 clones in each sample as colored bars while less abundant clones were relegated to a single low abundance bar shown in gray.

Figure 2.

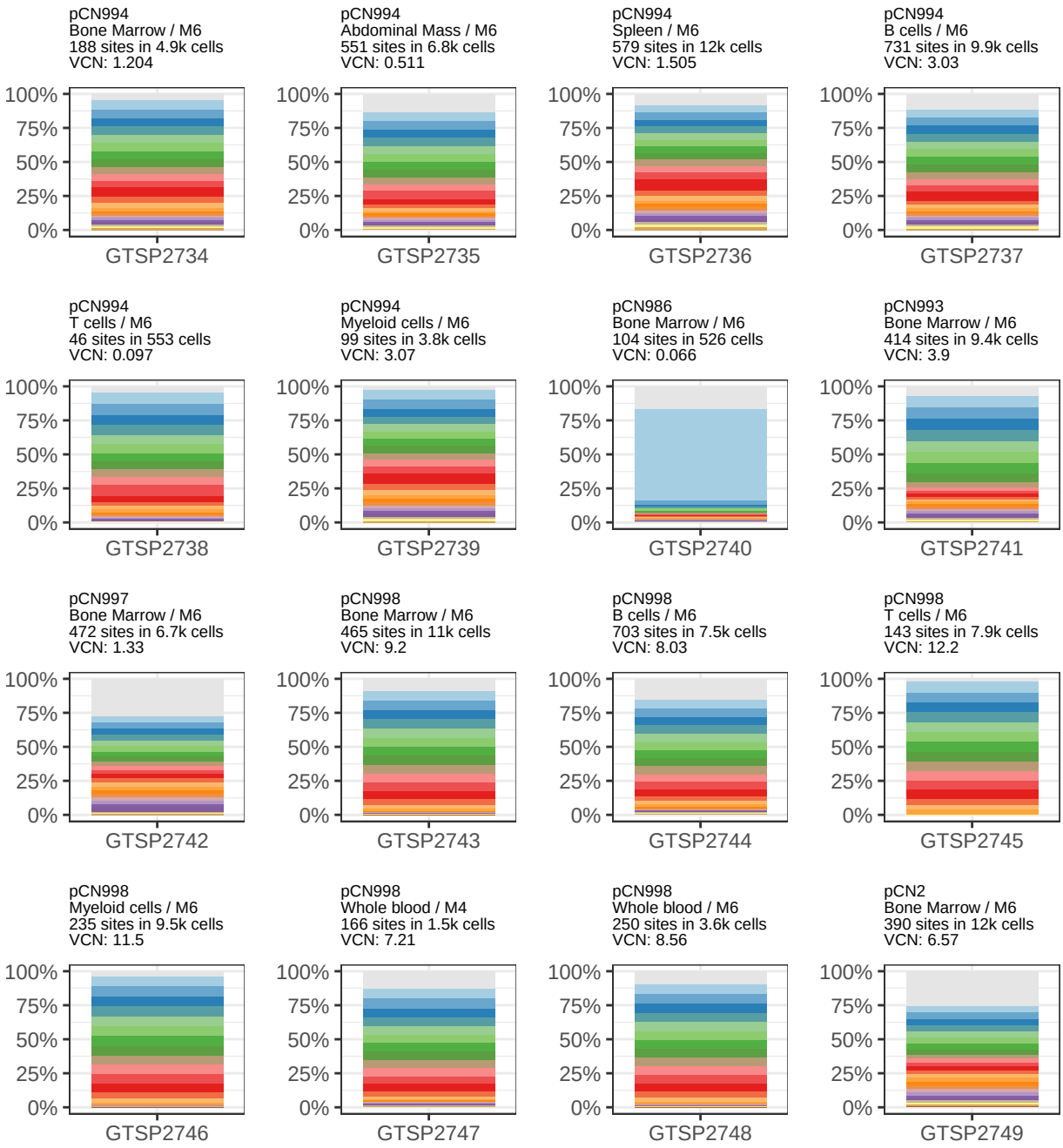
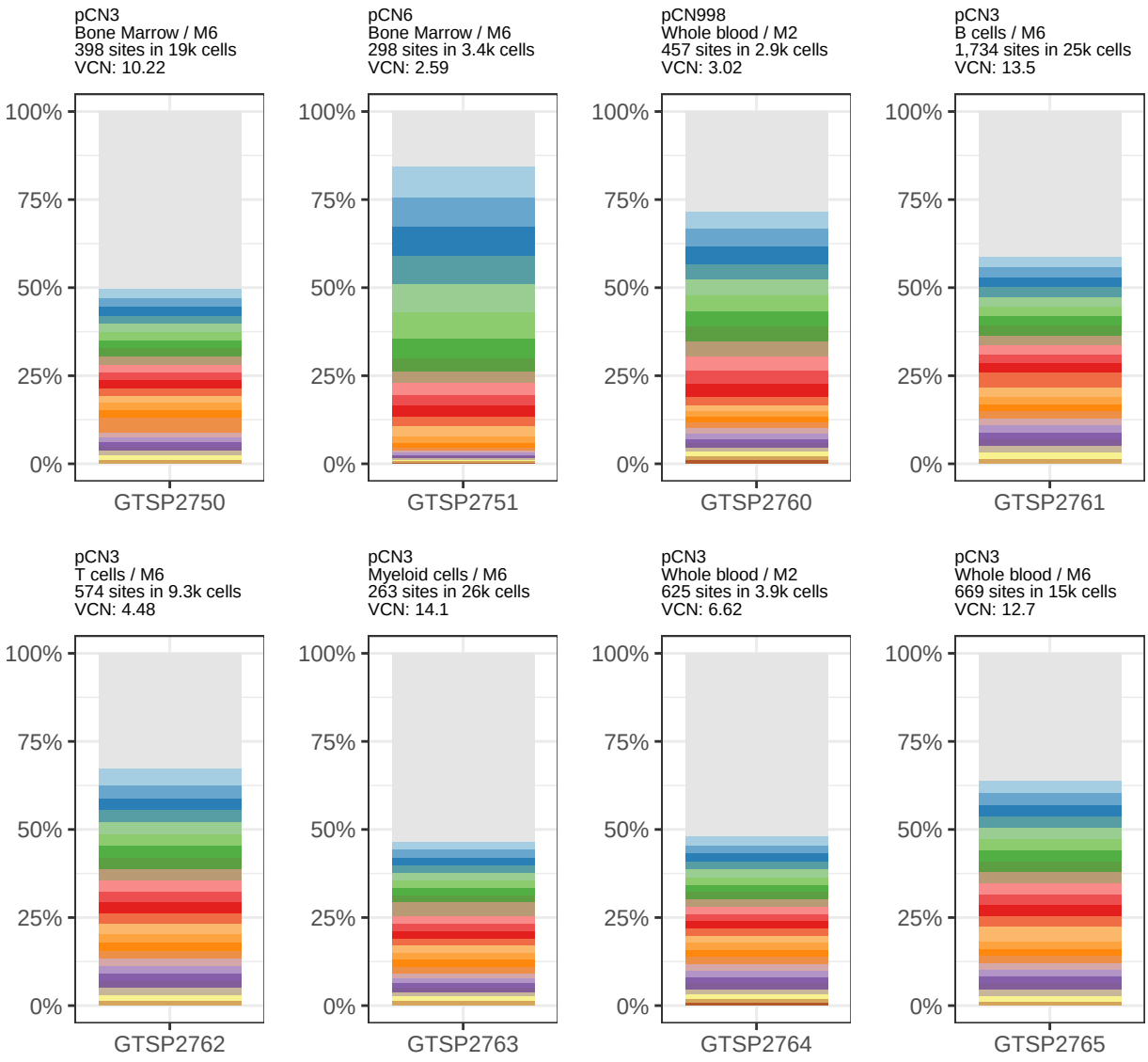


Figure 2 (continued).



Expanded clones

Table 2 below lists clones with relative clonal abundances $\geq 20\%$. The estimated number of cells harboring each integration (Abundance) is shown for context.

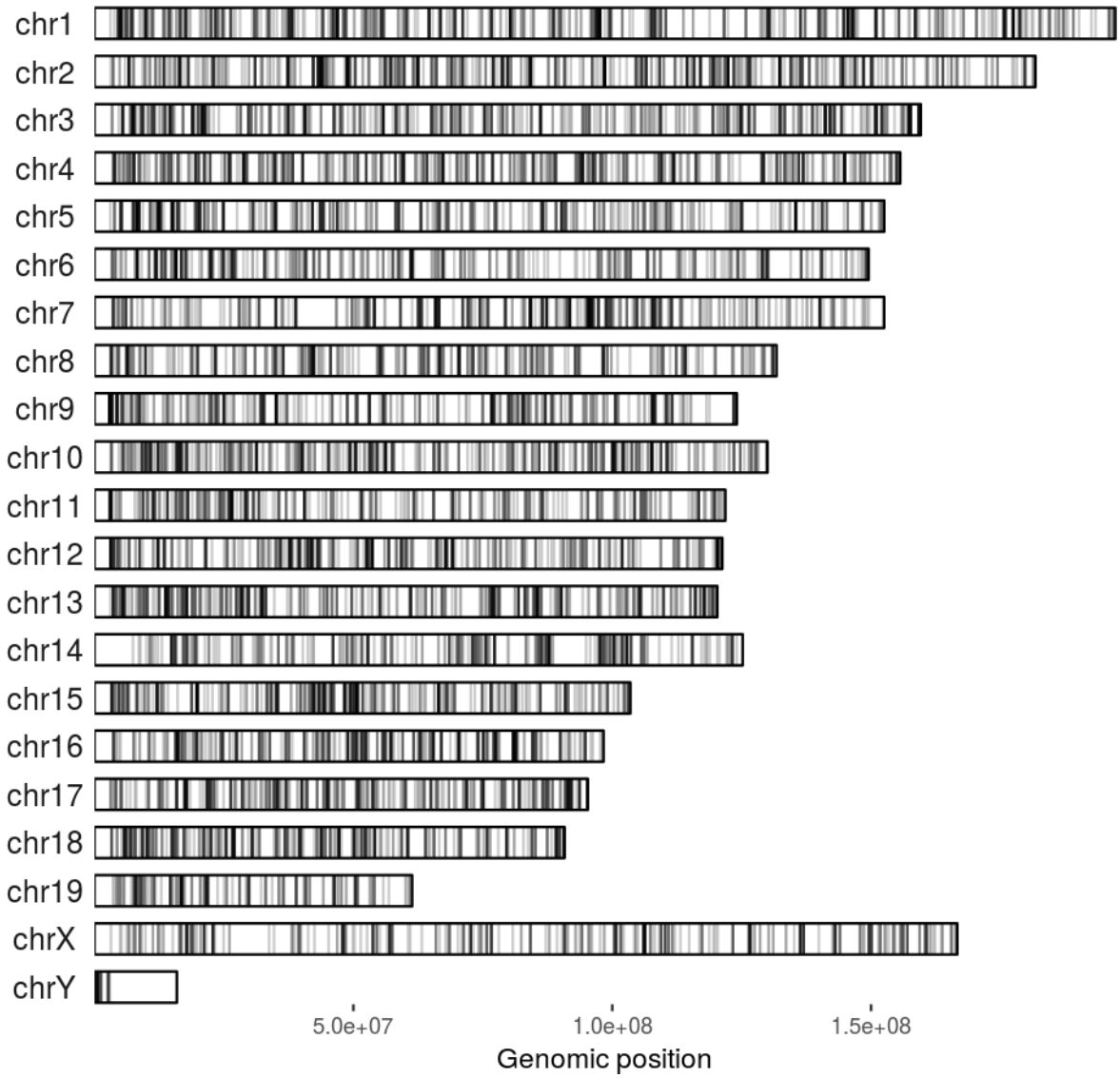
Table 2.

Subject	Organism	Time point	Cell type	Position	Relative abundance	Abundance	Nearest gene
pCN986	mouse	M6	Bone Marrow	chr9+66207164	66.92%	352	Herc1

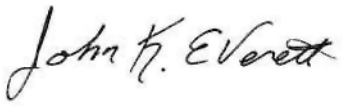
Mapping of integration site positions

Integration events were observerd across all mouse subject chromosomes (Figure 3).

Figure 3.



Analyst



John K. Everett, Ph.D.

Laboratory director

Frederic D. Bushman, Ph.D.

References

1. RTCGD: retroviral tagged cancer gene database. Akagi K, Suzuki T, Stephens RM, Jenkins NA, Copeland NG. Nucleic Acids Res. 2004 Jan 1;32(Database issue):D523-7.

2. Distribution of Lentiviral Vector Integration Sites in Mice Following Therapeutic Gene Transfer to Treat β-thalassemia. Ronen K, Negre O, Roth S, Colomb C, Malani N, Denaro M, Brady T, Fusil F, Gillet-Legrand B, Hehir K, Beuzard Y, Leboulch P, Down JD, Payen E, Bushman FD. Mol Ther. 2011 Jul;19(7):1273-86.

3. Estimating abundances of retroviral insertion sites from DNA fragment length data. Berry CC, Gillet NA, Melamed A, Gormley N, Bangham CR, Bushman FD. Bioinformatics. 2012 Mar 15;28(6):755-62.

4. INSPIIRED: A Pipeline for Quantitative Analysis of Sites of New DNA Integration in Cellular Genomes. Sherman E, Nobles C, Berry CC, Six E, Wu Y, Dryga A, Malani N, Male F, Reddy S, Bailey A, Bittinger K, Everett JK, Caccavelli L, Drake MJ, Bates P, Hacein-Bey-Abina S, Cavazzana M, Bushman FD. Mol Ther Methods Clin Dev. 2016 Dec 18;4:39-49.

Supplementary tables and figures

Numbers of inferred cells and integration sites identified in provided samples

Table S1.

Organism	GTSP	Subject	Cell type	VCN	Time point	Number inferred cells	Number of intSites
mouse	GTSP2734	pCN994	Bone Marrow	1.204	M6	4,881	188
mouse	GTSP2735	pCN994	Abdominal Mass	0.511	M6	6,838	551
mouse	GTSP2736	pCN994	Spleen	1.505	M6	12,114	579
mouse	GTSP2737	pCN994	B cells	3.030	M6	9,936	731
mouse	GTSP2738	pCN994	T cells	0.097	M6	553	46
mouse	GTSP2739	pCN994	Myeloid cells	3.070	M6	3,848	99
mouse	GTSP2740	pCN986	Bone Marrow	0.066	M6	526	104
mouse	GTSP2741	pCN993	Bone Marrow	3.900	M6	9,398	414
mouse	GTSP2742	pCN997	Bone Marrow	1.330	M6	6,697	472
mouse	GTSP2743	pCN998	Bone Marrow	9.200	M6	11,044	465
mouse	GTSP2744	pCN998	B cells	8.030	M6	7,501	703
mouse	GTSP2745	pCN998	T cells	12.200	M6	7,919	143
mouse	GTSP2746	pCN998	Myeloid cells	11.500	M6	9,486	235
mouse	GTSP2747	pCN998	Whole blood	7.210	M4	1,458	166
mouse	GTSP2748	pCN998	Whole blood	8.560	M6	3,558	250
mouse	GTSP2749	pCN2	Bone Marrow	6.570	M6	12,164	390
mouse	GTSP2750	pCN3	Bone Marrow	10.220	M6	18,701	398
mouse	GTSP2751	pCN6	Bone Marrow	2.590	M6	3,436	298
mouse	GTSP2760	pCN998	Whole blood	3.020	M2	2,931	457
mouse	GTSP2761	pCN3	B cells	13.500	M6	24,687	1,734
mouse	GTSP2762	pCN3	T cells	4.480	M6	9,251	574
mouse	GTSP2763	pCN3	Myeloid cells	14.100	M6	25,816	263
mouse	GTSP2764	pCN3	Whole blood	6.620	M2	3,863	625
mouse	GTSP2765	pCN3	Whole blood	12.700	M6	15,147	669

Sequencing depth

Identified integration site are shown as colored squares that are positioned by the number of reads leading to their identification.

Figure S2.