

Analysis of piggyBac mediated integration in the porcine genome

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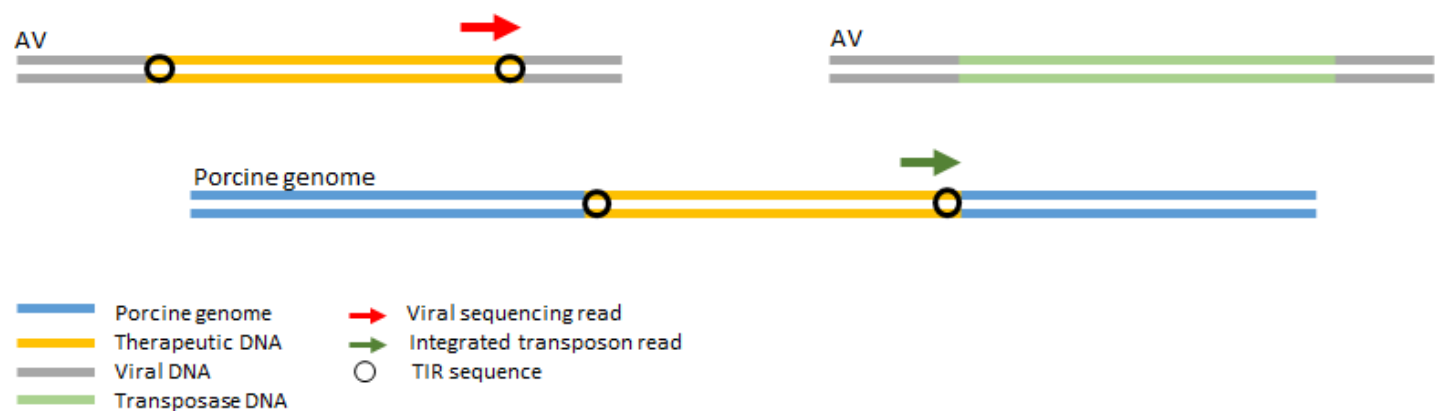
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Introduction

The primary focus of this analysis is to assess the integration efficiency of a piggyBac transposon system targeting the porcine genome where both the transposon and appropriate transposase are delivered via adenovirus vectors. Eleven (11) porcine tissue samples, each with three replicates, were analyzed with the INSPIRED¹ integration site pipeline where only sequencing from the 3' end of integrated transposons would yield porcine genomic sequences required to map integration positions (Figure 1).

Figure 1



Attrition of sequencing reads

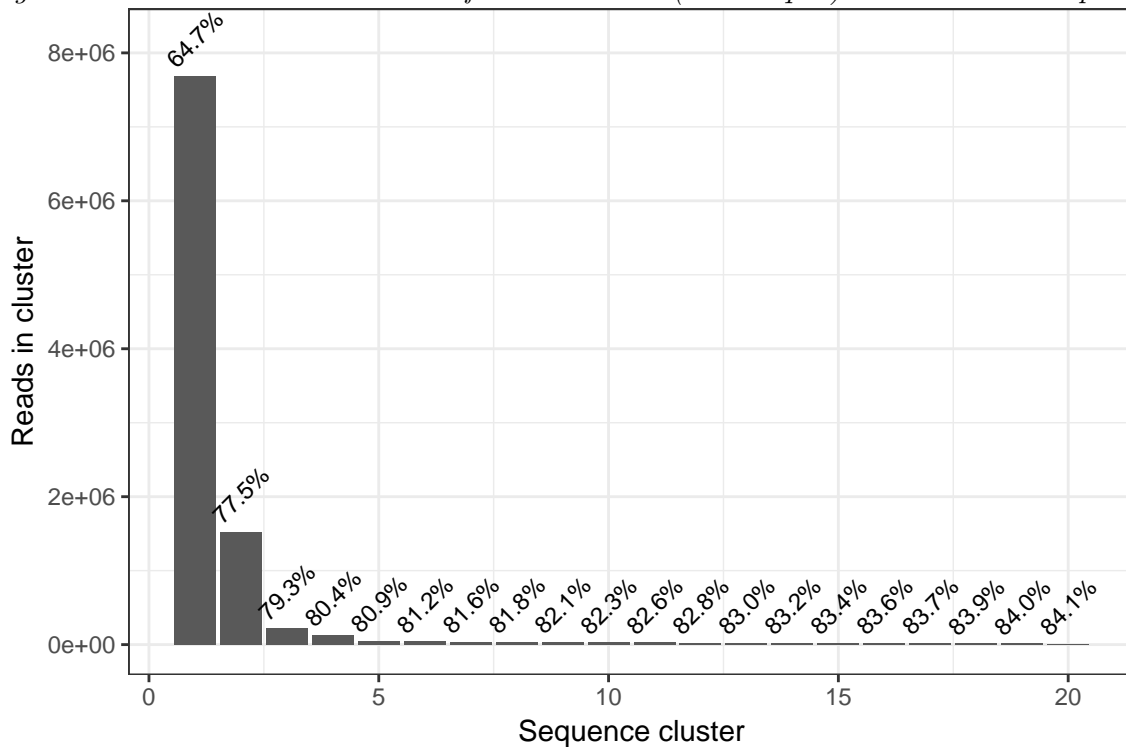
Table 1 below details the attrition of sequencing reads where technical replicates have been combined. The unusually high number of reads that do not match the transposon vector and which do not align to the susScr3 reference genome requires additional investigation.

Sequencing reads originating from the 3' end of the targeted transposons were clustered with a sequence identity threshold of 90% and the 20 most abundant clusters which include 84.1% of sequencing reads are shown in Figure 2. The 20 sequence clusters, except for cluster 18 (possibly from *Candidatus Fluviicola*) and cluster 20 (no clear source), map to different adenovirus sequence variants. The representative sequence for each cluster is provided in Table S1. Alternatively, this observation can be appreciated by reviewing the most frequent read sequences and how they align to one another (Table S2).

Table 1. INSPIRED pipeline read attrition

Subject	Sample	Filtered reads	Non-vector like reads	Reads aligning to genome	intSites (reps. combined)
p1166	Trachea	923939	29430	208	23
p1166	Lung	1319372	56023	50	44
p1168	Trachea	977030	355806	4	4
p1168	Lung	1201110	214530	16	15
p1169	Trachea	1289543	157285	8183	36
p1169	Lung	387593	14122	2677	7
p1171	Trachea	1052996	72905	5492	86
p1171	Lung	1130648	855182	31	13
p9846	Trachea	1130931	61204	1123	13
p9850	Trachea	680004	28962	491	19
p9851	Trachea	962963	145217	402	4

Figure 2. Most abundant clusters of similar reads (90% seq id) with cumulative percentages of all reads.



Characterization of identified integration sites

Figure 3 below shows the distribution of integration sites across the porcine genome while Figure 4 shows the upstream and downstream consensus sequence motifs adjacent to those sites. Differences between the genomic environments of identified sites and the same number of randomly selected sites from a published lentiviral trial to correct Wiskott-Aldrich syndrome (WAS) from which no adverse events have been reported is shown as a heat map in Figure S1².

The TTAA motif immediately following the identified sites (20.6% of sites) was expected given transposase's affinity for this sequence though the AGGG motif immediately upstream (33.7% of sites) was not expected. This finding suggests that a number of integration sites may be false positives arising from mis-priming against endogenous piggyBac/LOOPER elements in the porcine genome which are known to end in AGGG.

Figure 3. Distribution of identified integration sites.

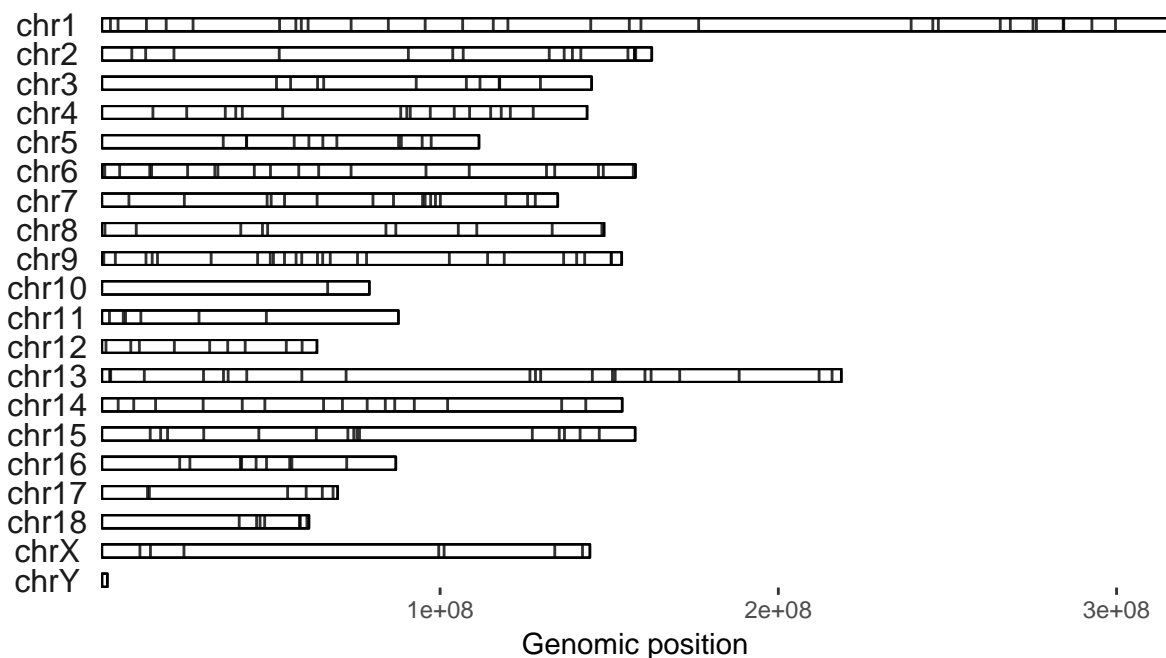
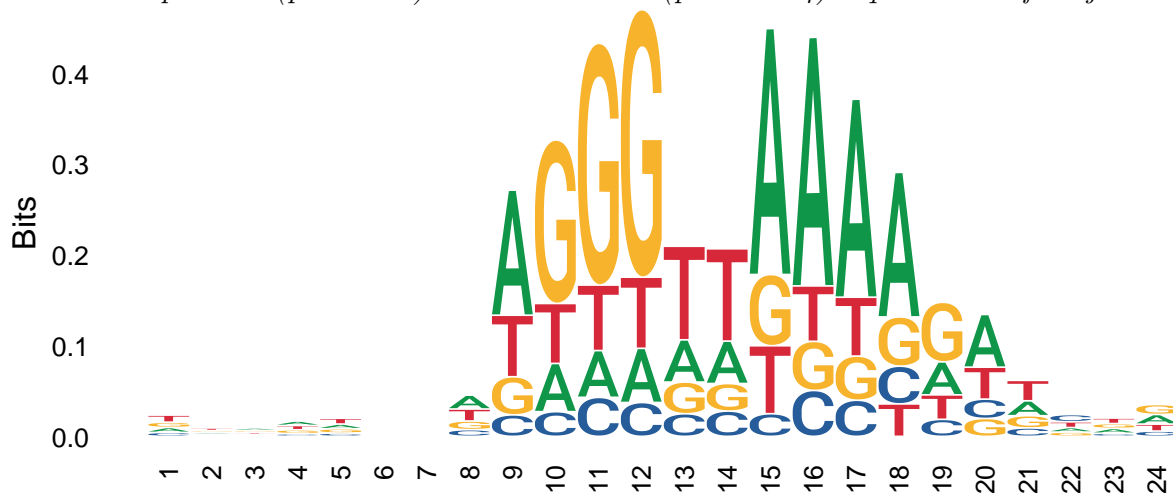


Figure 4.

Consensus upstream (pos. 1-12) and downstream (pos. 13-24) sequence motifs adjacent to integration sites.



Suplimentary figures and tables

Figure S1.
ROC heatmap comparing the genomic environments of integration sites found in this analysis to those found in a published lentiviral study

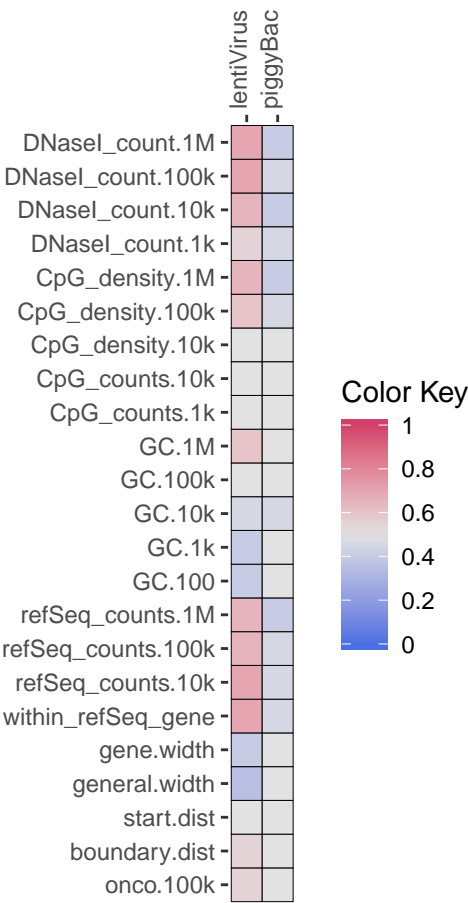


Table S1. Sequence representatives from the most abundant read clusters.

Cluster	Representative.sequence
Cluster 1	TTAAAAGATCTGGAAGGTGCTGAGGTCCGATGAGACCCGCACCAGGTGCA
Cluster 2	ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAG
Cluster 3	TTAAAAGATCTGGAAGGTGCTGAGGTACGATGAGAAGTCCCTTAAGCGGA
Cluster 4	TTAAAAGATCTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGTCCCT
Cluster 5	TTAAAAGATCTGGAAGGTGCTGAGGTACGAGTCCCTTAAGCGGAGGCTAC
Cluster 6	CTAAAAGAGCTGGAAGGTGCTGAGGTACGATAAGACCCGAACCAGGTGCA
Cluster 7	ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGGGTCCCTTAAGCG
Cluster 8	ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGAGACCCGGGTCCC
Cluster 9	TTAAAAGATCTGGAAGGTGCTGAGGTAAGTCCCTTAAGCGGAGTAAATCG
Cluster 10	TTAAAAGATCTGGAAGGTGCTGAGGTAGTCCCTTAAGCGGAGGTTCGGCCG
Cluster 11	TTAAAGGATCTGGAAGGTGCTGAGGTACGGTGAGACCAGCACCAGGTGCA
Cluster 12	TTAAAAGCTCTGGAAGGTGCTGCGGTACGATGCGACCAGCCCCAGGTGCA
Cluster 13	ATTAATACGCAGATCTGGAAGGTGCTGAGGTAGTCCCTTAAGCGGAGACC
Cluster 14	TTAAAAGATCCGGAAGGTGCTGAGGCAAGATGAGACCCGCACCTAGGTGCA
Cluster 15	TTAAAAGGTCTGTAAGGCGCTGAGGTACGCTGAGACCCGCACCAGGTGCA
Cluster 16	TTAAAAGATCTGGAAGGTGCTGAGGTACGAGGTCCCTTAAGCGGAGAGCC
Cluster 17	TTAAAAGACCTGGAAGGTGCTGAGGTACGCTGAGACTCGCCCCAGGTGCA
Cluster 18	AGGCTCCGGTTGATTTGACTGCCGACAATTACCATAGCGTCAGTCTGGT
Cluster 19	GTAACAGATCTGGAAGGTGCTGAGGGACGATGAGACCCGCACAAGGTGCA
Cluster 20	TTGTTGGCCGGGGCTGAGACTCGTTACATAGAACAATTACCATAGCGTCA

Table S2. Most abundant transposon 3' reads (ITR seqs removed)

Genomic sequence	nReads	Cumm. %reads	Transpon vector position	Transpos vector position
ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAG	1305206	67.12	NA	NA
TTAAAAGAG-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	36877	67.43	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCC-----AGTCCCTTAAGC	32231	67.70	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGTCCCT	31541	67.97	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCG	28122	68.21	2997	NA
CTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	27473	68.44	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGCACGATGAGACCCGCACCAGGTGCA	26538	68.66	2997	NA
TTAAAAGAT-----CTGGAGGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	26425	68.88	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGTA	26348	69.10	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGCGCA	25893	69.32	2997	NA
TTAAAGGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	25378	69.53	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGGCCCCGCACCAGGTGCA	24603	69.74	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACC-----AGTCCCTTAAGCG	23819	69.94	NA	NA
TTAAAAGAT-----CTGGAAGGTGCCGAGGTACGATGAGACCCGCACCAGGTGCA	23700	70.14	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGGGACCCGCACCAGGTGCA	22740	70.33	2997	NA
TTAAAAGCT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	22651	70.52	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTAGT	22234	70.71	NA	NA
TTAAAAGAT-----CTGGAAGGCGCTGAGGTACGATGAGACCCGCACCAGGTGCA	22124	70.90	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGC-----AGTCCCTTAA	21821	71.08	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	21630	71.26	2997	NA
TTAAAAGAT-----CCGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	21112	71.44	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCGCCAGGTGCA	20804	71.62	2997	NA
TTACAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	20361	71.79	2997	NA
TTAAAAGGT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	20337	71.96	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGACGAGACCCGCACCAGGTGCA	20153	72.13	2997	NA

Table S2. Most abundant transposon 3' reads (ITR seqs removed) (continued)

Genomic sequence	nReads	Cumm. %reads	Transpon vector position	Transpos vector position
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGAG	19495	72.29	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTGCGATGAGACCCGCACCAGGTGCA	18282	72.44	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAG-----AGTCCCTTAAGCGGAG	18216	72.59	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGGGGTACGATGAGACCCGCACCAGGTGCA	17542	72.74	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGAC-----AGTCCCTTAAGCGG	17337	72.89	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCG-----AGTCCCTTAAG	16814	73.03	NA	NA
TTAAAAGAC-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	16457	73.17	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGGTGAGACCCGCACCAGGTGCA	16137	73.31	2997	NA
TTAGAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	15674	73.44	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAAGTCCC	15558	73.57	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGAGTC	14669	73.69	NA	NA
AGGCTCCGGTTGATTGACTGCCGACAATTACCATAGCGTCAGTCCTGGT	14610	73.81	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCACACCAGGTGCA	14587	73.93	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACTCGCACCAGGTGCA	14204	74.05	2997	NA
TTACAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	13511	74.16	2997	NA
TTAAAAGAT-----CTGGAAGGTGTTGAGGTACGATGAGACCCGCACCAGGTGCA	12890	74.27	2997	NA
TTAAAAGAT-----CTGGGAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	12311	74.37	2997	NA
TTAAAAGAT-----CTGGAAGGTACTGAGGTACGATGAGACCCGCACCAGGTGCA	11687	74.47	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGTACCAGGTGCA	11611	74.57	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTATGATGAGACCCGCACCAGGTGCA	11530	74.67	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGAGTCC	11391	74.77	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTACA	11172	74.86	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCTGCACCAGGTGCA	10666	74.95	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGATCCGCACCAGGTGCA	10293	75.04	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACAATGAGACCCGCACCAGGTGCA	10289	75.13	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACG-----AGTCCCTTAAGCGGAGTTACA	10067	75.21	NA	NA
TCAAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	9960	75.29	2997	NA
TTGTTGGCCGGGGCTGAGACTCGTTACATAGAACAATTACCATAGCGTCA	9338	75.37	NA	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGAGACCCGG-----GTCCC	9275	75.45	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGATGCA	8587	75.52	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCA---AGTCCCTTA	8561	75.59	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCATCAGGTGCA	8504	75.66	2997	NA
TTTAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	8096	75.73	2997	NA
TTCAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	7748	75.80	2997	NA
TTGAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	7705	75.86	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAAGTACGATGAGACCCGCACCAGGTGCA	7639	75.92	2997	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGAGA-----AGTCCCTTAA	7471	75.98	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCAC--AGTCCCTT	7430	76.04	NA	NA
TTAAGAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	7412	76.10	2997	NA
TTAAAGATC-----TGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCAG	7287	76.16	2998	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGAGACCCGC-----AGTCC	7174	76.22	NA	NA
TTATAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	7074	76.28	2997	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGACGAGACCCGCACCAG	6883	76.34	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGA-----AGTCCCTTAAGCGGA	6807	76.40	NA	NA
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TTAAAAGAT-----CTGGAATGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	6611	76.52	2997	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGAG-----AGTCCCTTAAG	6453	76.57	NA	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGAGACCCG-----AGTCCC	6401	76.62	NA	NA
ATTAATACGCAGATCTGGAAGGCGCTGAGGTACGATGAGACCCGCACCAG	6265	76.67	NA	NA
TTAAAAGAT-----TTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	6098	76.72	2997	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGAGAC-----AGTCCCTTA	6001	76.77	NA	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGCACGATGAGACCCGCACCAG	5965	76.82	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGTGGTACGATGAGACCCGCACCAGGTGCA	5924	76.87	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAAGTGCA	5582	76.92	2997	NA

Table S2. Most abundant transposon 3' reads (ITR seqs removed) (*continued*)

Genomic sequence	nReads	Cumm. %reads	Transpon vector position	Transpos vector position
TTAAAAGAT-----CTGGAAGATGCTGAGGTACGATGAGACCCGCACCAGGTGCA	5421	76.97	2997	NA
TTAAAAGAT-----CTGGAAGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	5359	77.02	2997	NA
TTAAAAGAT-----CTGAAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	5265	77.06	2997	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGAT-----AGTCCCTTAAGCGG	5225	77.10	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCTGGTGCA	5028	77.14	2997	NA
ATTAATACGCAGATCTGGAGGGTGCTGAGGTACGATGAGACCCGCACCAG	4972	77.18	NA	NA
ATTAATACTCAGATCTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAG	4970	77.22	NA	NA
ATTAATACGCAGATCCGGAAGGTGCTGAGGTACGATGAGACCCGCACCAG	4866	77.26	NA	NA
TTAAACGAT-----CTGGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	4796	77.30	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGATACGATGAGACCCGCACCAGGTGCA	4778	77.34	2997	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGAGGCCCGCACCAG	4775	77.38	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTAAGGTACGATGAGACCCGCACCAGGTGCA	4681	77.42	2997	NA
TTAAAAGAT-----CTAGAAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	4647	77.46	2997	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGTACGATGAGACC-----AGTCCCTT	4608	77.50	NA	NA
ATTAATACGCAGATCTGGAAGGTGCCGAGGTACGATGAGACCCGCACCAG	4595	77.54	NA	NA
TTAAAAGAT-----CTGGTAGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	4551	77.58	2997	NA
ATTAATACGCAGATCTGGAAGGTGCTGAGGTAAGATGAGACCCGCACCAG	4512	77.62	NA	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATGCGACCCGCACCAGGTGCA	4469	77.66	2997	NA
TTAAAAGAT-----CTGGACGGTGCTGAGGTACGATGAGACCCGCACCAGGTGCA	4431	77.70	2997	NA
TTAAAAGAT-----CTGGAAGGTGCTGAGGTACGATAAGACCCGCACCAGGTGCA	4373	77.74	2997	NA

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