

Virus Analysis in Head and Neck and Bladder Cancers

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Viral Infections in Carcinogenesis

- **Head and Neck squamous cell carcinomas**

- the **sixth most common** cancer worldwide; annual burden of 355,000 deaths and 633,000 incident cases.

- **60–80 %** of oropharyngeal cancers, **~20%** of oral and laryngeal cancers are caused by *human papillomavirus (HPV)*.

- HPV-mediated cancers have significantly **improved outcomes**.

- **Bladder cancer**

- the **second most commonly** occurring **genitourinary cancer** in adults.

- moderate **association** between *HPV* and *BK polyomavirus infection* and tumors.

Detected Viral Genomes

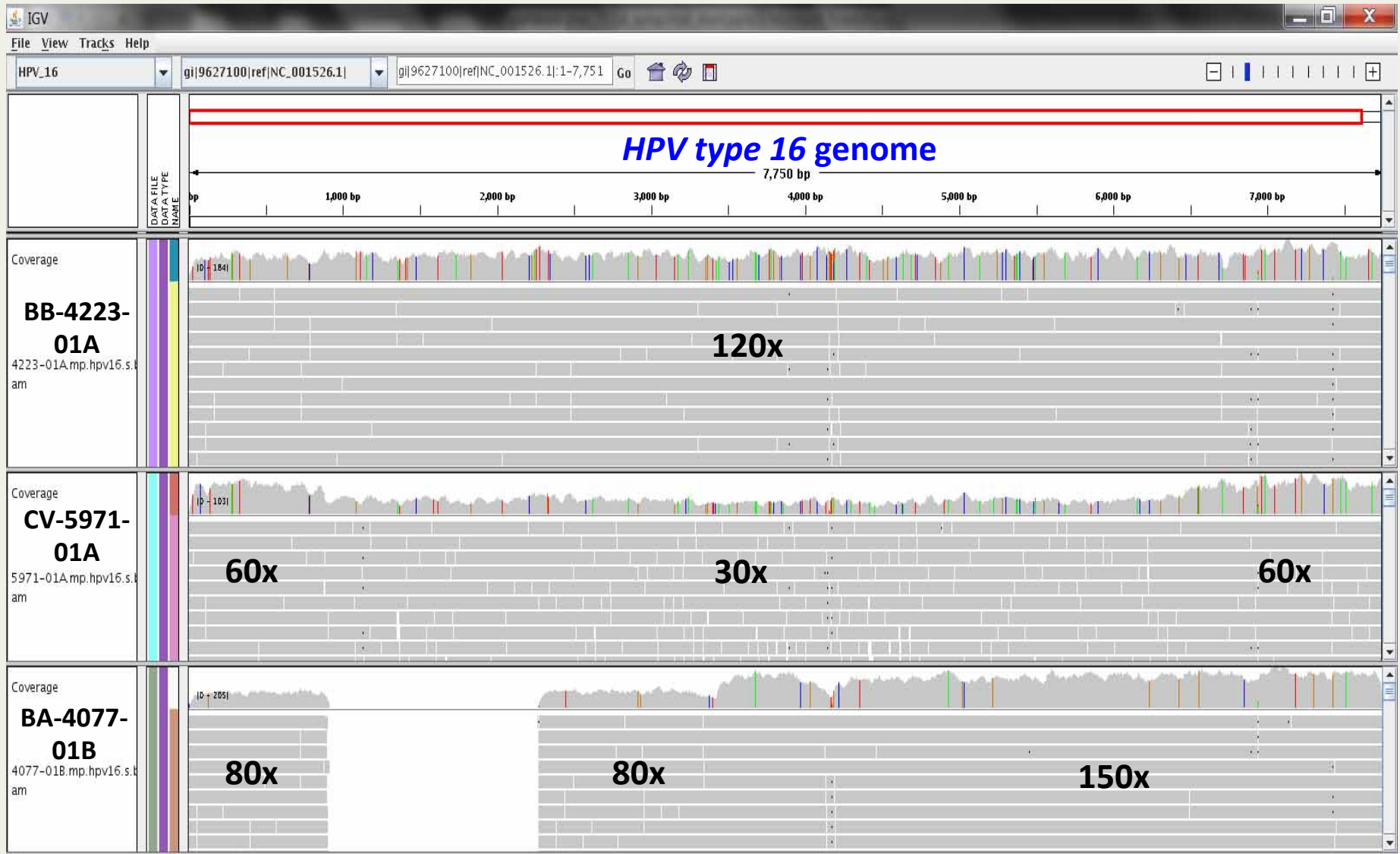
virus	tumor samples		control samples	
	Head&Neck (n=113)	Bladder (n=105)	Head&Neck (n=113)	Bladder (n=105)
<i>HPV type 16</i>	7 (6.2%)	2 (1.9%)	0	0
<i>HPV type 33</i>	2 (1.8%)	0	1* (0.8%)	0
<i>HPV type 90</i>	0	0	1 (0.8%)	0
<i>HPV type 56</i>	0	1 (1%)	0	0
<i>HPV type 6</i>	0	1 (1%)	0	0
<i>Human herpesvirus 1</i>	3 (2.7%)	0	1* (0.8%)	0
<i>Human herpesvirus 5</i>	1 (0.9%)	2 (1.9%)	0	0
<i>Human herpesvirus 6A</i>	1 (0.9%)	0	1* (0.9%)	0
<i>Human herpesvirus 7</i>	1 (0.9%)	0	1 (0.9%)	0
<i>BK polyomavirus</i>	0	1 (1%)	0	0

* has a virus positive tumor pair

HPV Positive samples

cancer type	sample	virus	% of covered viral genome	number of HPV copies per cell
Head & Neck	BA-5153-01A	<i>hpv 16</i>	100	30
	BB-4225-01A	<i>hpv 33</i>		20
	CV-6939-01A	<i>hpv 33</i>		4
	CN-4741-01A	<i>hpv 16</i>		26
	CV-5971-01A	<i>hpv 16</i>		5
	BB-4223-01A	<i>hpv 16</i>		19
	CN-5361-01A	<i>hpv 16</i>		4
	BA-5559-01A	<i>hpv 16</i>		1
	BA-4077-01B	<i>hpv 16</i>	82.9	17
	CV-6951-11A	<i>hpv 90</i>	31.5	<1
	CV-6939-11A	<i>hpv 33</i>	13.8	<1
Bladder	FD-A3B4-01A	<i>hpv 56</i>	48.2	<1
	BT-A20T-01A	<i>hpv 16</i>	87.2	<1
	GC-A3I6-01A	<i>hpv 16</i>	100	18
	FD-A3N6-01A	<i>hpv 6</i>		5

HPV 16 Positive Samples. Genome Visualization

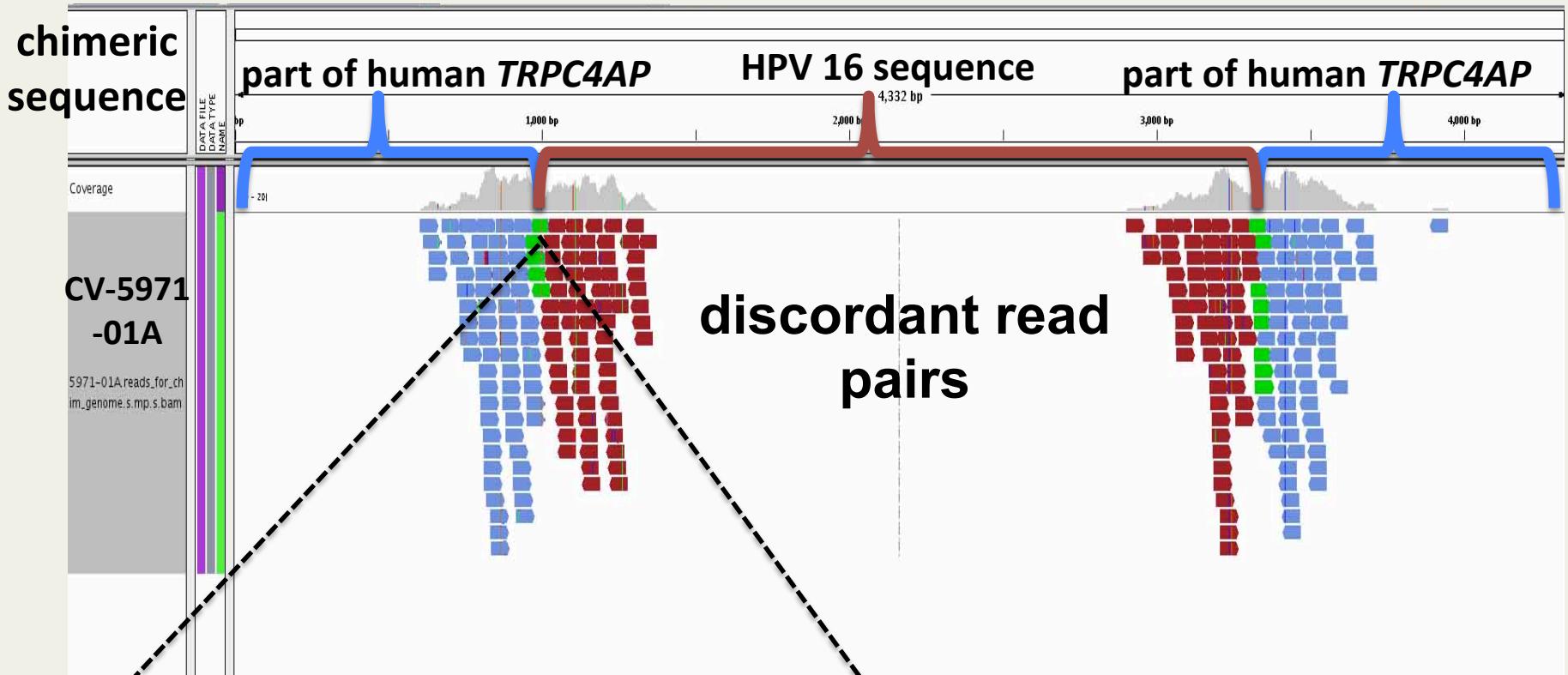


coverage varies not only across samples but also within the same sample

Virus Integration Events

Detection of Integration Events

➤ **HPV integrates in the gene *TRPC4AP***



GAGGCTGAGGTGAGAGGACCGCTGGGATTATTATTAAAGGCTCTGGGTC

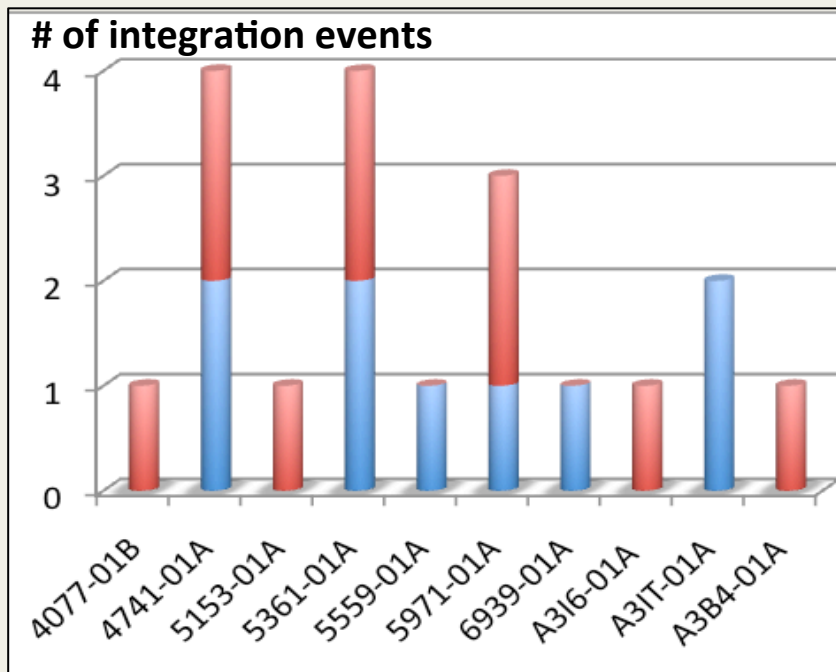
➤ **chimeric read**

Virus Integration Events in the Positive Samples (examples)

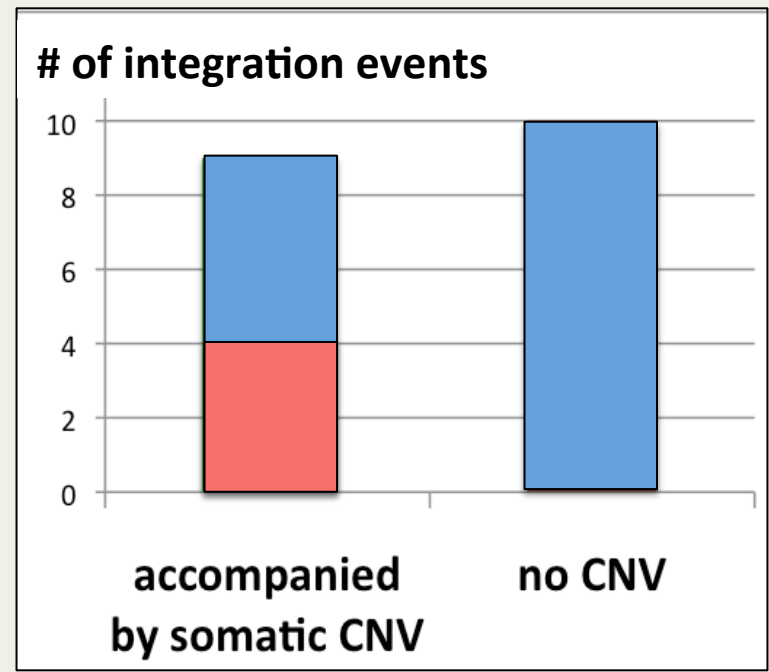
sample/ virus	discordant read pairs	gene/ chr region	gene function	related to cancer	CNV
5971-01A <i>hpv16</i>	128	<i>TRPC4AP</i>	cell cycle control	✓	✓
4077-01B <i>hpv16</i>	120	<i>RAD51B</i>	DNA repair by homologous recombination	✓	✓
4741-01A <i>hpv16</i>	38	<i>KLF5</i>	transcription factor	✓	✓
	7	100kb from <i>TP63</i>	member of the p53 family of transcription factors	✓	✓
A3I6-01A <i>hpv16</i>	65	<i>BCL2L1</i>	anti/pro-apoptotic regulator	✓	✓
A3B4-01A <i>hpv56</i>	20	<i>SEC16A</i> <i>NOTCH1</i>	protein transport Notch signaling network	✓	✓
A3IT-01A <i>BK</i> <i>polyomavirus</i>	29	5kb from <i>FIGN</i>	mitosis regulation	-	-
4726-01A <i>HHV 6A</i>	26	<i>telomeres</i>			

Summary of Integration Events in the *HPV* or *Polyomavirus* positive samples

sample type	# of integration positive samples (%)	# of integration negative samples (%)
cancer	10 (71.4%)	4 (28.6%)
control	0 (0%)	2 (100%)



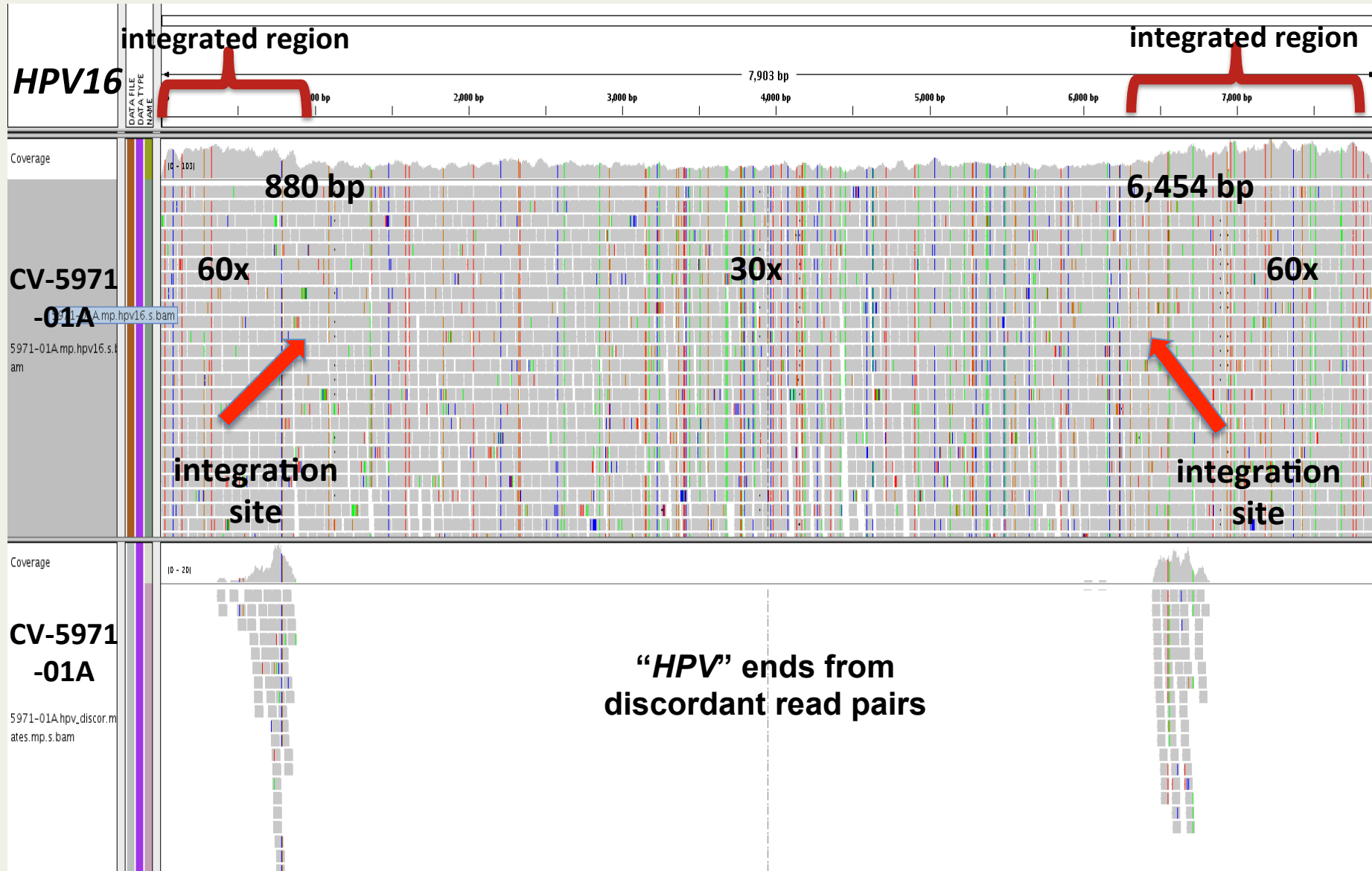
■ genes associated with cancer
■ genes without known association with cancer



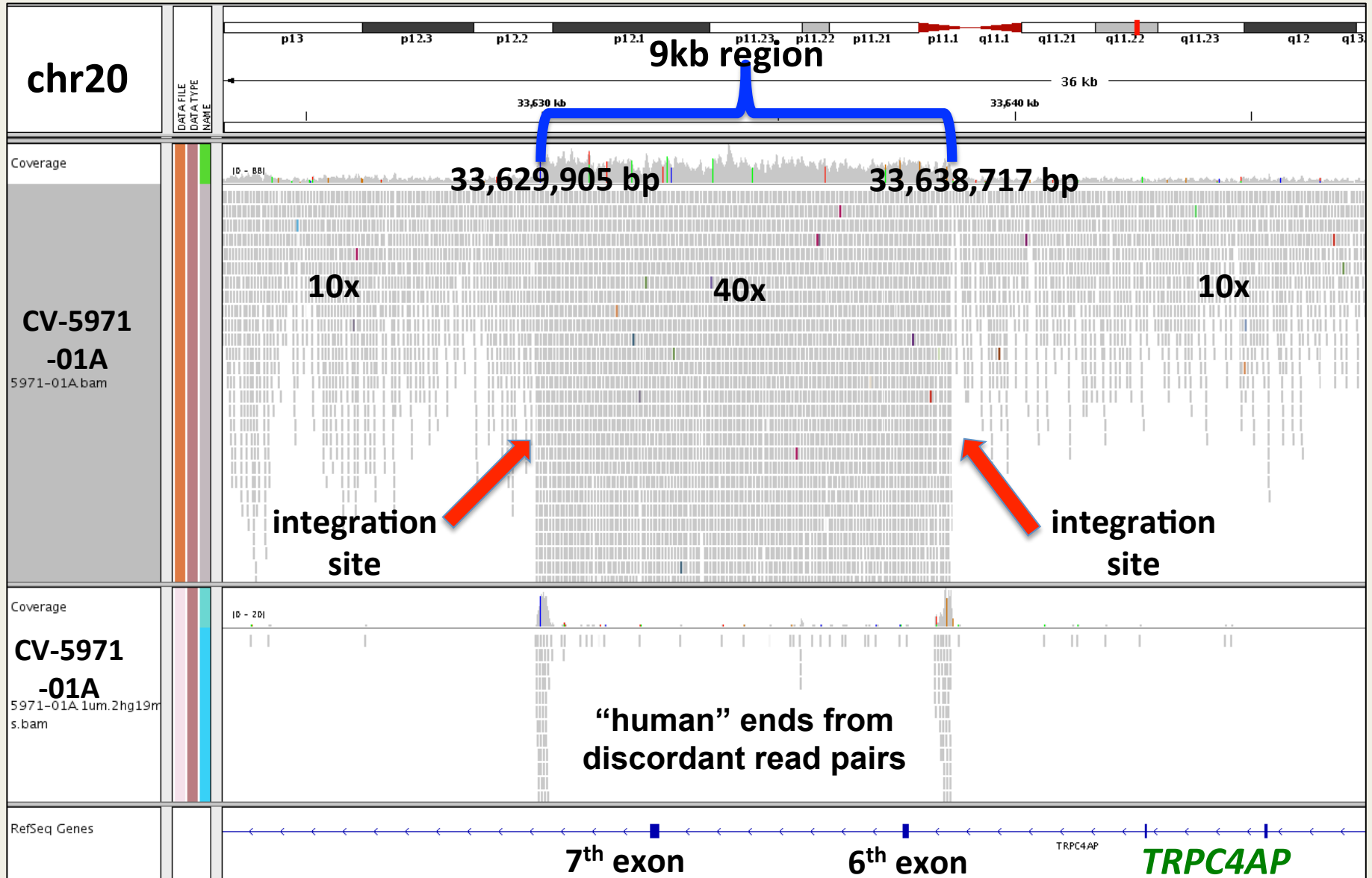
■ formation and amplification of chimeric episomes

Integration Events
Accompanied by Possible Formation
of Chimeric Episomes

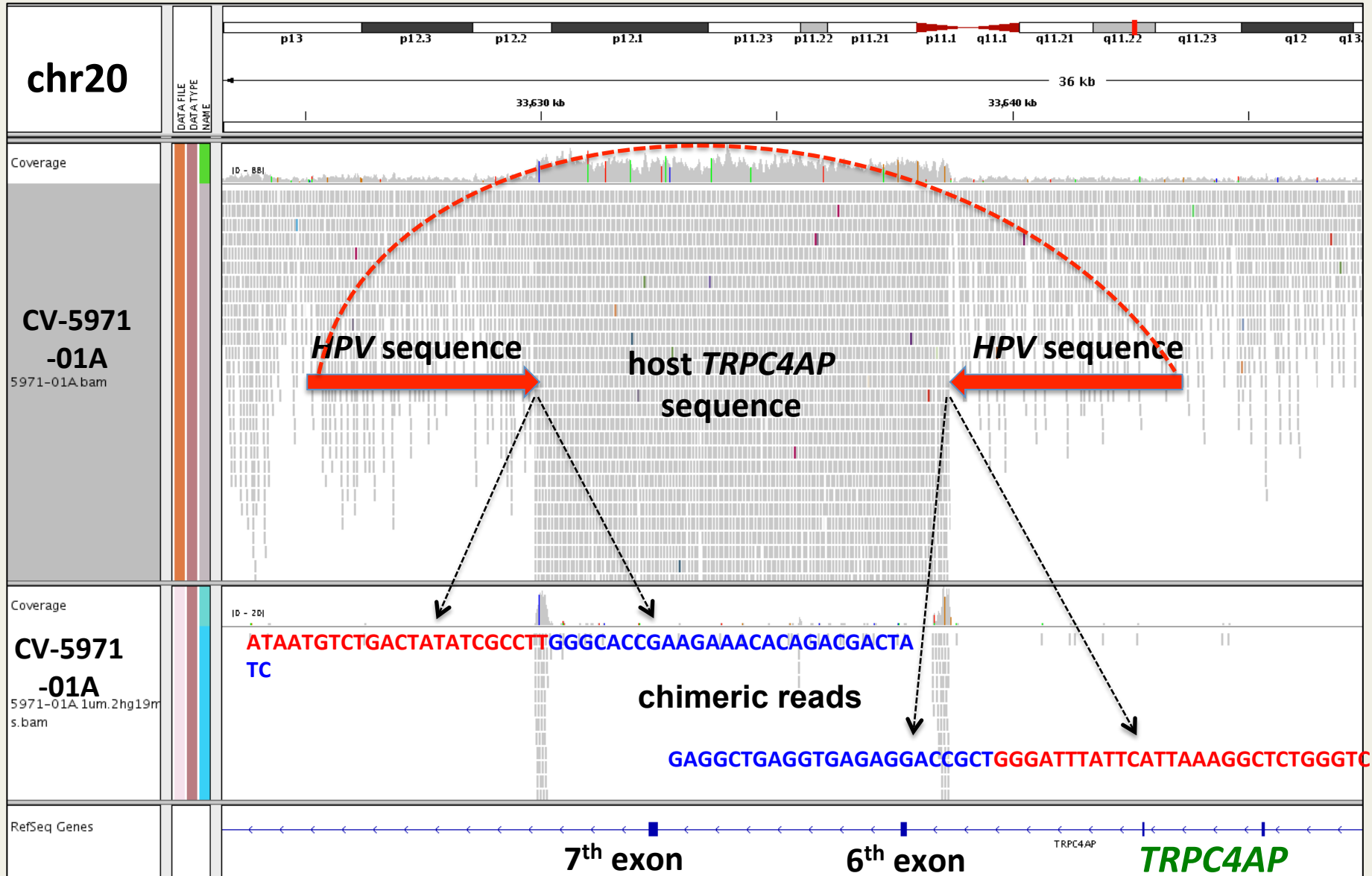
HPV Integration in *TRPC4AP*. Sample CV-5971-01A. HPV Genome.



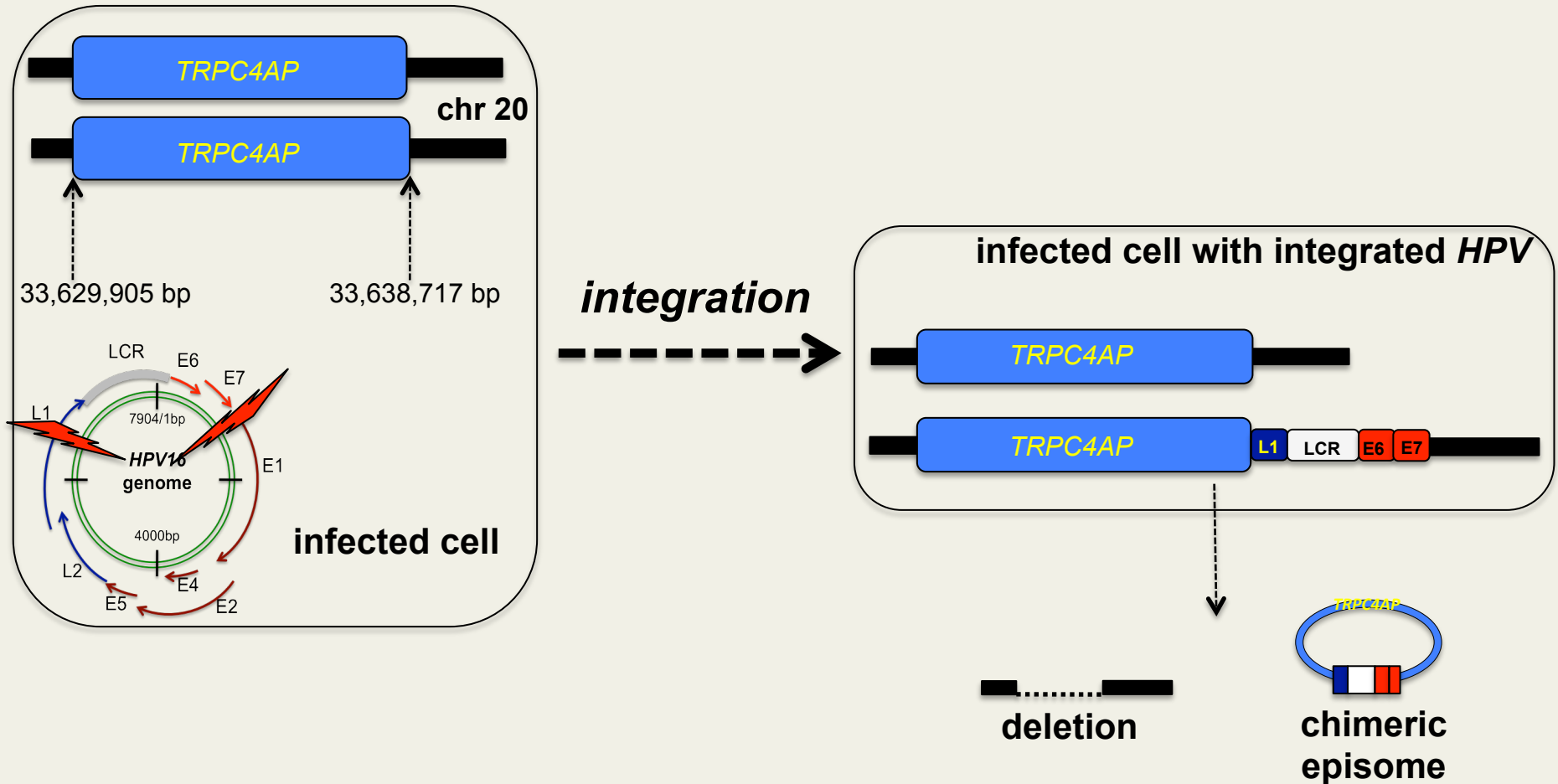
HPV Integration in *TRPC4AP*. Sample CV-5971-01A. Human Genome.



HPV Integration in *TRPC4AP*. Sample CV-5971-01A. Human Genome.



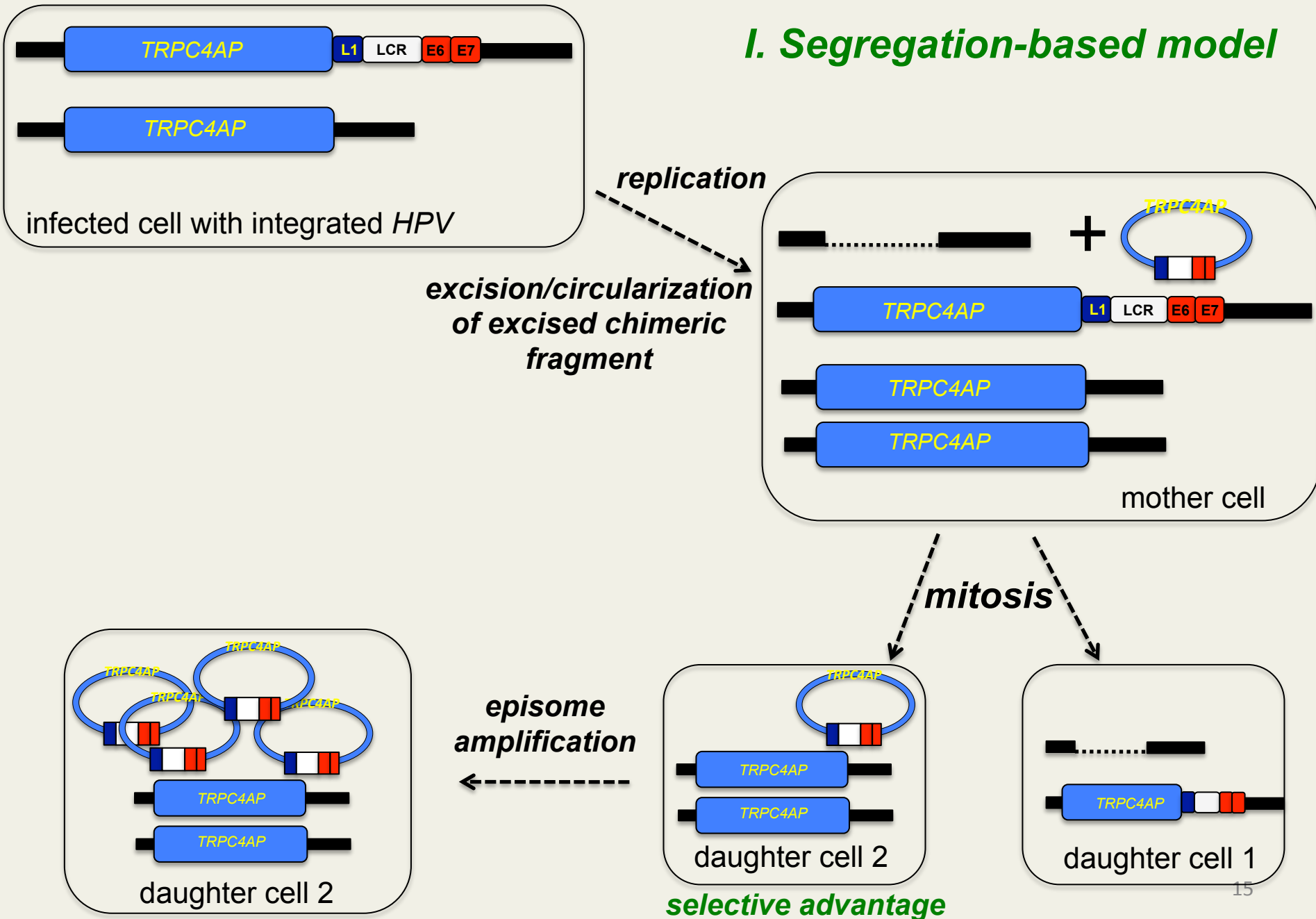
Suggested Model of the Integration Event



*Where is the
chromosome scar?*

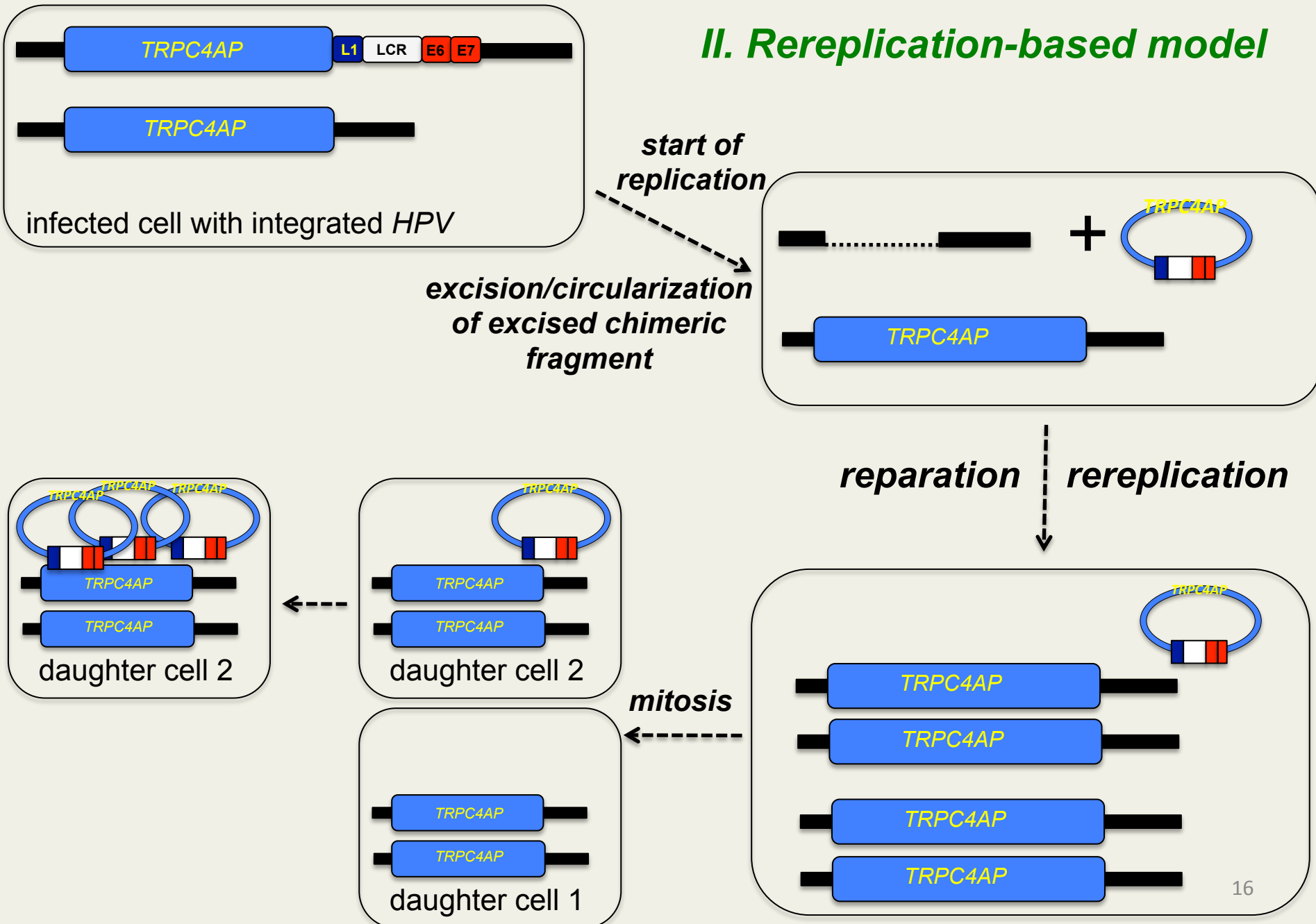
Suggested Model of the Integration Event

I. Segregation-based model



Suggested Model of the Integration Event

II. Rereplication-based model



Conclusions

- The presence of viral sequences and their cellular status can be detected effectively from low pass whole genome sequencing data.
- 8% of head&neck and 4% of bladder tumors are *HPV* positive.
- 9 tumors out of 13 *HPV* positive samples, as well as 1 *BK polyomavirus*, and 1 *HHV 6A* tumors have at least one integration event.
- Our results suggest that integration events might directly contribute to carcinogenesis through both viral gene expression and modification of cellular tumor suppressor or oncogenes.
- Based on our data we suggest that in about quarter of all *HPV* integration events the integration was followed by excision of fused host and viral regions that form circular minichromosomes that present in multiple copies within the cancer cells.

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