Integration Site Analysis of Latently Infected Cell Lines: Evidence of Ongoing Replication

Abha Chopra[1], Jori Symons[2], Shay Leary[1], Don Cooper[1], John Blinco[1], Eva Malantinkova[3] , Ward Spiegelaerde[3] , Linos Vandekerckhove[3], Sharon R Lewin[2,4], Paul Cameron[2,4] Simon Mallal[1,5]

[1]Institute for Immunology and infectious diseases (IIID), Murdoch University, Perth, WA, Australia. [2]Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, VIC, Australia. [3]HIV Translational Research Unit, Department of Internal Medicine, Faculty of Medicine and Health Sciences, Ghent University and Ghent University Hospital, Ghent, Belgium. [4]Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne, Australia, [5]Dept of Infectious Diseases, Vanderbilt University Nashville, USA.

Introduction

HIV cure is limited by persistence of long lived latently infected CD4+ T cells. Latently infected cell lines are widely used *in vitro* to study HIV latency. We identified and tested the stability of HIV integration sites in latently infected cell lines, obtained from NIH AIDS reagent program, using a newly developed high throughput method

Aims

In this study we aimed to:

- Develop a high throughput HIV integration site analysis method.
- Determine HIV integration sites in latently infected cell lines frequently used in HIV latency studies to determine sensitivity and specificity.

Methodology

HIV latently infected cells were obtained from NIH and were passed 10 times in a 1:6 dilution. 150,000 cells from passage 0,2,4,6,8 and 10 were analysed for HIV integration sites by robotic processing (Figure 1). HIV integration sites were called (Figure 2).

Schematic Workflow

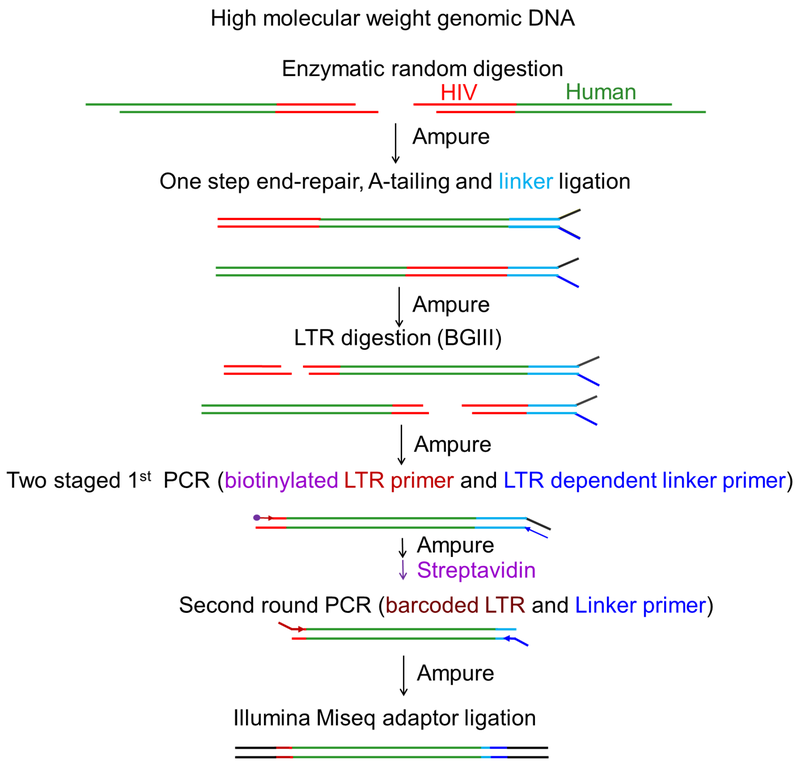

Figure 1. Schematic overview of high throughput HIV integration site analysis method suitable for robotic processing.

Analysis pipeline

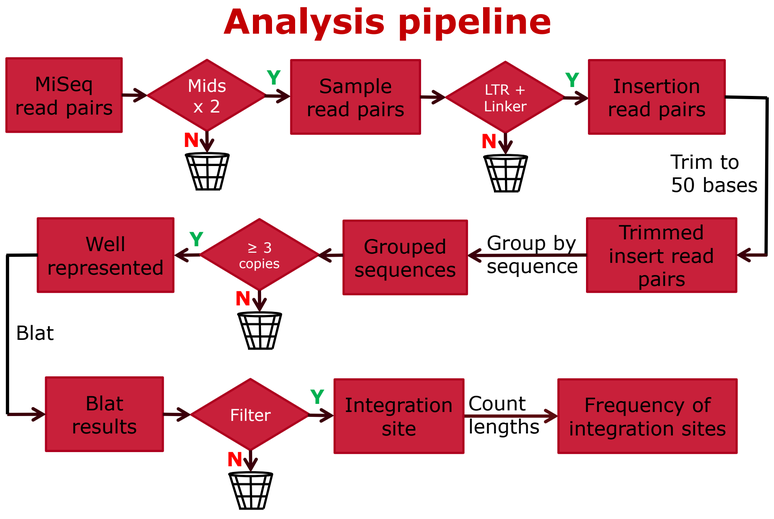

Figure 2. The MiSeq reads are paired and must contain a Mid sequence on either end, plus LTR sequence and linker sequence. The 50 base pairs are trimmed and kept. Chromosomal alignment is determined using the Blat-UCSC Genome Browser (GRCH38/hg38). Frequency is determined by a length difference of ≥2 nucleotides

Results

Stability of HIV integration sites

We analysed the HIV integration sites that are always present during the ten passages. The HIV integration site in all the J-Lat cell lines remained single and stable. Whereas in J1.1, U1 and ACH-2 multiple sites were continuously detected (Table 1).

Cell line	Replication competent HIV	Chromosome	Position	Gene	Gene orientation	HIV orientation	% events
J-Lat 8.4	NO	1	77946384	FUBP1	-	-	100%
J-Lat 9.2	NO	19	46381104	PPP5C	+	+	100%
J-Lat 10.6	NO	9	136468579	SEC16A	-	+	100%
J-Lat 15.4	NO	19	34441293	UBA2	+	+	100%
J-Lat tat-GFP 82	NO	10	39936068			+	100%
J-Lat tat-GFP A1	NO	X	34073326			-	100%
J-Lat tat-GFP A72	NO	2	171821429	SLC25A12	-	+	100%
J-Lat tat-GFP H2	NO	X	45038538	KDM6A	+	-	100%
J1.1	YES	11	685243	DEAF1	-	-	~35%
		12	54257973	CBX5	-	-	~37%
U1	YES	2	48177527	AC079807.4	-	+	~35%
		X	38811467			-	~50%
		19	34452847	UBA2	+	+	~4%
ACH-2	YES	7	33019791	NT5C3A	-	-	~35%
		9	128111651	SLC25A25-AS	-	-	~11%

Table 1. Key integration sites by cell line.

During the ten passages the number of unique integration sites in ACH-2 cells tended to increase, whereas it remained stable in J1.1 cells and tended to decrease in U1 cells by linear regression (Figure 3).

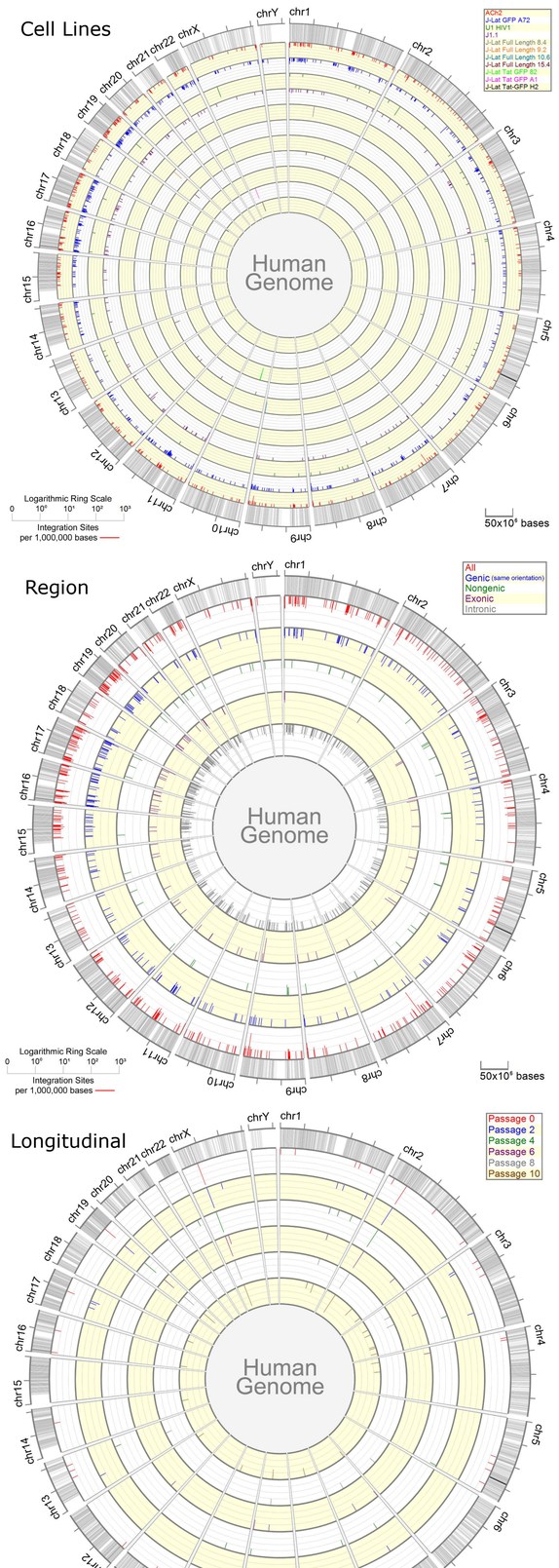

Figure 4. Integration site distributions on the Human genome map.

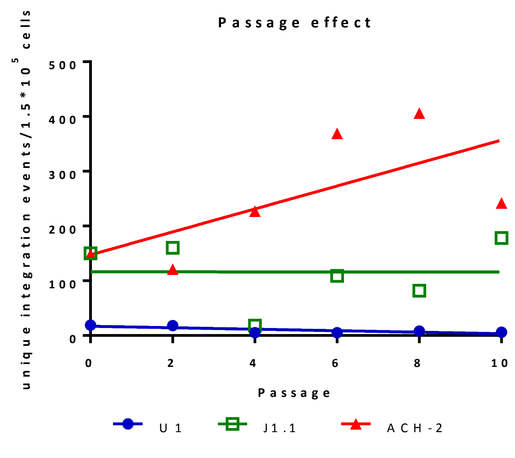

Figure 3. Effect of passage on integration events by cell line.

Residual replication in ACH-2 cells

Whole proviruses of ACH-2 cells were deep sequenced by a two long fragment PCR (Figure 5) and sequenced by internal HIV primers. Mutations were scored if the mutation was >1% of the total reads.

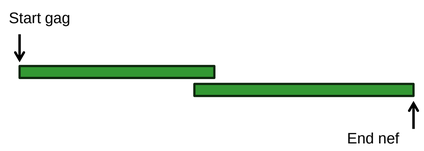

Figure 5. Schematic overview sequence strategy of ACH-2 provirus.

Deep sequencing of the provirus in ACH-2 demonstrated multiple polymorphisms indicative of reverse transcriptase activity (Figure 6).

Mutation type	Number of mutations
Transition	282
Transversion	229
Insertions	820
Deletions	473

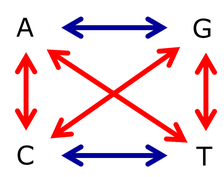

Figure 6. Mutation type distribution of the ACH-2 provirus.

Conclusions

- We have developed a high throughput assay for integration sites suitable for robotic processing.
- Cell lines infected with replication competent HIV have multiple unique HIV integration sites, not consistent with latent infection.
- Changes in Integration sites observed in ACH-2 cells and U1 cells over time are consistent with low level replication.
- Most of the Integration sites seen in the ACH-2 cell line are in intronic regions of the genes.
- These findings have implications for the use of some latently infected cell lines as models of HIV latency.

Acknowledgements

We would like to thank all staff at IIID for their support.