

Pursuing a Cure: Enhancing Limits of HIV Detectability



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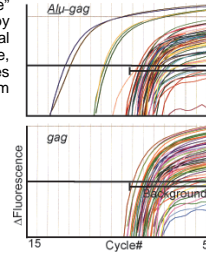


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Project Background

- HIV combines its DNA with its host's, using cellular machinery to produce viral particles. When integrating and copying, the virus is active. It also assumes a latent form where viral DNA enters the host genome without reproducing. While highly active antiretroviral therapy (HAART) can reduce viral populations below detectable limits, levels increase when therapy ceases. Our method measures the amount of virus in a blood cell subset previously thought to be HIV-resistant. A successful strategy will better illuminate therapeutic efficacy, HIV integration mechanisms, and the virus's behavior *in vivo*.
- Our process uses two gene sequences, *Alu* and *gag-1*, that are unique to human (*Alu*) and HIV (*gag-1*) DNA. When close to each other in the genome, indicating integration, they can be fluorescently tagged to create a "positive" signal. Older numerical methods characterized positives by average *Alu* and *gag-1* signals but broke down at low viral copy, where averages were statistically similar (table, below), but the results visually revealed clear positives above the background signal (figure, right). This problem motivated our new approach.

Patient	Cell Type	Alu-gag cycle threshold	Gag-only cycle threshold	p-value
ON HAART 1	Naive rCD4	34.92	34.99	0.451
	aCD4	34.50	35.84	0.132



Project Framework

Goal:

Assess which cell populations harbor latent HIV infection so that more research can be done to rid these cells of HIV DNA in an effort to find a cure.

Objective:

Develop a cell sorting method in combination with a more sensitive kinetic PCR method to accurately measure HIV DNA integration in PBMC subsets of HIV infected individuals.

Constraints:

Time: The PCR used for the assay requires 4-5 hours. The cell sorting must be performed on a limited schedule and takes 5-7 hours.

Finances: The sorting costs \$150/hour. The antibodies used for cell sorting and the primers and enzymes employed in PCR are also expensive.

Resources: Blood of HIV patients must be collected within a limited schedule.

Specifications:

Increase assay sensitivity 10-fold such that 0.1 provirus per 15,000 cells can be measured.

Increase accuracy of cell sort 4-fold such that contaminants are reduced to 0.5% of the specified population.

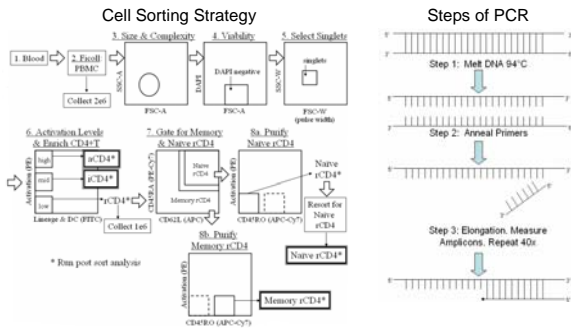
Risks:

There may be no integration in naive cells, or it may be below detection limits even with an improved assay.

If memory cell subsets are more than 100- to 200-fold more susceptible to HIV DNA integration than naive cells, the assay will not be able to prove that positive signals are not due to contaminating cells.

It may be very challenging to build a data set of patients on and off HAART within the time constraints.

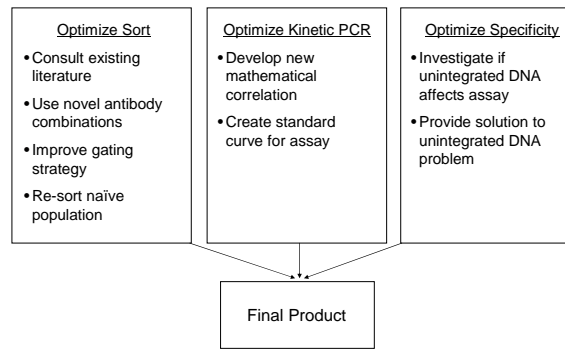
Project Illustration



Prior methods (steps 1-7) used only positive selection and resulted in high levels of contamination. Addition of a negative selection step (8a) and a second sort yielded enhanced purity of the naive population.

PCR measures HIV DNA integration in HIV infected individuals.

Block Diagram



Optimize Sort

- Consult existing literature
- Use novel antibody combinations
- Improve gating strategy
- Re-sort naive population

Optimize Kinetic PCR

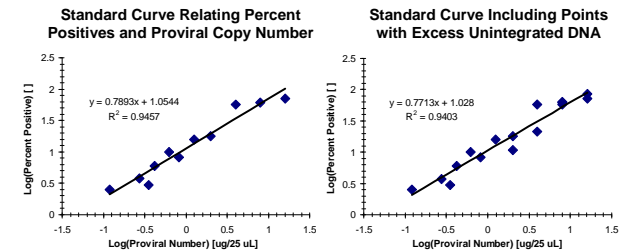
- Develop new mathematical correlation
- Create standard curve for assay

Optimize Specificity

- Investigate if unintegrated DNA affects assay
- Provide solution to unintegrated DNA problem

Final Product

Results: Standard Curve



A log-log correlation describes the relationship between proviral number at low levels and percent positives ($R^2=0.95$). This correlation can be used to detect integration events in patients with as few as 0.12 provirus per 15,000 cells.

Data assayed in the presence of unintegrated DNA shows a similar correlation ($R^2=0.94$), suggesting that unintegrated DNA does not significantly affect integration measurements at low proviral levels using percent positives.

Results: Sort Improvements

Patient	Cell	Contaminants
Uninfected 1	Memory	< 0.012%
	aCD4	< 0.0091%
ON HAART 3	Memory	0.016%
	aCD4	< 0.0034%
ON HAART 1	Memory	0.050%
	aCD4	0.031%
OFF HAART 1	Memory	0.011%
	aCD4	< 0.0066%
Uninfected 2	Memory	0.011%
	aCD4	< 0.013%

Prior cell sorting methods resulted in 2% contamination of the naive rCD4 population with memory rCD4. Shown in the table above are the percent contaminants of the naive population using the new sorting strategy on a number of patient samples. Both memory and activated (aCD4) cell contaminants are shown.

Specifications vs. Actual Performance

Specification	Promised	Delivered	Test Method
Cell sort specificity	<ul style="list-style-type: none"> 4-fold improvement in purity 0.5% or fewer contaminating cells 	<ul style="list-style-type: none"> 40-fold improvement in purity 0.0034% -- 0.050% contaminating cells 	After cell sorting, contaminants determined by flow cytometry.
Assay sensitivity	<ul style="list-style-type: none"> Increase 10-fold 0.1 provirus per 15,000 cells 	<ul style="list-style-type: none"> Increased 8.3-fold 0.12 provirus per 15,000 cells 	After dilution of viral stock, sensitivity determined by PCR identification of positive signals over background.
Assay vulnerability to unintegrated DNA	<ul style="list-style-type: none"> Developed during project: team-specified Old method skewed by excess unintegrated DNA 	<ul style="list-style-type: none"> Percent positives method unaffected by unintegrated DNA (<2.5% change in standard curve relationship) 	After standard curve completion, vulnerability to unintegrated DNA determined by adding excess DNA, re-assaying, and supplementing curve.

Conclusions and Recommendations

Detection sensitivity at dilute viral load increased ten-fold. Since time, not technology, constrained the assay, future investigations can evaluate therapeutic success in patients at even lower copy.

Cell sort specificity improved forty-fold. By isolating blood subsets with greater purity, we may better understand HIV's effects on resistant cell types.

In addition, the new mathematical correlation protects assay results against skew by unintegrated DNA. Thus the percent positives method amplifies the clinical applicability of the repetitively sampled assay.

Overall, enhanced detection of rare integration events in specific cell populations affords insight into HIV life cycle mechanisms. Ongoing research can apply this understanding to eliminate infection, rather than simply decrease it below measurable levels, with the aim of arriving at a cure.

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