Exploring the HIV reservoir with full-length provirus sequencing

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Outline

1. Background to HIV provirus sequencing
2. Origin and use of the “FLIPS” assay
3. Sequence assembly workflow
4. Provirus annotations: Intact vs defective

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Cell Reports
Resource

Identification of Genetically Intact HIV-1 Proviruses in Specific CD4+ T Cells from Effectively Treated Participants

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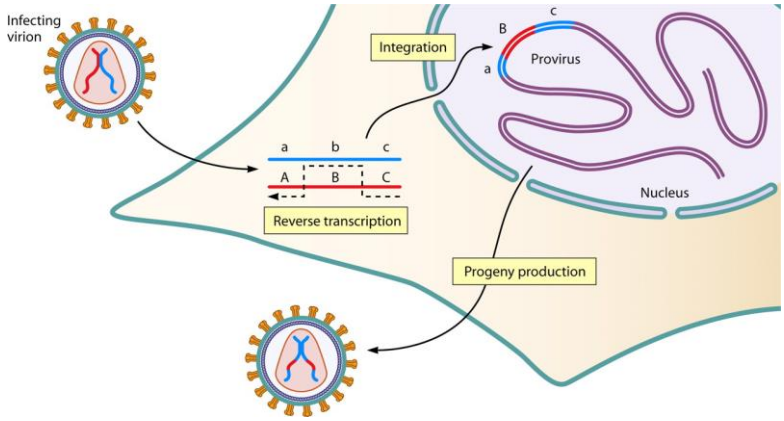
What is a Provirus?



Adewunmi Onafuwa-Nuga & Alice Telesnitsky
Microbiol. Mol. Biol. Rev. (2009) 73:451-480

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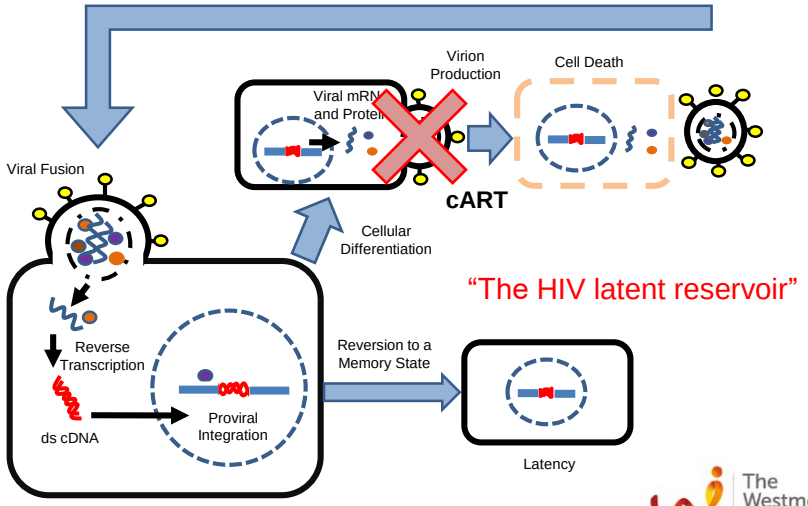
HIV Lifecycle



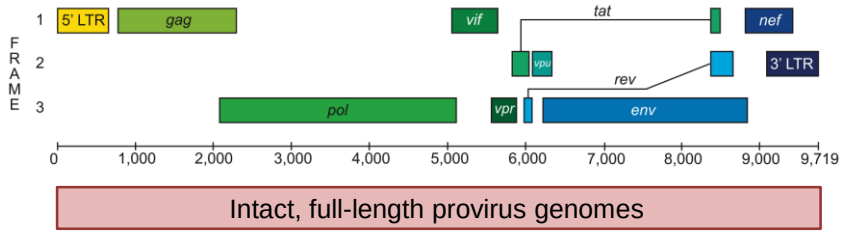
HIV Reservoir

For a cure, we need to know where the virus hides

- For example, we know that the virus remains dormant in resting, memory CD4+ T-cells.
- But...
  - which particular cell subsets?
  - where are the viable "replication-competent" viruses?
  - And, in what proportion?

Provirus sequencing

How do we known if a virus is replication-competent?



Intact, full-length provirus genomes

High-throughput sequencing looking for intact proviruses

No mutations, no deletions, no frameshift, no stop codons, no inversions

Vast majority will be defective!

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The FLIPS assay

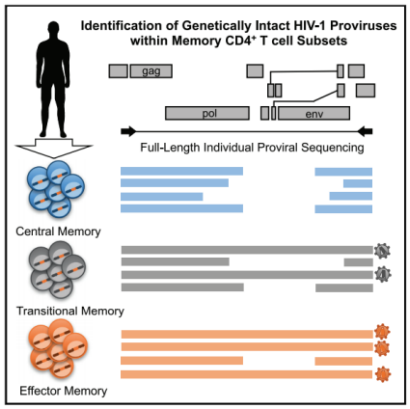
Full

Length

Individual

Provirus

Sequencing



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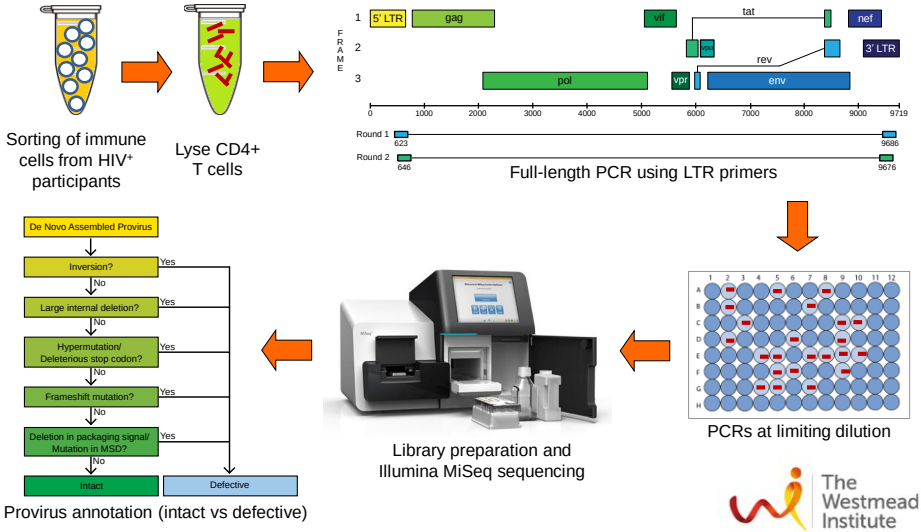
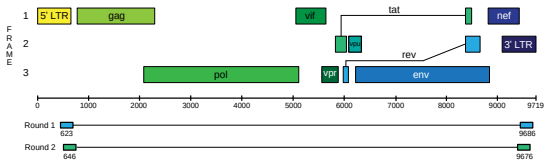
The FLIPS assay

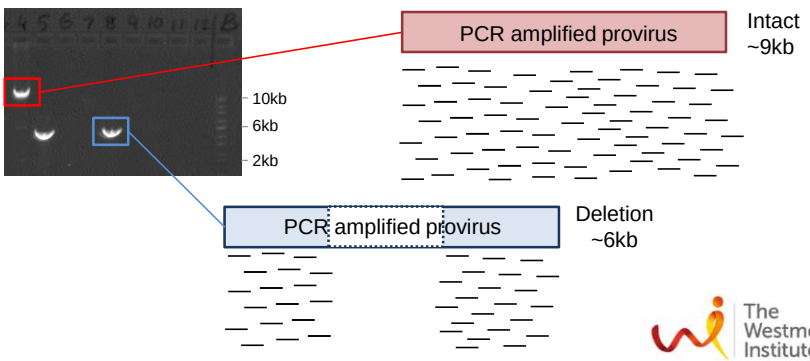


PCR using LTR primers



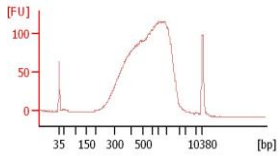
"Limiting dilution"

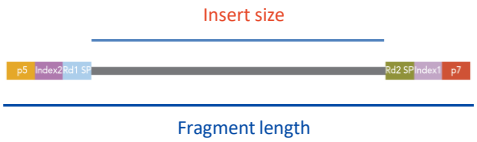
One PCR amplicon = One provirus



Illumina MiSeq sequencing

- DNA 'Tagmentation' (Tagging + Fragmentation)





- Sequence "barcodes" allows multiple samples per run
- One MiSeq run = 20 million sequence reads (2x150nt)
- **60 to 80 "proviruses" per run**

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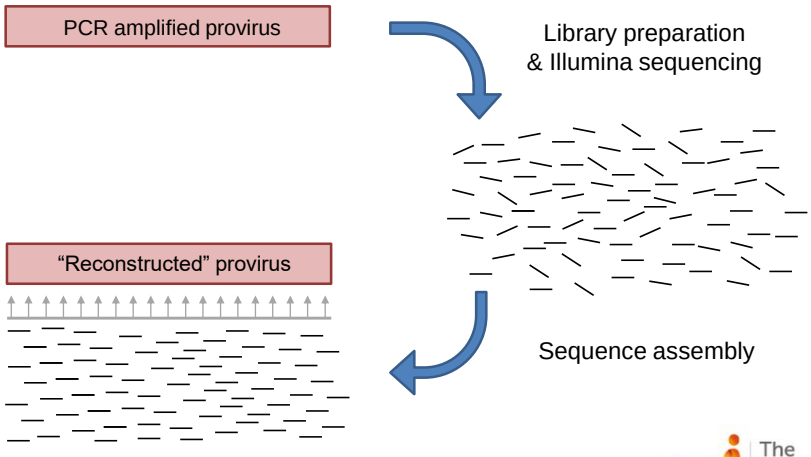
Need for sequence assembly



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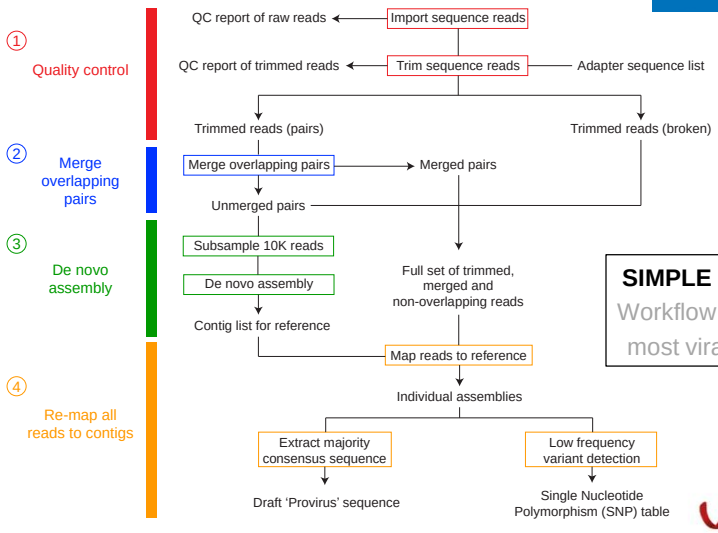
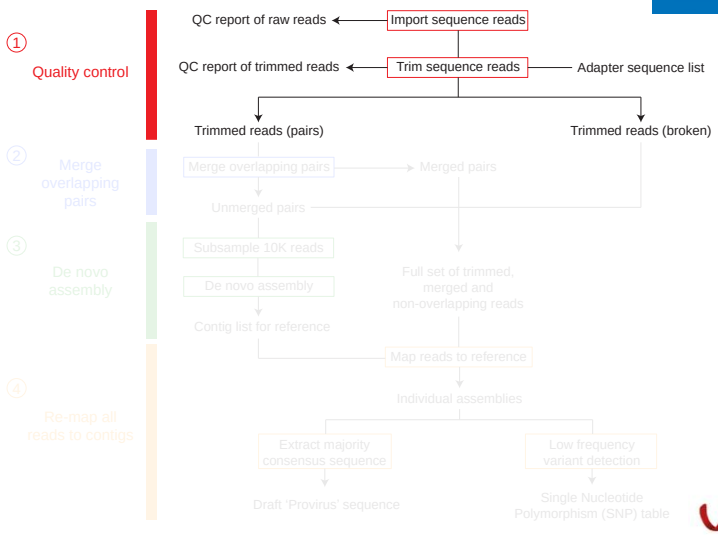
Assembly workflow



SIMPLE & RELIABLE
Workflow works well for most viral assemblies

Assembly workflow



1. Quality control

- Assess quality of the data
  - Sequencing provides per base “quality” scores
  - Score reflects a probability of error
- Trim sequences
  - Based on quality threshold (1%)
  - Of any sequence artifacts like primer or adapters
- Filter reads by length (>50bp)

Open source = FastQC, Trimmomatic & Cutadapt

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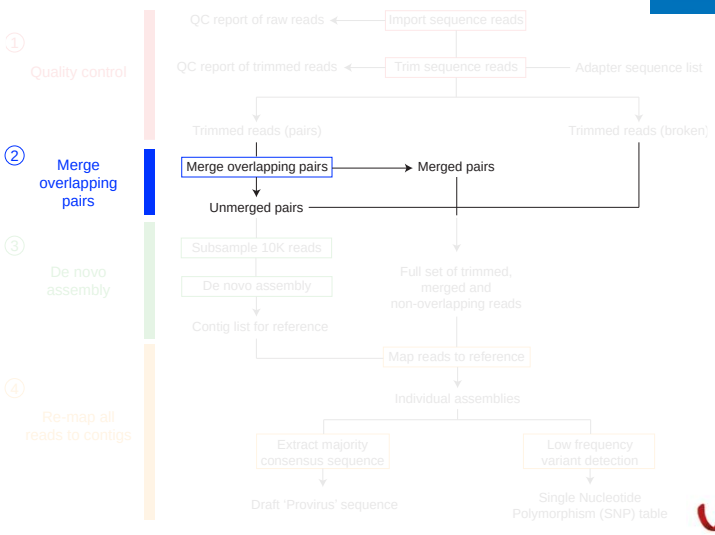
Assembly workflow

CLC Genomics Workbench



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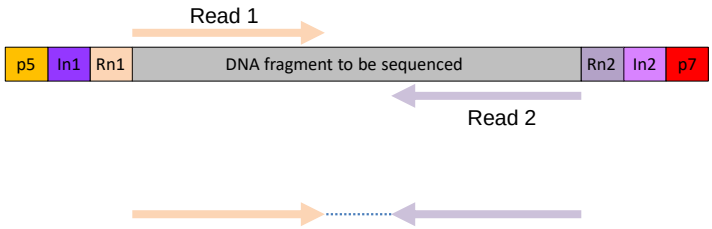
2. Merge overlapping pairs

- Paired reads are from one fragment, sequenced from each end



Paired reads

Open source = Flash

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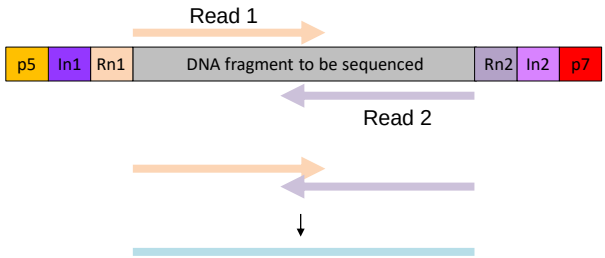
2. Merge overlapping pairs*

- If read length (2X150nt) more than fragment length, then reads will overlap and can be collapsed



Merged reads

Open source = Flash

*Somewhat optional

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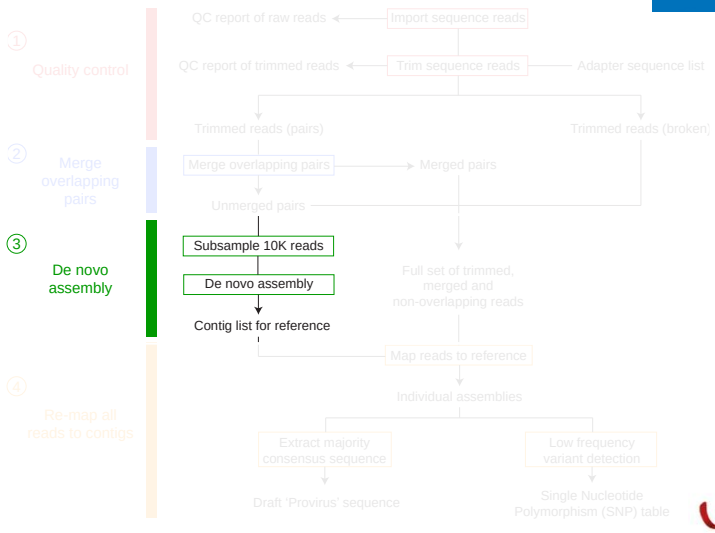
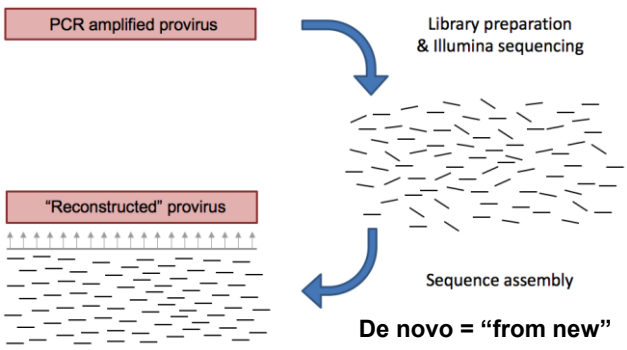
Assembly workflow



3. De novo assembly

Reconstructing the sequence from short reads



De novo = "from new"

No guides… No prior info…

Just the reads!

3. De novo assembly

- Cuts reads into smaller bits (“words”), then looks for overlap and then extends to make longer “contigs”
- Each set should contain amplified target (i.e. provirus)
- Downsample reads to 10K to **run on typical desktop**
  - Full set of 300,000 reads = slow, needs larger memory
- Provirus should be an assembled contig
  - Most abundant & matched to PCR product size

Open source = Trinity, Spades, Velvet

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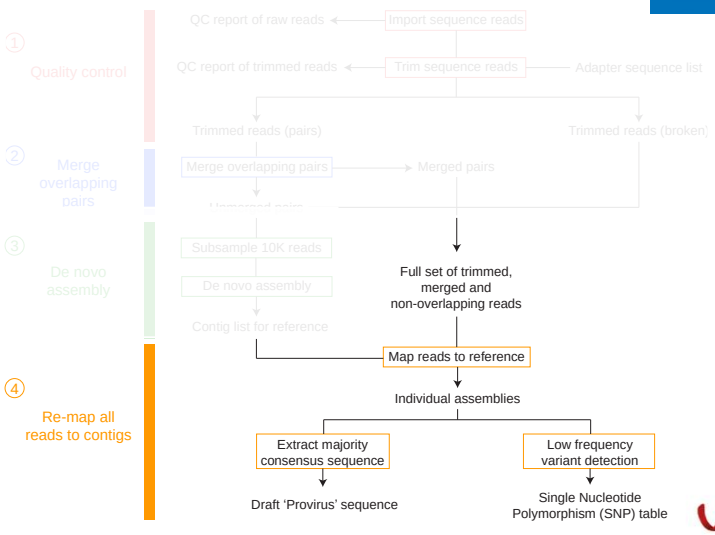
Assembly workflow

CLC Genomics Workbench



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4. Final provirus sequences

- Identify HIV provirus from list of *de novo* contigs
- Map the full set of reads and take a final “consensus”
- Look for heterogeneity in data
  - One amplicon = One provirus = No SNPs or variation
- The final ‘provirus’ must then be annotated/analysed

Intact or Defective?

Open source = Bowtie, Samtools, Freebayes

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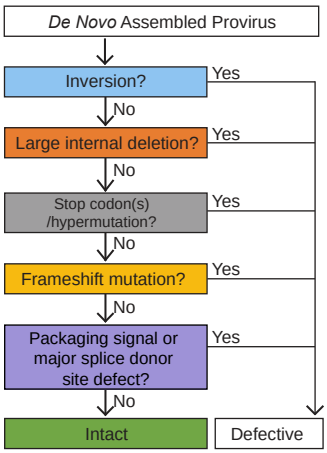
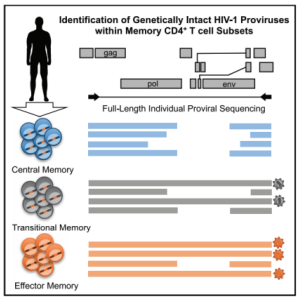
Provirus annotation



Based on notion that the virus needs complete, intact gene sets for functional replication



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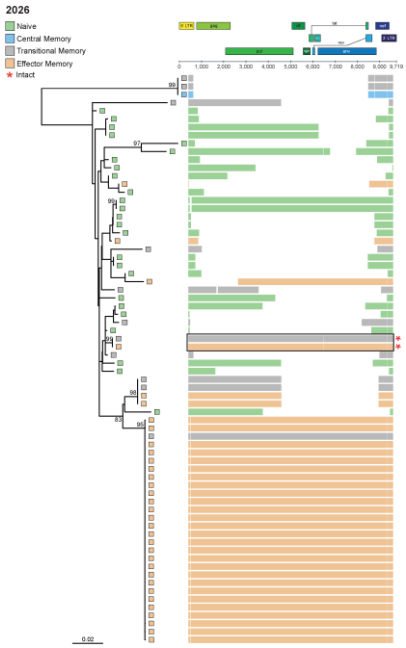

Provirus Phylogeny

- Compare sequences for each patient
- Map proviruses to cell subsets
- Identical sequences suggest clonal expansions
- Where intact genomes?
- Must check non-coding regions!

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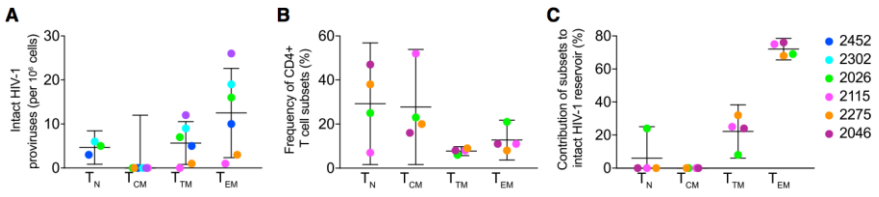
Where are the intact proviruses?



- Intact proviruses were unequally distributed between T-cell subsets
- Effector memory cells containing the largest proportion of genetically intact HIV-1 proviruses

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Pros and Cons

- Previous methods targeting *env* or *gag-pol* overestimated frequency of intact proviruses
- High-throughput sequencing screen more provirus
- The context of provirus integration cannot be determined using FLIPS
- Single copy assays prone to contamination
- PacBio single molecule seq (i.e. 1 provirus = 1 read)

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Summary

- FLIPS utilizes NGS to sequence and characterize HIV-1 proviruses
- FLIPS can identify genetically intact and likely replication competent HIV-1 proviruses
- Identical HIV-1 proviruses suggest maintenance of reservoir by cellular proliferation & importance of reservoir in *Effector Memory T Cells*

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