# Lentiviruses:

Slow and Steady wins the race

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## Outline

- The "Slow" virus
- Turning the Tables, bad guy makes good
  - Using a lentivirus to stop a lentivirus
  - Making a lentivirus vector
- Evading detection
- Breaking the retrovirus "rules"

## The Slow Virus

- Subclass of Retrovirus
  - Replicates exclusively in nucleus
  - Complex gene expression/regulation vs. simple retrovirus
- "lenti" Latin for SLOW
  - Persistent infection, incubation could take years
  - Lysogenic in differentiated cells
    - Differentiated cell factors

LOOK FAMILIAR???


## Bad guy virus makes good

Don't judge a virus by it's epidemic.

Phil Sharp Nobel Laureate

- Lentivirus vector induced siRNA inhibits HIV pol expression by selective binding and disruption of gag gene

## Making a Lentivirus Vector I

- 3 Plasmid Co-Transfection

1) Most trans-acting viral genes Plasmid
2) VSV psudeotyped env gene Plasmid**
3) YOUR FAVEORITE GENE and cis-acting elements for packaging, RT, and integration Plasmid

## Making a Lentivirus Vector II

**RECIPIE REVIEW**

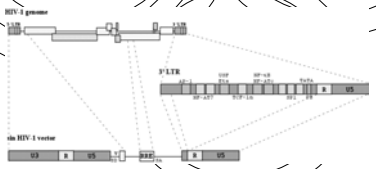
- 3 essential cis-acting elements
  - Packaging signal "Ψ" TAKE ME!!!
  - Reverse Transcription COPY ME!!!
    - primer binding site (PBS)
    - terminal repeat (R) guides RT from RNA-DNA
    - Purine rich 5' of 3' LTR region
- LTR Intergation sequences INTEGRATE ME!
  - Internal Promoter or IRES

## CLAIM TO FAME: Gene Therapy

- Lentiviruses are the ONLY retroviruses able to integrate into chromosome of Non-Dividing Cells!!!
  - **Matrix proteins hijack nuclear import machinery gets into nucleus**
  - **Uses cellular nuclear replication machinery**
- Up to 6 months sustained expression

## Reducing the Risks

- Non-Replication-Competent Recombinant (non RCR) vectors
  - Piecemeal Plasmids for viral vector genome
- Self-inactivating (SIN) vectors
  - 3' LTR deleted to remove transcriptional promoter.
  - Does that eliminate free ds proviral DNA?




## Can't Catch Me!!

- Low antigen and viral expression
  - Incomplete integration
  - Requires factors from differentiated non-mitotic cells for lytic phase
- Lots of mRNA splice variants, more than retroviruses
  - ANTIGENIC VARIATION
  - Restricted Expression pathways
- High env mutation
- Immune response increases severity of infection

## Breaking the retrovirus "rules"

1) Active cell cycle required
   - Lentivirus gets into nucleus and replicates slowly in quiescent NON-MITOTIC differentiated cell
   - Lentiviral unique effectors for hijacking nuclear localization and replication machinery

2) Integrated viral DNA is the most efficient template for retroviral RNA transcription
   - Majority of Lentiviral provirus genome NOT integrated
     - Free ds linear DNA in nucleus of infected cell

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