Transposable Elements in Genomes: *i) effect of drugs on mobilization; ii) identification of interactors; and iii) cloning of zebrafish elements*

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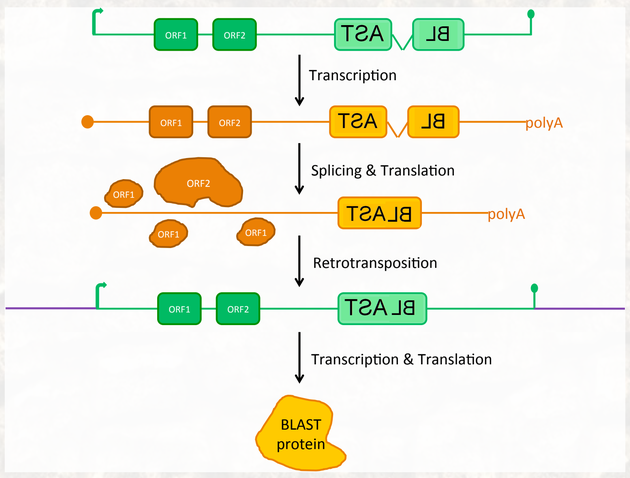
Introduction

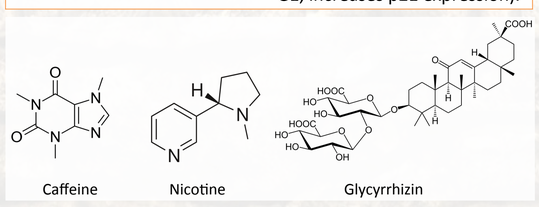
Transposable elements (TEs) are DNA sequences which can change its location within the genome. At least 45% of the human genome is made of TEs, and currently only LINE-1 retrotransposons continue to move in the population. LINE-1s are copy and paste TEs, and each cell in our body contains 80-100 active LINE-1s. LINE-1 insertions in humans occur randomly through the genome, and new insertions can be a source of mutations.

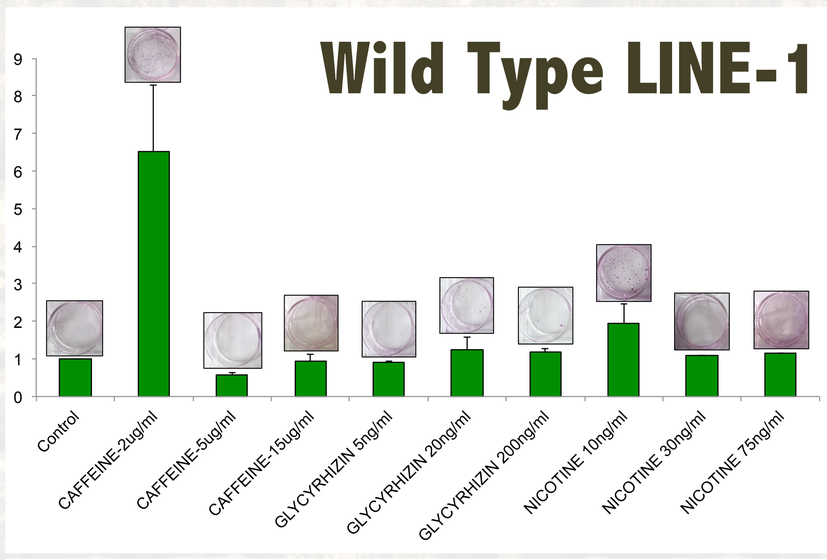
Experiment I: Effect of drugs on L1 mobilization.

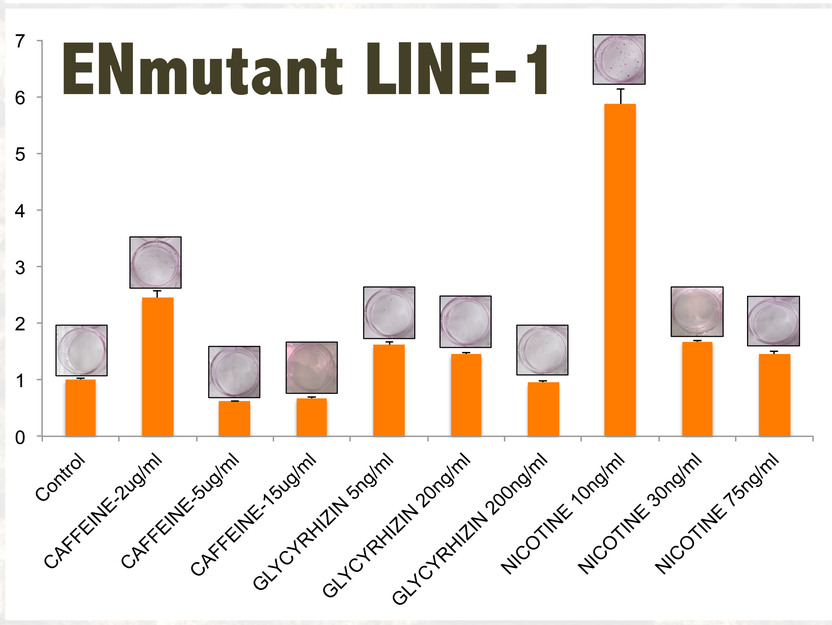
Scientific Question: Do commonly used drugs which influence human's physiological activity such as nicotine, caffeine and glycyrrhizin affect L1 retrotransposition in HeLa cells?



Drug	Influence on cells
Caffeine	Has an affect on regulatory proteins (apoptosis, DNA repair and cell division)
Glycyrrhizin	Inhibits phosphokinase P -> inhibits virus replication
Nicotine	Inhibits cell growth (arrest in G1, increases p21 expression).







- Retrotransposition of wild type LINE-1 is increased by:
  - Caffeine in concentration 2 µg/ml
  - Nicotine in concentration 10 ng/ml
- Retrotransposition of an EN-mutant LINE-1 is increased by:
  - Glycyrrhizin in concentration 5 ng/ml and 20 ng/ml
  - Caffeine in concentration 2 µg/ml
  - Nicotine in all tested concentrations

Acknowledgements

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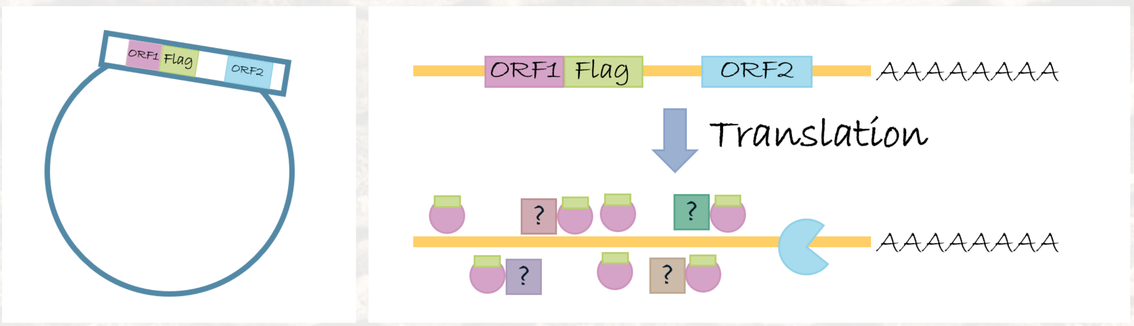
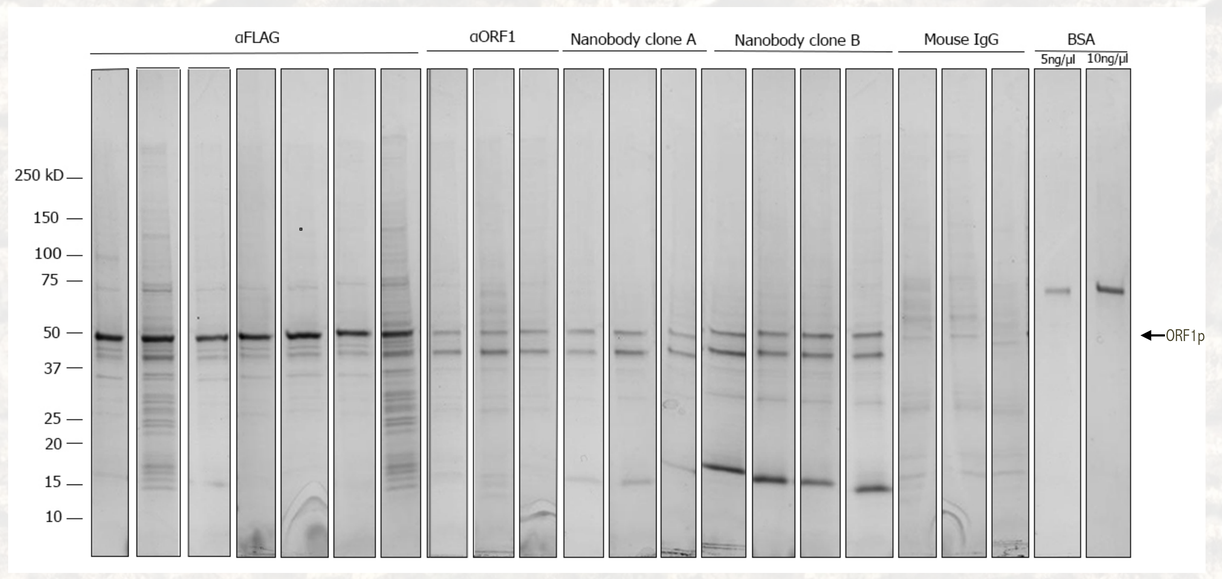
Experiment II: Identification of host factors that interact with L1.

Scientific Goal: Identify a good antibody to pull-down L1 interactors.

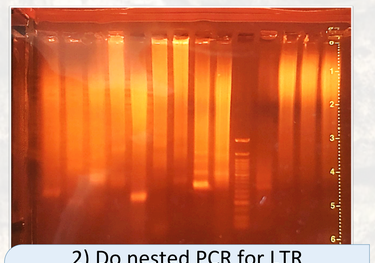


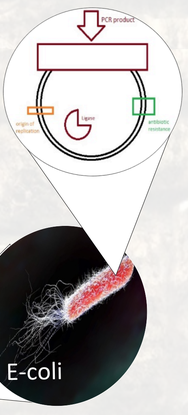
- Conclusions:
  - The three antibodies tested successfully immunoprecipitated L1-ORF1p and interacting proteins.
  - Nanobody clone B antibody precipitates the most ORF1p.



Experiment III: Cloning active LTR-retrotransposons from zebrafish.

Scientific Goal: Clone and sequence active LTR retrotransposon from the zebrafish genome.





3) Purify DNA with LTR-retrotransposons

4) Cloning and transformation in *E.coli*

5) Sequencing plasmids; identify active elements that will be tested in zebrafish