

IBUPROFEN INHIBITS LINE ONE RETROTRANSPOSITION

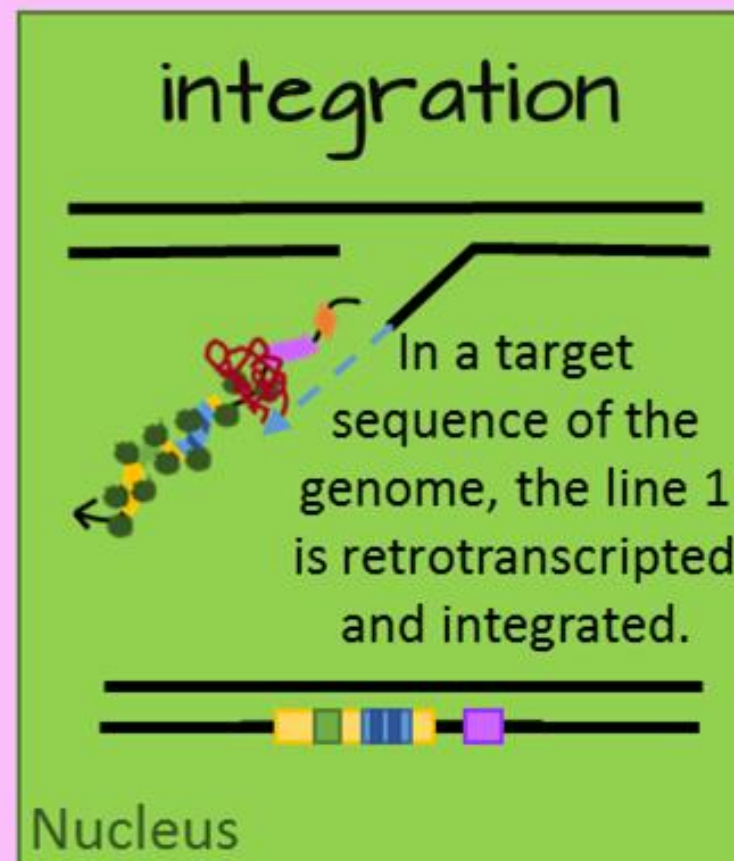
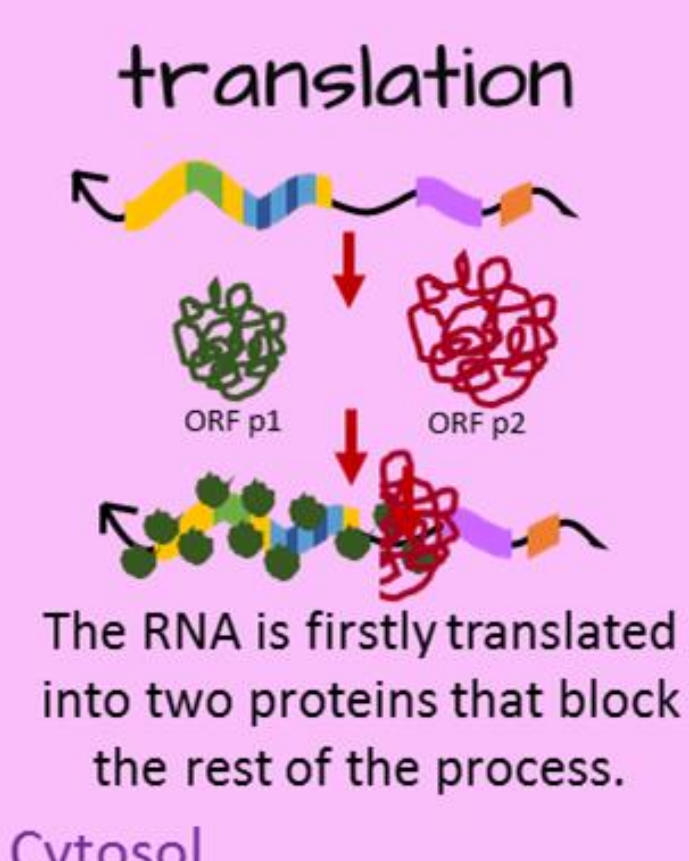
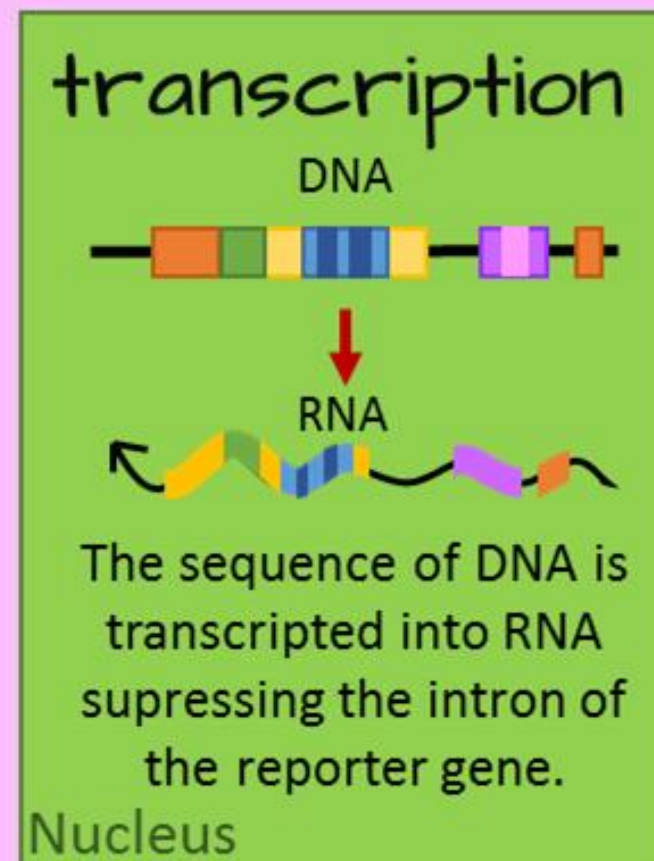
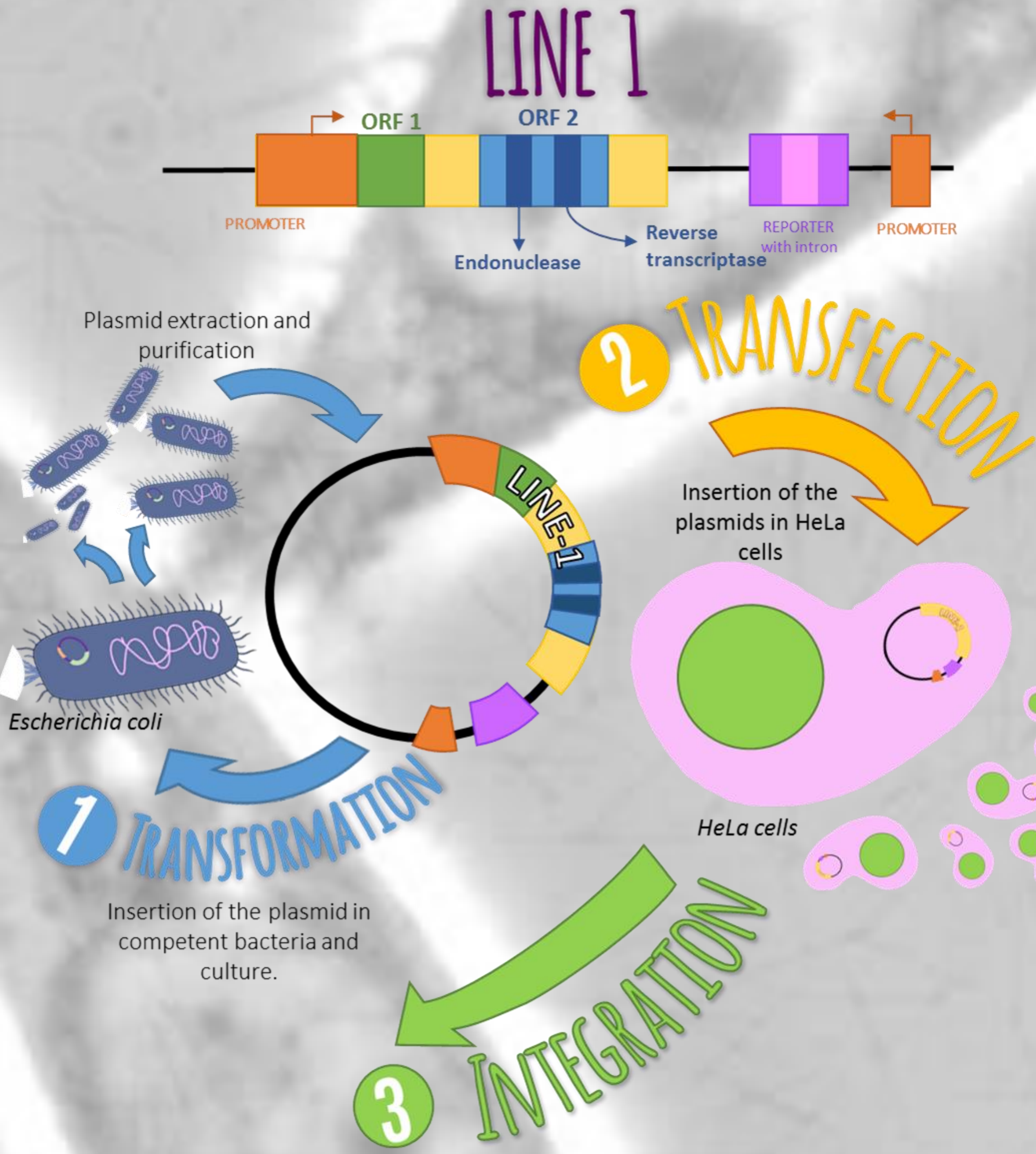
ABSTRACT

Transposons are repetitive DNA sequences with the capacity of copying themselves into different loci in our genome. There are two types: DNA transposons, that cut and paste themselves between chromosomes, and Retrotransposons, that use a retrotranscriptase to reconvert their mRNA into DNA in another place. From this second type the only active one in the human genome, nowadays, is LINE-1. New insertions have an important role in evolution but can also cause mutations, being implicated in a lot of genetic diseases. In order to prevent non-desired mutations, for example in iPSCs cells used in regenerative medicine, our lab is interested in finding chemical inhibitors for retrotransposition activity. We tested whether common drugs like Ibuprofen and Cetirizine could affect the retrotransposition rate of LINE-1. With the purpose to achieve this goal we transfected HeLa cells with a LINE-1 plasmid, and exposed them to the drugs. Co-transfection with GFP served as transfection efficiency control. The results show that Ibuprofen is a potential inhibitor while Cetirizine doesn't affect retrotranscription.



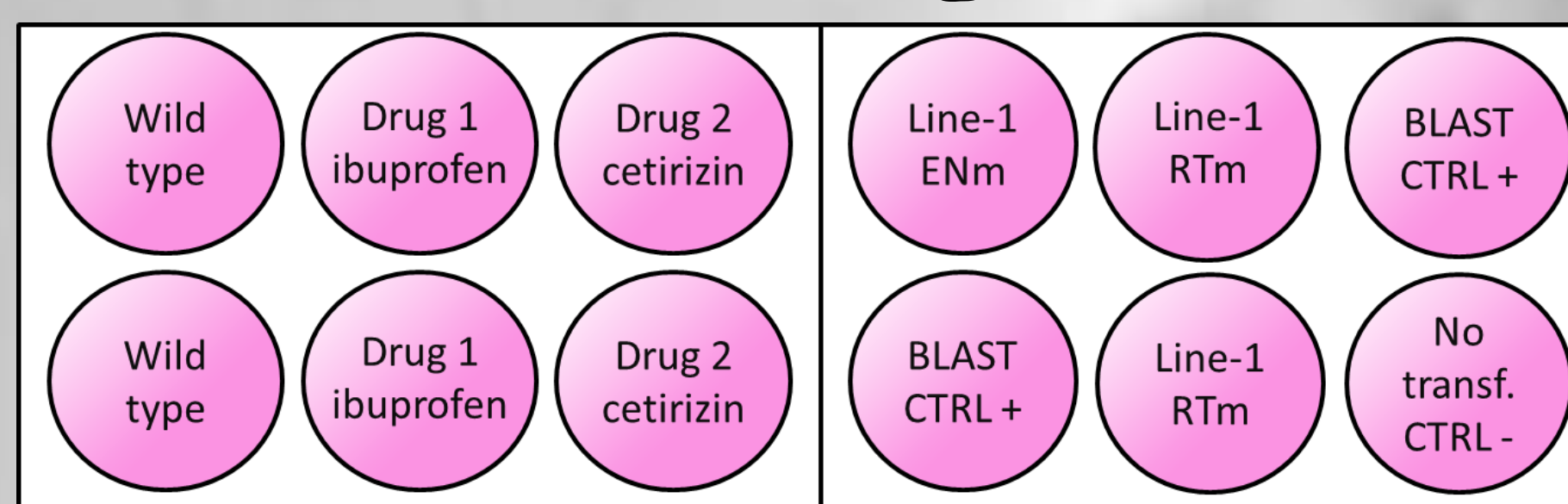
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MATERIAL & METHODS

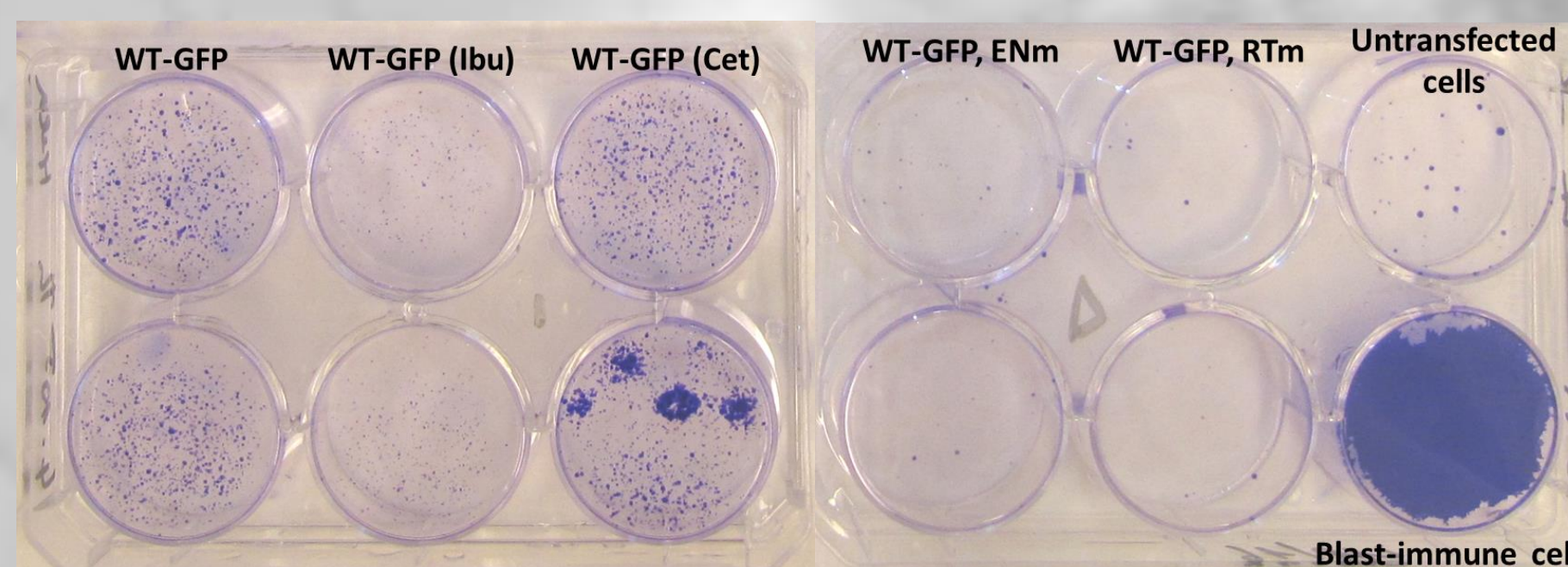


RT RATE TEST

Effect of drugs/mutations on RT rate

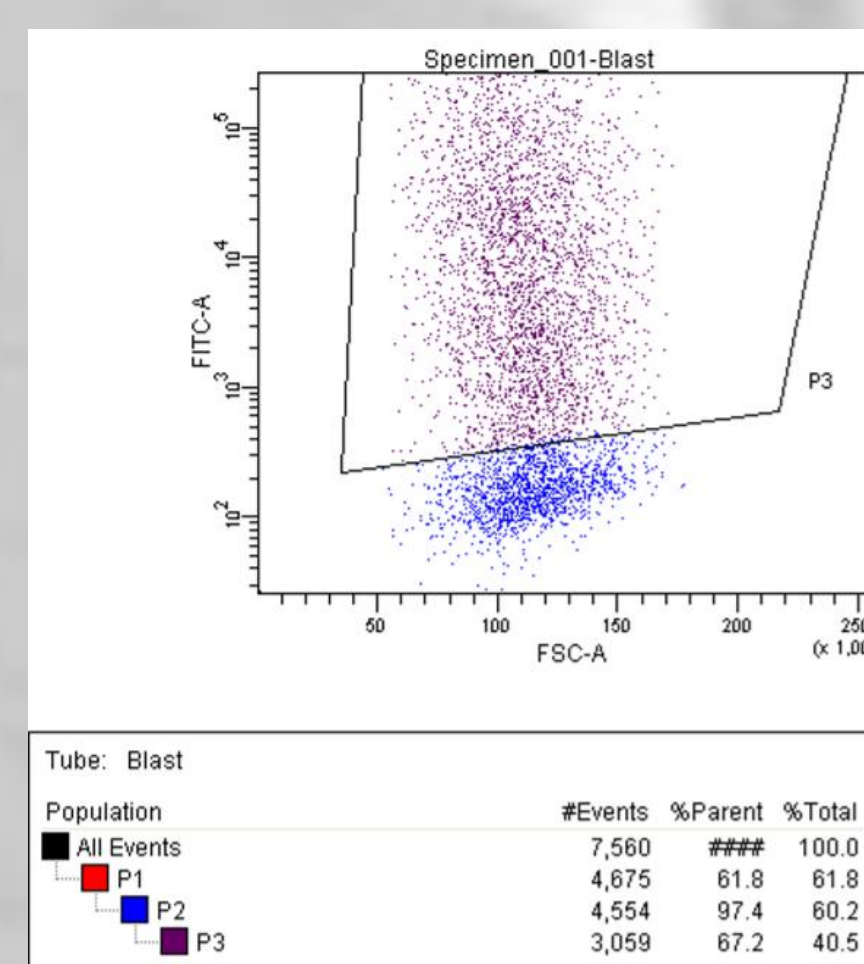


HeLa cells transfected with plasmides as indicated. (wells: n=2). L-1 WT survival rate is 100%.

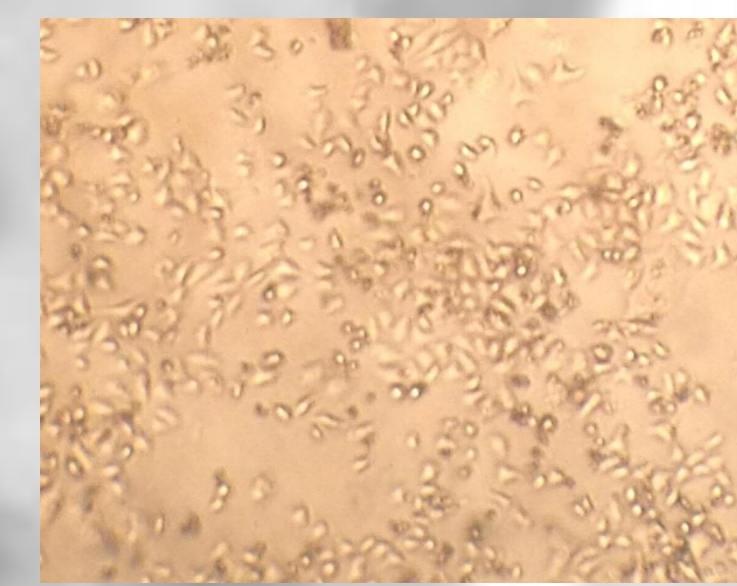


HeLa cell colonies resistant to blasticidine and stained with crystal violet dye. Ibuprofen, and RT and EN mutations inhibit L-1 RT.

Transfection efficiency test

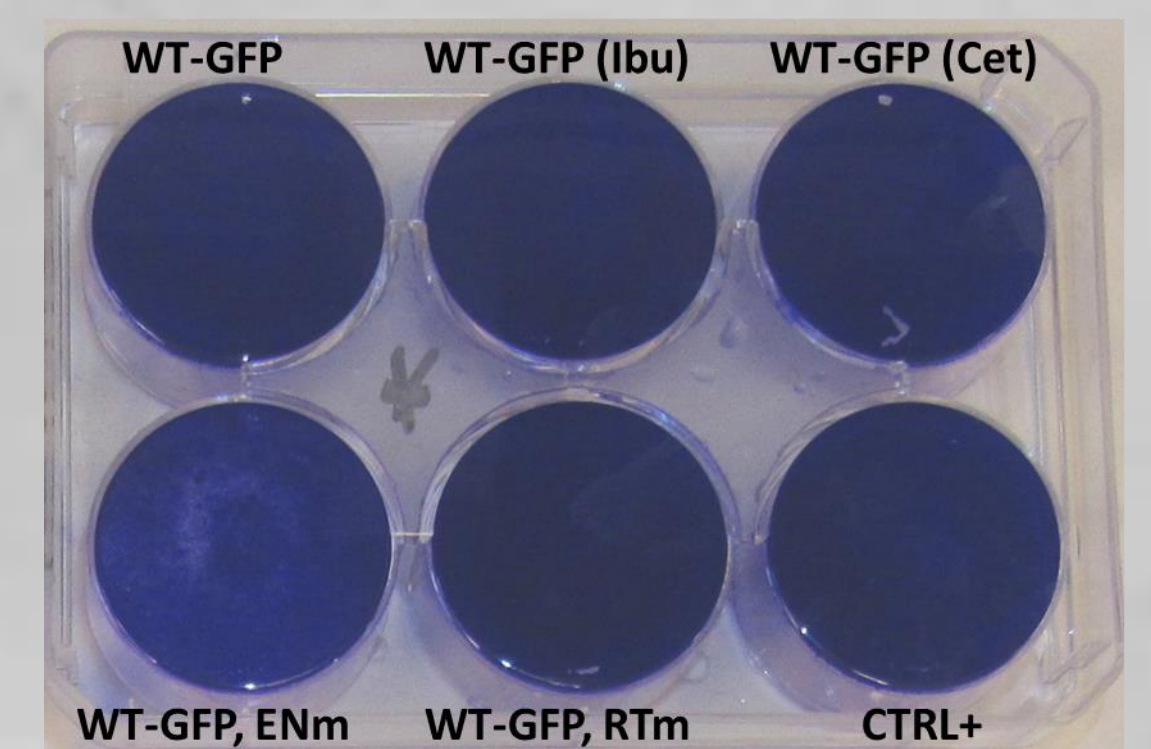


HeLa Cells co-transfected with L-1 plasmides and GFP, analysed with cytometer.



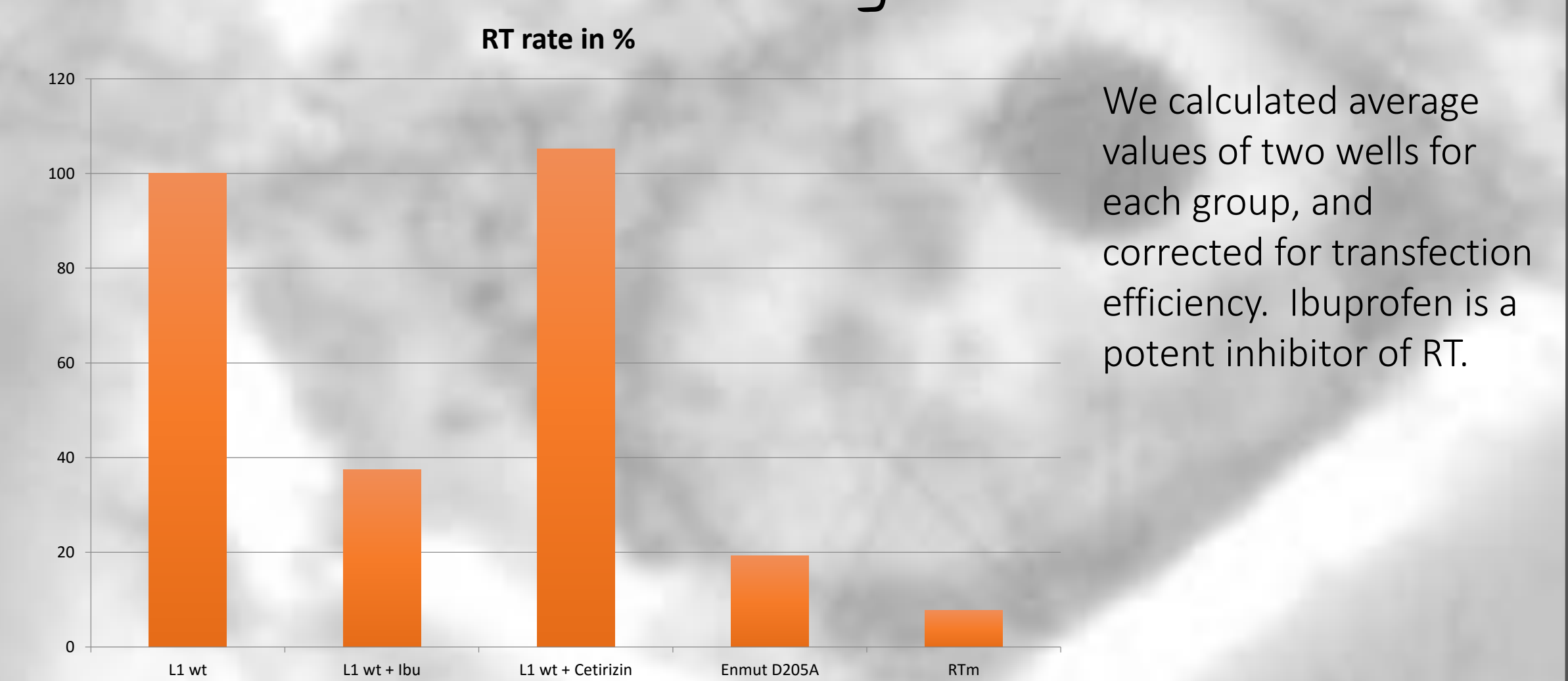
HeLa cells in the field of a microscope.

Toxicity test



HeLa cells cotransfected with L-1 plasmides and BLAST cassette show no toxicity.

The Retrotransposition rate of L-1 WT and L-1 with drugs/mutations.

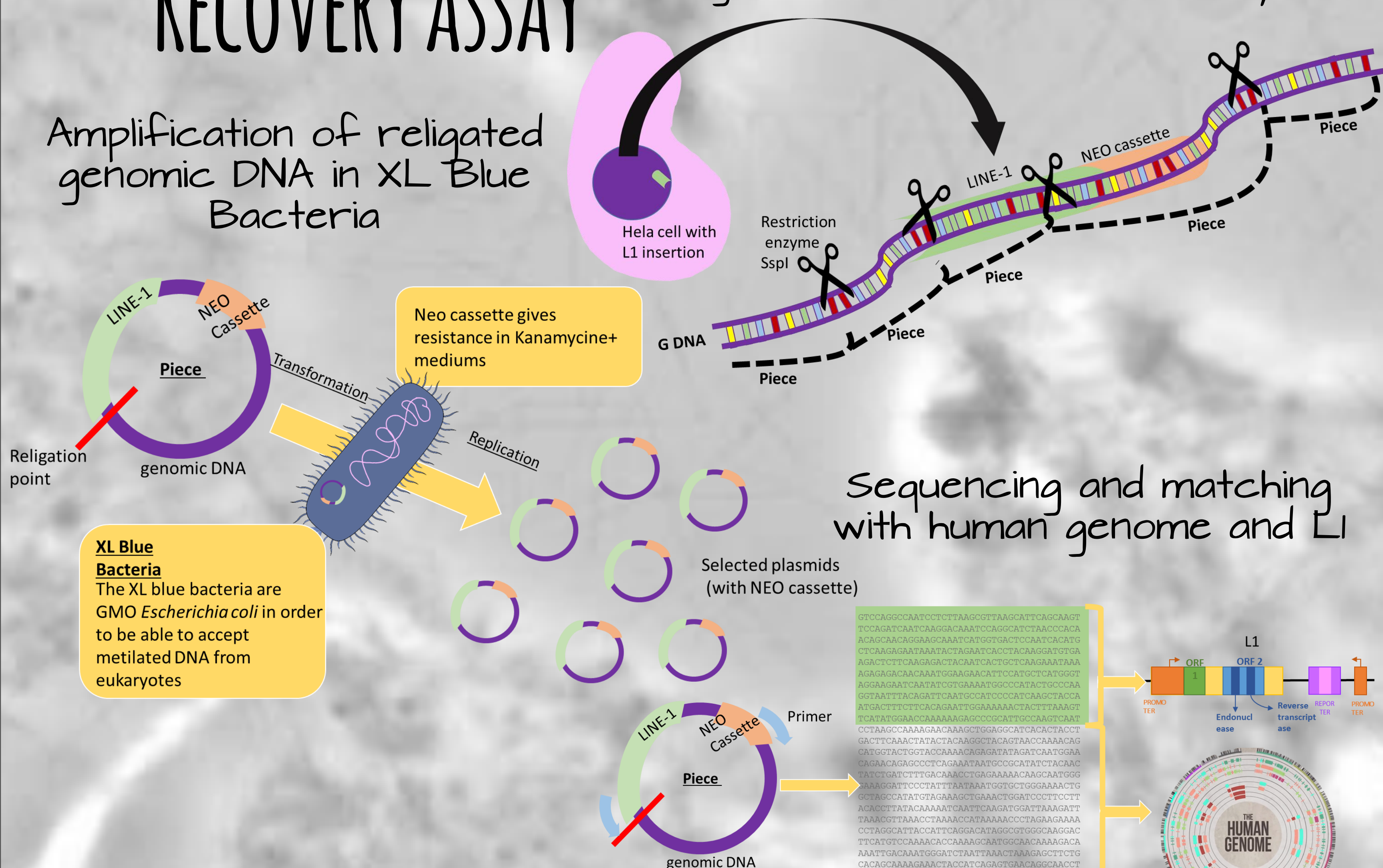


We calculated average values of two wells for each group, and corrected for transfection efficiency. Ibuprofen is a potent inhibitor of RT.

RECOVERY ASSAY

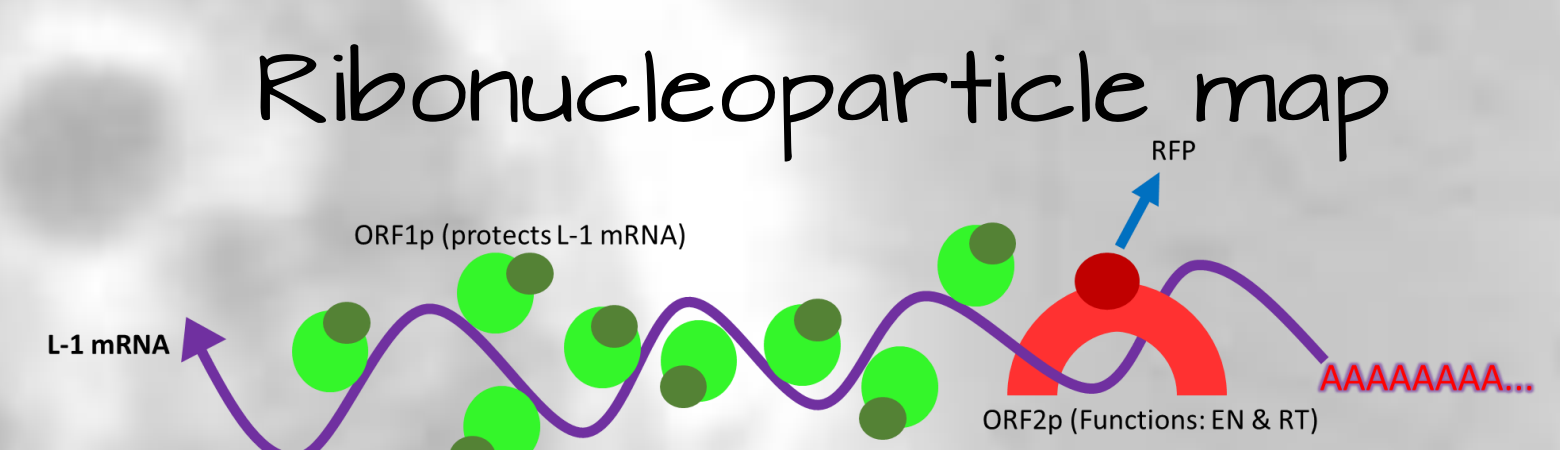
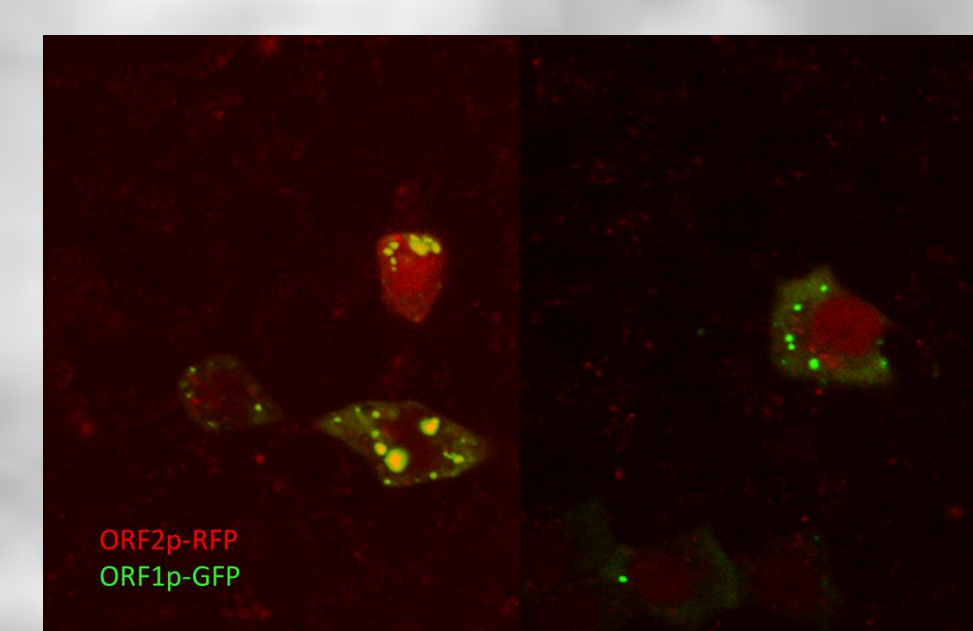
Amplification of religated genomic DNA in XL Blue Bacteria

Extraction of genomic DNA and digestion with restriction enzymes



Colony	Chromosome locus
pRAM36	Chromosome 15
pRAM07	Chromosome 3 or 4 (inserted in another transposon)
pRAM41	Chromosome 4

RIBONUCLEOPARTICLE LOCALIZATION



FUTURE DIRECTIONS...

After verifying the inhibitory activity of Ibuprofen in HeLa cells, our next step will be to repeat the experiment in order to confirm the results. Next, we will test whether there is a concentration-dependent effect on retrotransposition. On the other hand we will repeat the experiment with different cancer cell lines like U2OS or 293T, stem cells like iPSC, and neural progenitor cells. Finally, our aim will be to find out the reason why Ibuprofen inhibits LINE-1 retrotransposition. This could be done by studying the possible inhibitory effect of other compounds with a similar structure as Ibuprofen.