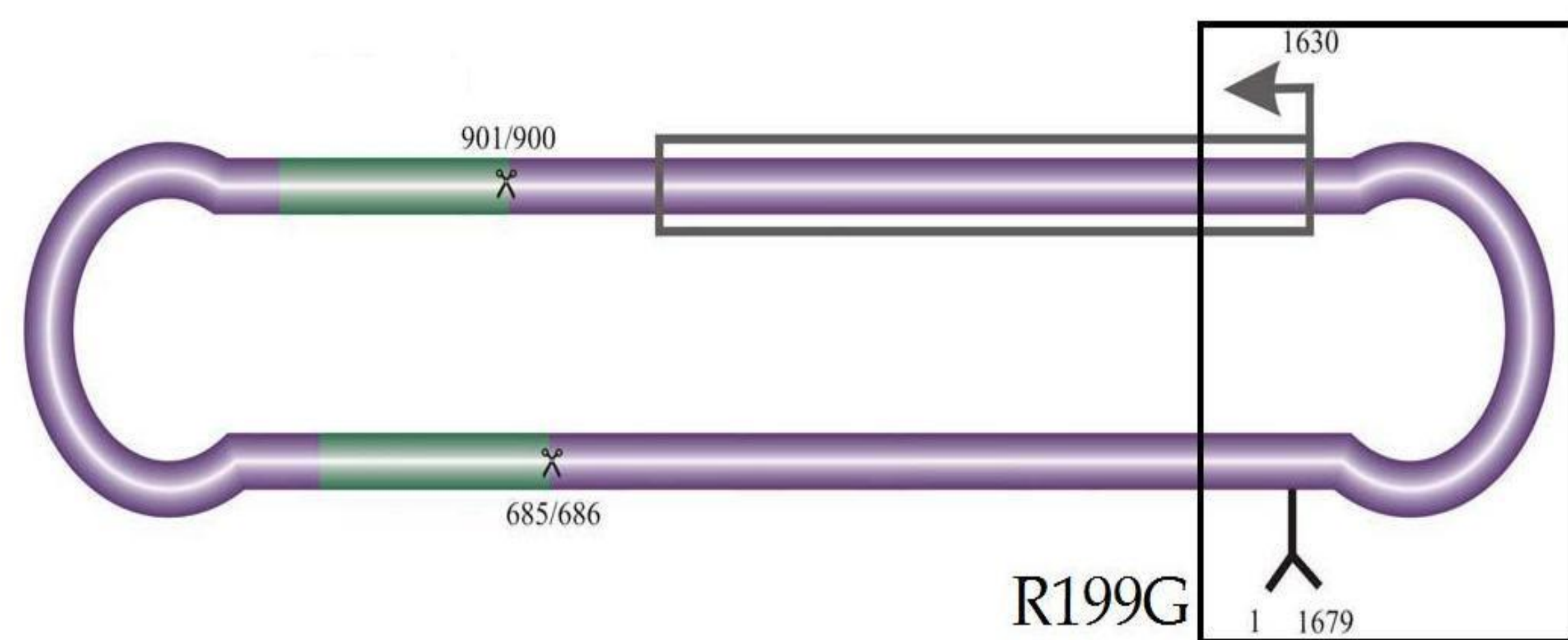


Introduction

The hepatitis *delta* virus (HDV) causes one of the most serious and rapidly progressive forms of viral hepatitis, often leading to fulminant hepatocellular carcinoma. HDV consists of a small, single-stranded, circular RNA molecule encoding a single open reading frame. It needs to manipulate host proteins to replicate due to its minimalist nature. However, mechanisms by which HDV infection contributes to hepatitis are poorly understood.

Recently, the polypyrimidine tract-binding protein-associated splicing factor (PSF) was identified as a protein interacting with the HDV genome. PSF mutations have been identified in both cervical cancer and papillary renal carcinoma cell lineages, and PSF dysregulation is associated with induction of several oncogenes. It was shown that noncoding RNAs can bind and reverse PSF-mediated repression of several proto-oncogenes, promoting cell proliferation and oncogenesis. Preliminary results of research done at the Pelchat lab indicate an increase of the oncogene GAGE6 in cells expressing HDV RNA. Therefore, the hypothesis is that by interacting with PSF, HDV RNA can reverse PSF repression and promote liver tumour development. The objective of this research project is to obtain indications linking the ability of HDV RNA to bind PSF with the induction of Gage6, by determining the GAGE6 mRNA content in human embryonic kidney (HEK) 293 cells after transfection with R199G – a HDV-derived RNA fragment known to bind PSF.



Materials & Methods

RNA Synthesis, Transfection and RNA Extraction

To synthesize R199G RNA, a standard polymerase chain reaction (PCR), gel extraction, in-vitro transcription and ethanol precipitation were performed as previously described (Abraham & Pelchat, 2008; Sikora *et al.*, 2009). The quality and size of the RNA was verified by agarose gel electrophoresis. HEK 293 cells were then cultured and transfected with R199G RNA using Lipfectamine 2000 transfection reagent (Invitrogen), and mRNA was extracted using Trizol (Invitrogen), as per manufacturer protocols. mRNA was also extracted from cells transfected with a plasmid containing a trimer of HDV genomes (pSVLD3), and non-transfected cells, as positive and negative controls respectively.

Reverse Transcription and Quantitative PCR

Complimentary DNA (cDNA) was reverse transcribed from the mRNA using iScript Select cDNA Synthesis Kit (Bio-Rad). qPCR was performed using iQ SYBR Green Supermix (Bio-Rad) on all the cDNA samples using separate primers for: the GAGE6 gene, the actin gene, and R199G. Manufacturer protocols were followed for both reverse transcription and qPCR. Finally, qPCR results were compared to determine if there was an increase in Gage6 expression in cells transfected with R199G.

Results

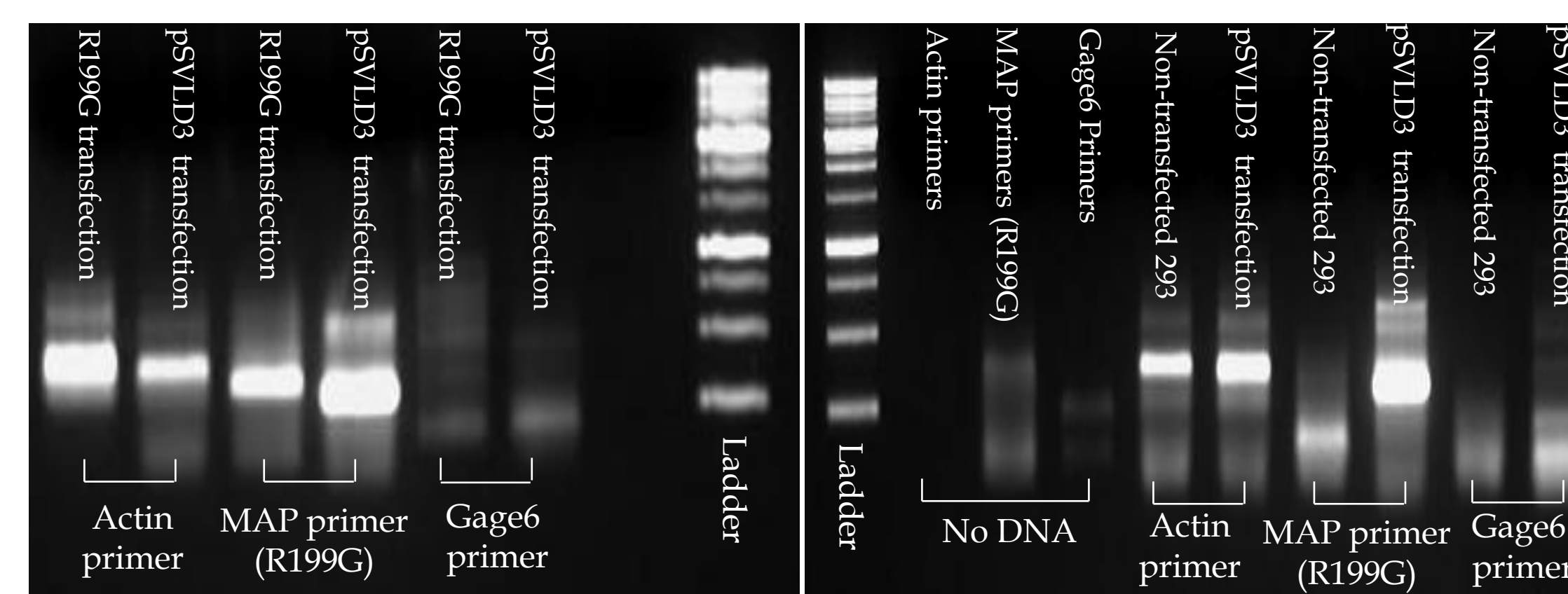


Figure 1: Agarose gel electrophoresis of the qPCR products. Bands present for the actin and the R199G samples indicate the presence of mRNA for those genes after transfection. Smears produced in each lane are indicative of amplified primers or non-specific amplification during qPCR. However, no bands are present that indicate the presence of Gage6 mRNA after transfection.

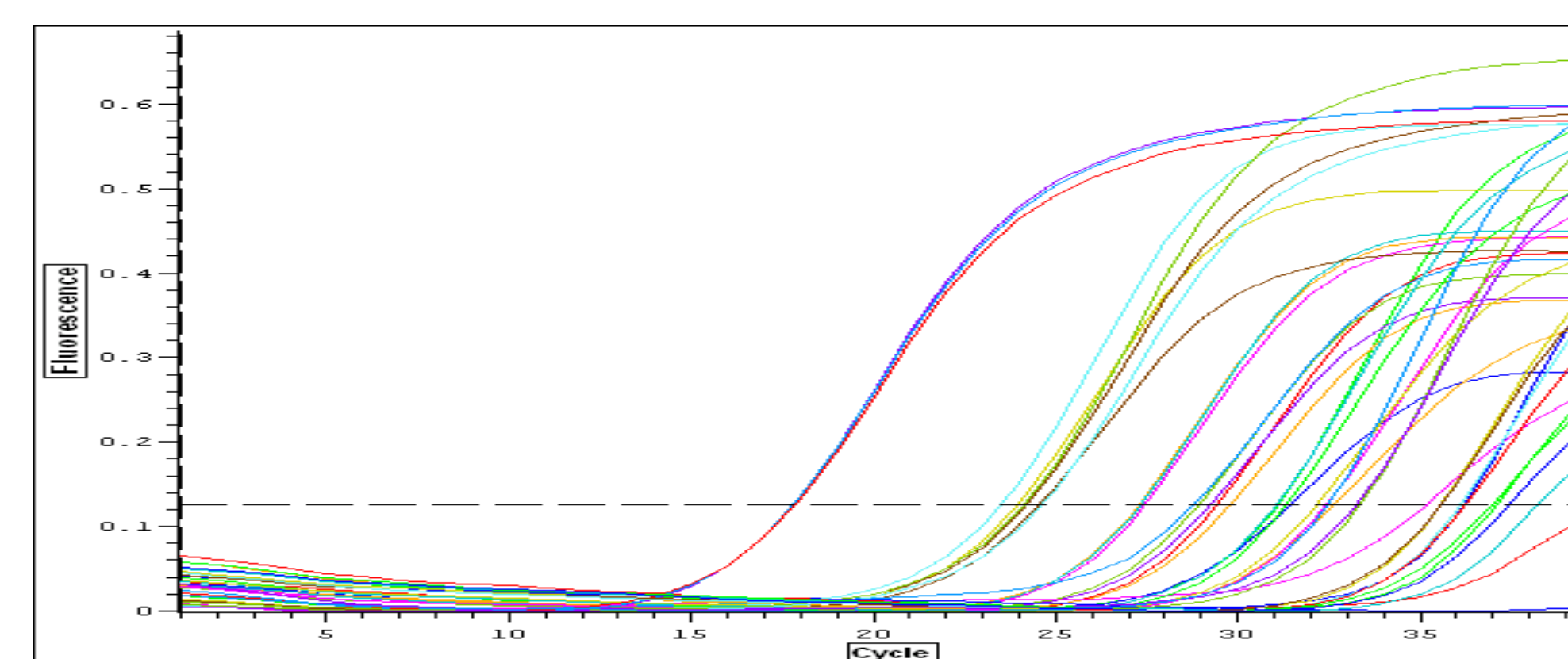


Figure 2: qPCR Ct curves. A positive qPCR reaction is detected by accumulation of a fluorescent signal. Ct (cycle threshold) is the number of cycles (x-axis) required for the signal to exceed the background level. Ct levels are inversely proportional to the amount of target nucleic acid in the sample. Thus, a low Ct for samples transfected with R199G or pSVLD3 and prepared for qPCR with Gage6 primers would indicate the presence of Gage6 in those samples and thus, an increase in Gage6 expression in those cells. However, no such significant increase was observed.

PCR, Transcription & mRNA Extraction

Figure 3: Agarose gel electrophoresis of R199G PCR product. The dark bands at the bottom correspond to R199G DNA. The preceding bands are most likely a result of non-specific amplification during PCR.

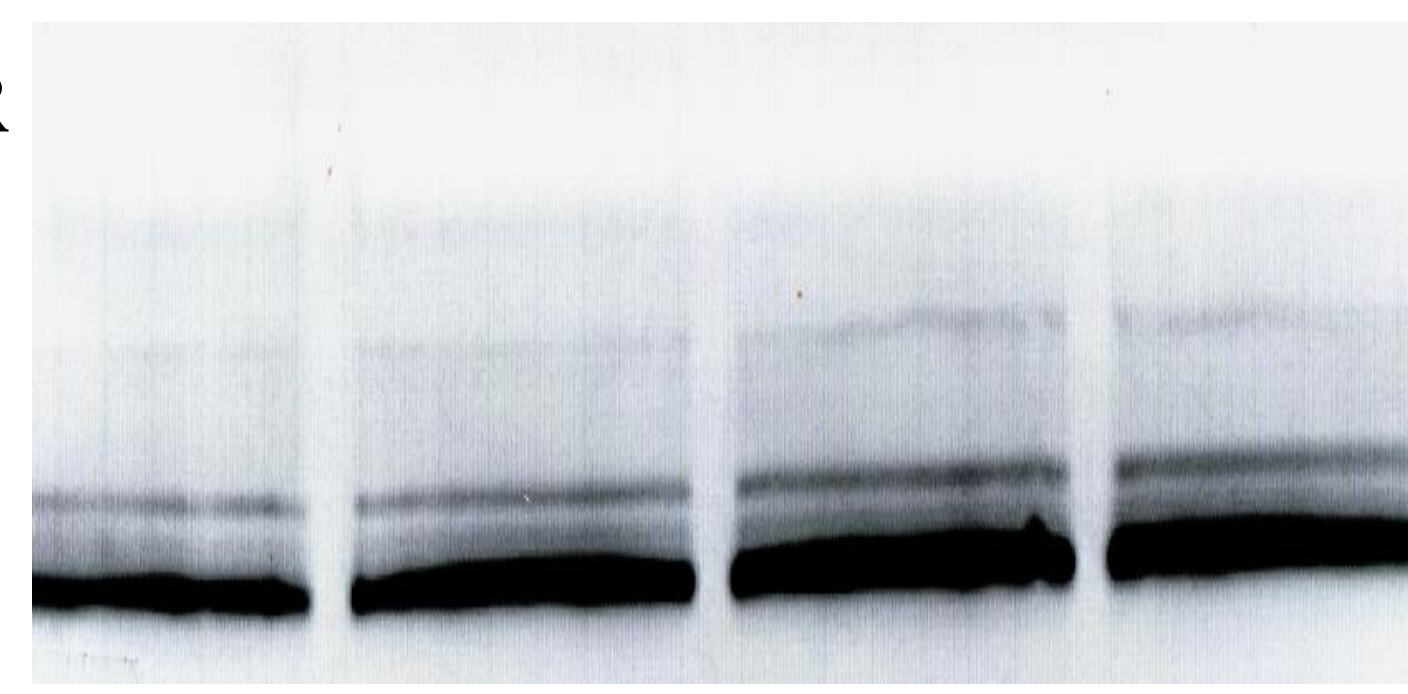


Figure 4: Agarose gel electrophoresis of purified and non-purified R199G RNA. The band for purified RNA has a higher intensity because the purified RNA is more concentrated.

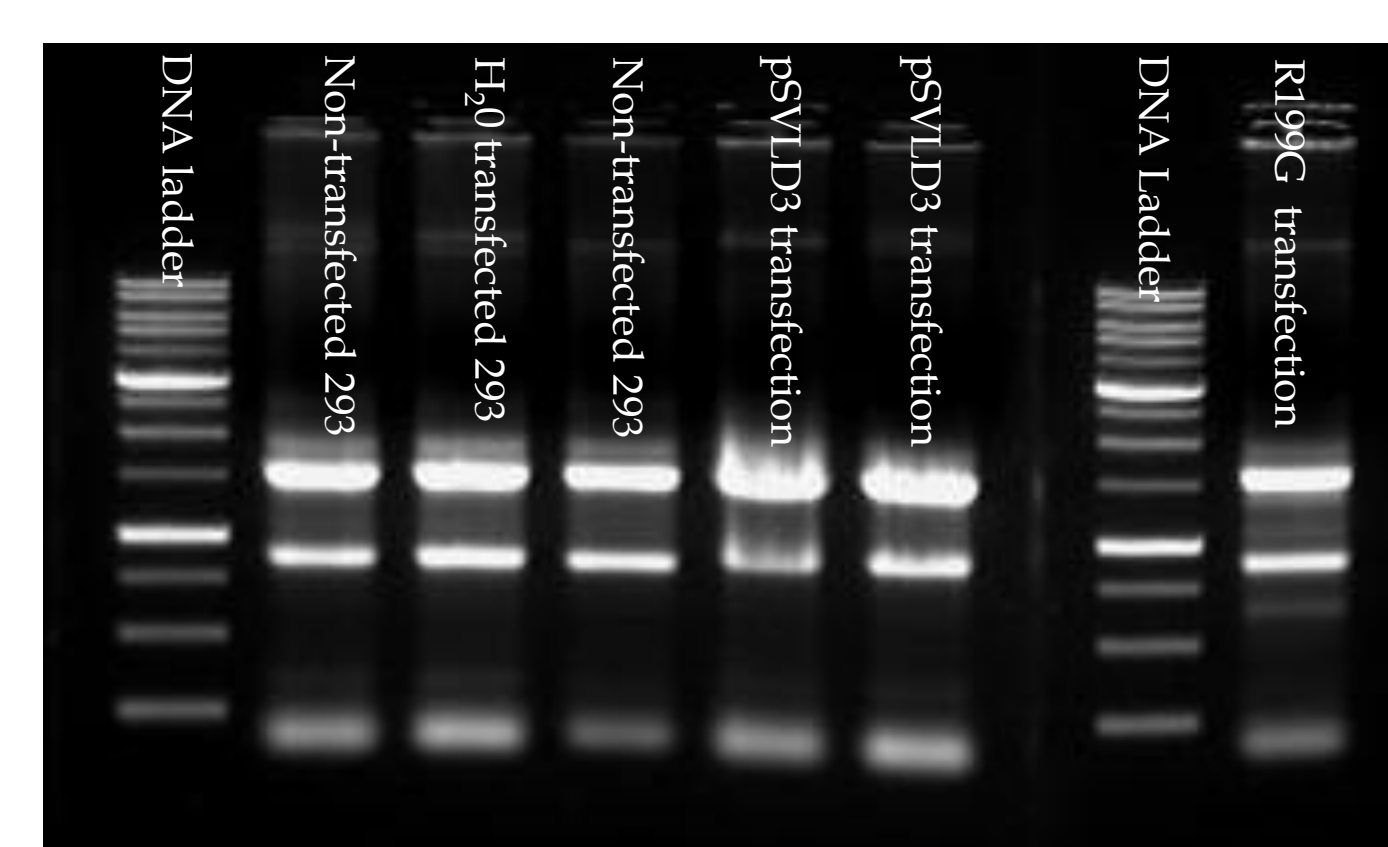


Figure 5: Agarose gel electrophoresis of the mRNA extracted from transfected cells. The bands correspond to the 28s, the 18s and the 5s ribosomal RNA respectively. rRNA is used to assess the quality of cellular RNA as the mRNA content of cells are usually very low.

Discussion

As mentioned previously, qPCR was performed on mRNA extracted from the R199G-transfected, pSVLD3-transfected and non-transfected cells using primers for GAGE6, R199G and actin. R199G is a control which indicates the presence of viral RNA in cell cultures transfected with R199G or pSVLD3. To compare qPCR results, it is necessary to have the same amount of cDNA present in the experimental treatment and the controls initially. Thus, the housekeeping actin gene is used as a normalizer as its expression level should be the same for all samples. For example, a higher amount of GAGE6 and actin in the qPCR of a R199G-transfected sample than non-transfected sample may indicate that more mRNA was extracted from the latter, as opposed to increased expression. However, qPCR results and gel electrophoresis of the qPCR samples did not indicate any notable increase of GAGE6 mRNA in R199G-transfected cells upon comparison with the controls.

Conclusion

Throughout this research project, important molecular biology techniques such as PCR, gel extraction, in-vitro transcription, transfection, RNA extraction, reverse transcription and qPCR were successfully performed. Although the final results obtained did not indicate any significant increase in the expression of GAGE6 mRNA in HEK 293 cells transfected with R199G RNA and HDV genomes, the preliminary results obtained previously for a similar experiment did provide such an indication. Therefore, no specific conclusion can be made on whether or not HDV RNA induces expression of GAGE6 - the experiment should be repeated with optimized conditions for induction of the GAGE6 oncogene. Possible aspects of the project that can be experimented with include: optimization of the amount of RNA to be used for transfection; optimization of the melting temperature of DNA for qPCR; and development of more specific primers to be used for GAGE6 amplification in qPCR.

References

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- Sikora D, Greco-Stewart VS, Miron P, Pelchat M. [The hepatitis delta virus RNA genome interacts with eEF1A1, p54\(nrb\), hnRNP-L, GAPDH and ASF/SF2](#) *Virology*. 390:71-78 (2009).

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