Examination of Effects of Simulated Cosmic Radiation on Epstein Barr Virus Reactivation to Model Reactivation During Space Travel

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Abstract

Astronauts are exposed to many stressors during space travel which may cause severe health issues. It is has been shown that during space travel, astronauts exhibit an increased reactivation of latent viruses, including Epstein-Barr Virus (EBV). EBV is a double stranded DNA gammaherpes virus that infects lymphocytes and epithelial cells, mainly in the oral cavity, before establishing latency in B cells. 90% of human adults are infected with EBV, often without noticeable symptoms. This thesis focuses on the hypothesis that different forms of radiation such as iron, carbon, and high energy proton radiation, as well as inducers such as immunosuppression and microgravity, may trigger reactivation. This project was aimed at determining the quantitative effects of different types and amounts of radiation (0.1, 0.5, 1.0, and 2.0 Gy) on EBV reactivation by looking for activation of two EBV lytic RNAs (immediate early- BZLF1, late- BLLF1), and a cellular control RNA (GAPDH) in EBV-latently infected Akata Cells at specific time points over several weeks. Using reverse transcription, quantitative PCR, and statistical analysis, it was observed that there was an increase in overall viral lytic transcripts, suggesting that radiation is a significant factor in the reactivation of the virus. Carbon and iron reactivation had higher viral transcript values of both the intermediate early and late gene markers, although intermediate early transcript values were higher than late transcript values overall. High energy proton radiation caused an earlier noticeable activation at around 4 days, whereas carbon radiation and iron radiations caused greatest activations at around 18 days. These results suggest that cosmic radiation’s effects on the microbiome of astronauts, thus it is an important factor to consider for future long term space travel.
**Common Abbreviations / Acronyms Used in This Thesis**

EBV: Epstein Barr Virus
DNA: Deoxyribonucleic Acid
RNA: Ribonucleic Acid
PCR: Polymerase Chain Reaction
GAPDH: Glyceraldehyde 3-Phosphate Dehydrogenase
BZLF1, also known as Zta, EB1: intermediate-early viral gene of Epstein Barr Virus
BLLF1: late viral gene of Epstein Barr Virus, envelope glycoprotein gp350
NASA: National Aeronautics and Space Administration
RT: Reverse Transcription
Introduction

Epstein-Barr Virus (EBV), also known as human herpesvirus 4, is one of the most common human viruses, and can be found in humans worldwide. EBV is a double-stranded, linear DNA virus, which spreads via body fluids like saliva, and is known to cause illnesses such as infectious mononucleosis (Odumade 2011). Most initial infections of EBV occur in the oral cavity among the lymphocytes and epithelial cells. From there, EBV binds to B cells and can trigger fusion with a membrane, allowing endocytosis of the virus into vesicles and fusion with the vesicle membrane, which releases the nucleocapsid into the cell’s cytoplasm. The virus’s genome is then easily transported to the nucleus and replicated within the cell. Once this initial infection has occurred, EBV can establish a lifelong infection, interchanging between lytic and latent phases. The latent phase occurs when there is a persistent viral infection without new active viral production. The number of infected B cells decreases over time after the primary infection, but are never entirely eliminated. It is thought that one in a million B cells continues to carry the EBV genome after the host recovers from the acute infection (Bornkamm 2001). During latency, limited protein gene products are expressed. Under certain conditions such as stress, latent cells can be stimulated to reactivate, producing new viral particles that can infect new B cells, thus becoming lytic. This life cycle can be seen in Figure 1.

![Diagram of EBV replication cycle](image)

Figure 1: Diagram of EBV replication cycle, showing initial infection, latency, and reactivation. (Mehta, 2017)

With respect to space travel, latent virus reactivation may be a note-worthy threat to crew health during longer missions, especially if those missions are far from Earth’s protective atmosphere. Increased reactivation of latent herpes viruses such as EBV was previously shown in astronauts during short-term space shuttle flights (Mehta 2014). EBV DNA copies were elevated during flight as compared with pre- and post-flight levels. While there are a number of factors that may have influenced this, including stress from the journey, immunosuppression, and microgravity, increased exposure to cosmic radiation while in space likely had a crucial role in this reactivation.

The goal of this thesis project was to determine the effects of three components of cosmic radiation in varying amounts by examining how they contribute to triggering reactivation as measured by activation of EBV genes involved in reactivation and productive viral replication. There are three temporal classes of viral lytic gene products—intermediate early, early, and late. This experiment makes use of BZLF1, an intermediate early gene product which acts as a transactivator of viral lytic replication, and BLLF1, which
is a late gene that codes for a structural protein. Additionally, GAPDH was monitored, as it is a common cellular housekeeping gene. By measuring the ratio of a viral RNA/GADPH RNA it is possible to normalize data to determine if changes in viral gene transcription are significant (Geiser 2011).

The cells used were EBV+ Akata cells which are an immortalized B cell line derived from a Japanese patient with Burkitt’s lymphoma, a tumor induced by EBV. These cells are known to stably maintain latent EBV genomes which makes them useful to study reactivation. While astronauts do reactivate other viruses such as varicella-zoster virus and cytomegalovirus, Akata cells are a good model because the reactivation of the EBV viral genomes can readily be measured (Mehta 2017).

Methods

Samples:

Samples were obtained directly from NASA (Mehta lab, Johnson Space Center) for workup and analysis. Table 1 shows the NASA samples used, grouped by radiation type and amount. There were also a number of shipping controls included to ensure the reliability of the samples.

NASA replicated space by exposing the different groups of cells to different types of radiation before sending them for workup and analysis. For example, the high energy proton radiation group was exposed to ionizing radiation at Brookhaven National Laboratory at the different amounts (0.1, 0.5, 1.0, or 2.0 Gy) as seen in Figure 1. Similar procedures were done for the carbon and iron radiation samples. The radiation for these samples is reported in units of grays (Gy), which represent the absorption of one joule of radiation energy per kilogram of matter.

<table>
<thead>
<tr>
<th>Table 1: Samples provided from NASA for work up and analysis.</th>
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<tr>
<td><strong>High Energy Proton radiation</strong></td>
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<td><strong>0.1 Gy (# of biological replicates)</strong></td>
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<td><strong>Carbon radiation</strong></td>
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Extraction of RNA and DNA Samples using Trizol

The experiments followed a protocol written by Adit Dhummakput in 2015 for the isolation of the EBV-infected Akata cells.

**Sample preparation**: For each sample, after centrifuging at 4 °C to separate the cells from RNAlater (Qiagen) and removing the RNAlater, 1mL of Trizol reagent and 0.200 mL of chloroform was added, and the 1.5 mL microcentrifuge tube was mixed vigorously. The tube was incubated for 3 minutes at room temperature, then centrifuged for 15 minutes at 12,000 x g at 4 °C. The aqueous (upper) phase was removed and transferred to a new microcentrifuge tube. The organic (lower) phase was saved, and labeled the microcentrifuge tube “DNA”. In these steps, Trizol serves as a mixture of phenol and guanidine isothiocyanate, which is used to separate proteins from the RNA and DNA by denaturing them. The guanidine salts also reduce the nuclease activity. The pH dependence on the phenol in this process determines the portioning of DNA and RNA between the organic and aqueous phases. In this experimental setup, the upper aqueous phase is almost entirely RNA, as the pH is 4.8 (Zumbo 2012). Most proteins and small DNA fragments fall to the organic phase, and some of the larger proteins may remain at the interphase between the two phases.

**RNA Isolation**: For each aqueous layer sample, using the new microcentrifuge tube, 0.5 µL of glycoblue (ThermoFisher Scientific) and 0.500 mL of 100% isopropanol was added. The microcentrifuge tube was mixed vigorously and incubated for 10 minutes at room temperature. The microcentrifuge tube was then centrifuged at 12,000 x g for 10 minutes at 4 °C. The supernatant was removed, leaving a blue RNA pellet in the bottom of the microcentrifuge tube. The pellet was washed three times with 1mL of 75% ethanol, vortexed and centrifuged at 7,500 x g for 5 minutes at 4 °C between each wash. The ethanol was removed and the pellet was air-dried for 10 minutes to allow the remaining ethanol to evaporate. Next, the pellet was resuspended in 45.0 µL of RNase-free water. Next, the microcentrifuge tube was incubated at 60°C for 15 minutes. Next, 5.0 µL 10x DNase buffer (Ambion by Life Technologies), 1.0 µL Turbo DNase (Ambion by Life Technologies), and 0.5 µL RNasin (20 Units/µL, ThermoFisher Scientific) from
the Turbo DNA-Free kit (Ambion by Life Technologies) was added and the tube was incubated for 30 minutes at 37°C. Then, 5.0 μL DNase inactivation solution was added, and mixed occasionally for 2 minutes at room temperature. Finally, the sample was centrifuged at 10,000g for 90 seconds at room temperature and the supernatant was transferred to a new tube, labeled “RNA”.

*Reverse Transcription Reaction*: Each microcentrifuge tube now contained the RNA needed for reverse transcription. Using ThermoFisher Scientific Nanodrop 2000, the concentration of RNA in each sample was determined. Then calculations to determine the amount of sample to use for each RT reaction such that the total concentration of RNA is 1 μg for each 20.0 μL reverse transcription reaction were completed. In a new tube, labeled “cDNA”, the correct amount of RNA was added to 2.0 μL 10x RT buffer (500 mM Tris-HCl, pH 8.3, 750 mM KCl, 30mM MgCl₂, 50.mM DTT), 2.0 μL 5mM dNTPs, 2.0 μL Random Decamers, 0.5 μL RNasin (20 Units/μL), and 1.0 μL omniscript RT (50-70% glycerol). I then added nuclease free water such that the total volume of each sample is 20.0 μL. The samples were incubated for 1 hour at 37°C, then stored at -20°C. Each tube now contained cDNA needed for qPCR. In these steps, RNAsin is a protein-based ribonuclease inhibitor that binds noncovalently to inactivate many RNases over the temperature range, which keeps contamination low. The buffer provides the correct pH environment for the random decamers to prime the strands of RNA such that deoxynucleotide triphosphates (dNTPs) to be added to the growing strand to create the cDNA.

*Controls*: For the set of control samples, a set of “RT minus reactions,” were made, which means that they have undergone sample preparation and RNA isolation, but no reverse transcription reaction. The appropriate volume of RNA from the end of the RNA isolation process so that the corresponding volume for 0.5 μg RNA plus nuclease free water had a final volume of 10.0 μL per sample was added to each sample. These tubes contain just RNA, and are used to ensure that there is no contamination of the original samples that may be amplified in the PCR step.

**qRT-PCR**

*Background information*: The primers (designed by Adit Dhummakput) are TaqMan Gene Expression Assays, ordered from Applied Biosystems by Thermo Fisher Scientific, and are specific for BZLF1, BLLF1, or GAPDH. Aliquots of the primers were created upon arrival such that there would be limited amount of freeze-thaw disruption, and one aliquot is used for each PCR. Standards were created by Dhummakupt in 2015 using a stable EBV value in a series of dilutions from 10⁻¹ to 10⁻⁶. The dilutions are ten-fold, and were made using TE buffer. The standards are stored at -20°C when not in use. These are used to help quantify the value of the samples based on a known, predictable standard, diluted to cover the possible range of amplification Cₗ values of the actual samples.

**TaqMan PCR Protocol**: For each PCR plate, the TaqMan PCR protocol on the 40 minute “fast” setting was used on a StepOne Plus Real-Time PCR machine located in the lab of Dr. David Bloom. Each PCR plate layout was set up on the computer, using triplicate wells for each standard and sample and 1 well of water instead of sample as a negative control. For example, one plate contained all of the 0.1 Gy iron radiation days 0-22 for the primer GAPDH. To prepare the plate, to each well, 5 μL Taqman PCR Fast Universal Master Mix, 3 μL Nuclease-free water, .5 μL primer (18 μM), and 1.5 μL of the cDNA sample or control was added. The protocol was run on the PCR machine for the fast cycle of around 40 minutes, or 40 cycles of the preprogramed, basic PCR cycling protocol of denaturing and annealing. The program records Cₗ values and quantity means for each sample during this time. After the cycles were completed, any extraneous amplifications (based on human pipette error) were removed, and the threshold value was set to the ideal value to eliminate any noise from the stray amplifications, and then saved the data for analysis. Next, a PCR plate using the same standards and the corresponding “RT minus” samples with the
same cocktail of PCR ingredients was set up and run. Any amplifications of these samples (as there should be none) were noted.

**Total:** These two PCR plate processes for each amount/type of radiation and each primer probe were completed. In total, there were 3 (radiation type) x 4 (radiation amount) * 3 (primer probes) * 2 (RT and RT minus) = 72 plates completed.

**Analysis**

The data was analyzed using Excel and GraphPad Prism. The data was grouped by type of radiation, either high energy proton, carbon, or iron. For each set (radiation type and amount), the quantity means for each sample of BLLF1 and BZLF1 was divided by its corresponding quantity mean value for GAPDH. For example, the 0.1 Gy iron day 0 BLLF1 sample quantity mean was divided by the 0.1 Gy iron day 0 GAPDH sample quantity mean to yield value normalized to the cellular control. Once that normalization was complete, each sample was divided by its corresponding day 0 value to yield a fold change from the day 0 time point. For example, the 0.5 Gy carbon day 8 BZLF1/GAPDH value was divided by the 0.5 Gy carbon day 0 BZLF1/GAPDH value to yield a fold change in normalized mean quantity value between day 0 and day 8 for this sample. Statistical analysis were performed using the RM Two-Way ANOVA Matched Values and Tukey’s Multiple Comparisons Test on GraphPad Prism to determine significance of these values as compared to other levels of each type of radiation. Other groupings, such as by amount of radiation, were also done, following the same steps as above to graph and determine significance.

**Results**

**Scatterplot data of normalized PCR samples affirm data quality**

Viewing the results as scatterplots shows each normalized sample’s quantity mean for the intermediate early gene. As seen in **Figure 3**, the sample for each type of radiation, dose of radiation, and time point are for the most part fairly close together, allowing averages to be used for future analyses. Comparison of the y axis between [(a) and (b)] and [(c) and (d)] suggest a much larger amount of BZLF1 compared to BLLF1 expression in the H+ radiation samples at all levels of radiation as well as the carbon radiation samples at all levels of radiation. This suggests that the early intermediate gene product is expressed at a much higher rate throughout reactivation.
Figure 3: Scatter plots for high energy proton, carbon, and iron radiation samples’ normalized mean quantities. These graphs show the mean normalized value of the three technical PCR replicates for each sample per type of radiation, dose of radiation, and time point. The x axis shows the time point, measured in days, which represents the days post infection at which each sample was collected after receiving their respective radiation treatments. The y axis shows the normalized value for each of the points, meaning the sample’s BZLF1 or BLLF1 quantity value divided by the corresponding GAPDH quantity value. This normalizes the data for the amount of viral particles and cells in the sample well. There were three samples for each time point and radiation dose for high energy proton radiation, whereas the carbon and iron radiation only have two biological replicates per time point and radiation dose, as seen on the graph.
Statistical analyses of PCR means show temporal differences in transcript inductions at different radiation doses within radiation types

After doing the statistical analysis described in the methods above, it is clear that certain days have statistically significantly different levels of gene product expression. As seen in Figure 4 (a), the value for H+ at 0.1 Gy is significantly different than 0.5 Gy, 1.0 Gy, and 2.0 Gy at day 4. Additionally, at day 8, the value for H+ at 0.1 Gy is significantly different than 1.0 Gy and 2.0 Gy. This suggests that the lower level of radiation is responsible for reactivation at a larger relative amount for BZLF1. In (b), the value for H+ at 1.0 Gy is significantly different than 0.1 Gy and 0.5 Gy at day 4 for normalized BLLF1, although the actual transcript value is much less than the values in (a). This suggests that late genes may need larger dose of radiation to reach significantly more activation. In (c), there seems to be a larger amount of transcript values at around 18 days, yet nothing is statistically significant. In (d), the carbon radiation caused transcript values at 0.1 Gy to be statistically significant at 10 and 22 days, and it seems that all radiation doses had greater transcript values at day 10. In (e), there is a noticeable peak in transcript values around day 18 for the iron radiation normalized BZLF1 values, yet none are statistically significant at p=.05 level. Iron radiation’s trend with BLLF1 as seen in (f) suggests a messier trend in the iron radiation BLLF1 transcript values. While neither (e) or (f) had significant increases of one dose of radiation over another at any particular day, it does continue the same trend of increased activation of gene markers with radiation.
Figure 4: Transcript values over time for H+, carbon, and iron radiation samples at various levels of radiation. These graphs show the normalized (to GAPDH) means of the quantity means of the qPCR samples at each level of radiation over the time course. The values of significance are shown on the graphs. The x axis shows the time point, measured in days, which represents the days post infection at which each sample was collected after receiving their respective radiation treatments. The y axis shows the mean of the normalized value for each of the points, ie the sample’s BZLF1 or BLLF1 quantity value divided by the corresponding GAPDH quantity value, averaged per sample group. The error bars shown represent the standard error of the mean for each sample. Statistical analysis were performed using the RM Two-Way ANOVA Matched Values and Tukey’s Multiple Comparisons Test on GraphPad Prism to determine significance of these values as compared to other levels of each type of radiation. The p values for the significant points are noted on the graphs, as well which comparisons were done to determine significance.
Combined data sets show higher levels of gene expression in carbon and iron radiation samples

By combining the BZLF1 and BLLF1 normalized transcript values of expression into graphs grouped by radiation type, it is easier to compare the relative abundances of the intermediate early and late genes between the three types of radiation. As seen in Figure 5 (a), the high energy proton has the greatest gene expression of BZLF1 at the 0.1 Gy value at day 4, whereas in (b), carbon radiation has the greatest BZLF1 at 0.5 Gy later in the timecourse, at around 18 days. Similarly in (c), iron radiation has increased BZLF1 expression at day 18 at 0.1, 0.5, and 1.0 Gy radiation. This suggests that the type of radiation impacts the peak expression in the time course, with H+ peaking earlier around day 4 and carbon and iron peaking later, around day 18. Overall, all of the carbon and iron radiation had higher relative levels of gene expression for both the intermediate early and late genes.

![Combined BZLF1 and BLLF1 normalized transcript values over time at various amounts of the three types of radiation. These graphs show the combined BZLF1 and BLLF1 normalized (to GAPDH) values for each type of radiation over the time course. The x axis shows the time point, measured in days, which represents the days post infection at which each sample was collected after receiving their respective radiation treatments. The y axis shows the mean of the normalized value for each of the points, i.e. the sample’s BZLF1 or BLLF1 quantity value divided by the corresponding GAPDH quantity value, averaged per sample group. The error bars shown represent the standard error of the mean for each sample.](image-url)
Fold change comparisons show greater comparative changes in later time points

In order to examine the change of each sample of the time course compared to the original values, the fold change graphs where created (Figure 6) and grouped by radiation type. As seen by the trends in (a), H+ radiation has the largest fold change in normalized BZLF1 transcript values in 0.1 Gy at day 4, whereas normalized BLLF1 transcript values have the greatest fold change at day 12 in 0.5 Gy, which is consistent with earlier ideas that late genes are take more time for similar reactivations. By examining carbon radiation in (b), it is clear that most of the transcripts peak from day 10 to 14, with 1.0 Gy for BLLF1 being the exception. The results for iron radiation in (c) are less useful in this view, as 0.1 Gy and 2.0 Gy for BLLF1 seem to have a large fold change earlier in the time course, whereas the rest of the values seem to peak around day 18, as suggested with other data comparisons. Overall, these graphs show that there is in fact a great change from day 0 to the other days in the time course due to the effects of the radiation at various doses.

Figure 6: Fold change comparisons for H+, carbon, and iron radiation. This figure shows the fold change of each sample group mean compared to the day 0 sample group mean for the three types of radiation at various levels of radiation (as listed in the legend, all in units of Grays) over time. The x axis shows the time point, measured in days, which represents the days post infection at which each sample was collected after receiving their respective radiation treatments. The y axis shows the fold change of the mean transcript value for each group of samples per radiation dose and time point divided by the corresponding day 0 mean transcript value. This allows for a comparison to the original value to be more easily visualized.
Examination by radiation dose shows larger reactivations at lower dosages

Grouping by radiation dose allows an easier comparison of the expression levels of the genes by amount of radiation. As seen in Figure 7, the iron radiation has the most robust reactivation at the 0.1 Gy, 0.5 Gy, and 1.0 Gy radiation amounts, peaking at day 18 in the time course. In (d), at 2.0 Gy, there is less overall transcript values for the iron, and the iron and carbon radiation values are closer together. This suggests that at higher doses of carbon and iron radiation, the virus responds more similarly, whereas H+ has much lower transcript values.

![Graphs showing transcript values by radiation amount](image)

**Figure 7: Comparison of normalized transcript values by radiation amount.** This figure shows the different types of radiation grouped by amount of radiation. The x axis shows the time point, measured in days, which represents the days post infection at which each sample was collected after receiving their respective radiation treatments. The y axis shows the mean of the normalized value for each of the points, ie the sample’s BZLF1 or BLLF1 quantity value divided by the corresponding GAPDH quantity value, averaged per sample group. Note that (a) and (b) have the same y axis values, but (c) and (d) have smaller scales. The error bars shown represent the standard error of the mean for each sample.

**Discussion**

Radiation that astronauts would encounter in space is mostly composed of high energy proton radiation (85%), with about 1% from prominent ions such as carbon and iron (Schimmerling 2011). Although the carbon and iron are a small proportion, their higher charge and energy are just as important in terms of potential biological impact as high energy protons (Schimmerling 2011). It has been shown that proton radiation on its own can cause DNA damage (Hada 2006). Such damage can kill cells and lead to short term effects such as nausea, or long term effects such as nervous system damage.

Chemically, the radiation experienced in space may contribute to somatic or genetic injury within astronaut DNA. Somatic injuries would include sickness, as suggested above, while genetic injury includes damage to the reproductive cells as a result of the exposure to the different forms of radiation.
The DNA exposed during space travel may experience direct action, when alpha or beta particles or x-rays create ions which break the sugar phosphate backbones or base pairs of DNA, or indirect action via the creation of free radicals that are highly reactive due to unpaired electrons (Khanna, 2001). These indirect effects can initiate harmful chemical reactions within the cell and lead to altered cell function or even death. Luckily, DNA repair mechanisms such as excision may prevent these consequences of radiation, at least to a certain extent.

Thus, as the potential for long-term space travels continues to be discussed, these impacts of DNA damage, viral reactivation, and other possible unknown consequences must be considered. Most people, even if they do not have EBV, the virus studied in this experiment, probably have one of the other prevalent latent viruses such as Herpes Simplex Virus, which could each pose different potential risks in a high radiation environment. In fact, Mehta’s 2017 *Nature* article noted increased frequency of reactivation of Epstein-Barr virus, varicella-zoster virus, and cytomegalovirus in a 60-180 day mission of astronauts at the International Space Station.

Experiments by Mehta have demonstrated that gamma radiation is another common type of radiation encountered by astronauts in space. Cell samples exposed to gamma radiation tend to have similar cell viabilities as samples exposed to high energy proton radiation, but differ in latent virus reactivation. In experiments by Mehta, he showed that gamma radiation peaks early around day 4, similar high energy proton radiation, but with about double the viral load amounts (Mehta, unpublished).

Since the shortest estimates of travel time to Mars are 150-300 days, future experiments that would be helpful in fully understanding this subject of radiation’s role on reactivation in long-term space travel would be to collect samples from astronauts who have been on the International Space Station or on exploratory missions for at least a year. Other key ground experiments to perform would be to expose the samples to multiple types of radiation, such as both carbon and iron, iron and hydrogen, carbon and hydrogen, and all three, to determine how the more likely situation of multiple radiation types affects the cells. One can predict that multiple types of radiation would cause greater reactivation overall for both intermediate early and late transcripts. This, and similar studies, would help scientists determine the viability of long-term space missions in the future.

**Conclusion**

There are important differences in the transcript values for the immediate early and late gene based on both the type and amount of radiation applied to the samples. The immediate early gene tended to experience higher levels of reactivation (as seen via normalized transcript values) earlier in the time course as compared to the late gene. Additionally, the late gene seemed to require larger amounts of radiation for its transcript values to substantially increase, compared to the immediate early gene which was seen in large quantities at just 0.1 Gy of radiation. There were also stark differences by type of radiation. High energy proton radiation caused overall lower levels of normalized transcript values as compared to carbon and iron radiation, which had similar levels of transcript values for the respective gene markers.
Acknowledgements

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UF College of Medicine Dept. of Molecular Genetics and Microbiology – David Bloom, PhD
References


Appendix A: Information about the solutions used during this project, in order of appearance

All descriptions taken from the manufacturer’s website.

**Trizol reagent**: Catalog Number: 15596018 from Thermo Fisher Scientific

“TRIzol® Reagent is a complete, ready-to-use reagent for the isolation of high-quality total RNA or the simultaneous isolation of RNA, DNA, and protein from a variety of biological samples. This monophasic solution of phenol and guanidine isothiocyanate is designed to isolate separate fractions of RNA, DNA, and proteins from cell and tissue samples of human, animal, plant, yeast, or bacterial origin, within one hour.”

**Glycoblue**: Catalog number AM9515 from ThermoFisher Scientific.

“GlycoBlue™ Coprecipitant consists of a blue dye covalently linked to glycogen, a branched chain carbohydrate, which is useful as a nucleic acid coprecipitant. The attached dye increases visibility of the pellet.”

**TURBO DNA-free kit**: Turbo DNase, DNase reaction buffer, DNA inactivation Reagent, Catalog number AM1907 from Ambion by Life Technologies.

[https://tools.thermofisher.com/content/sfs/manuals/cms_055740.pdf](https://tools.thermofisher.com/content/sfs/manuals/cms_055740.pdf)

**SUPERase-In RNase Inhibitor**: catalog number AM 2694 from ThermoFisher Scientific

“Protein-based ribonuclease inhibitor which noncovalently binds and inactivates a wide variety of RNases in a wide range of temperature and pH conditions.”

**Omniscript rt kit**: Omniscript reverse transcriptase, 5mM dNTPs, RT Reaction buffer. Catalog number: 205111 from Qiagen

“Omniscript RT is specially designed for all reverse transcription with any amount of RNA from 50 ng to 2 µg per reaction. Omniscript RT is also usually the enzyme of choice with viral RNA due to the presence of carrier RNA in most viral RNA preparations. In comparative experiments, Omniscript RT consistently outperforms other reverse transcriptases over a wide range of starting RNA amounts. Lot-to-lot reproducibility of Omniscript Kits is ensured by rigorous quality control at QIAGEN. The optimized Buffer RT, dNTPs, and water included in all Omniscript RT Kits are guaranteed RNase-free, and each lot of Omniscript RT is thoroughly tested for RT-PCR reproducibility.”

**Random decamers**: Catalog number AM 5722G from ThermoFisher Scientific

“Random Decamers are the same primers currently included in the RETROscript® Kit (Cat. No. AM1710). They are provided at a stock concentration of 50 µM, and are functionally tested using the RETROscript® Kit.”

**TaqMan Fast Universal PCR Master Mix (2x)**: Catalog number 4352042 from ThermoFisher Scientific.

“TaqMan® Fast Universal Master Mix (2x), No AmpErase® UNG delivers results in 40 minutes for 40 cycles of PCR in a 20 µL reaction volume using the Applied Biosystems® 7900HT and Fast 7500 Fast Real-Time PCR Systems. The optimized formulation contains AmpliTaq® Fast DNA Polymerase, UP, a highly purified DNA polymerase designed to allow instant hot start, minimizing non-specific product formation and allowing room temperature reaction setup. Additionally, a
proprietary ROX™ dye serves as a passive internal reference to normalize non-PCR–related fluorescence fluctuations, for superb precision on Applied Biosystems® real-time PCR instruments.”

*TE buffer:* 100mM Tris Cl + 10mM EDTA