The Efficacy of Tetramethylammonium Chloride and BioStab PCR Optimizer as Proposed Treatments for the Elimination of PCR Mediated Recombination in Unintegrated HIV-1 pDNA

Abstract

When DNA needs to be analyzed, polymerase chain reactions must occur in order to expand the DNA to a point that it becomes testable. PCR, however, has its drawbacks. PCR-mediated recombination occurs when the DNA anneals improperly during the PCR cycles. Tetramethylammonium chloride (TMAC) is an industrial chemical used in both hydrofracking and to increase specificity of PCR products. Biostab PCR Optimizer is a company-made chemical that claims to increase specificity of PCR products. This study aims to decrease the effects of PCR mediated recombination with varying levels of these two chemicals separately.

Introduction

When HIV-1 infects the body, it attempts to integrate into each cell to begin the replication process. When viral load assays are performed on HIV-1 patients to assess their level of HIV-1 infection, it takes into consideration the HIV-1 that has successfully managed to integrate into cells. However, there is a great deal of HIV-1 DNA not being considered when these viral loads are performed. This is the unintegrated DNA, the genetic material that failed to integrate into a cell and lives its short lifespan without a host. While studies have shown that this unintegrated DNA can cause health issues, it cannot replicate on its own. Furthermore, it has been found that the measure of unintegrated DNA, and not the measure of integrated DNA, is a more accurate

look at the level of infection a patient experiences. Therefore, the viral load assay used today is not as accurate as one that may take into account the unintegrated HIV-1 DNA in a patient (Zicari 2019). The DNA in question must be studied through PCR and electrophoresis analysis to one day create a more accurate viral load assay of unintegrated DNA. Unfortunately, a phenomenon known as PCR mediated recombination occurs when the unintegrated DNA is extended in PCR; specifically, 2-LTR unintegrated HIV-1 DNA. This changes the results and invalidates them (Shi, 2022). In this study, to attempt to remedy the PCR mediated recombination issue, I introduced the chemicals tetramethylammonium chloride (TMAC) and BioStab PCR Optimizer to the PCR mixture. These chemicals could be used to eliminate PCR mediated recombination and these results could one day aid in creating a more effective viral load assay.

Hypothesis:

TMAC and the PCR Optimizer will have differing but positive effects on the PCR products and a clearer result will be shown in the gel electrophoresis.

Materials and Methods:

In order to determine the proper number of cycles to run the PCR for, I tested out how each different cycle number affected the results seen in the gel electrophoresis. I tested this both with the 1-LTR and 2-LTR plasmid DNA separately to determine the proper number of cycles for each of them. In addition, testing early in the experiment had to be done to determine the different number of plasmids in each microliter of PCR liquid. It was decided that the best results (to avoid under or over saturated) were measured when 1 million copy per microliter was used

for the 1-LTR, and 2.5 million copies per microliter was used for the 2-LTR. Table 1 shows the ingredients for one tube's yield of DNA mixture that would be put through PCR for a total of 25 microliters. However, I many times made batches of 4, which I would then split up into 4 small PCR tubes.

10x Buffer	2.5 μL
25 mM $MgCl_2$	2 μL
dNTP	2 μL
Reverse Primer	0.5 μL
Forward Primer	0.5 μL
Taq Polymerase Enzyme	0.25 μL
DNA	5 μL
H_2O	12.25 μL

Table 1

The DNA was not added until every substance was mixed together in the large batch and then smaller amounts of the mixture pipetted to their smaller respective tubes. While making batches of 4, the DNA would be added only after that batch of 4 was split into the four tubes; subsequently, 20 microliters was never added to the big batch – 5 microliters were instead added to each of the four batches. This guarantees that each tube has the exact same amount of DNA. When TMAC or PCR Optimizer was added to the experiment, the H_2O levels would decrease by the amount of microliters of TMAC or PCR Optimizer added in order to maintain the balance of a total of 25 μL of liquid in each PCR tube.

To prepare the area, distilled water was poured over the counter area in the PCR clean room. This room is specifically designed to keep DNA away from other experiments and away from PCR products being run through gel electrophoresis. The area was then wiped down with a paper towel. Most of the products used to create the mixture are stored in the freezer, so I must melt everything but the Taq polymerase enzyme by using a dry bath. When the ice has melted, the pipetting can commence after it is vortexed to ensure the products are mixed well before

commencing. Each product is pipetted as detailed above into one large tube. If a batch larger than a yield 1 has been made, it then gets split into smaller PCR tubes before the DNA is added. The sample is spun in a centrifuge for 30 seconds. The PCR is run for a specific number of cycles, depending on the experiment. While the tubes undergo PCR, the gel is made in which the gel electrophoresis is run. The gel is made using 30 mL of 25x stock solution and mix it with 3 g of agarose, which then gets microwaved for 1 minute. I take it out to mix before microwaving it for 10 more seconds. The gel is cooled slightly before adding 3 microliters of GelGreen, which is used for gel staining. The purpose of GelGreen is to allow the results to show up during the gel electrophoresis stage. GelGreen was pipetted straight into the solution of agarose. After it is cool enough to hold for just a few seconds, it gets poured into the mold. 1-μL dots of loading buffer are pipetted onto the surface of a small square of ParaFilm. It is mixed with 5 μL of the mixture pipetted out of the post-PCR tube, and then 5 μL of the new solution containing the loading buffer is pipetted into a well. This is done for each tube containing mixtures that have already undergone PCR. DNA ladder is added to the first well so that we can compare sizes of DNA to this. Before anything can be loaded, however, buffer must be poured to cover the gel mold. Once each PCR product has been loaded into its prospective well, it is plugged in to run for 45 minutes. At the end of those 45 minutes, the electrophoresis machine is turned off, unplugged, and the gel is carefully removed before being placed on the molecular imager machine, which is where the image of the results are obtained.

- List of materials
    - Bio Rad Molecular Imager ChemiDoc XRS+ with Image Lab Software
    - Applied Biosystems PCR machine

    - Eppendorf Centrifuge
    - Kimtech Kimwipes
    - Scale
    - PCR Optimizer
    - Tetramethylammonium Chloride (TMAC)
    - 6x Loading Buffer
    - Pipette tips
    - Pipettor
    - Buffer solution
    - $MgCl_2$ Solution
    - Applied Biosystems dNTP mix
    - Forward primer
    - Reverse Primer
    - H_2O
    - AmpliTaq DNA Polymerase (enzymes)
    - Buffer Solution
    - SeaKem LE Agarose
    - GelGreen
    - DNA ladder
- Study site: BioSafety Lab Level 2 at the Albany College of Pharmacy and Health Sciences, Albany, NY
- PCR methodology

Results

Fig. 1

To begin, we see the quite evident PCR mediated recombination in the 2-LTR DNA, with the band that lines up with 1-LTR. This supports the prior knowledge of the occurrence of PCR mediated recombination in 2-LTR pDNA.

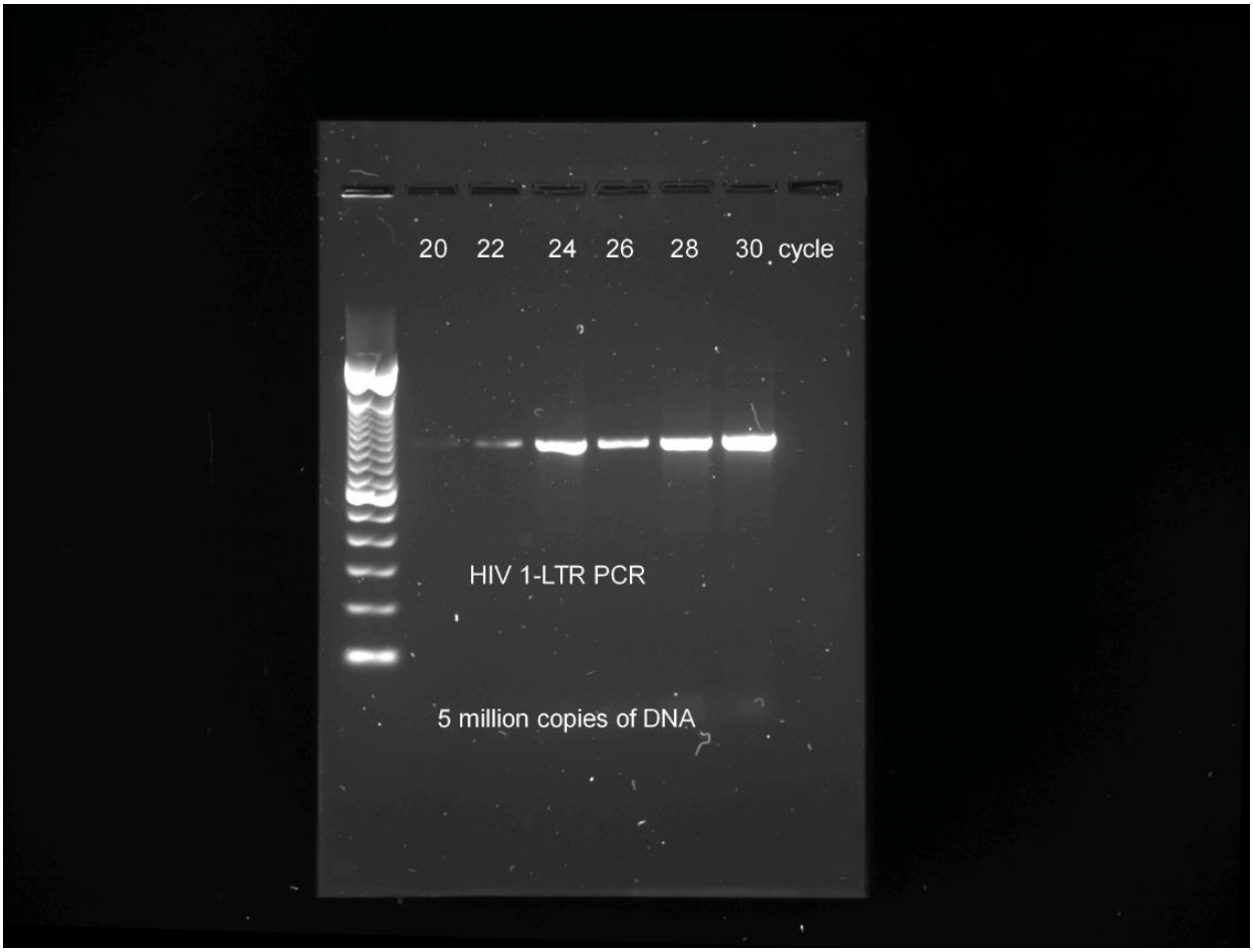

Fig. 2

A total of 5 million copies of DNA was placed in each PCR tube (1 million copies for each microliter) and cycle numbers are tested. Between cycle 24 and cycle 26, there is a huge discrepancy. It was decided that, because 24 appears so overly saturated and cycle 26 was much more stable looking, they may have gotten mixed up while loading into wells. This is an error we took into account and decided that 24 cycles seemed to be a good fit for getting results with the 1-LTR plasmid DNA.

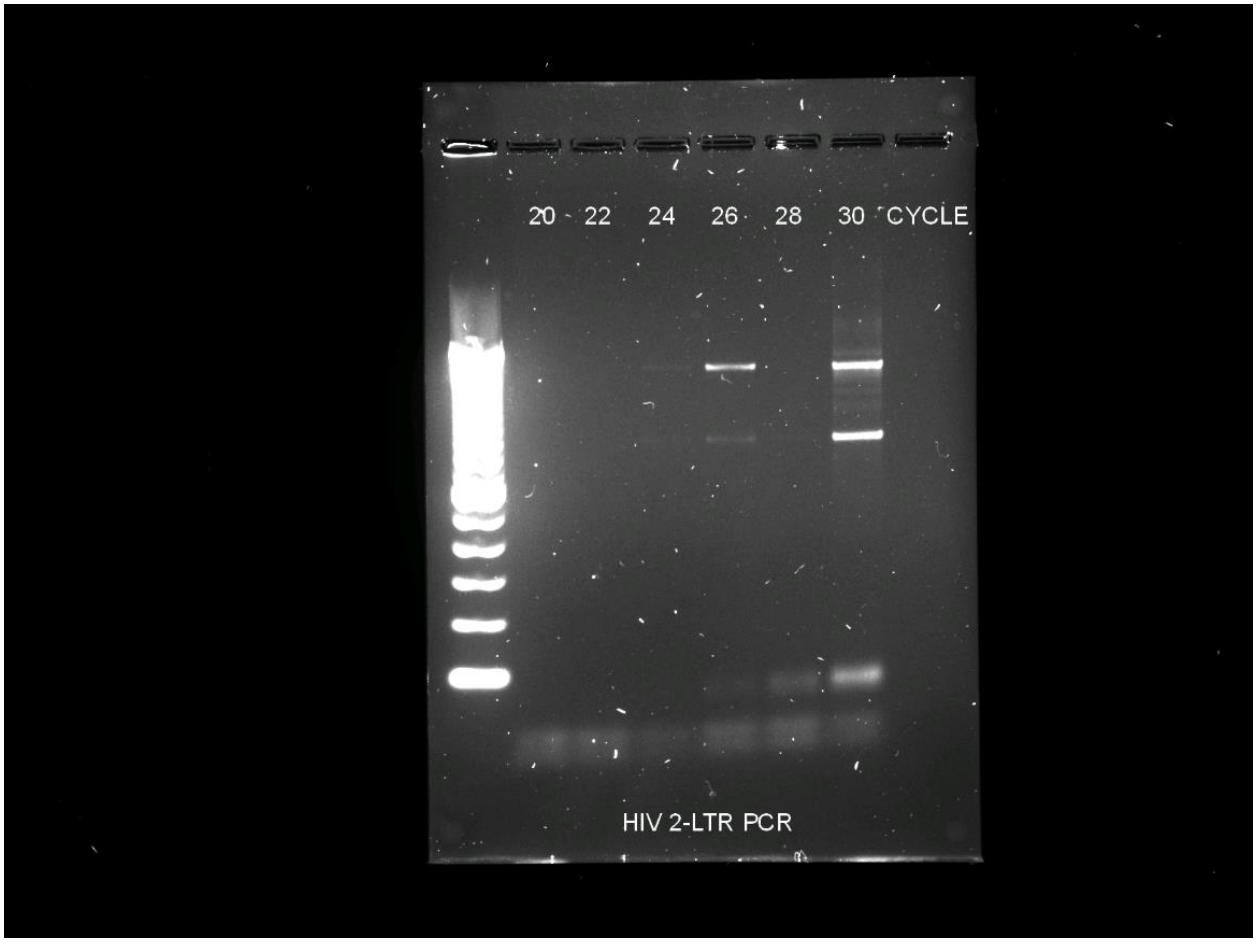

Fig. 3

Similar to the experiment with1-LTR detailed in Fig. 2, 1 million copies per microliter of 2-LTR DNA were used. However, the results were not the same. Instead they were incredibly undersaturated, which tells us that there was not enough DNA present to show up in the gel. That means for 2-LTR, we need more than 1 million copies per microliter.

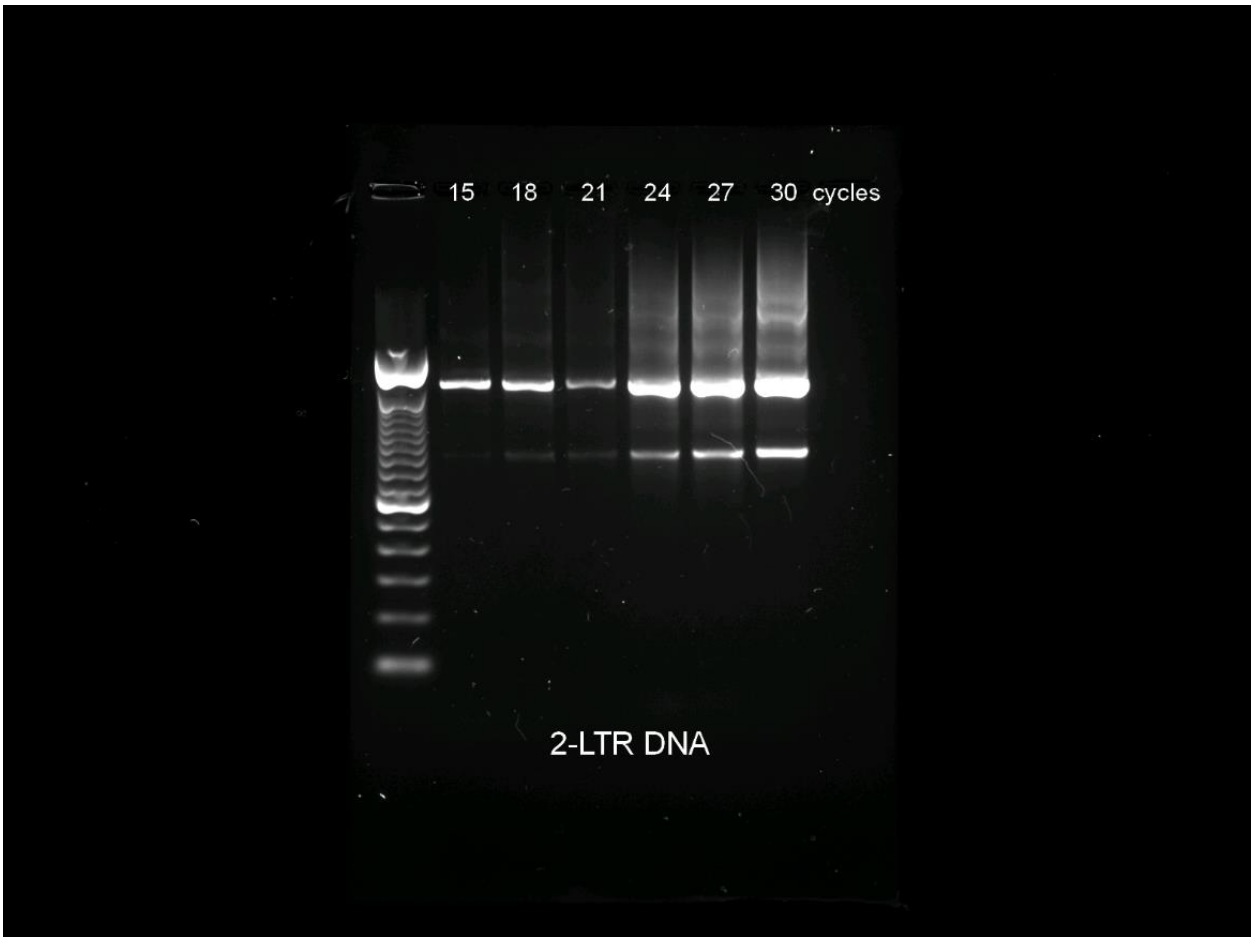

Fig. 4

The experiment was attempted again, this time with 2.5 million copies of DNA per microliter. As shown, the picture is much more well-saturated and the signal is stronger. It was decided that 25 cycles was a sufficient number because, at 24, the PCR-mediated recombination line still looked weak. We want the line to look strong so we can see if any of the treatments we will do are truly going to work on it. We cannot get a false sense of something working just because we didn't run it for enough cycles.

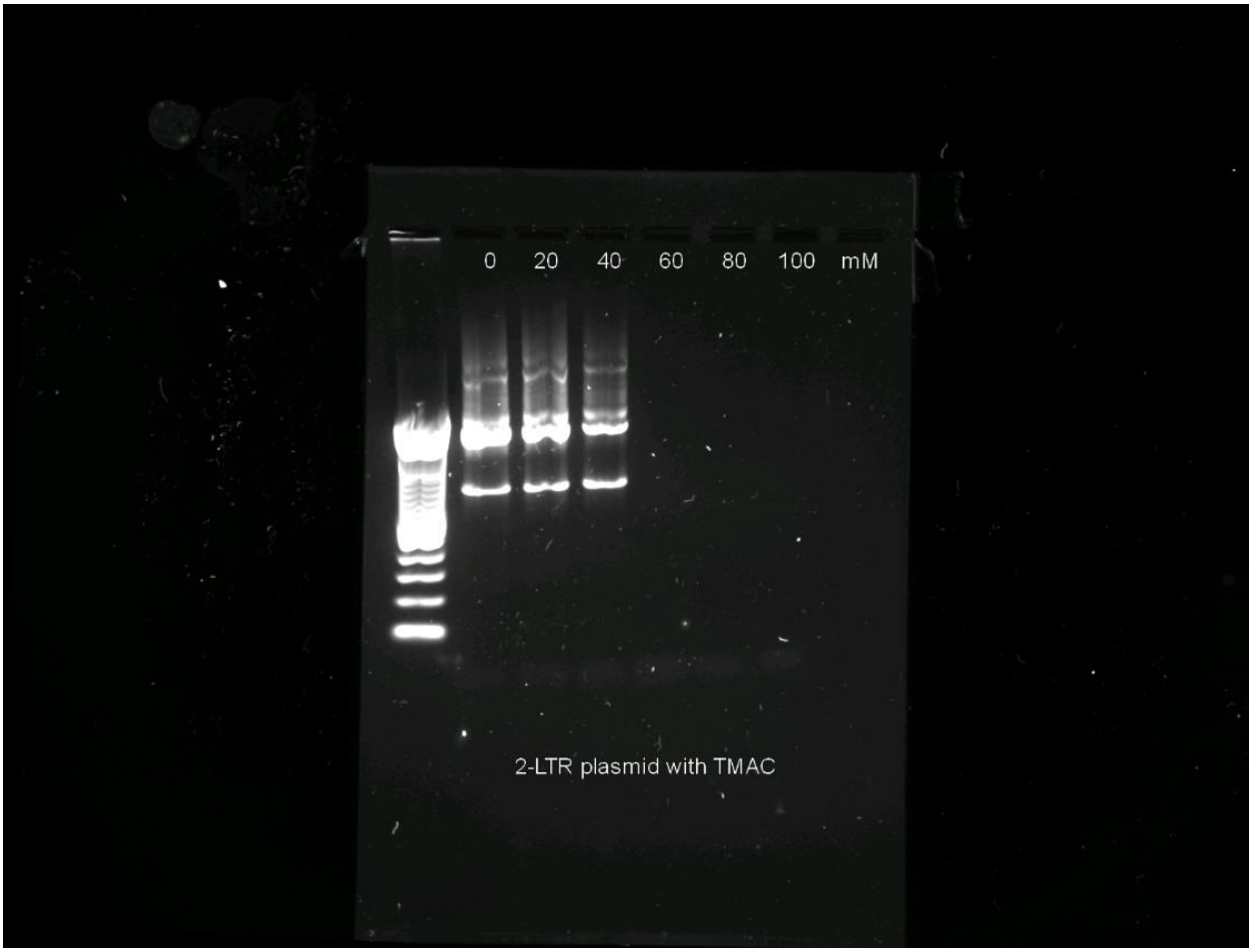

Fig. 5

TMAC was added in this experiment. As TMAC is an inhibitor of PCR, the bands can be seen growing weaker and weaker until they are completely inhibited at 60 microliter concentration. Because the band of PCR mediated recombination did not get significantly weaker in comparison to the 2-LTR band that is supposed to be there, it can be inferred that TMAC does not work in fixing the problem of PCR mediated recombination in unintegrated 2-LTR HIV-1 DNA.

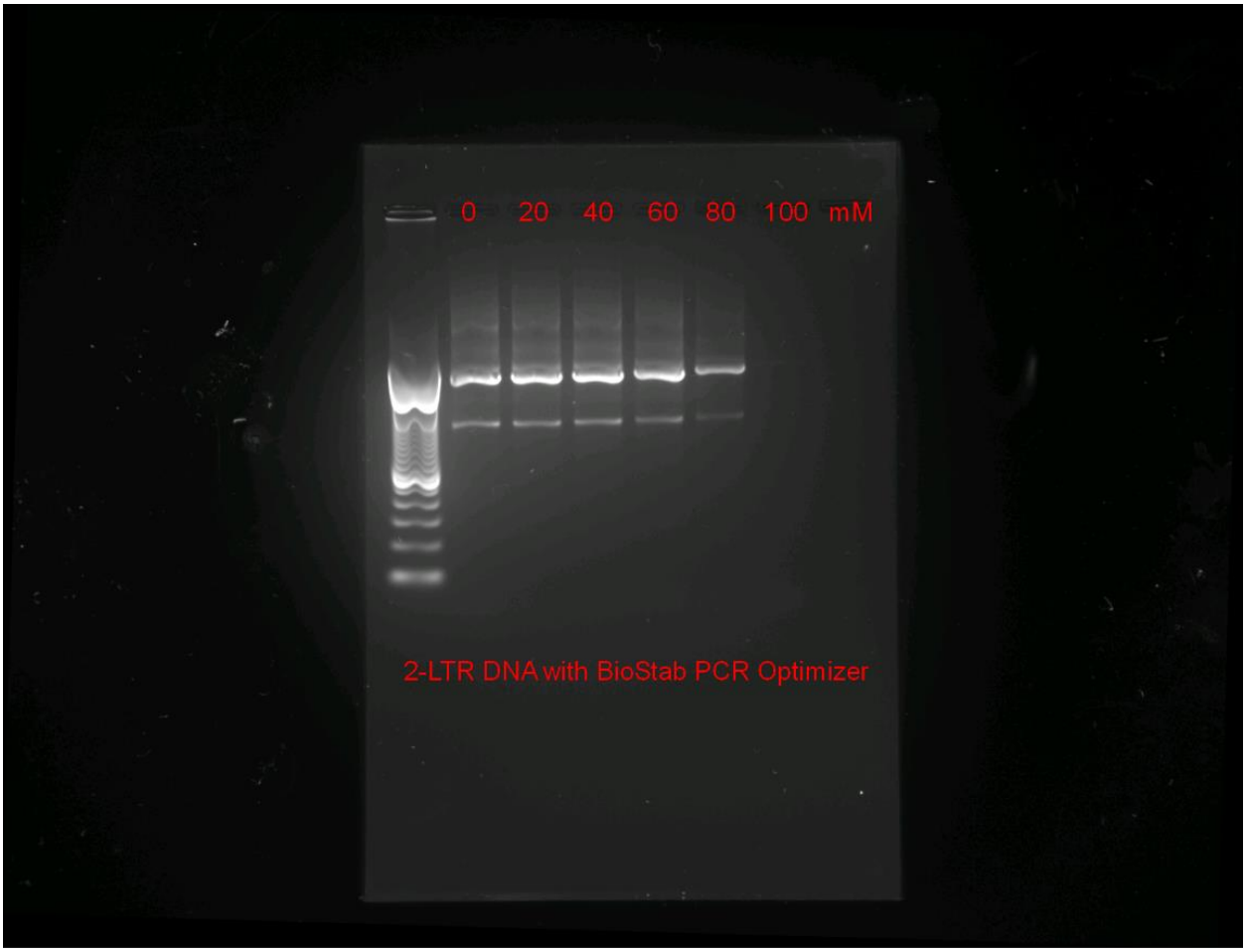

Fig. 6

In this experiment, PCR Optimizer was used. By the faintness of the results obtained, it can be inferred that PCR Optimizer is an inhibitor to PCR. As can be seen, the PCR Optimizer significantly lessens the strength of the bands of the PCR mediated recombination. However, it also lessens the strength of the bands of the 2-LTR DNA, which means that it is not necessarily eradicating the PCR-mediated recombination.

Discussion

While Tetramethylammonium chloride solution and PCR Optimizer did not give us the results we looked for, these results still have important implications. This was the first time that TMAC and PCR Optimizer were used on HIV-1 1-LTR and 2-LTR plasmid DNA. It was discovered before that TMAC does resolve some PCR-mediated recombination in other instances of PCR-mediated recombination. While it is known that TMAC is a sort of PCR inhibitor, there have been successes in using the inhibition to an advantage of simply inhibiting PCR mediated recombination (Chevet, 1995). In my experiment, however, TMAC did not work as intended. From 60 microliters on, TMAC fully inhibited the PCR. Between 0 and 40 microliters, some inhibition is observable both in the good band and the one that's not supposed to be there. This is most likely because TMAC works by increasing the stability of AT DNA bonds, which can either increase specificity by preventing improper annealing or inhibit the entire PCR due to the new strength of the AT bonds. In the future, there is certainly a chance that TMAC combined with another chemical, such as PCR Optimizer or betaine, which has also seen recent success, could successfully inhibit PCR mediated recombination.

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Bibliography:

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