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Capturing HIV-1 Envelope Diversity: Preventing Recombination in Bulk PCR using Droplet Digital PCR (ddPCR) and Q5 High-Fidelity Polymerase

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Introduction

- Human Immunodeficiency Virus (HIV-1) is a **retrovirus** with genetic diversity, especially within the **envelope** gene.
- An individual with HIV-1 can harbor **multiple** variants.
- Amplification is done to replicate the envelope diversity.

Name of Method	Expensive?	Labor-intensive?	Recombination?
Single Genome Amplification (SGA)	Yes	Yes	No
Bulk PCR with Taq	No	No	Yes

Figure 1. Analysis of current methods for envelope amplification

- Our research focuses on testing two new methods, **Droplet Digital PCR** (ddPCR) and **Bulk PCR with Q5 High-Fidelity Polymerase**, on their ability to prevent recombination within the envelope.
- We predict that these methods will lower recombination frequency. Samples will be sent to be PAC-BIO sequenced.

Methods

- ddPCR with Taq was done on three cDNA patient samples.
- **Nested PCR** was run on an Agarose Gel Electrophoresis.
- PCR products from the 1st cycle were used as template for the 2nd cycle of PCR.

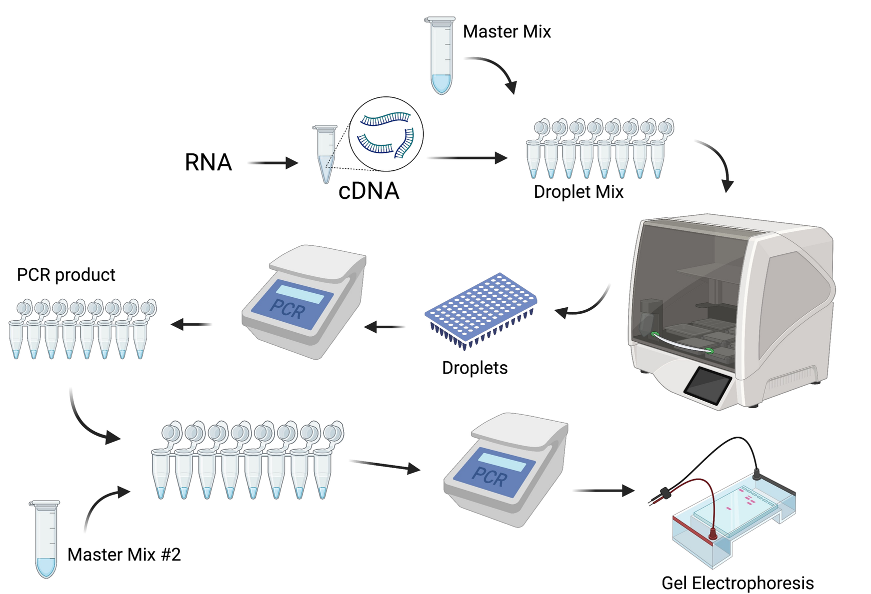

Figure 2. Demonstrating ddPCR methods

- ddPCR allowed for amplification of **one template strand** of DNA that exists in each droplet to reduce recombination.
- Agarose Gel Electrophoresis was done to detect target sequences with the correct kB for amplification.

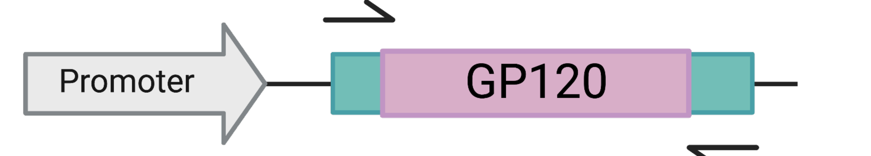

Figure 3. Amplification of GP120

- Bulk PCR used both expression plasmids and Δ5' LTR NL43 (Viral Construct) plasmids.
- Bulk PCR with Q5 High-Fidelity Polymerase was done with **2 sets of primers**.

Old (O) - Δecto + envIF	Δecto	5'-AAGCCTCCTACTATCATTAT-3'
	envIF	5'-AGAAAGAGCAGAAGACAGTGGCAATGA-3'
New (N) - Δecto (long-4) + envIF (short-3)	Δecto (long-4)	5'-AACCTACCAAGCCTCCTACTATCATTAT-3'
	envIF (short-3)	5'-GCAGAAGACAGTGGCAATGA-3'

- New primers were used to equalize melting temperatures since Q5 is sensitive to large differences in melting temperature.

Results

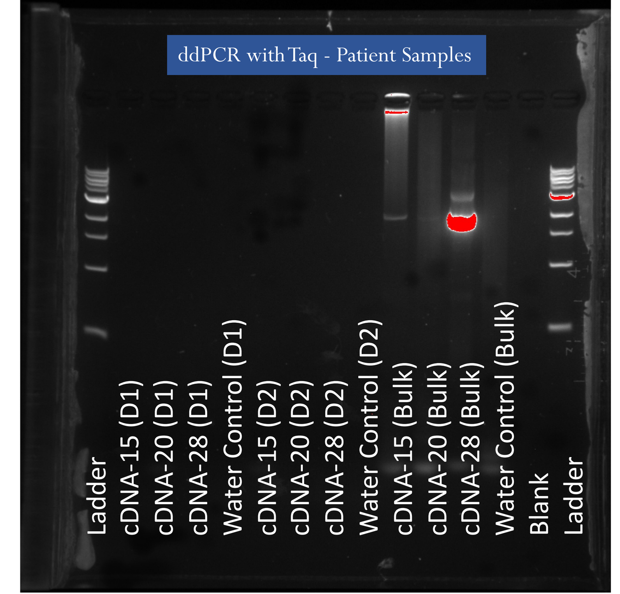

Figure 4. ddPCR was done on **3 patient samples** with two different droplet mixtures (**D1: laboratory-made mix, D2: ddPCR-specific mix**). Droplets only formed in ddPCR-specific mixture. Bulk PCR was done as control and water was used as the negative control in all variations. Bulk PCR did amplify as expected.

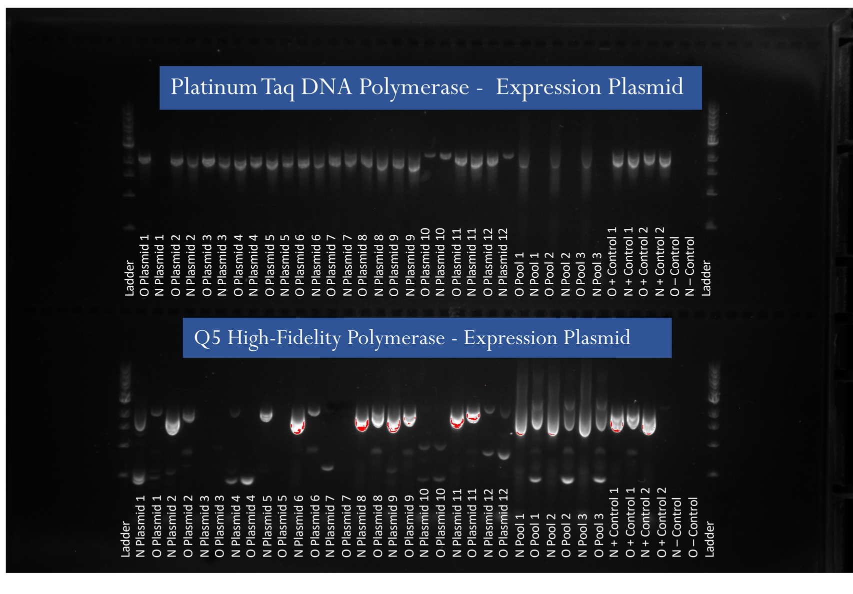

Figure 5. Bulk PCR with Platinum Taq DNA Polymerase was completed to compare sequencing to Bulk PCR with Q5 High-Fidelity Polymerase. **12 expression plasmid samples** were used with 2 sets of primers (**O: Δecto + envIF, N: Δecto (long) + envIF (short)**). Pools were made containing all plasmids. Bulk PCR with Taq amplified all samples except **Plasmid 1 (N)** and **primer pools (N)** did not amplify. Bulk PCR with Q5 had large streaking and off-target binding with lack of amplified portions.

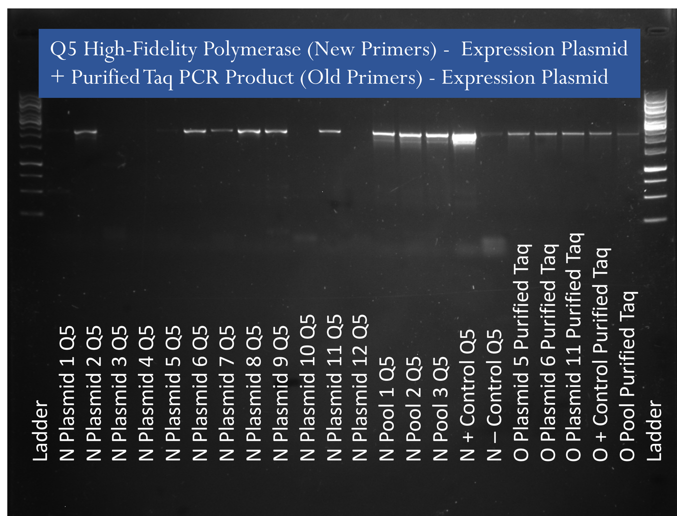

Figure 6. Certain plasmid samples (1, 3, 4, 5, 7, 10, 12) did not amplify with Q5. Plasmids 2, 6, 8, 9, and 11 amplified. Random Taq samples (5, 6, 11, pool, positive control) were **purified** in order to detect DNA concentration in spectrophotometry.

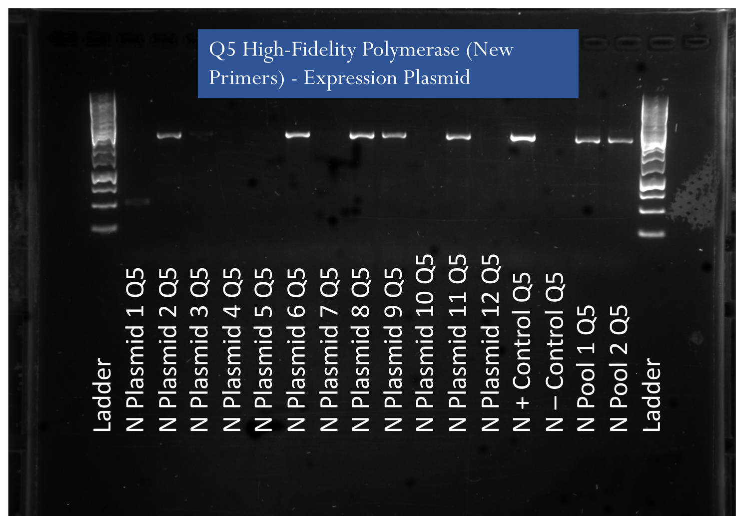

Figure 7. Unamplified samples were plasmids 1, 3, 4, 5, 7, 10, 12. This is similar to **Figure 6** when comparing Q5 samples.

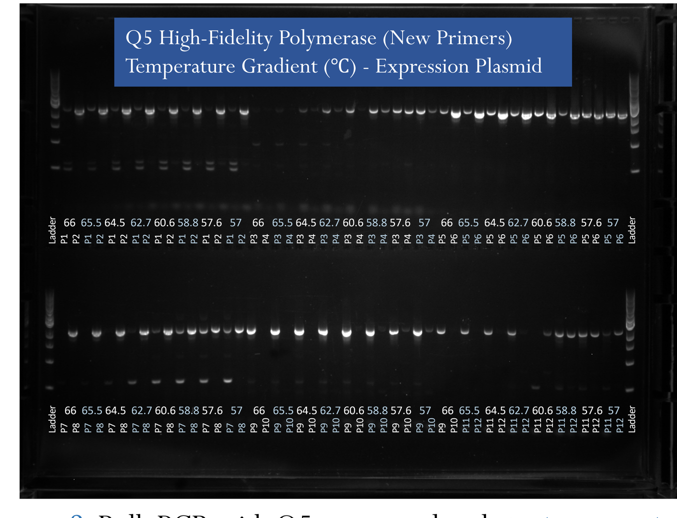

Figure 8. Bulk PCR with Q5 was completed at a **temperature gradient** (57 ℃ - 66 ℃) in order to detect a temperature that matched Q5's high-sensitivity. Certain plasmids amplified better at **lower** temperatures (Plasmid 2, 3, 7, 8, 12). Other plasmids amplified at **higher** temperatures (Plasmid 5, 6, 11). Plasmid 9 worked well and **consistently** at all temperatures. Plasmid 1 and 10 worked **poorly** at all temperatures.

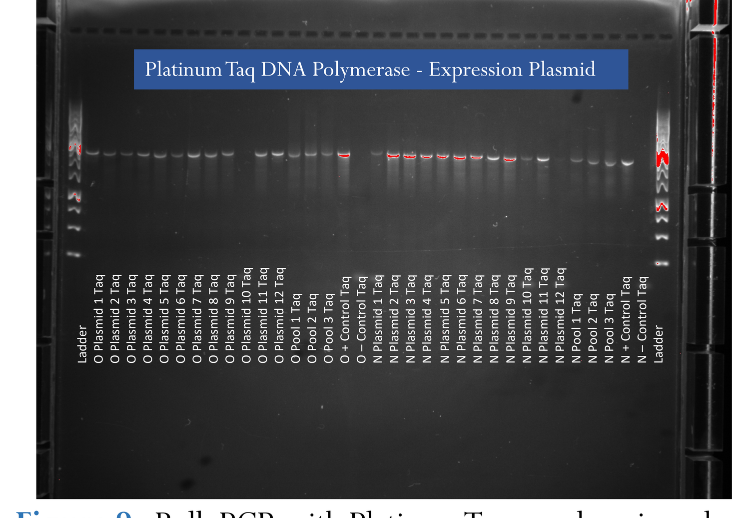

Figure 9. Bulk PCR with Platinum Taq was done in order to see if plasmid pools and plasmid 1 with new primers would amplify. Plasmid 1 (N) and plasmid pools (N) did amplify but **faint** compared to the rest. This PCR went through 35 cycles, so amplification may have occurred previously but **not enough to produce band until this run**.

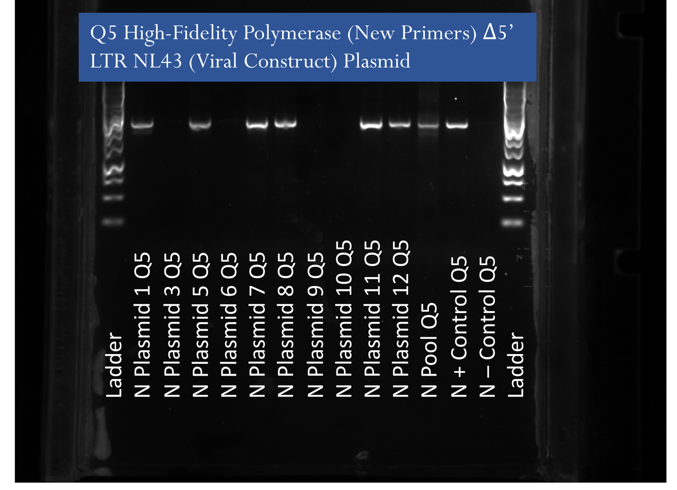

Figure 10. Δ5' LTR NL43 (Viral Construct) plasmid samples were used in this Q5 PCR. The old primers **placed the envelope** gene within the plasmid initially which indicates that all primer sets should be able to amplify, considering the new set of primers is edited from the old set. Only plasmids 1, 5, 7, 8, 11, and 12 amplified which **does not match** the amplified expression plasmids in **Figures 5-8**.

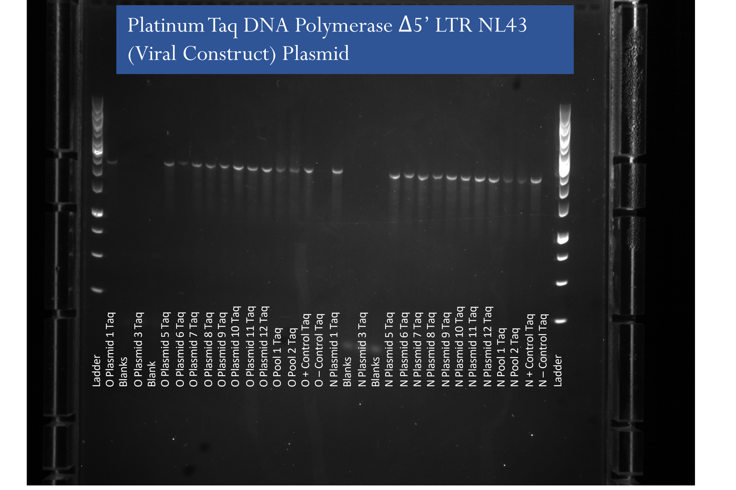

Figure 11. Δ5' LTR NL43 (Viral Construct) Plasmid Samples were used in this PCR with Taq. All plasmids amplified except Plasmid 3 which **does not match** the unamplified expression plasmids in **Figures 5 and 9**.

DNA Concentration (ng/μL) of Purified Taq PCR Product (New Primers)		DNA Concentration (ng/μL) of Purified Q5 PCR Product (New Primers)		DNA Concentration (ng/μL) of Purified Taq PCR Product (Old Primers)	
Plasmid 5	2.9	Plasmid 5	N/A	Plasmid 5	5.3
Plasmid 6	3.2	Plasmid 6	11.4	Plasmid 6	5
Plasmid 11	4.3	Plasmid 11	10.6	Plasmid 11	4.6
Plasmid Pool	N/A	Plasmid Pool	36.8	Plasmid Pool	13.2

Figure 12. The Purified Taq PCR Product for both sets of primers came from **Figure 5** gel and the Purified Q5 PCR Product came from **Figure 6** gel. These PCR Products were PCR-cleaned up and purified to remove primers, enzymes, and nucleotides to properly measure DNA concentration through spectrophotometry. All Q5 (N) concentrations were **higher** than the Taq (N) concentration. Plasmid 5 in Q5 and the plasmid pool in Taq (new) were not done due to the lack of amplification. All of this demonstrates the **increased amount of DNA** produced with the Q5 enzyme compared to the DNA produced with the Taq enzyme.

Conclusions

- Envelope sequences from patient samples did not amplify with ddPCR (**Figure 4**).
- The off-target binding in **Figure 5** affirms the annealing temperature necessary for Q5 when compared to **Figure 6.**
- Both **Figures 6 and 7** indicate Q5's inability to amplify certain plasmids. Primers work for Taq which demonstrates that it is not the cause. Q5 uses a premade master mix which **reduces variability**.
- Temperature gradient for Q5 demonstrated that each plasmid best amplified at various temperatures.
  - Only two plasmids worked poorly under all conditions.
- Bulk PCR with Platinum Taq was done to compare with Q5. **Figure 9** indicates that Taq works for most samples.
- Δ5' LTR NL43 (Viral Construct) plasmids were used to test if they were better suited for Q5 amplification. However, amplified samples were different compared to expression plasmids.
  - There is still not an **overarching condition** that can be used to amplify envelopes for Q5.
- Random samples of Taq (N), Taq (O), and Q5 (N) were purified in Figure 12 which indicated that Q5 samples **generated more DNA** when compared to Taq samples.
- Most Taq samples in expression and Δ5' LTR NL43 plasmids amplified. Plasmids will be matched with Q5 amplified samples and both will be purified.
  - Purified samples will be sent to be **PAC-BIO sequenced** to investigate the **rates of recombination and template switching** when comparing it to known protocols.
  - Q5's high-fidelity is predicted to have **lower recombination** when compared to Taq.

Future Directions

- **SGA** will be conducted as a **control** to compare with Q5 and ddPCR.
- We hope to eventually use **patient samples** (unknown envelope) instead of plasmids.
- These results will contribute to future experiments when forming viruses through **homologous recombination** and testing envelope function and antibody susceptibility.
  - Neutralizing-resistant antibodies (HIV-Tuberculosis co-infection)
  - Maternal-fetal antibodies and **vertical transmission of HIV-1**

Acknowledgements

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