Droplet Single Genome Amplification Allows for Characterization of Autologous HIV Envelope Sequences

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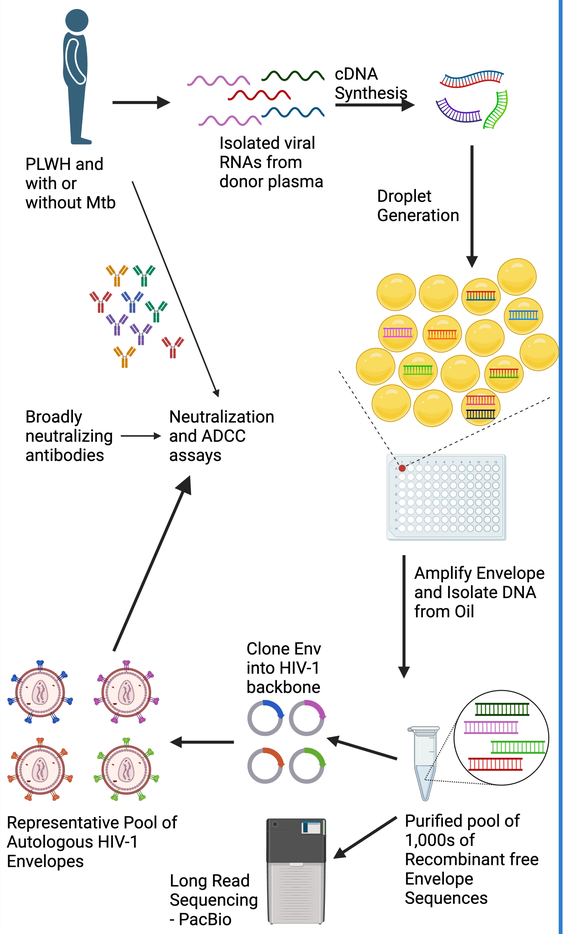
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Objective

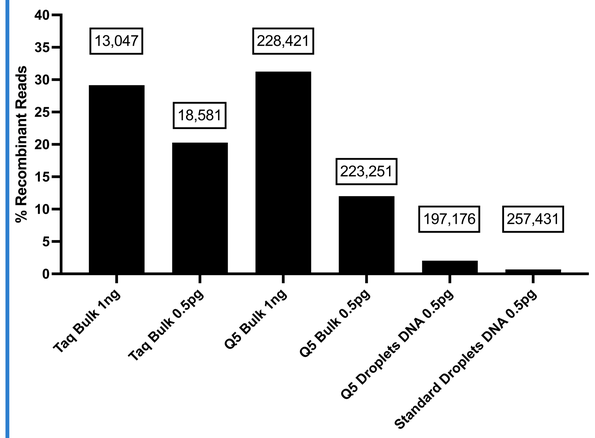
High rates of mutations within HIV-1's envelope (Env) gene is a primary mechanism by which the virus evades the adaptive immune response. Methods to assess HIV-1 Env diversity within an individual living with HIV-1 remain extremely limited due to the inability to remove recombinant artifacts generated during PCR. We aimed to develop a digital droplet-based PCR (ddPCR) approach to overcome these hurdles.

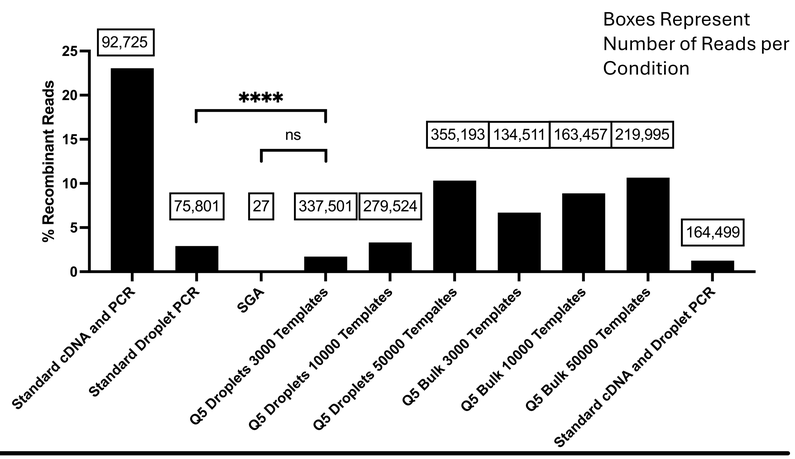
Methods



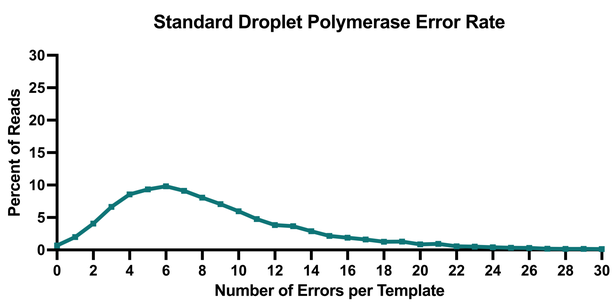
Results

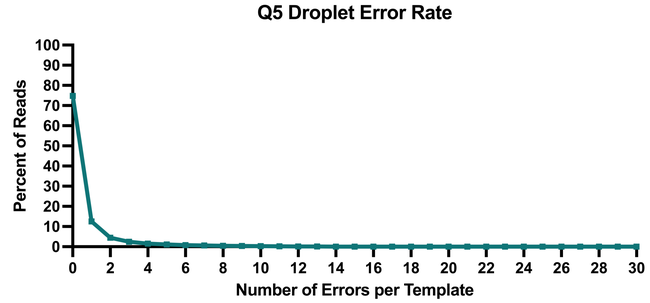
Recombination rates are significantly reduced in droplet emulsion PCR, and generate drastically more reads than SGA



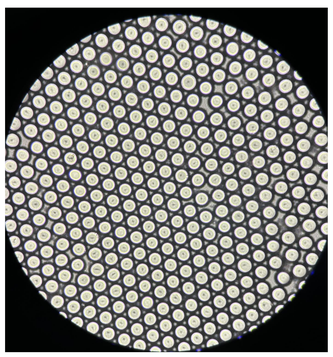


Our novel Q5 droplet PCR has a significantly lower error rate than standard droplet emulsions

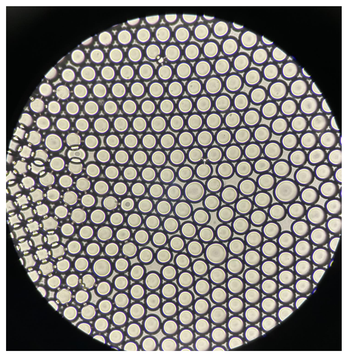




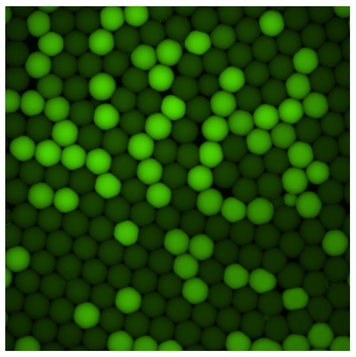
Our droplets form similarly to standard droplets and amplification occurs within each droplet



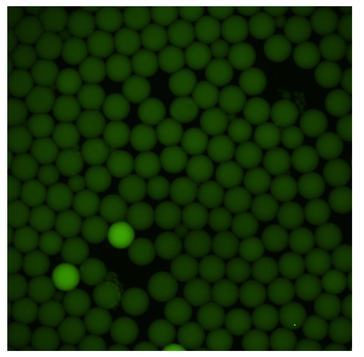
Standard ddPCR Droplets



Q5 Optimized Droplets



Q5 Optimized Droplets with SYBR Green Post Amplification



SYBR Green Negative Control

Conclusions

- **We developed a novel approach to use a one step Q5 master mix in an oil emulsion, which allows virtually recombination free amplification of thousands of HIV-1 envelope sequences directly from RNA in a single reaction.**
- Digital droplet PCR significantly reduces recombination rates compared to bulk PCR by sequestering an individual template into an individual droplet.
- Recombination occurs in bulk PCR even when as little as 8 different templates are present.
- The error rate of the standard polymerase used in ddPCR is too high to be useful for downstream applications. In our droplet master mix using the Q5 polymerase had 75% of templates had 0 errors with 92% of templates having 2 or less errors.
- Amplification of templates occurs within each droplet.

Future Directions

To clone HIV-1 envelopes amplified with our novel approach into a subtype C backbone allowing us to generate a pool of replication competent virus. These viruses will be used in neutralization and ADCC assays, allowing us to directly determine which mutations lead to sensitivity or resistance to neutralizing autologous antibodies in a high throughput screen.

Acknowledgements

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