

Introduction

- Human Immunodeficiency Virus (HIV-1) is a **retrovirus** that attacks the immune system.
- Every person with HIV-1 has a **different** strain and **specific** variant.
- An individual with HIV-1 can harbor multiple variants: **superinfection**.
- Our goal is to find out if a baby has a superinfection that was established through **vertical transmission**: breastfeeding from the mother.
- Vertical transmission is a good representation because the mother passes on **antibodies and virus**.

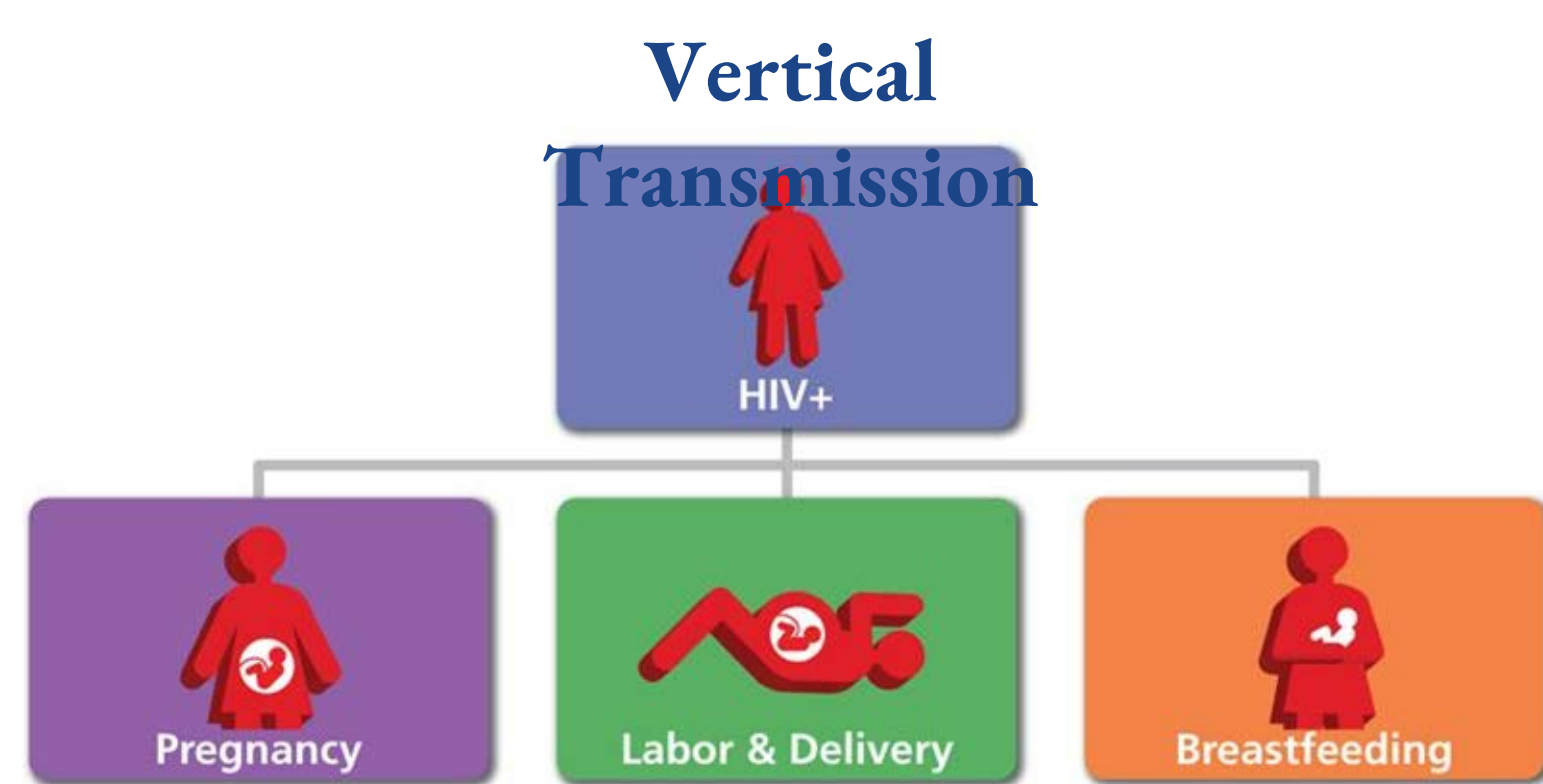


Figure 1. The three ways of vertical transmission

Methods

- We used **Single Genomic Amplification (SGA)**
- The protocol consisted of diluting the mother and baby's cDNA in order to have one template in each well following two rounds of **nested Polymerase Chain Reaction (PCR)** to amplify small sections of the diluted cDNA.
- Following PCR, **gel electrophoresis** detected the positivity of the templates.

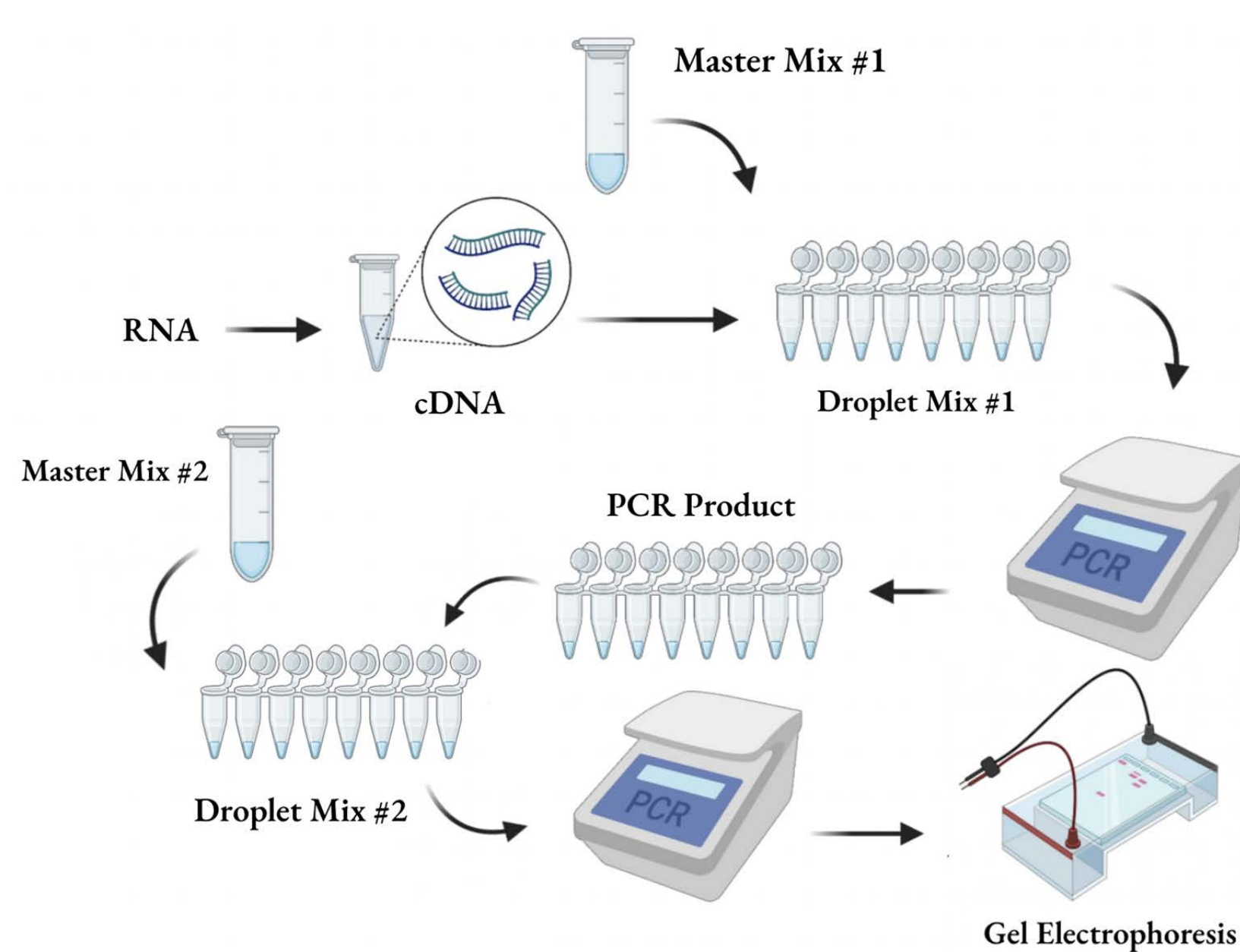


Figure 2. Demonstrating SGA methods

- Once positive samples were detected, we performed **cell isolation** on the to get the DNA sequences
- Sequences are from Nanopore

Results

- Our goal was to have **30% positive feedback** on both agarose gels: mother and baby
- This symbolizes that we diluted the cDNA correctly, meaning there would be only **one template** in each well. This is significant because recombination did not occur in these wells.

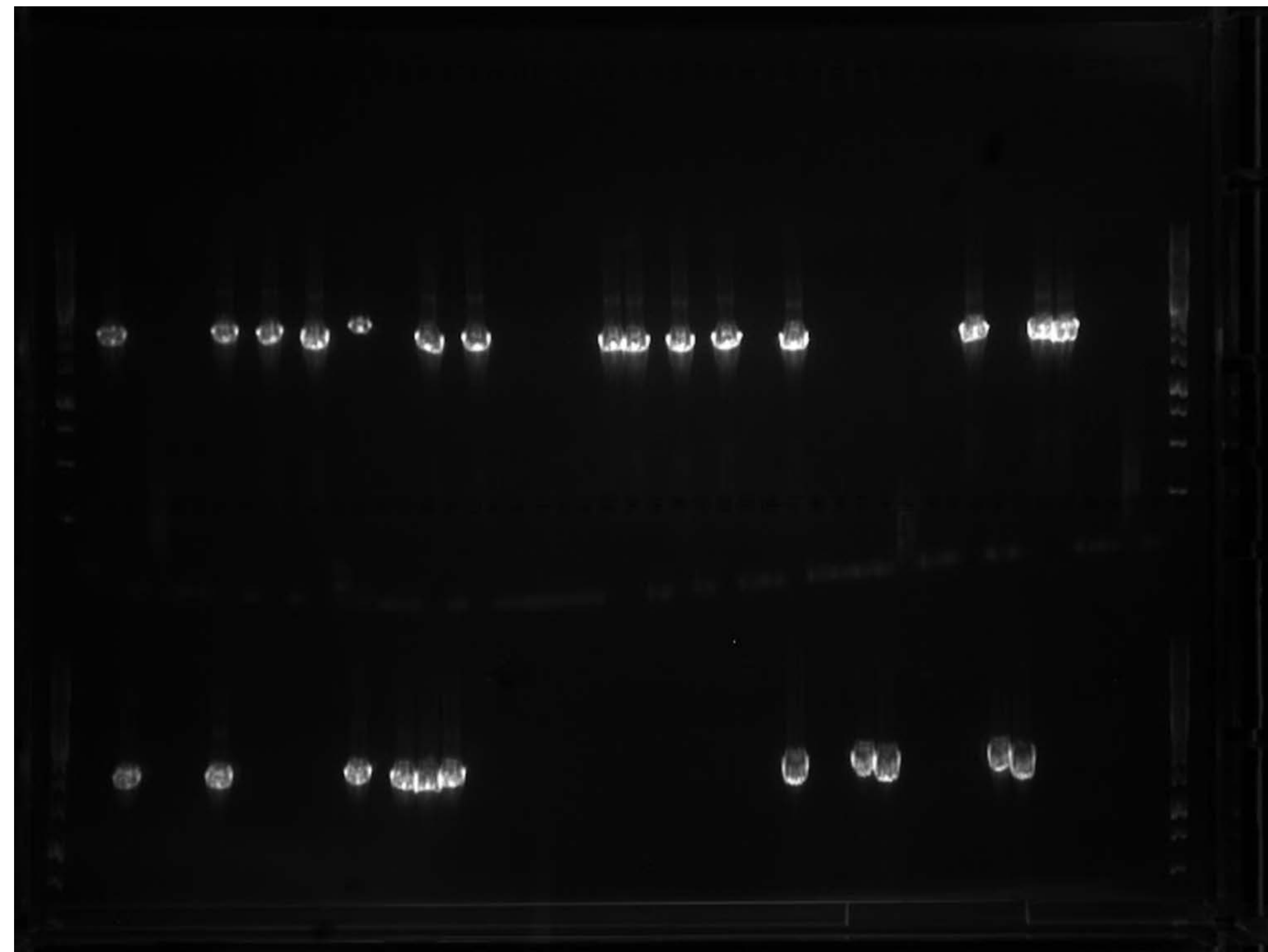


Figure 3. SGA was done on the mother's cDNA and it had **26 positive wells**. The positive wells show that there is only one template in each and **no recombination** occurred. The positive wells also amplified correctly. The **baby (not displayed) was the same**, but with 22 positive wells.

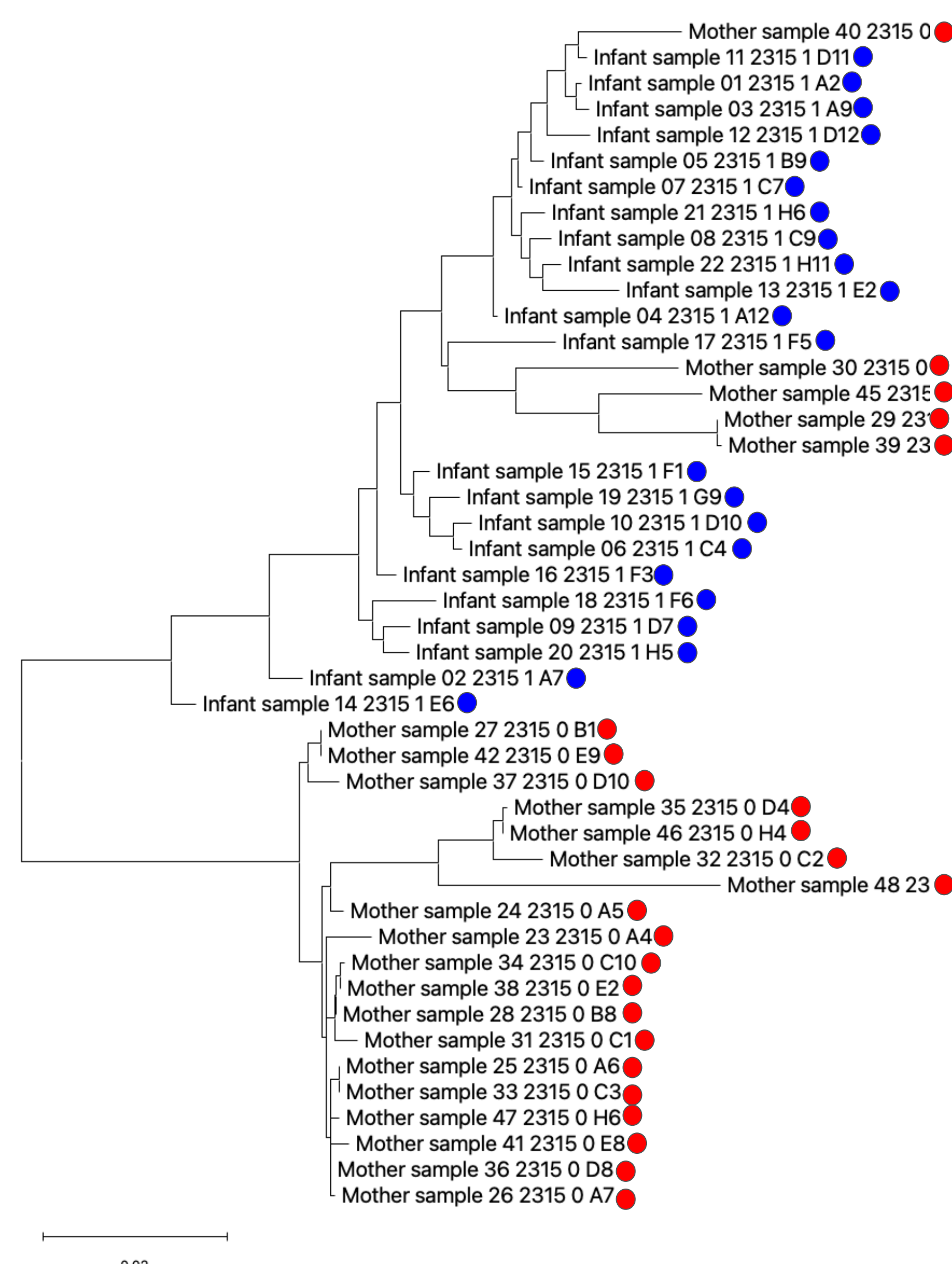


Figure 4. Phylogenetic tree of the mother and baby DNA sequences. We computed it using nucleotide differences. The program used the Maximum Likelihood Method and Tamura-Nei method. Trees were constructed using MEGA11. The mother samples were indicated using a red dot and the infant was indicated using the blue dot.

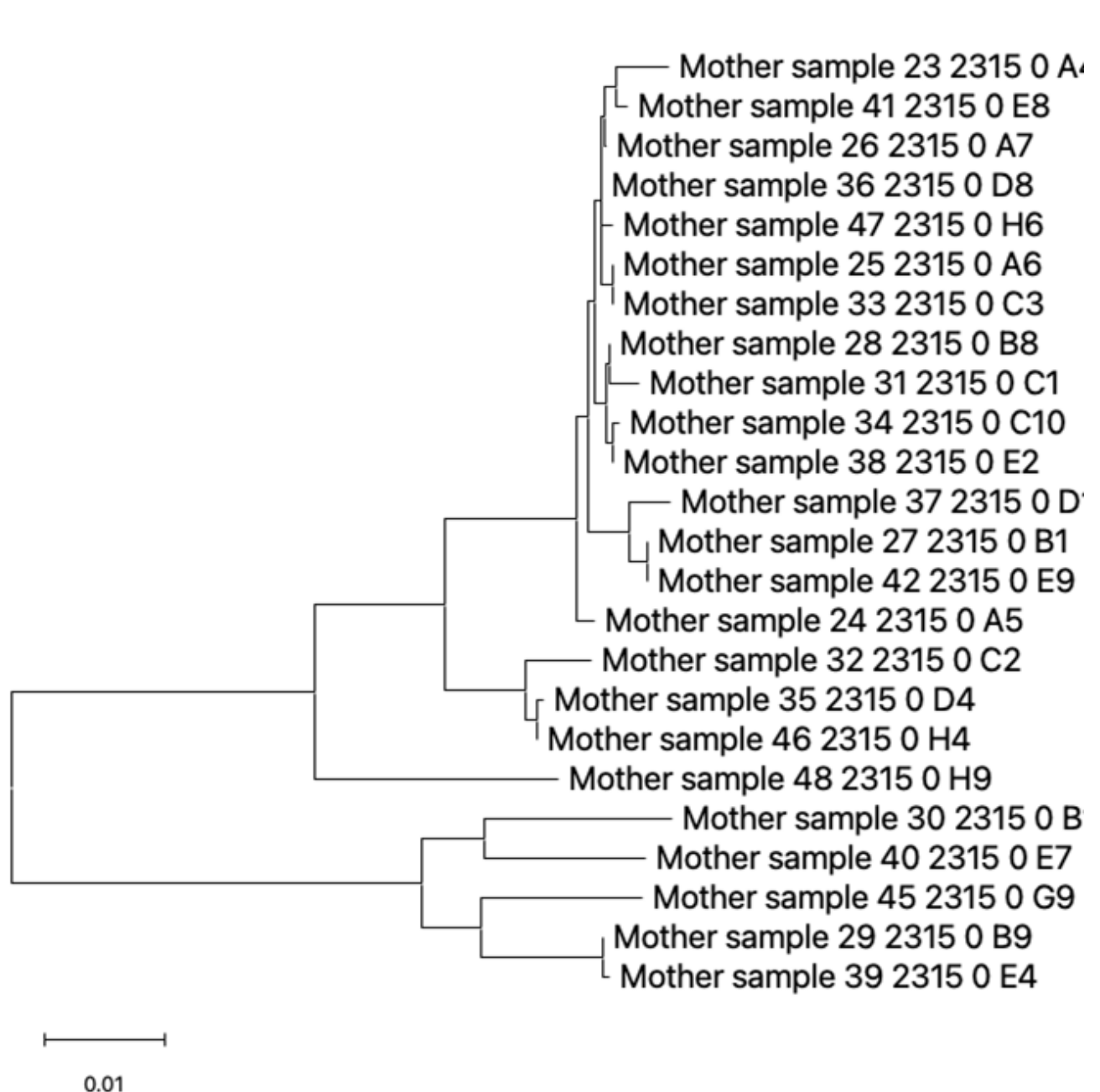


Figure 5. Mother phylogenetic tree

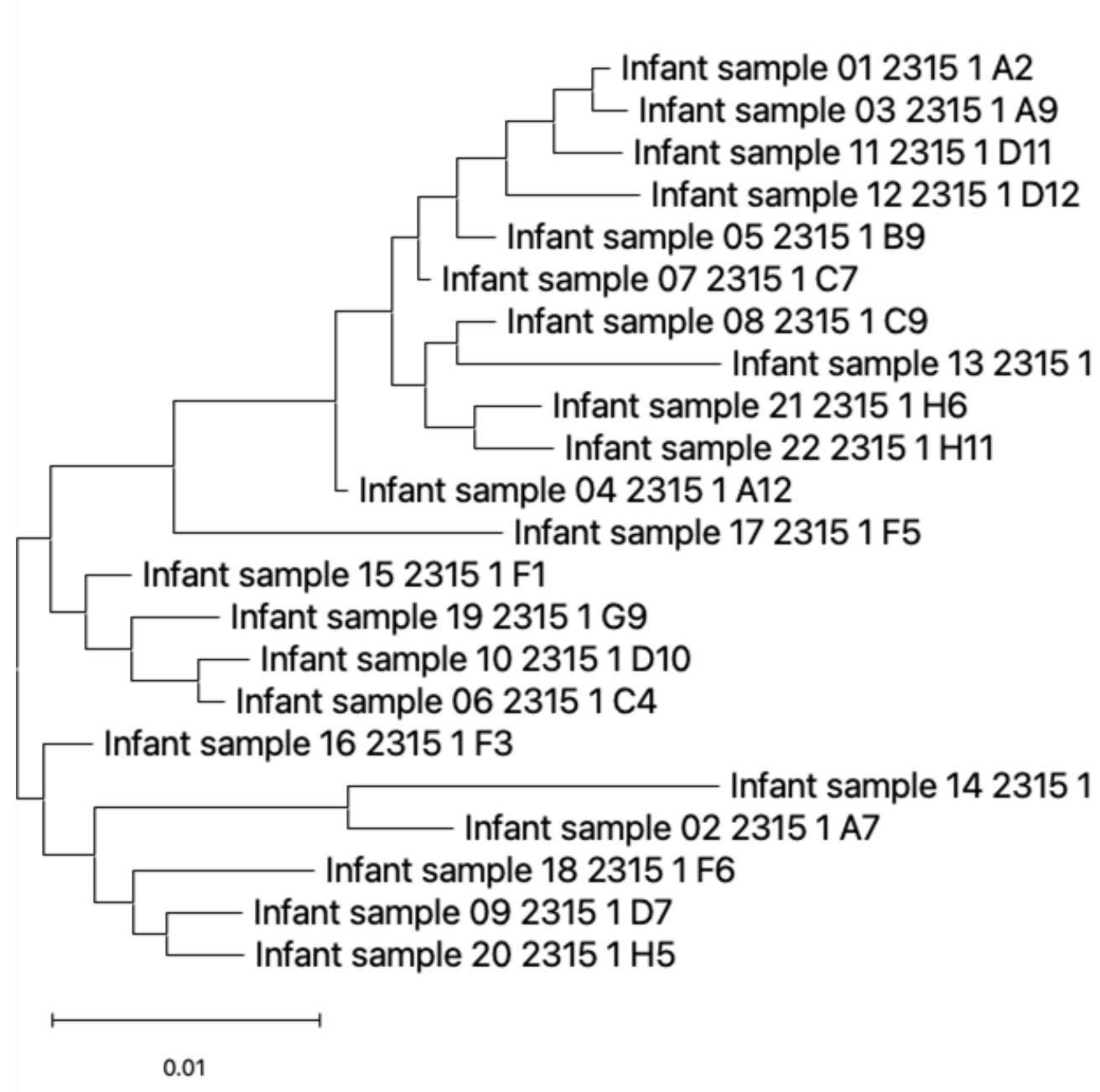


Figure 6. Infant phylogenetic tree

Conclusions

- The phylogenetic tree shows that the infant and mother are **mostly clustered in specific areas**.
- This trend is not perfect, indicating that this **does not conclusively suggest a superinfection**, but it does not rule it out.
- Some of the infant samples are **equally similar** to some mother samples and other infant samples.
- This could indicate a **missing mother sample** or could be a sign of **recombination**, producing new sequences that are unlike any sequence.
- Recombination shows that the samples we received are **not biologically relevant**.
- These recombined sequences can lead to making of viruses that do not exist in a person.
- Trees **could** display evidence of **superinfection**.
- SGA is a tedious process and optimally, we will find another method that is more efficient.
- This leads to our future directions, like **Droplet Digital PCR**

Future Directions

- The results will be applied to **Droplet Digital PCR (ddPCR)**.
- If PCR is done in a single tube, **template switching** can occur.
- It provides altered clones that are misrepresenting the original virus.
- ddPCR mitigates the alteration through the thousands of **oil droplets** in each tube.
- This approach will allow us to have a more **efficient way of detecting superinfections** and may replace SGA.

References

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