Perfecting the Polony Method to Quantify Marine Viruses

Shelby Mahank[1], Natalie Sawaya[2], Nava Baran[3], Debbie Lindell[3], and Mya Breitbart[2]

1. *University of South Florida, St. Petersburg*
2. *University of South Florida, College of Marine Science*
3. *Technion – Israel Institute of Technology*

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Introduction

Gokushoviruses remain understudied

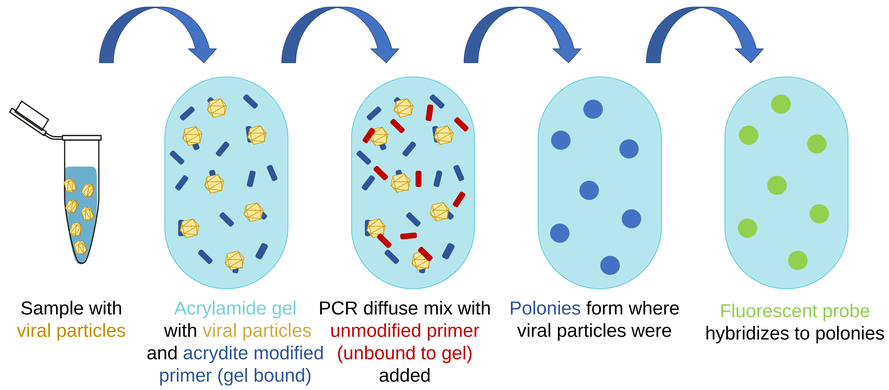
- They are a type of **Bacteriophage (aka phage)** - *this means that they are a virus that only infects bacteria*
- Gokushoviruses are:
  - Found in all environments: Marine, Soil, Digestive System
  - A subfamily of *Microviridae*
  - Small single-stranded DNA phage with a circular genome
  - Known to infect intracellular bacteria like *Chlamydia*
    - We hypothesize they could infect *Endozoicomonas*, found in coral

Why use polonies?

- Polony stands for *Polymerase colony- because it amplifies DNA on a single spot on a gel, forming a DNA "colony"*
- Polonies can quantify diverse groups of viruses
- It is the only method that can quantify single stranded DNA[1]:
  - **Staining** doesn't work because these viruses are too small to be seen when stained
  - **Sequencing** works best on double stranded DNA
  - **Rolling circle amplification** is biased toward small circular DNA
- Polonies allow for **absolute quantification** of viruses

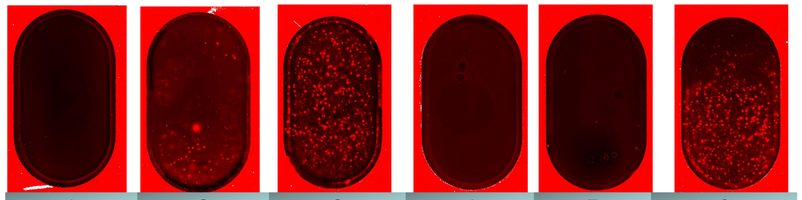
Method

Polony Protocol[2]:



Results

Comparison of efficiency among replicates – all from same positive control



Replicate	1	2	3	4	5	6
Extrapolated polony/slide	0	363	841	0	0	56
Expected polony/slide	2176	2176	2176	2176	2176	2176
Efficiency	0	16.7	38.6	0	0	2.6
Average efficiency			18.44			18.75

Comparison of contamination in no template controls (NTC)

No contamination | Contamination

These 2 are NTCs made with sterile lab water (PCR Water)

Gokushovirus polonies from Bayboro environmental samples

0.02 µm filtered | 0.22 µm filtered

These 2 samples were made from bay water that was run through a filter

Conclusions & Future Work

- Positive control and environmental samples result in polonies but still need optimization
- Currently we are having issues with contamination and consistency. This is our plan to improve the method:

Consistency-

1. Test whether primers/probe only pick up gokushoviruses
2. Test if the salt in marine water increases the efficiency of polonies
   - Find alternative to bay water
3. Test if it matters whether the DNA plasmid is used or a phage capsid

Contamination-

1. Identify where the contamination is coming from
   - Gel component is likely contaminated
   - PCR water has been ruled out

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Reference-

1. Székely AJ, Breitbart M (2016) Single-stranded DNA phages: from early molecular biology tools to recent revolutions in environmental microbiology. *FEMS Microbiology Letters*, **363**.

2. Baran N, Goldin S, Maidanik I, Lindell D (2018) Quantification of diverse virus populations in the environment using the polony method. *Nature Microbiology*, **3**, 62–72.