Detecting and Quantifying Bacteriophage M13 on Human Skin

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Rapid evolution of antibiotic resistant bacteria

Population of Pathogenic Bacteria
Rapid evolution of antibiotic resistant bacteria

Population of Pathogenic Bacteria

Antibiotics

Selection of Antibiotic Resistant Bacteria
Rapid evolution of antibiotic resistant bacteria

Population of Pathogenic Bacteria

Antibiotics

Selection of Antibiotic Resistant Bacteria

Carbapenem-Resistant Enterobacteriaceae
Bacteriophages: natural viruses that infect bacteria

Non-Resistant

Antibiotics

Resistant

Population of Pathogenic Bacteria

Selection of Antibiotic Resistant Bacteria

Bacteriophages
Bacteriophages: natural viruses that infect bacteria

Population of Pathogenic Bacteria

Bacteriophages

Antibiotics

Selection of Antibiotic Resistant Bacteria

Successful Treatment of Pathogenic Bacteria
Characterizing the microbial community of wounds
Characterizing the microbial community of wounds

Diabetic Foot Ulcers  Surgical Wounds
Characterizing the microbial community of wounds

Diabetic Foot Ulcers
Surgical Wounds
Characterizing the microbial community of wounds

- Diabetic Foot Ulcers
- Surgical Wounds

Bacteria:
- *Acinetobacter baumannii*
- *Escherichia coli*
- *Streptococcus pneumoniae*
- *Klebsiella pneumoniae*
- *Burkholderia cepacia*
- *Staphylococcus aureus*
Using phage M13 to develop the skin swab protocol
Using phage M13 to develop the skin swab protocol

Bacteriophage M13

Escherichia coli
Using phage M13 to develop the skin swab protocol

Bacteriophage M13

Escherichia coli

How much phage can we recover by swabbing a human skin?
Recovering phage M13 from human skin swab

1. Skin Swab Sample Collection
Recovering phage M13 from human skin swab

1. Skin Swab Sample Collection
Recovering phage M13 from human skin swab

1. Skin Swab Sample Collection

Cotton Swab

Phage M13
Recovering phage M13 from human skin swab

1. Skin Swab Sample Collection

Phage M13

Cotton Swab

Sterilized Tweezers
Recovering phage M13 from human skin swab

1. Skin Swab Sample Collection

Phage M13

Cotton Swab

PBS Buffer

Vortex

Sterilized Tweezers

Cotton
Recovering phage M13 from human skin swab

1. Skin Swab Sample Collection

Phage M13

Cotton Swab

Sterilized Tweezers

PBS Buffer

Vortex

Centrifuge
Recovering phage M13 from human skin swab

1. Skin Swab Sample Collection

- Use Sterilized Tweezers to collect cotton swab
- Vortex the PBS Buffer with cotton
- Centrifuge the solution to collect Phage M13 in supernatant
Recovering phage M13 from human skin swab

1. Skin Swab Sample Collection

- **Phage M13**
- **Cotton Swab**
- **Sterilized Tweezers**
- **PBS Buffer**
- **Cotton**
- **Vortex**
- **Centrifuge**
- **Phage M13 in supernatant**
- **Transfer the supernatant**
Recovering phage M13 from human skin swab

1. Skin Swab Sample Collection

- Collect skin swab sample
- Use sterilized tweezers
- Dip cotton swab in PBS buffer
- Vortex the mixture
- Centrifuge the sample
- Transfer the supernatant
- Add DNase
- Phage M13 in supernatant
Recovering phage M13 from human skin swab

1. Skin Swab Sample Collection

- Cotton Swab
- Sterilized Tweezers
- PBS Buffer
- Centrifuge
- Vortex
- DNase
- Transfer the supernatant
- Remove contaminating sequences
- Phage M13 in supernatant

Phage M13

Cotton

Sterilized Tweezers

PBS Buffer

Centrifuge

Vortex

DNase

Transfer the supernatant

Remove contaminating sequences
Recovering phage M13 from human skin swab

1. Skin Swab Sample Collection

- **Phage M13 in supernatant**
- **Transfer the supernatant**
- **Isolated Phage M13**
- **Remove contaminating sequences**
- **DNase**
- **Sterilized Tweezers**
- **Cotton Swab**
- **Cotton**
- **PBS Buffer**
- **Vortex**
- **Centrifuge**
- **Phage M13**

**Steps**:
1. Collect skin swab sample.
2. Use sterilized tweezers to remove cotton swab from the skin.
3. Place the cotton swab in PBS buffer and vortex it.
4. Centrifuge to separate the supernatant.
5. Transfer the supernatant to a new tube.
6. Use DNase to remove contaminating sequences.
7. Isolate the phage M13 from the supernatant.
Recovering phage M13 from human skin swab

2. Phage DNA Extraction

Isolated Phage M13
Recovering phage M13 from human skin swab

2. Phage DNA Extraction

Isolated Phage M13 → Phage M13 → Phage DNA → PureLink Viral DNA Extraction Mini Kit
Recovering phage M13 from human skin swab

2. Phage DNA Extraction

Isolated Phage M13 → Phage M13 Lysis

Phage DNA → PureLink Viral DNA Extraction Mini Kit

Isolated Phage M13
Recovering phage M13 from human skin swab

2. Phage DNA Extraction

Phage M13 Lysis

Isolated Phage M13

DNA Extraction

PureLink Viral DNA Extraction Mini Kit
Recovering phage M13 from human skin swab

2. Phage DNA Extraction

- Isolated Phage M13
- Phage M13 Lysis
- DNA Extraction
- PureLink Viral DNA Extraction Mini Kit
- Phage DNA

3. Quantification of Phage DNA

- Extracted Phage DNA
Recovering phage M13 from human skin swab

2. Phage DNA Extraction

- Isolated Phage M13
- Phage M13 Lysis
- DNA Extraction
- PureLink Viral DNA Extraction Mini Kit

3. Quantification of Phage DNA

- Extracted Phage DNA
- Quantitative-PCR
Recovering phage M13 from human skin swab

2. Phage DNA Extraction

Phage M13 Lysis

Isolated Phage M13

PureLink Viral DNA Extraction Mini Kit

Phage DNA

3. Quantification of Phage DNA

Extracted Phage DNA

Quantitative-PCR

gPCR Principles
Recovering phage M13 from human skin swab

2. Phage DNA Extraction

Isolated Phage M13

Phage M13 Lysis

DNA Extraction

PureLink Viral DNA Extraction Mini Kit

Extracted Phage DNA

Quantitative-PCR

3. Quantification of Phage DNA

qPCR Principles

n=1
Recovering phage M13 from human skin swab

2. Phage DNA Extraction

- Phage M13 Lysis
- DNA Extraction
- Isolated Phage M13
- PureLink Viral DNA Extraction Mini Kit

3. Quantification of Phage DNA

- Extracted Phage DNA
- Quantitative-PCR

qPCR Principles

\[ n = \text{number of cycles} \]

\[ 2^n \]

n = 1, 2, ...
Recovering phage M13 from human skin swab

2. Phage DNA Extraction

Isolated Phage M13

Phage M13 Lysis

PureLink Viral DNA Extraction Mini Kit

Extracted Phage DNA

3. Quantification of Phage DNA

Quantitative-PCR

qPCR Principles

n = number of cycles

n=1

n=2

... ... \(2^n\)
Recovering phage M13 from human skin swab

2. Phage DNA Extraction

- Phage M13 Lysis
- DNA Extraction
- PureLink Viral DNA Extraction Mini Kit

3. Quantification of Phage DNA

- Extracted Phage DNA
- Quantitative-PCR
- qPCR Principles

\[ 2^n \]

n = number of cycles
Identifying qPCR limit of detection for phage M13 DNA

Samples

M13KE 10-fold
Identifying qPCR limit of detection for phage M13 DNA

Samples

M13KE

Control

10-fold
Identifying qPCR limit of detection for phage M13 DNA

Samples

Control

M13KE

10-fold
Identifying qPCR limit of detection for phage M13 DNA

Samples

Control

M13KE

Skin Swab

10-fold
Identifying qPCR limit of detection for phage M13 DNA

**Samples**

- Control
- M13KE
- Skin Swab

10-fold
Identifying qPCR limit of detection for phage M13 DNA

Samples

Samples were assayed in triplicate
Identifying qPCR limit of detection for phage M13 DNA

Samples

Control

Skin Swab

Samples were assayed in triplicate

Absolute Quantification of M13KE Skin Swab Samples

$R^2 = 0.98$

No M13 Samples were assayed in triplicate
Identifying qPCR limit of detection for phage M13 DNA

- qPCR baseline of detection = \(2.36 \times 10^4\) copies

Samples

- M13KE
- Skin Swab
- Control

Absolute Quantification of M13KE Skin Swab Samples

\[ R^2 = 0.98 \]
Identifying qPCR limit of detection for phage M13 DNA

**qPCR baseline of detection = 2.36 x 10^4 copies**

### Samples

- **Control**
- **M13KE**
- **Skin Swab**

### Absolute Quantification of M13KE Skin Swab Samples

- $R^2 = 0.98$

![Graph showing absolute quantification of M13KE skin swab samples](image-url)
Identifying qPCR limit of detection for phage M13 DNA

- qPCR baseline of detection = 2.36 x 10^4 copies
- Limit of detection for human skin swab = 1.00 x 10^6 copies
- Contaminating sequences on human skin

![Diagram showing qPCR results and limit of detection](image-url)

**Samples**

- **Control**
- **M13KE**
- **Skin Swab**

**Absolute Quantification of M13KE Skin Swab Samples**

- **Limit of Detection** = 1.00 x 10^6 copies
- **Phage M13 detectable by qPCR**
- **Phage M13 not detectable by qPCR**

**R^2 = 0.98**

![Graph showing Cq vs 10^Y](image-url)
Establishing a protocol to quantify phages from wounds

qPCR limit of detection for human skin swab = $1.00 \times 10^6$ copies

Human skin wounds
Establishing a protocol to quantify phages from wounds

qPCR limit of detection for human skin swab = $1.00 \times 10^6$ copies

Human skin wounds
Establishing a protocol to quantify phages from wounds

qPCR limit of detection for human skin swab = $1.00 \times 10^6$ copies

Human skin wounds

Can quantify using qPCR

More than $10^6$ copies
Establishing a protocol to quantify phages from wounds

qPCR limit of detection for human skin swab = 1.00 x 10^6 copies

Human skin wounds

More than 10^6 copies

Less than 10^6 copies

Can quantify using qPCR
Establishing a protocol to quantify phages from wounds

qPCR limit of detection for human skin swab = $1.00 \times 10^6$ copies

Human skin wounds

- More than $10^6$ copies: Can quantify using qPCR
- Less than $10^6$ copies: Cannot quantify using qPCR
Establishing a protocol to quantify phages from wounds

qPCR limit of detection for human skin swab = $1.00 \times 10^6$ copies

Human skin wounds

More than $10^6$ copies

Can quantify using qPCR

Less than $10^6$ copies

Cannot quantify using qPCR
Sequencing phage M13 genome for quantification

Extracted Phage DNA
Sequencing phage M13 genome for quantification

- Mix with PhiX control plasmid
- Extracted Phage DNA
Sequencing phage M13 genome for quantification

- Mix with PhiX control plasmid
- Extracted Phage DNA
- Sequencing Library Preparation

Sequencing phage M13 genome for quantification
Sequencing phage M13 genome for quantification

- Extracted Phage DNA
- Mix with PhiX control plasmid
- Sequencing Library Preparation
- Illumina Next Generation Sequencing
Sequencing phage M13 genome for quantification

1. Extracted Phage DNA
2. Mix with PhiX control plasmid
3. Sequencing Library Preparation
4. Illumina Next Generation Sequencing
5. Galaxy Genome Assembly & Mapping
Sequencing phage M13 genome for quantification

1. Extracted Phage DNA
2. Mix with PhiX control plasmid
3. Sequencing Library Preparation
4. Illumina Next Generation Sequencing
5. Galaxy Genome Assembly & Mapping
6. Data Analysis
Applying the protocol to analyze human skin wounds

Extracted Phage DNA

Mix with PhiX control plasmid

Sequencing Library Preparation

Illumina Next Generation Sequencing

Galaxy Genome Assembly & Mapping

Data Analysis

Human skin wounds
Applying the protocol to analyze human skin wounds

Extracted Phage DNA

Mix with PhiX control plasmid

Sequencing Library Preparation

Illumina Next Generation Sequencing

Galaxy Genome Assembly & Mapping

Data Analysis

Apply the phage detection protocol

Human skin wounds
Applying the protocol to analyze human skin wounds

1. Mix with PhiX control plasmid
2. Sequencing Library Preparation
3. Illumina Next Generation Sequencing
4. Galaxy Genome Assembly & Mapping
5. Data Analysis

Data Analysis

- Apply the phage detection protocol
- Analyze the metagenomics data in the microbiome

Human skin wounds
Acknowledgments

Chen Group:
Celia Blanco
Gregory Campbell
Irene Chen
Michael Devano
Evan Janzen
Coulter Keeler

MARC U-STAR:
Arica Lubin
Stephanie Mendes
Joel Rothman

Thomas Nguyen
Abe Pressman
Ranajay Saha
Allison Tam
Claire Tran
Samuel Verbanic
Baoqing Zhou

Research reported here was supported by the National Institute Of General Medical Sciences of the National Institutes of Health under Award Number T34GM113848.
Quantifying phage M13 DNA by qPCR – Standard Curve

Serial dilution of M13mp18

- Baseline of detection = $1.3 \times 10^5$ copies/uL
- Linear equation, $R^2 = 0.98271$

This standard curve was used in the absolute quantification of our samples.
## Quantifying phage M13 DNA by qPCR – Control Samples

### 10-fold serial dilution of M13KE

![10-fold serial dilution of M13KE](image)

### Table: Expected vs. Observed

<table>
<thead>
<tr>
<th>Sample</th>
<th>Expected Total # of DNA</th>
<th>Observed Total # of DNA</th>
<th>Mean Cq</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>100x</td>
<td>5.0 x 10^{10}</td>
<td>4.63 x 10^{10}</td>
<td>2.87</td>
<td>0</td>
</tr>
<tr>
<td>10x</td>
<td>5.0 x 10^{9}</td>
<td>1.50 x 10^{9}</td>
<td>8.87</td>
<td>0.54</td>
</tr>
<tr>
<td>1x</td>
<td>5.0 x 10^{8}</td>
<td>3.57 x 10^{8}</td>
<td>11.38</td>
<td>0.76</td>
</tr>
<tr>
<td>0.1x</td>
<td>5.0 x 10^{7}</td>
<td>3.58 x 10^{7}</td>
<td>15.41</td>
<td>0.57</td>
</tr>
<tr>
<td>0.01x</td>
<td>5.0 x 10^{6}</td>
<td>2.60 x 10^{6}</td>
<td>19.99</td>
<td>1.31</td>
</tr>
<tr>
<td>0.001x</td>
<td>5.0 x 10^{5}</td>
<td>5.70 x 10^{5}</td>
<td>22.65</td>
<td>0.30</td>
</tr>
<tr>
<td>0.0001x</td>
<td>5.0 x 10^{4}</td>
<td>1.84 x 10^{5}</td>
<td>24.62</td>
<td>0.17</td>
</tr>
<tr>
<td>Negative Control (PBS)</td>
<td>0</td>
<td>2.36 x 10^{4}</td>
<td>28.22</td>
<td>2.82</td>
</tr>
</tbody>
</table>

### Graph: Log Starting Quantity vs. Cq (# of cycles)

- Baseline of detection for M13KE = 2.36 x 10^4 copies

Control samples were compared to skin swab samples.
Quantifying phage M13 DNA by qPCR – Swab Samples

10-fold serial dilution of M13KE

<table>
<thead>
<tr>
<th>Sample</th>
<th>Expected Total # of DNA</th>
<th>Observed Total # of DNA</th>
<th>Mean Cq</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>100x</td>
<td>$1.0 \times 10^{10}$</td>
<td>$2.86 \times 10^{10}$</td>
<td>7.75</td>
<td>0</td>
</tr>
<tr>
<td>10x</td>
<td>$1.0 \times 10^{9}$</td>
<td>$1.12 \times 10^{9}$</td>
<td>9.38</td>
<td>0.89</td>
</tr>
<tr>
<td>1x</td>
<td>$1.0 \times 10^{8}$</td>
<td>$1.31 \times 10^{8}$</td>
<td>13.13</td>
<td>0.65</td>
</tr>
<tr>
<td>0.1x</td>
<td>$1.0 \times 10^{7}$</td>
<td>$2.56 \times 10^{7}$</td>
<td>15.99</td>
<td>0.26</td>
</tr>
<tr>
<td>0.01x</td>
<td>$1.0 \times 10^{6}$</td>
<td>$7.68 \times 10^{6}$</td>
<td>18.09</td>
<td>0.13</td>
</tr>
<tr>
<td>0.001x</td>
<td>$1.0 \times 10^{5}$</td>
<td>$4.61 \times 10^{6}$</td>
<td>18.99</td>
<td>0.71</td>
</tr>
<tr>
<td>0.0001x</td>
<td>$1.0 \times 10^{4}$</td>
<td>$7.20 \times 10^{6}$</td>
<td>18.21</td>
<td>0.24</td>
</tr>
<tr>
<td>Negative Control (PBS)</td>
<td>0</td>
<td>$7.04 \times 10^{6}$</td>
<td>18.24</td>
<td>0.13</td>
</tr>
</tbody>
</table>

- Limit of detection for human skin swab = $1.0 \times 10^6$ copies
- Contaminating sequences on human skin