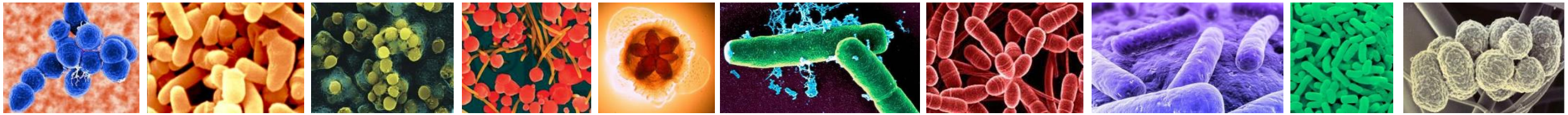


Detecting and Quantifying Bacteriophage M13 on Human Skin

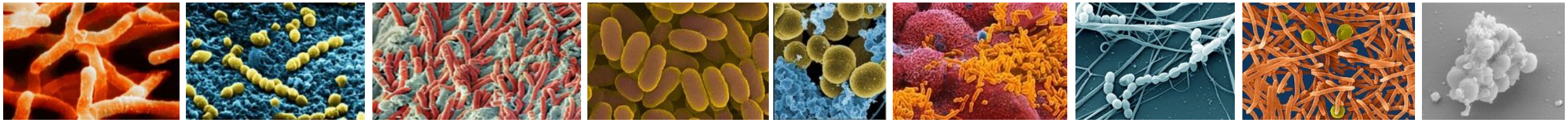
Young Hun (Colin) Kim

Baoqing Zhou

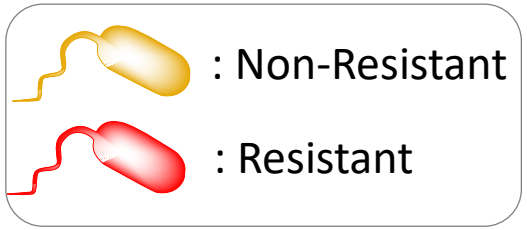
Biochemistry, College of Creative Studies

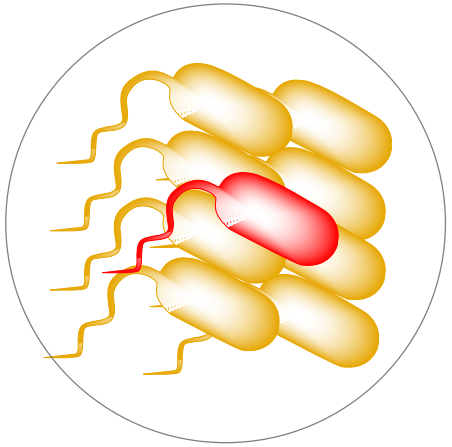
Chen Lab

Department of Chemistry and Biochemistry, UCSB



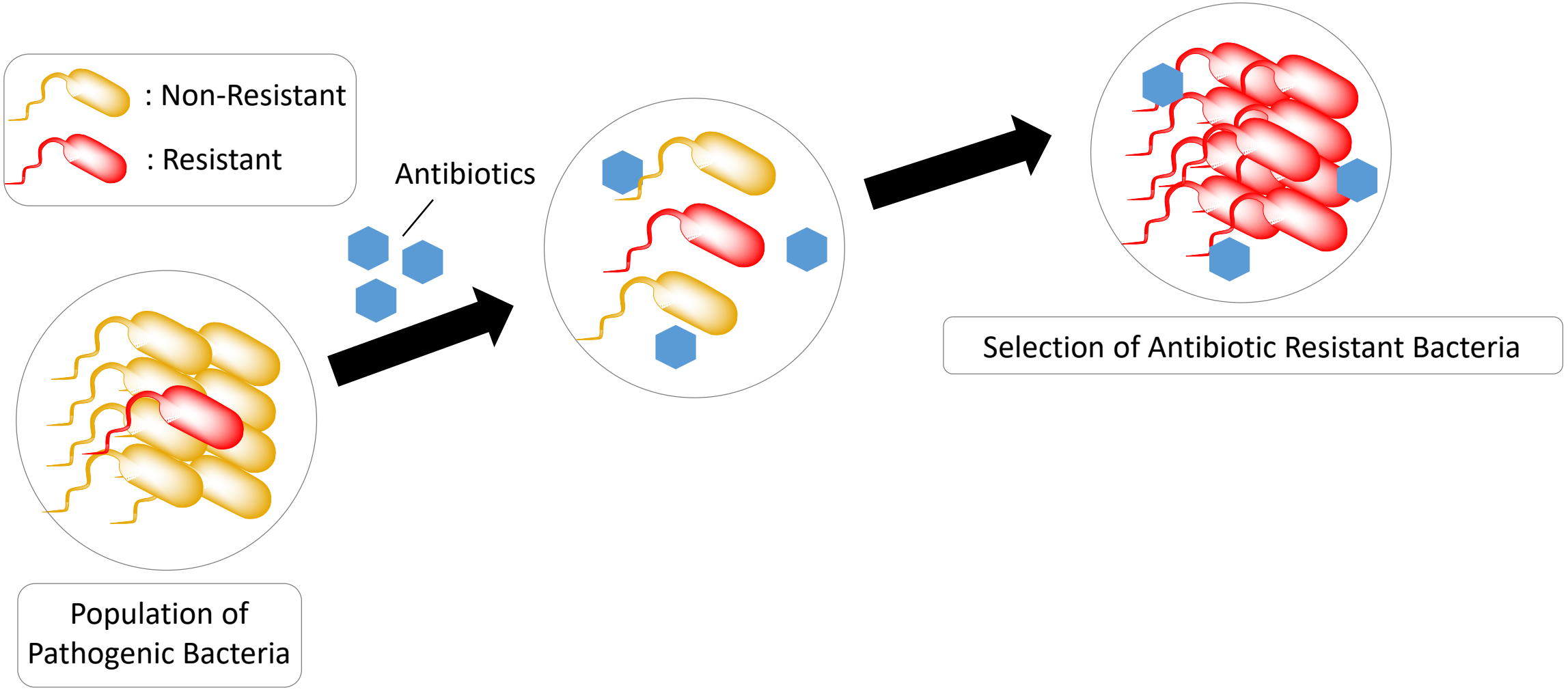
Rapid evolution of antibiotic resistant bacteria





Population of
Pathogenic Bacteria

Rapid evolution of antibiotic resistant bacteria



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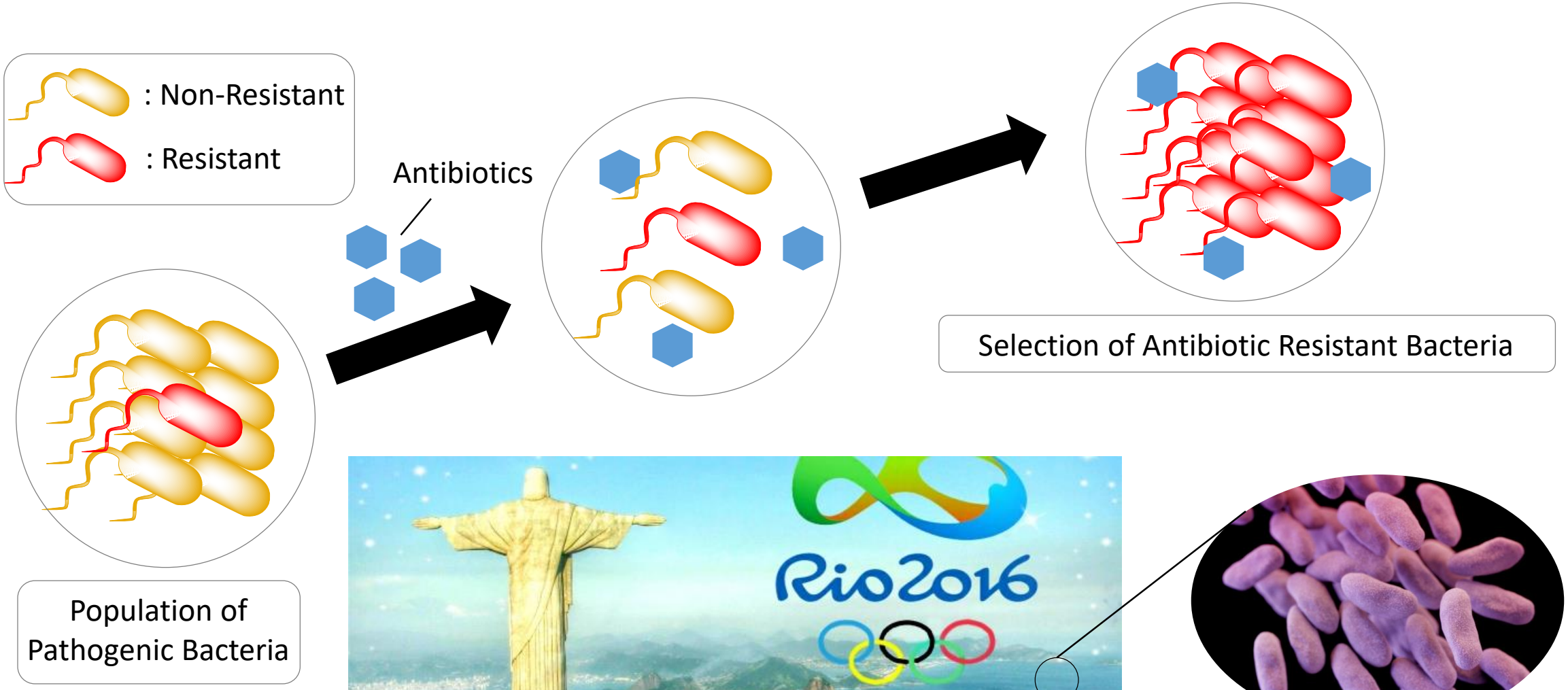
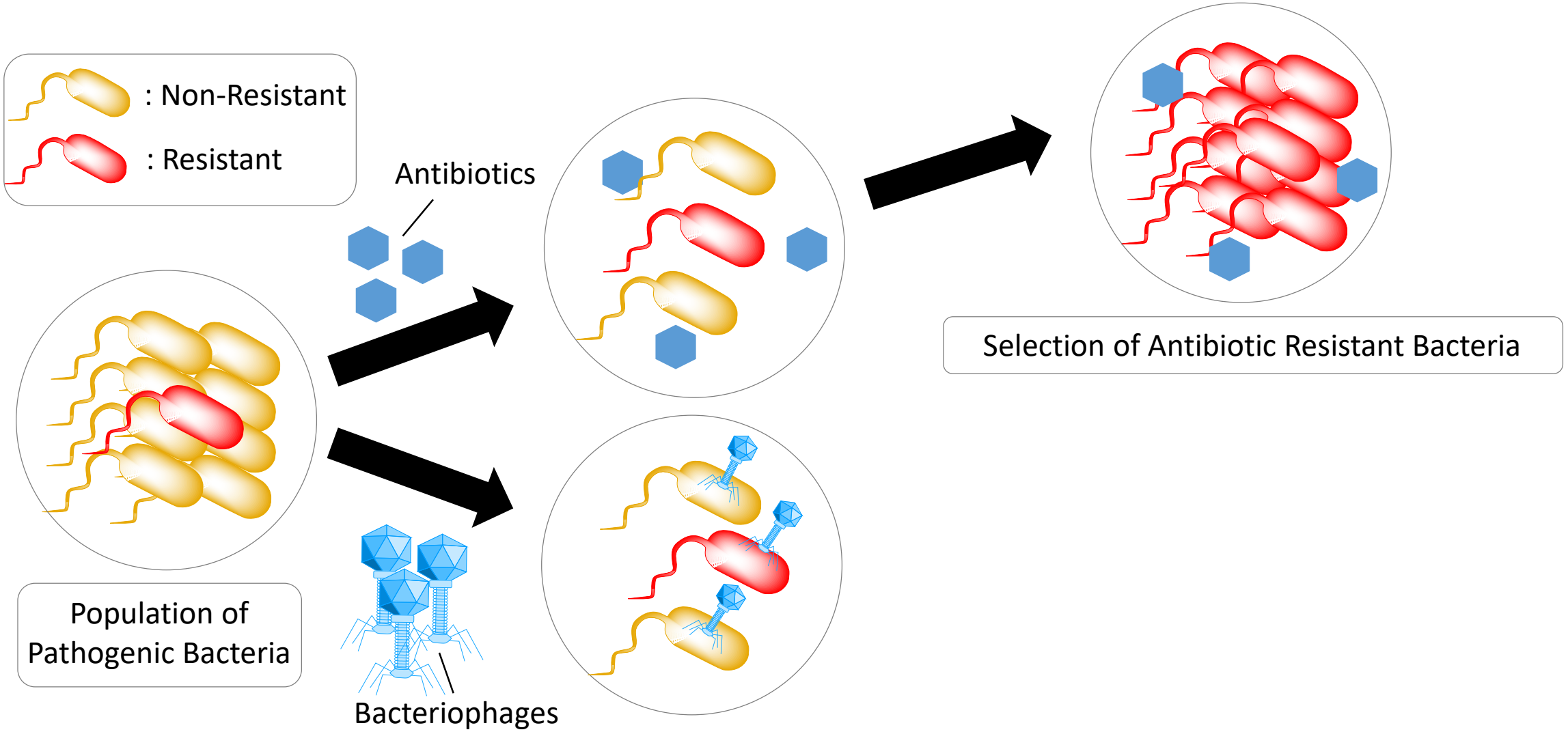


Bacteriophages: natural viruses that infect bacteria



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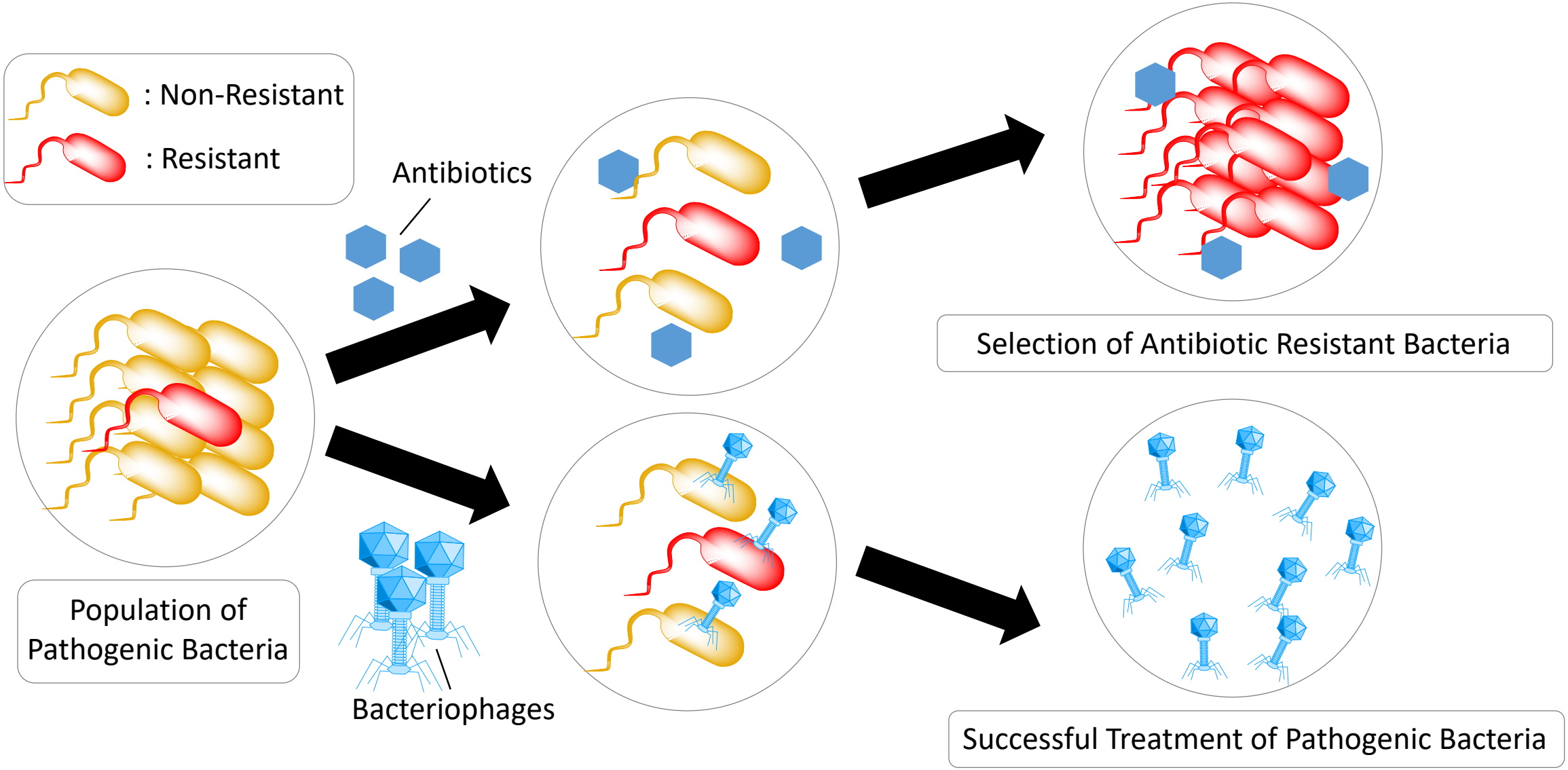

Characterizing the microbial community of wounds

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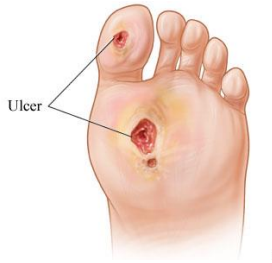

Diabetic Foot Ulcers

Surgical Wounds

Characterizing the microbial community of wounds

Diabetic Foot Ulcers

Surgical Wounds

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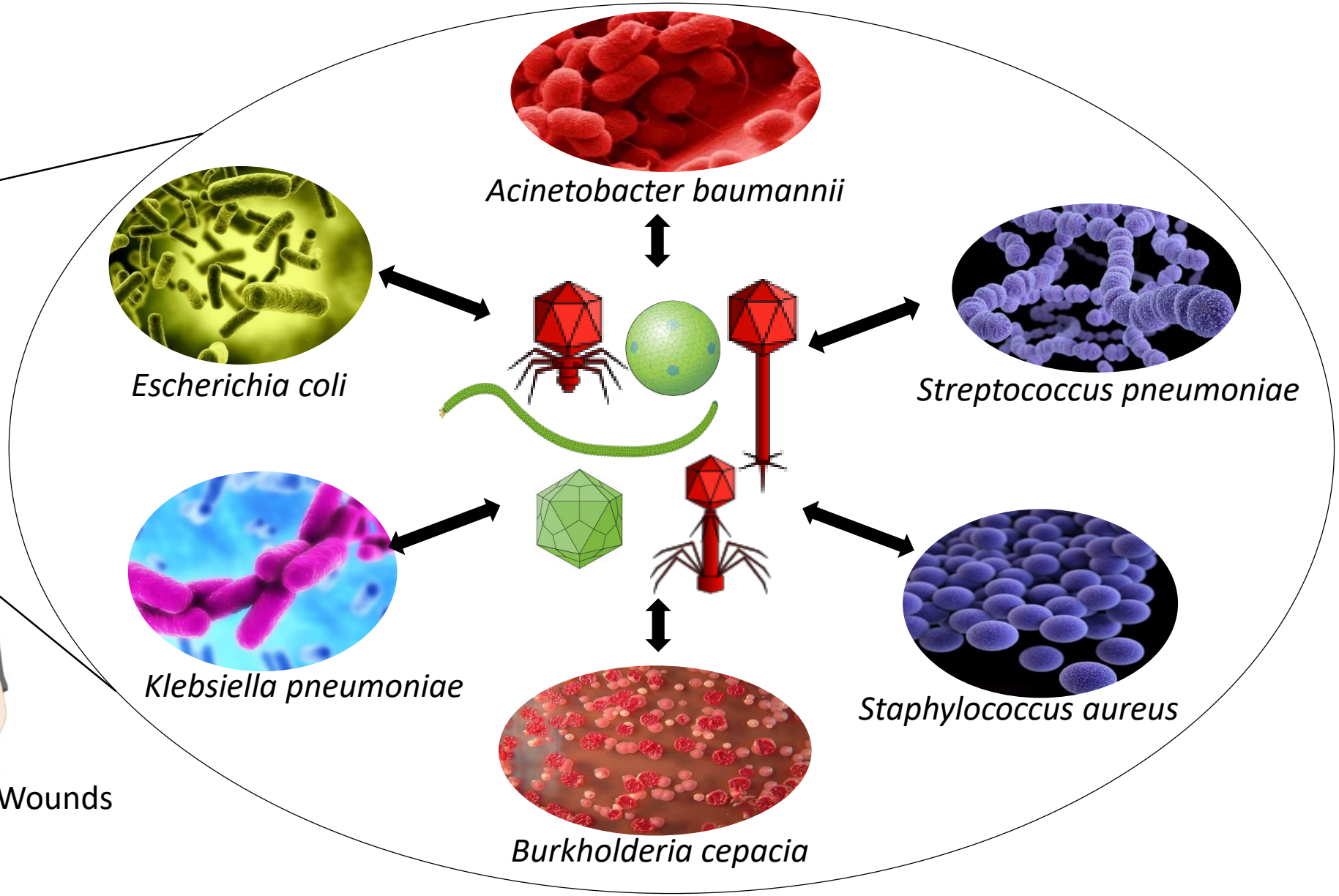
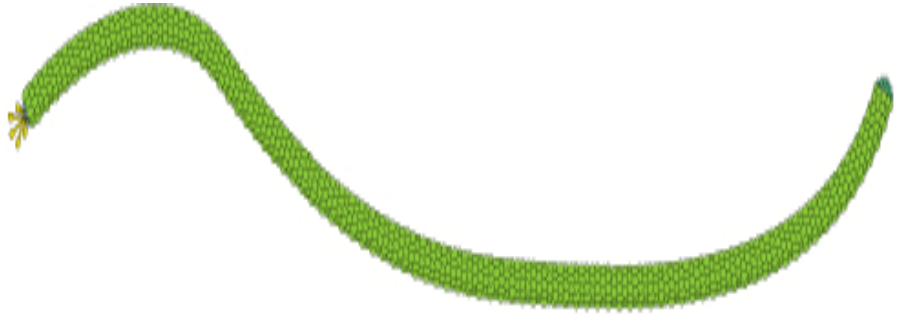
Characterizing the microbial community of wounds



Diabetic Foot Ulcers

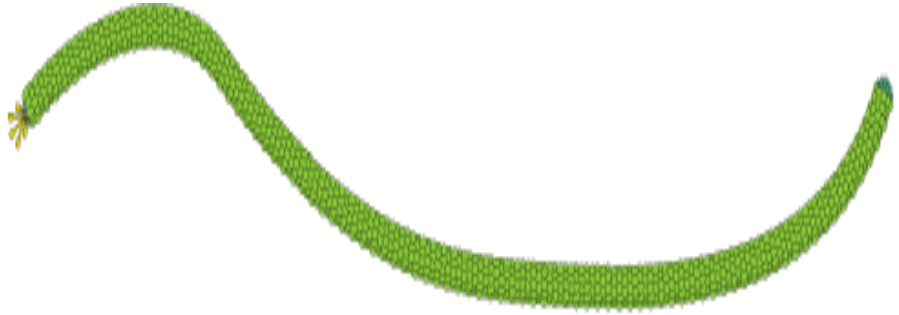
Surgical Wounds

Using phage M13 to develop the skin swab protocol



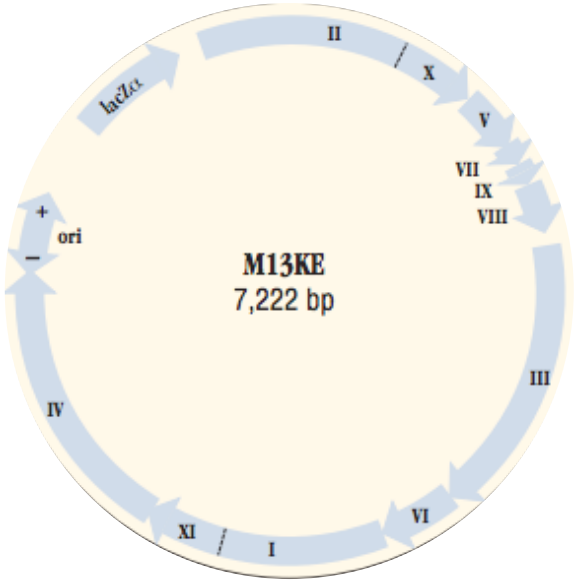
Bacteriophage M13

Using phage M13 to develop the skin swab protocol

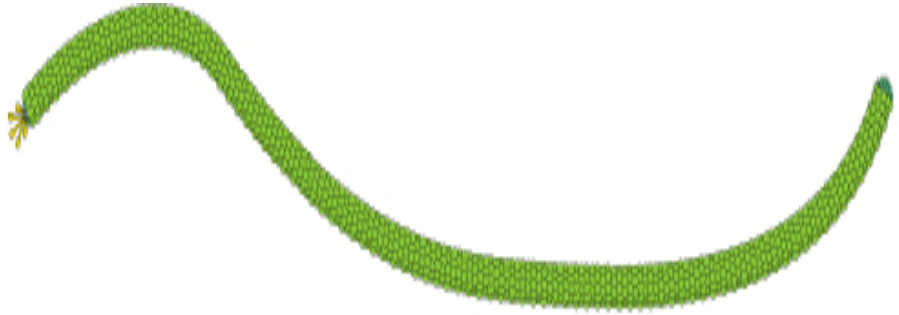


Bacteriophage M13

Escherichia coli



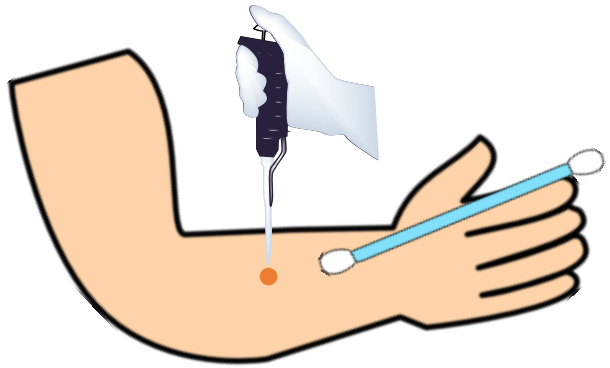
Using phage M13 to develop the skin swab protocol

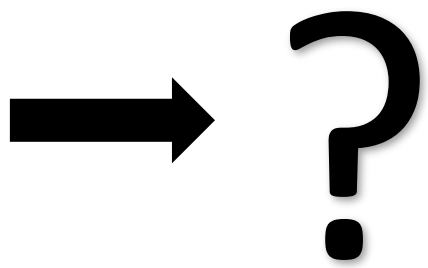


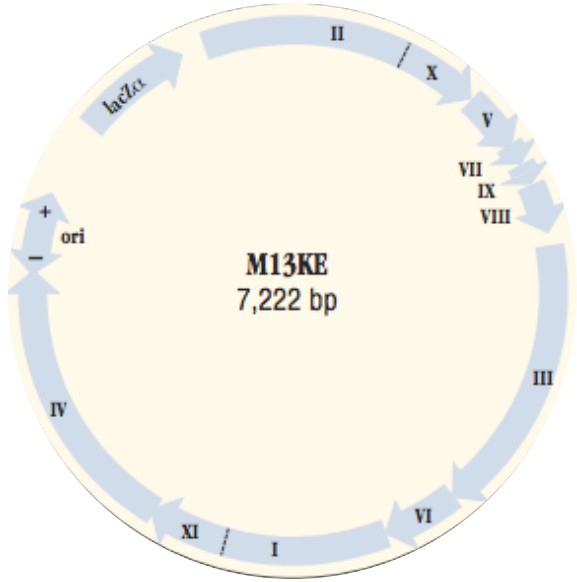
Bacteriophage M13

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

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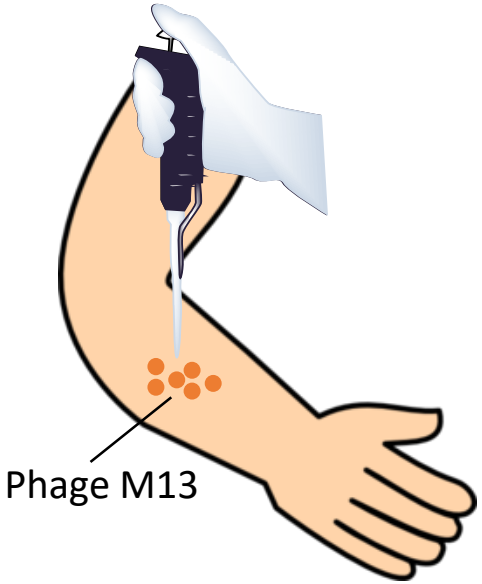

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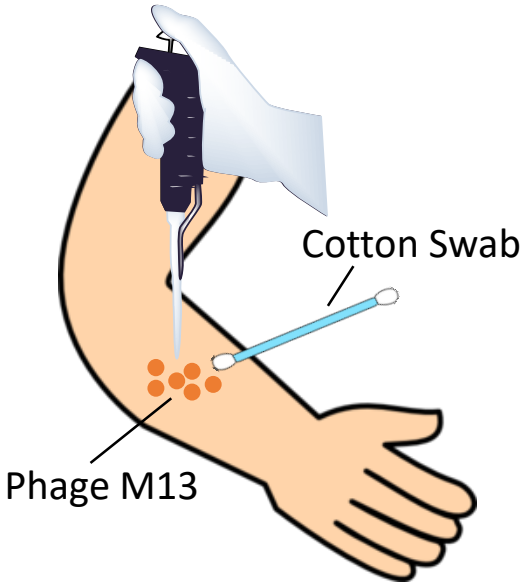

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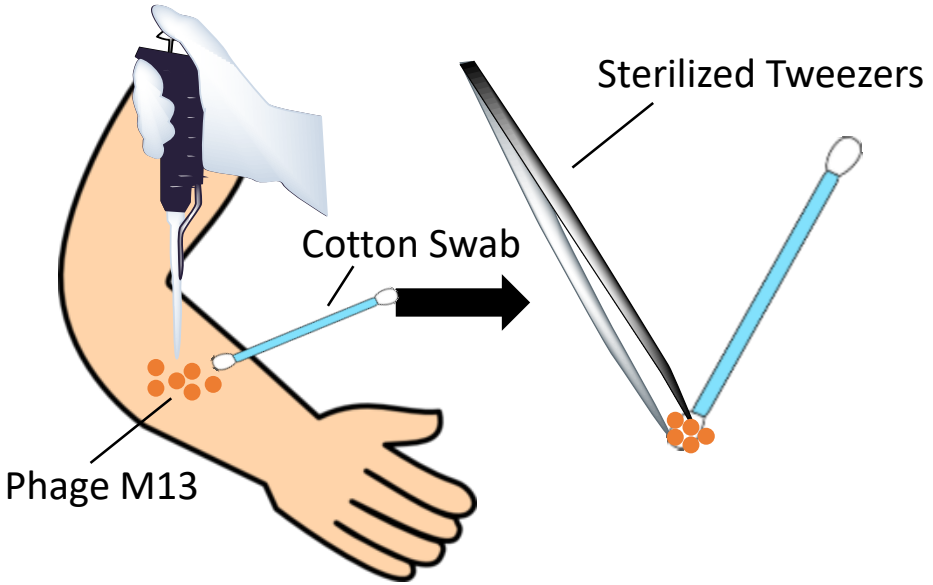

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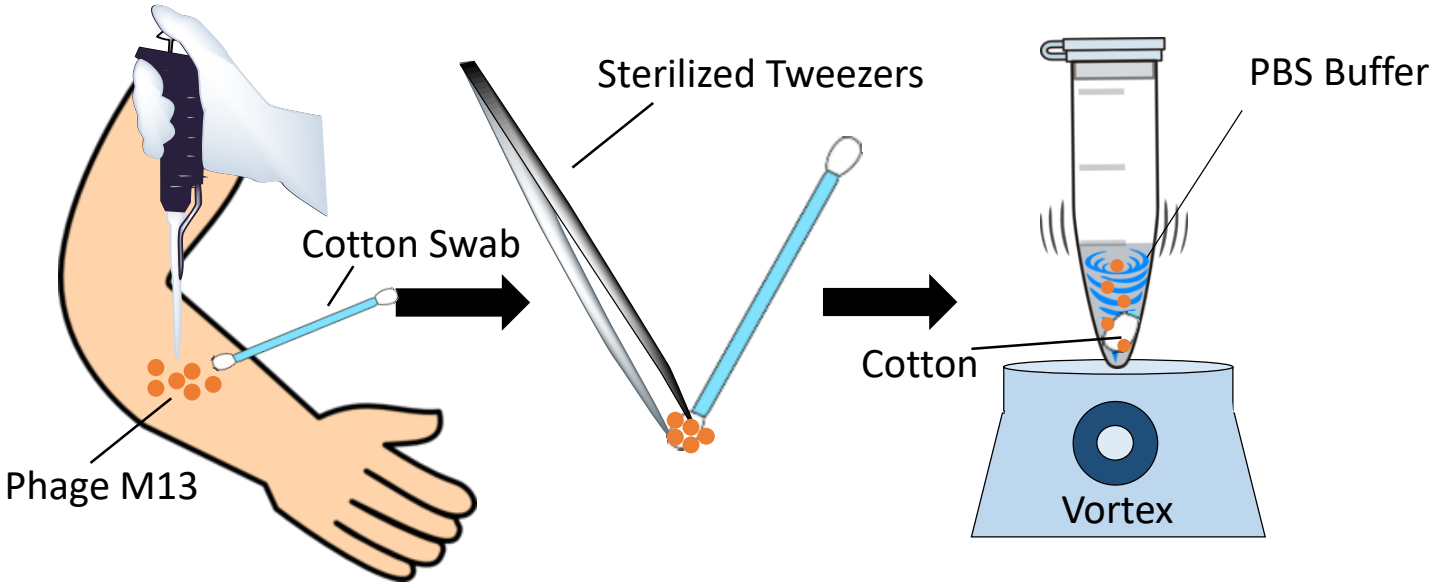

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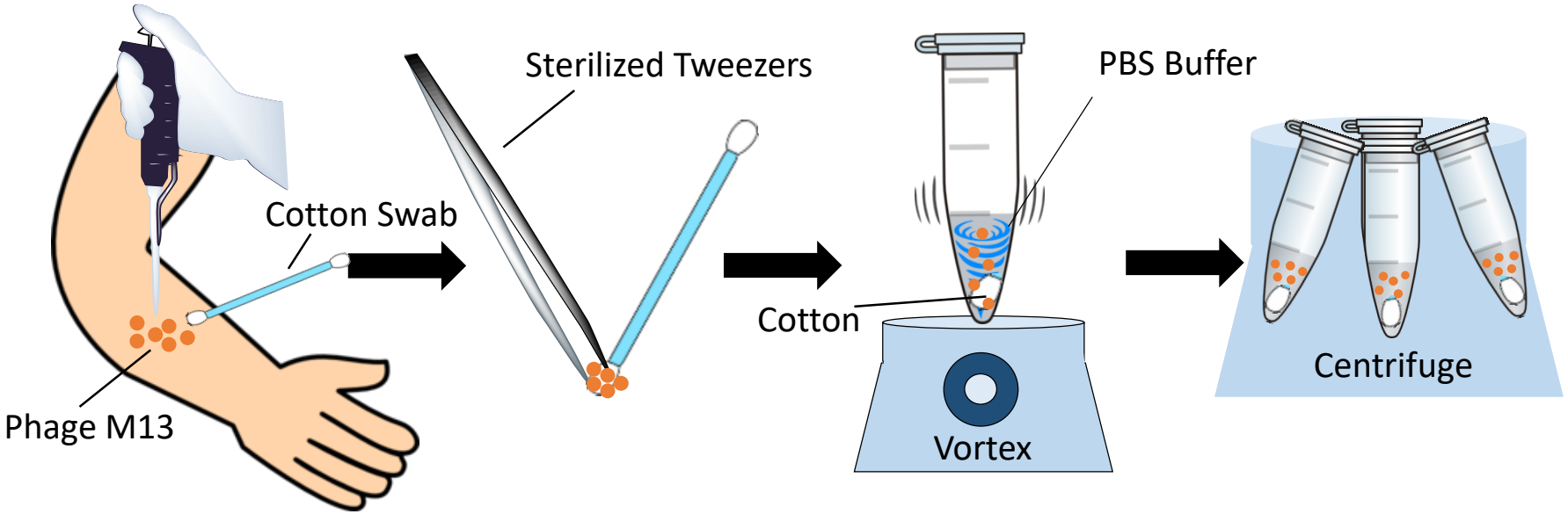

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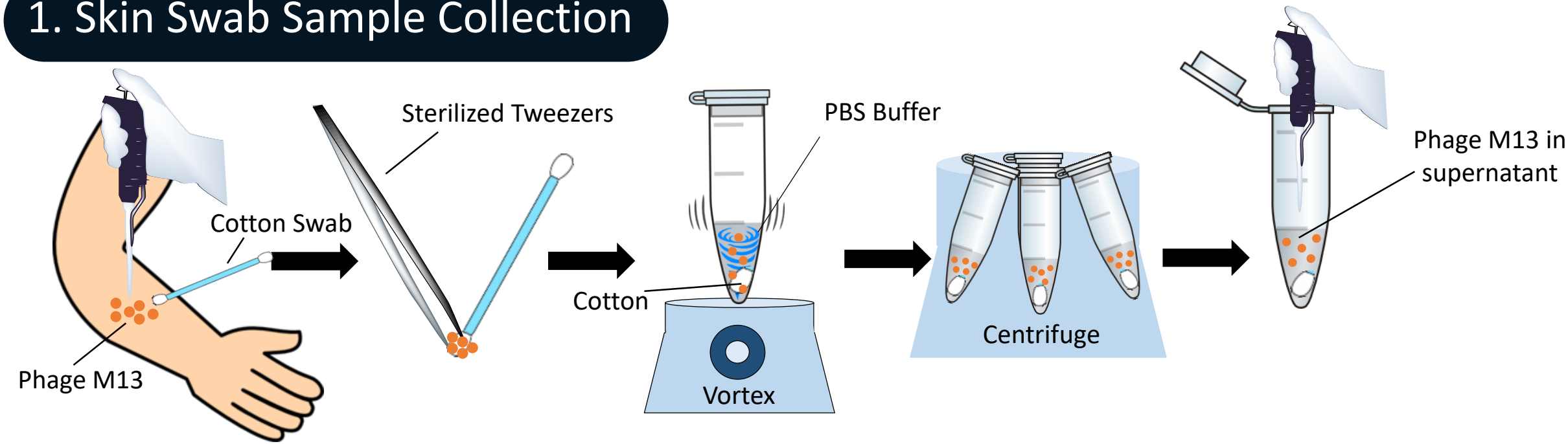

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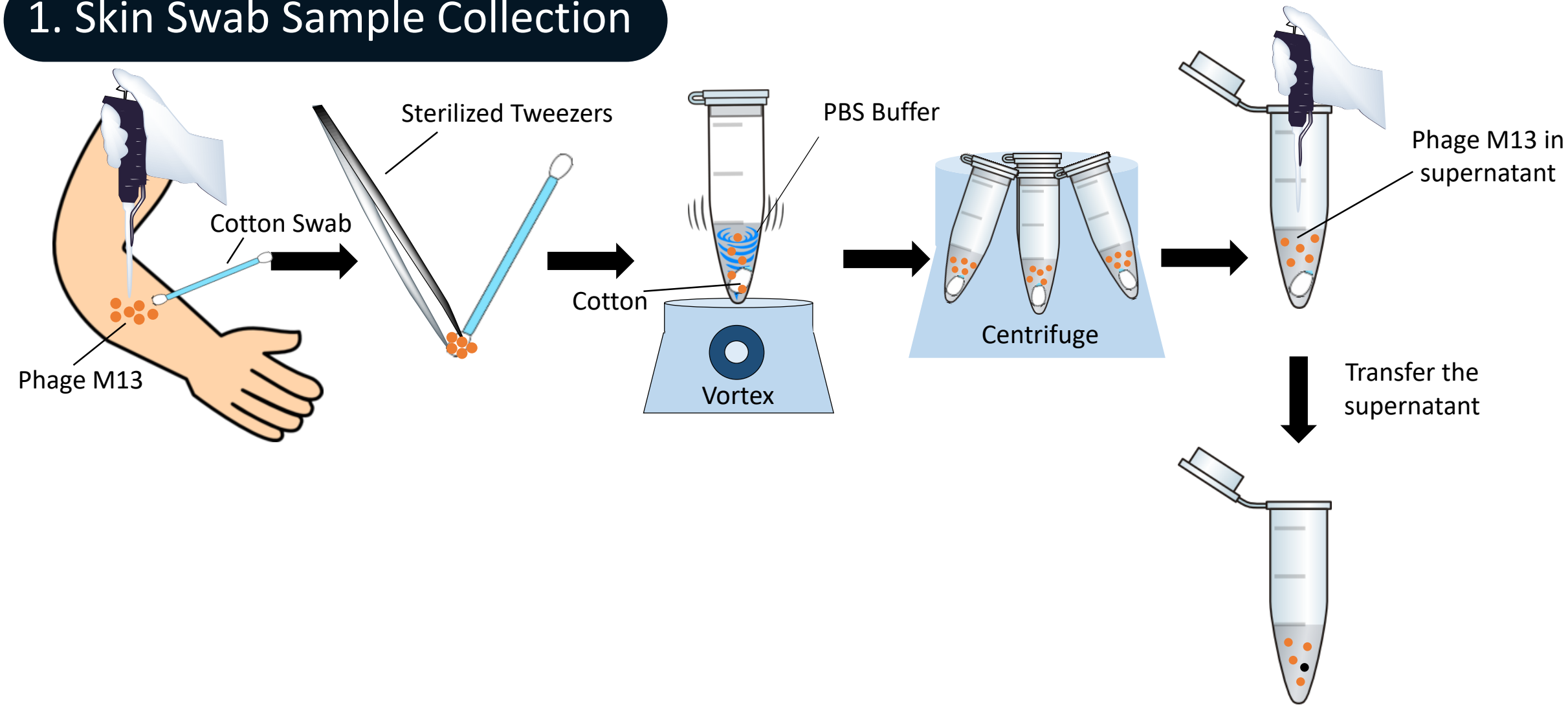

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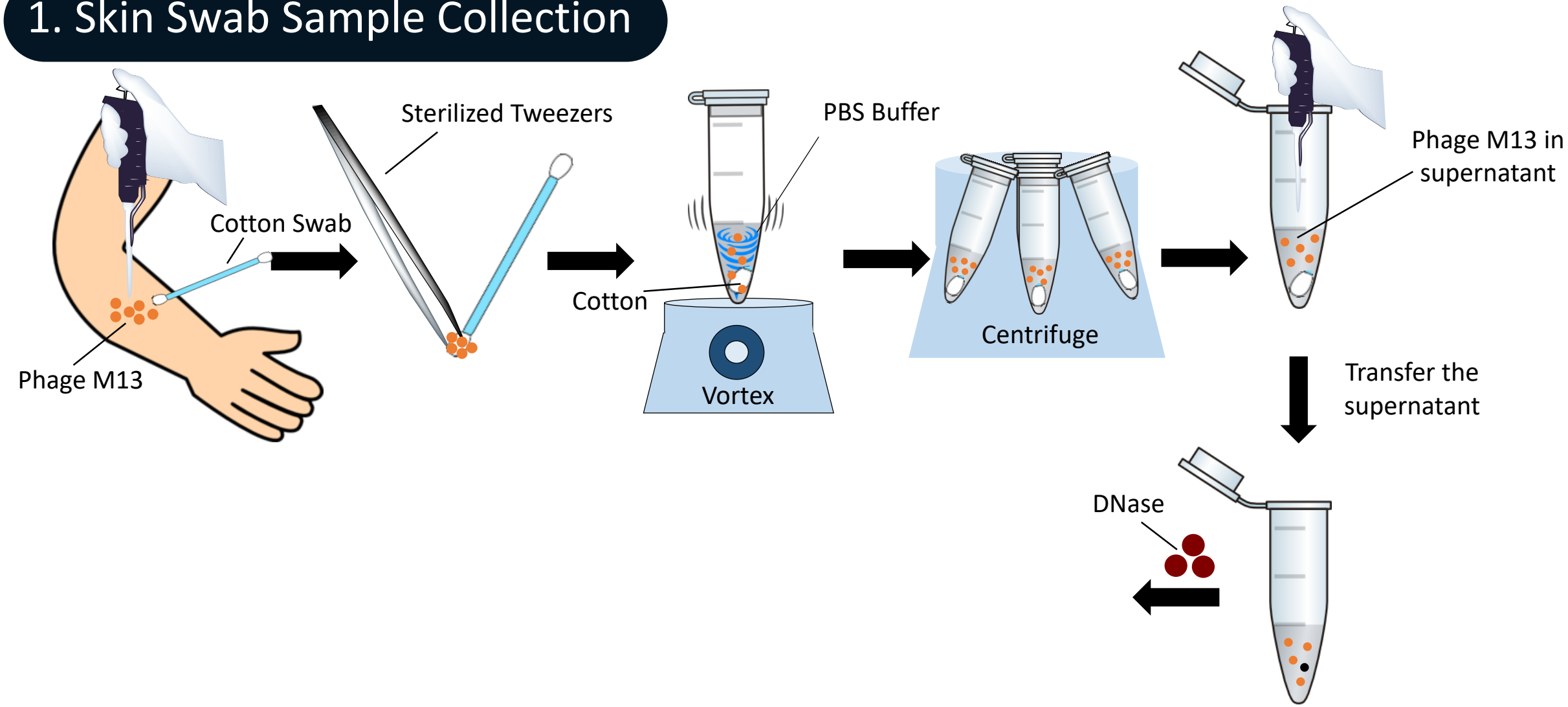

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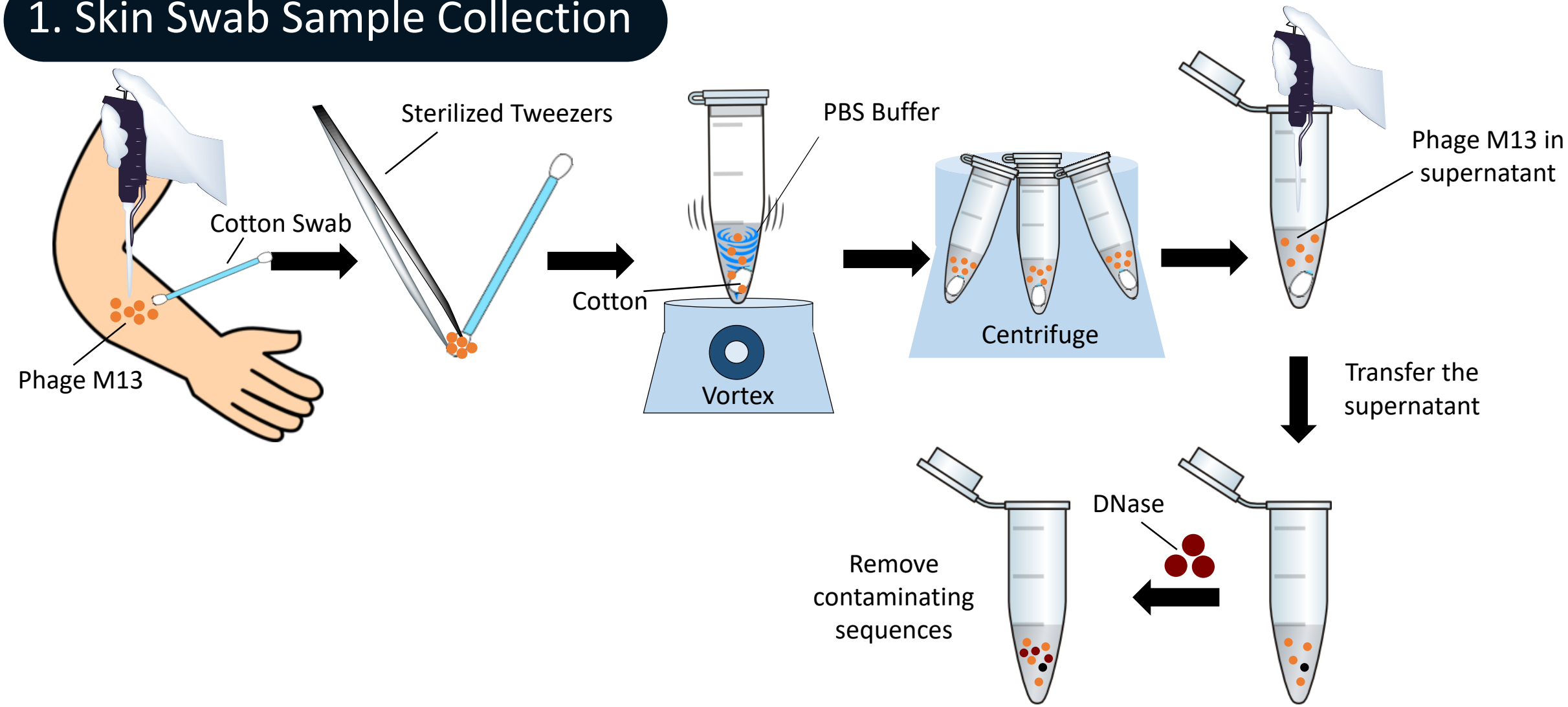

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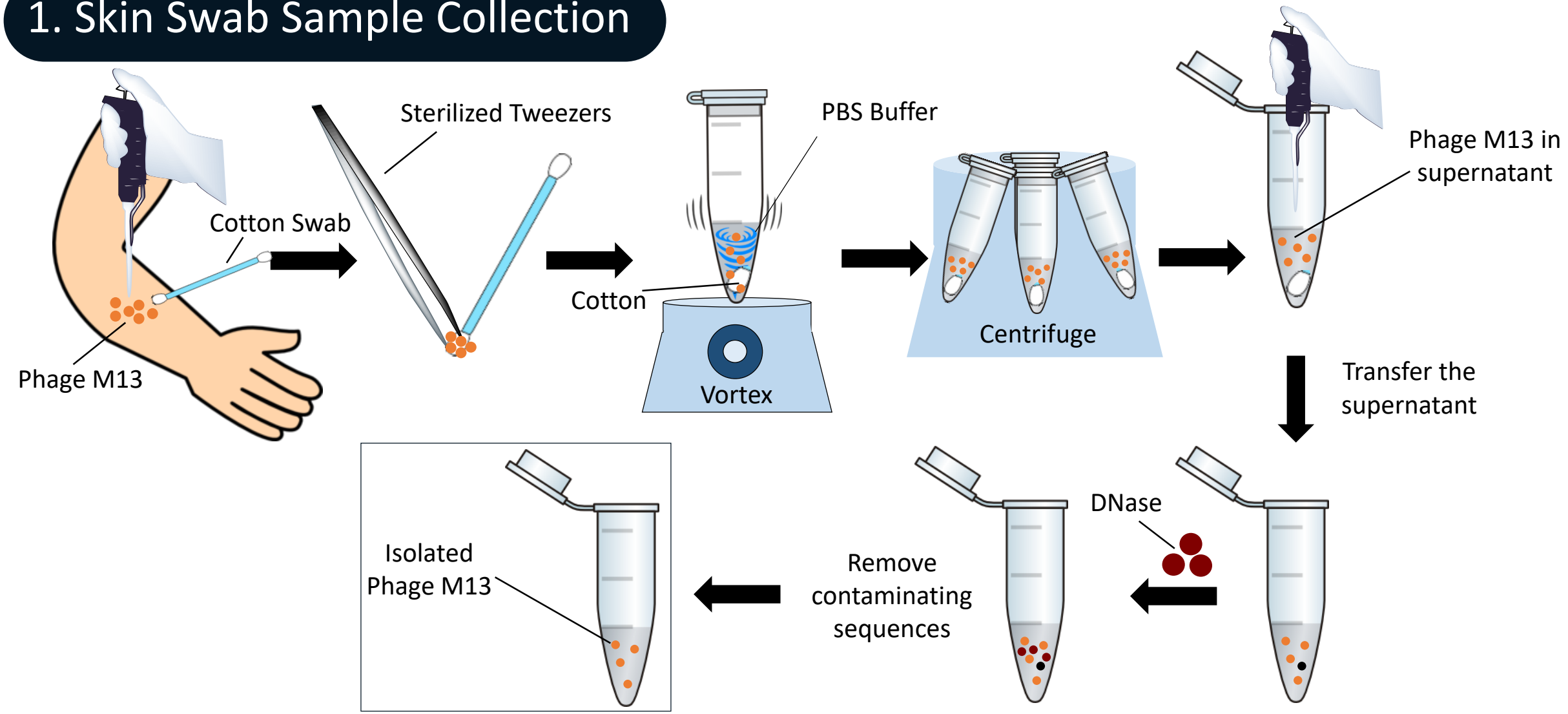
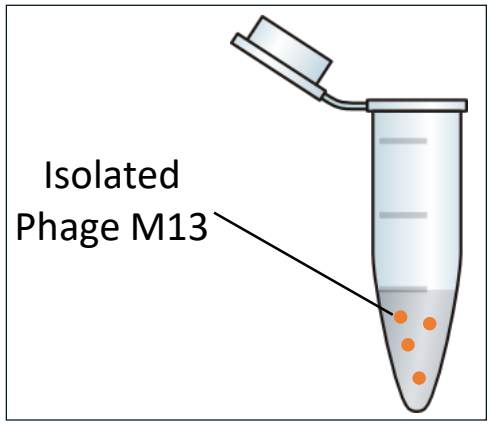


Recovering phage M13 from human skin swab

2. Phage DNA Extraction



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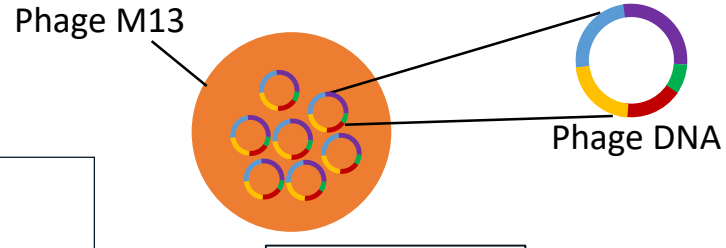

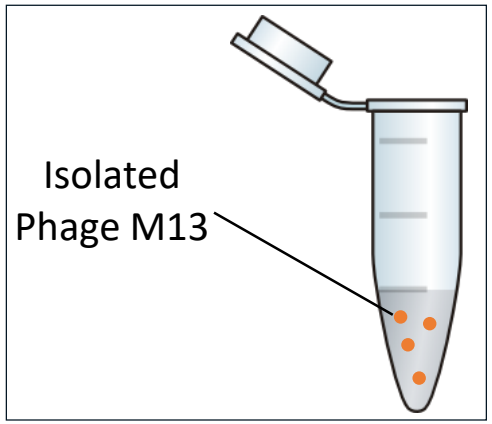

PureLink
Viral DNA
Extraction
Mini Kit

Recovering phage M13 from human skin swab

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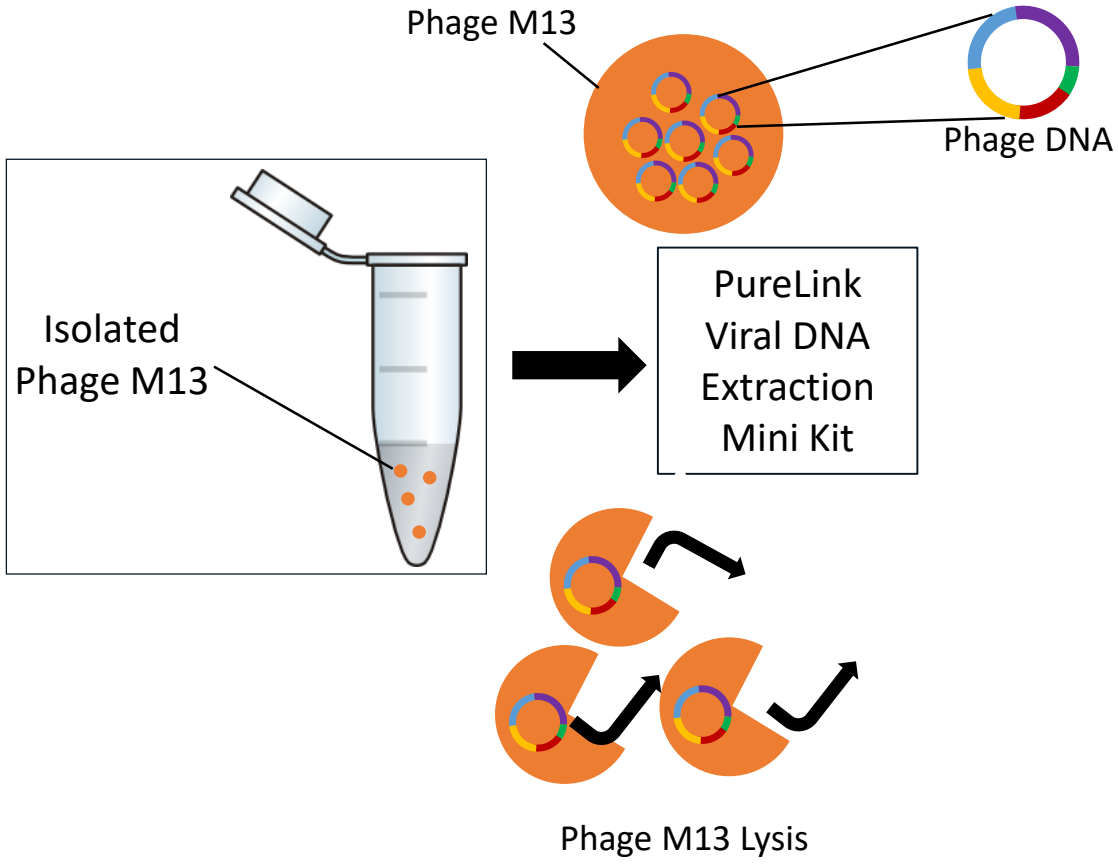

Recovering phage M13 from human skin swab

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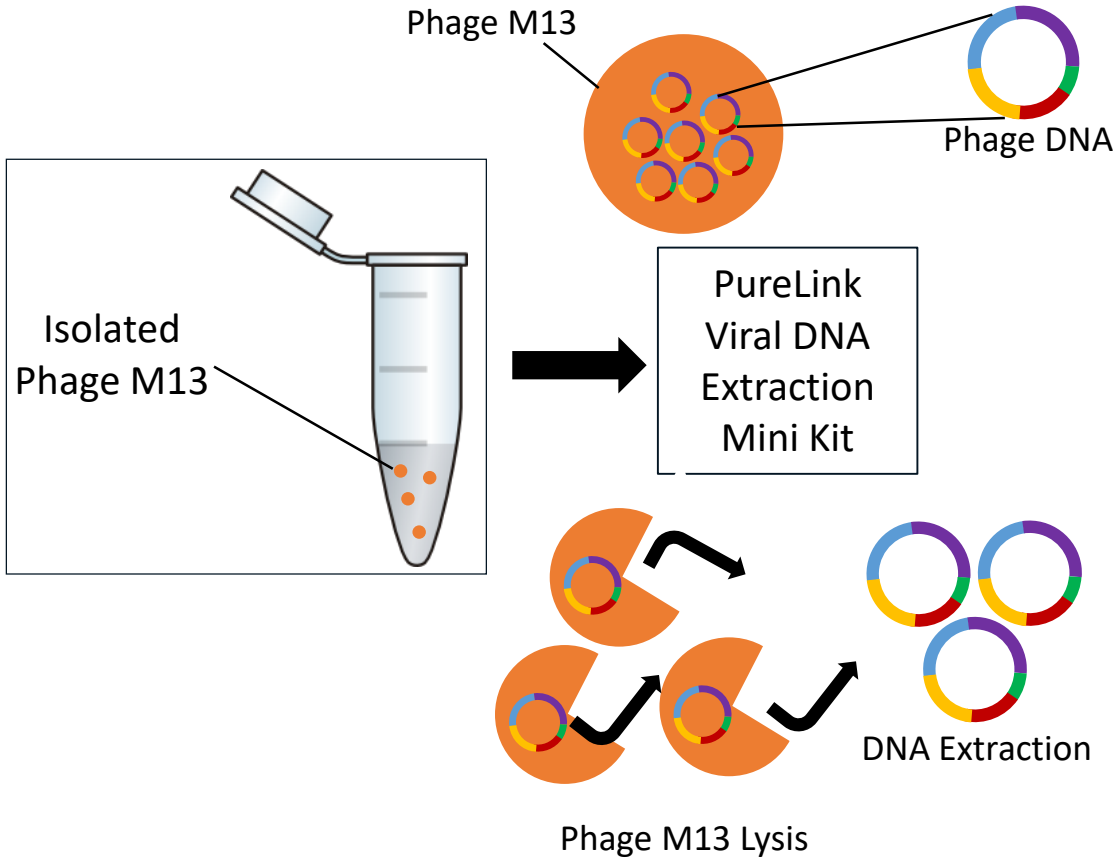

Recovering phage M13 from human skin swab

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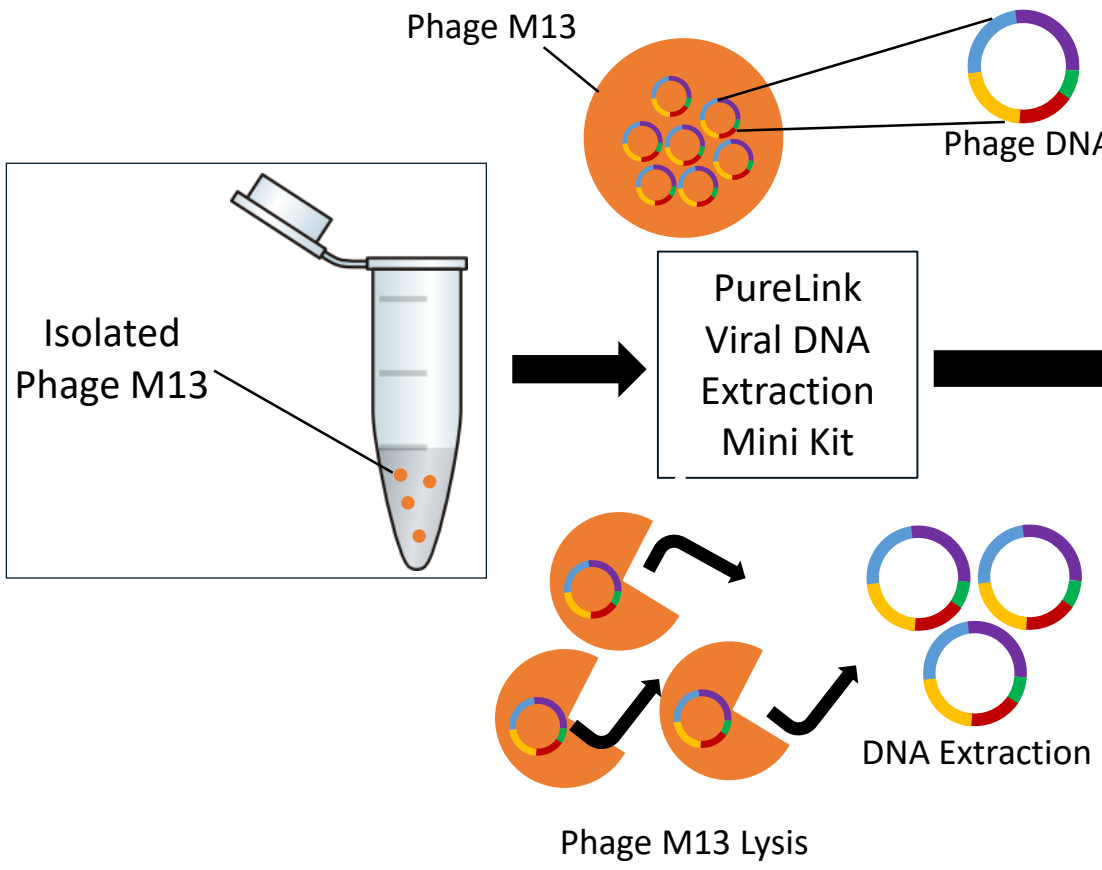
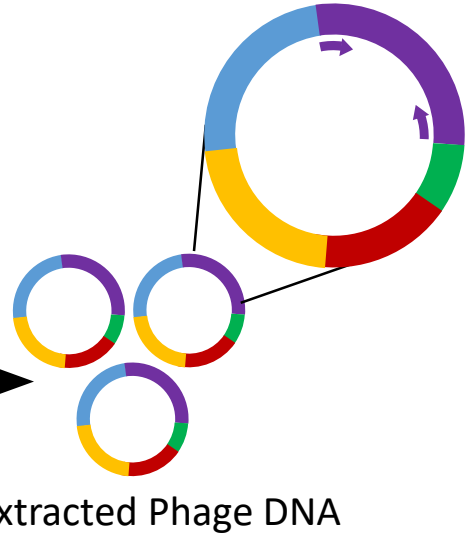


3. Quantification of Phage DNA



Recovering phage M13 from human skin swab

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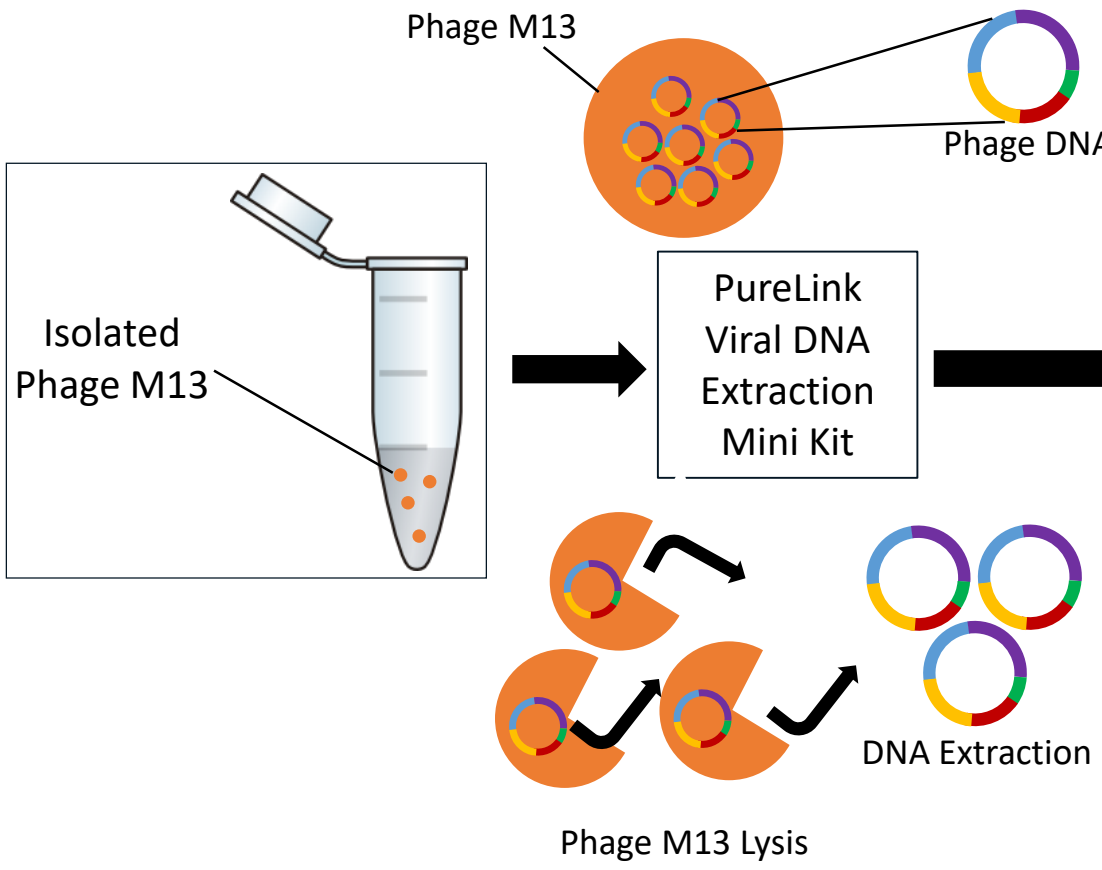

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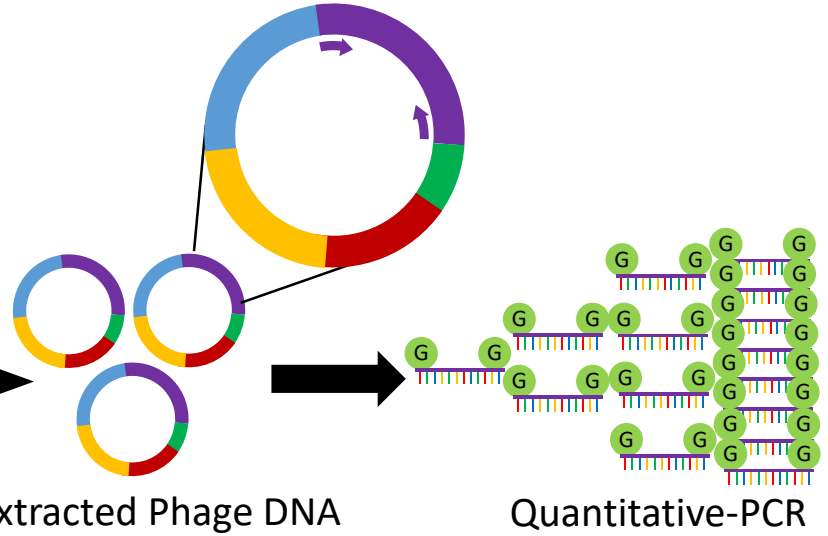

Recovering phage M13 from human skin swab

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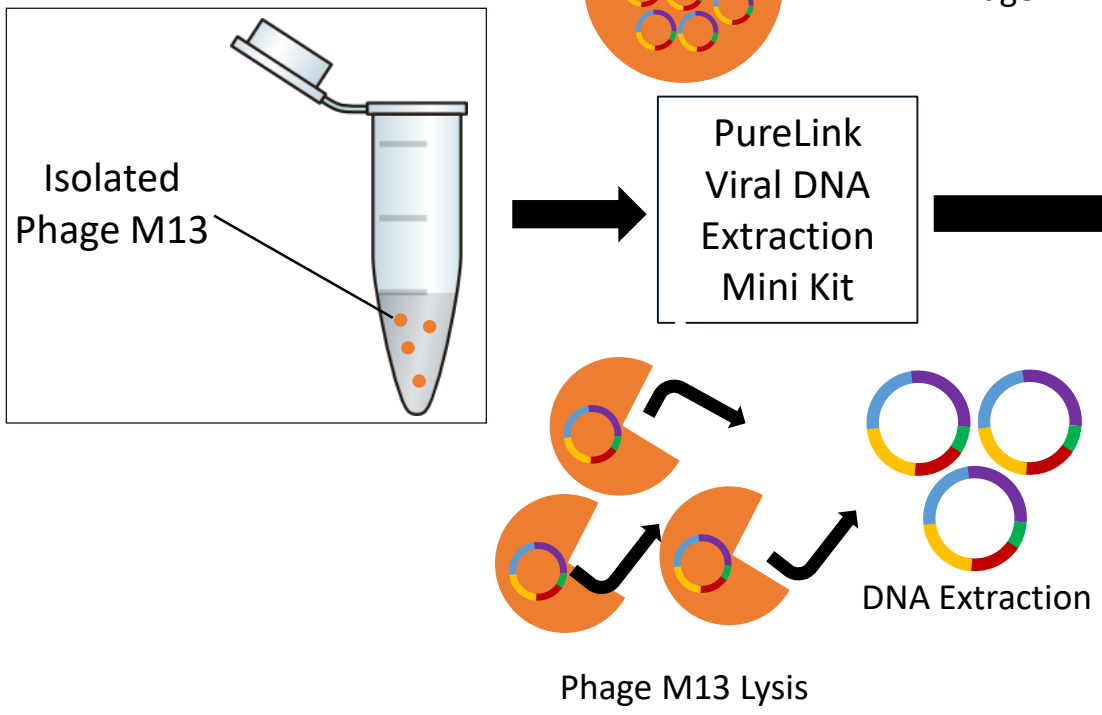

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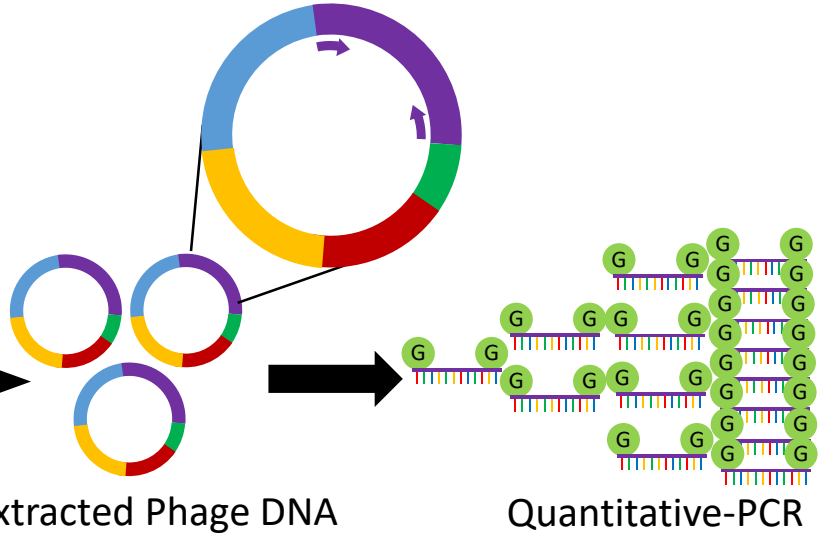

qPCR Principles

Recovering phage M13 from human skin swab

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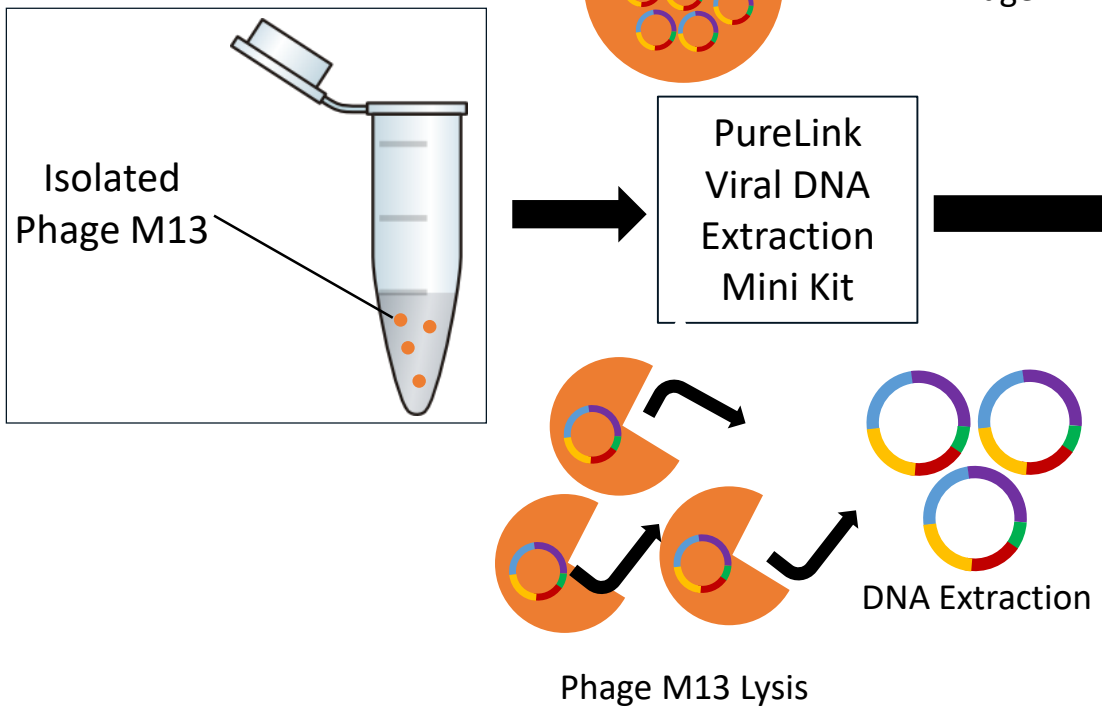

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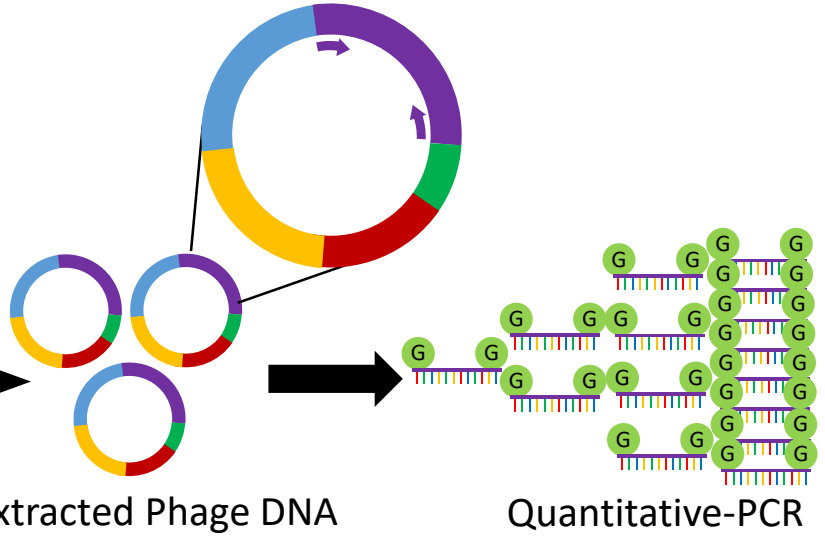
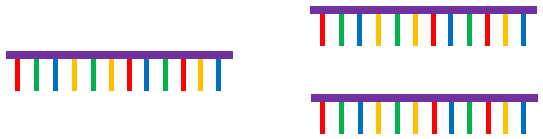


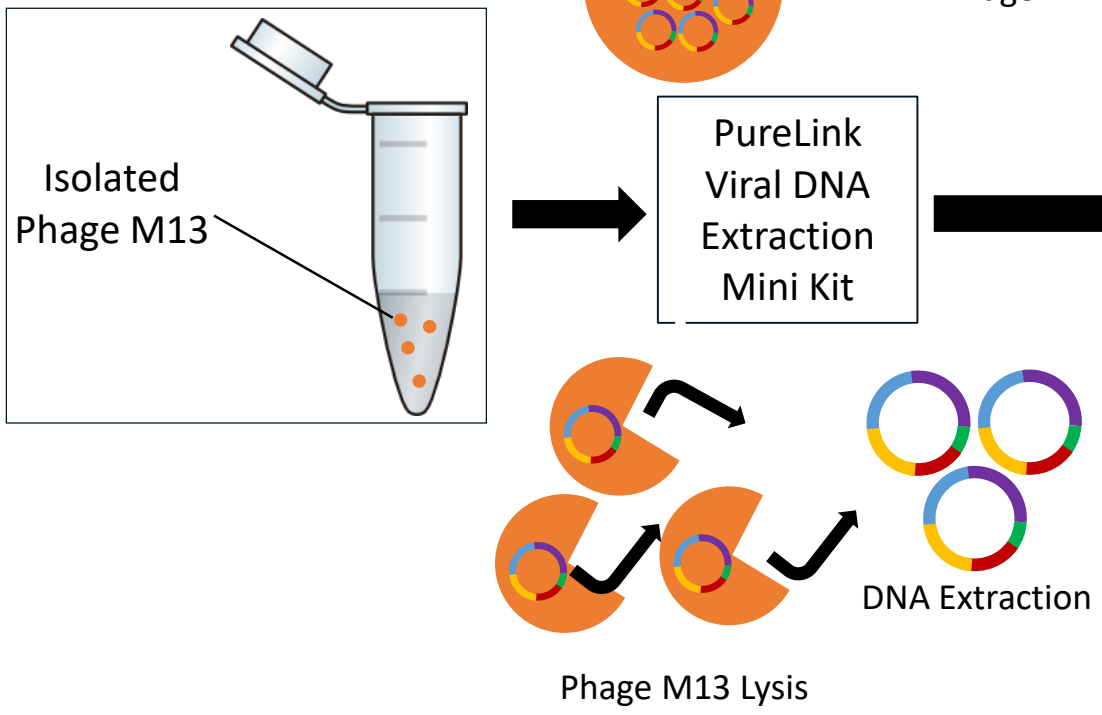
qPCR Principles



n=1

Recovering phage M13 from human skin swab

2. Phage DNA Extraction



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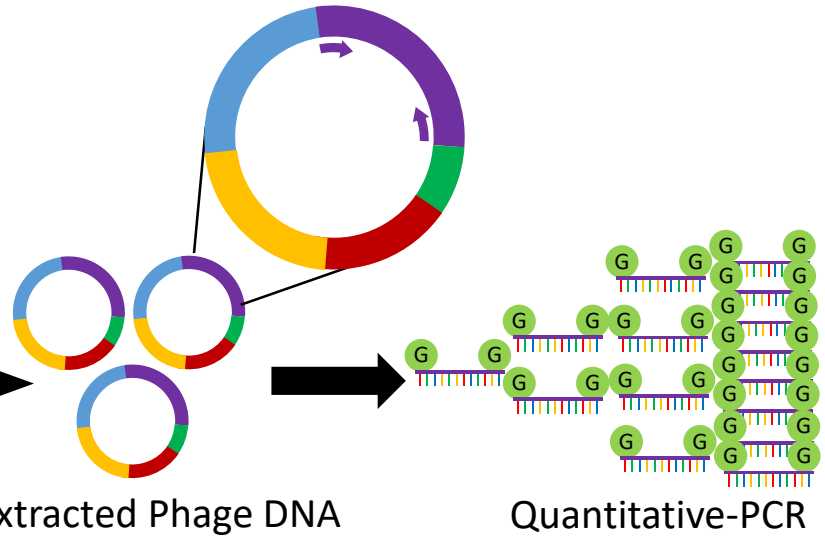

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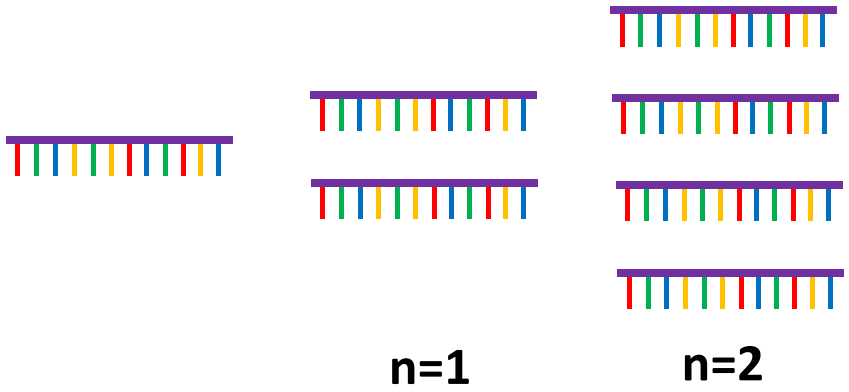
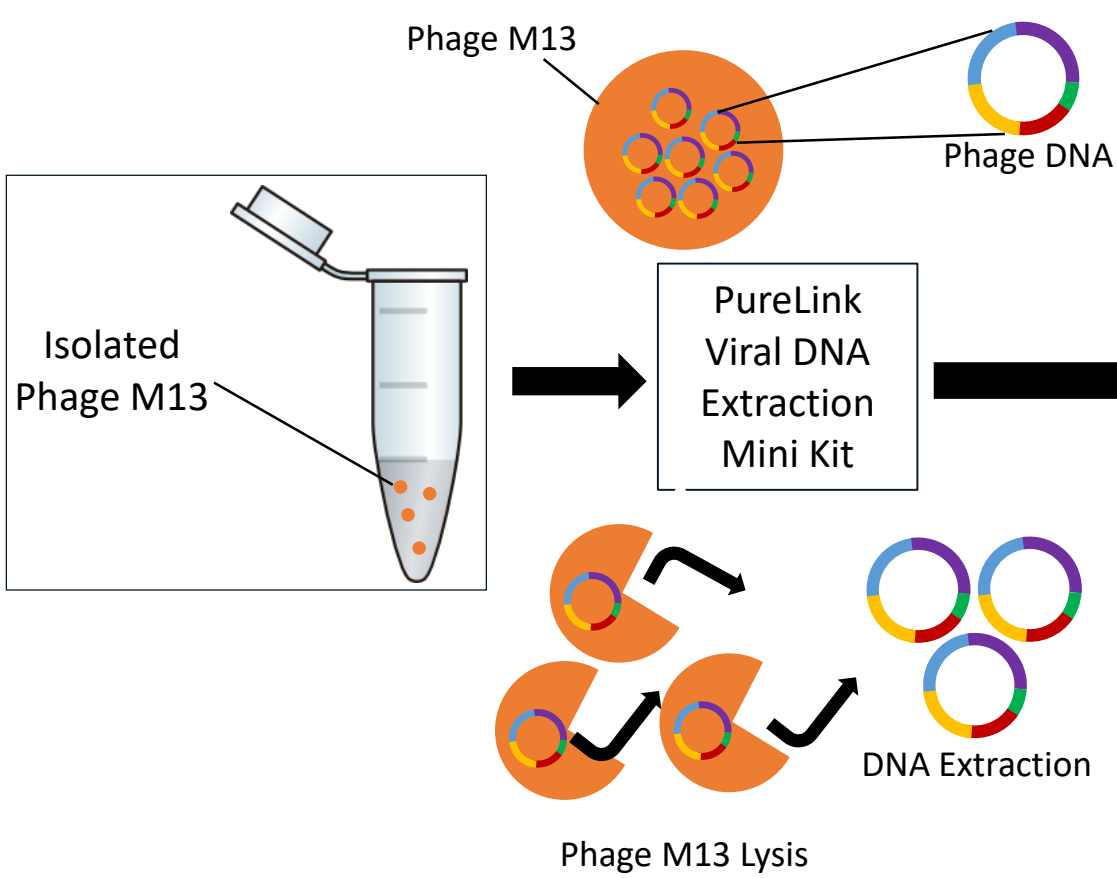


$\ldots\ \ldots\ 2^n$

n = number of cycles

Recovering phage M13 from human skin swab

2. Phage DNA Extraction



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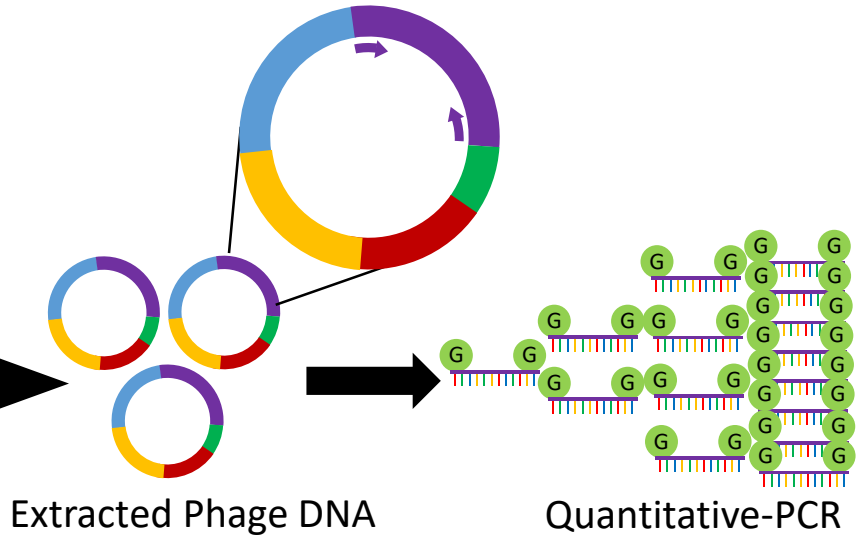
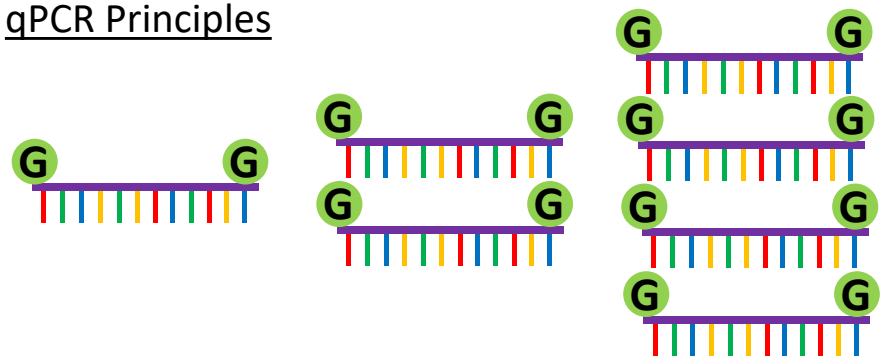


qPCR Principles



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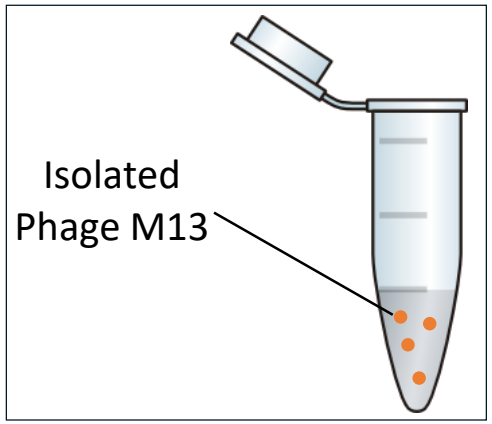
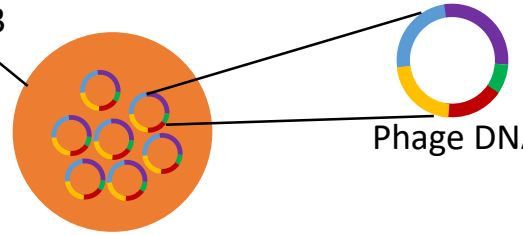
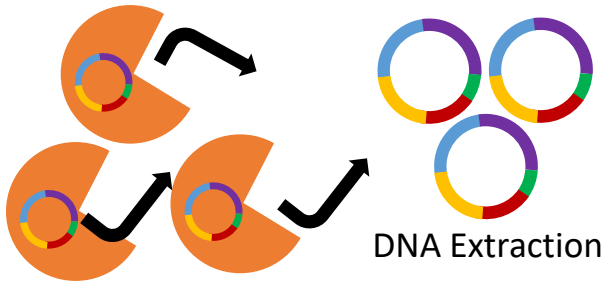
Recovering phage M13 from human skin swab

2. Phage DNA Extraction





PureLink Viral DNA Extraction Mini Kit



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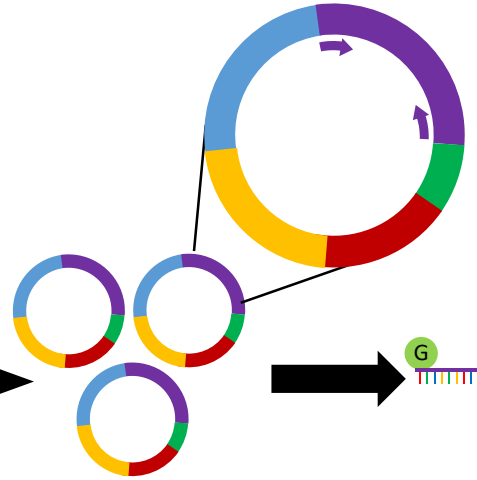
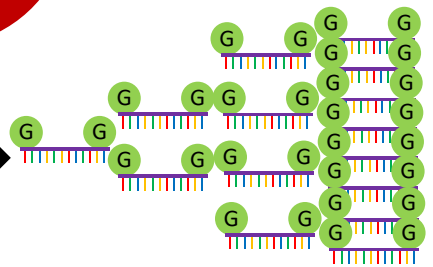
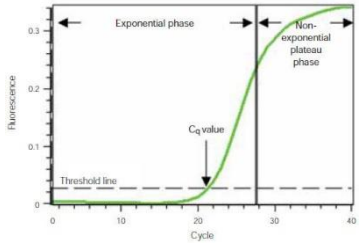


Extracted Phage DNA

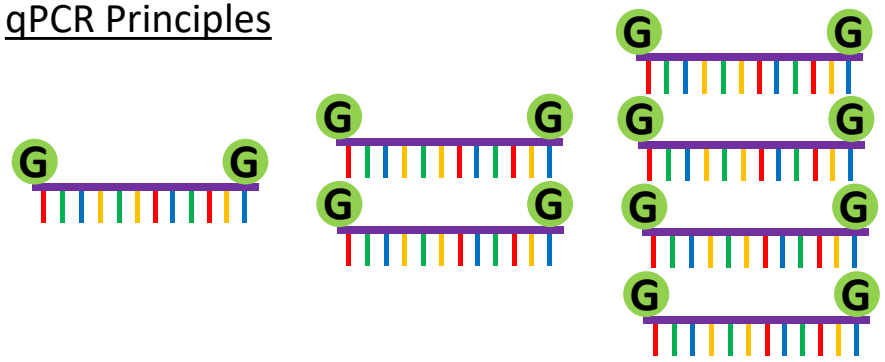


Quantitative-PCR



Data Analysis

<u>qPCR Principles</u>



$\ldots\ \ldots\ 2^n$

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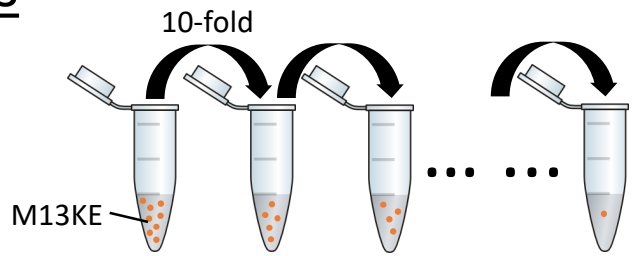
Identifying qPCR limit of detection for phage M13 DNA

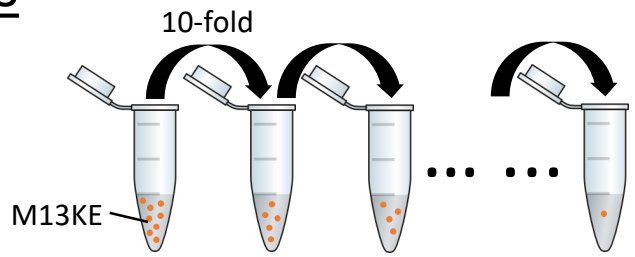
Samples



Identifying qPCR limit of detection for phage M13 DNA

Samples

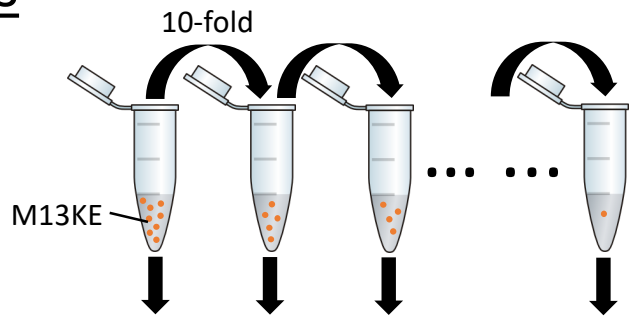
Control



Identifying qPCR limit of detection for phage M13 DNA

Samples

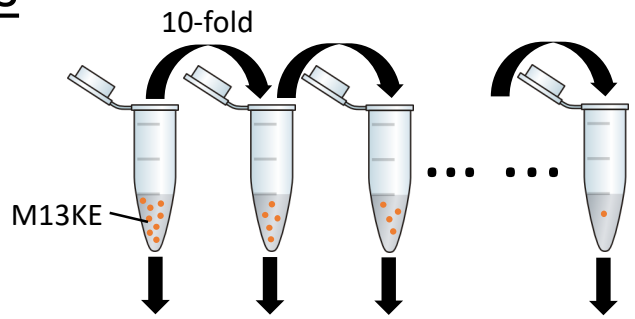
Control



Identifying qPCR limit of detection for phage M13 DNA

Samples

Control



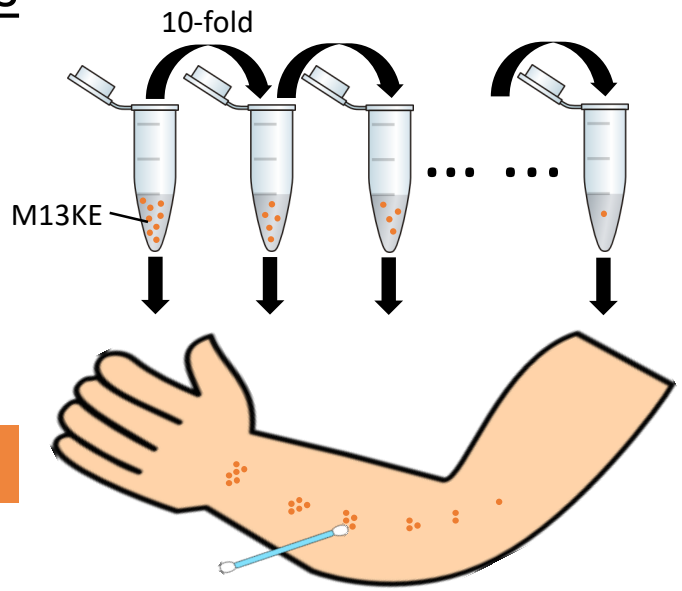
Skin Swab

Identifying qPCR limit of detection for phage M13 DNA

Samples

Control

Skin Swab

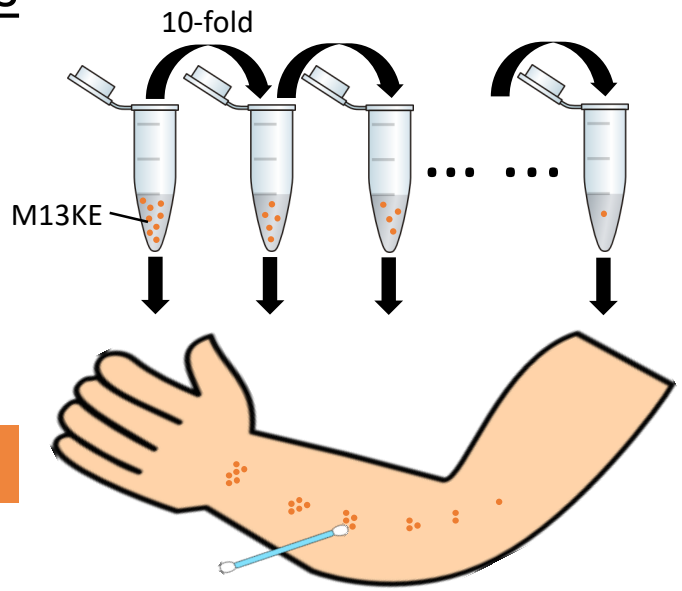


Identifying qPCR limit of detection for phage M13 DNA

Samples

Control

Skin Swab



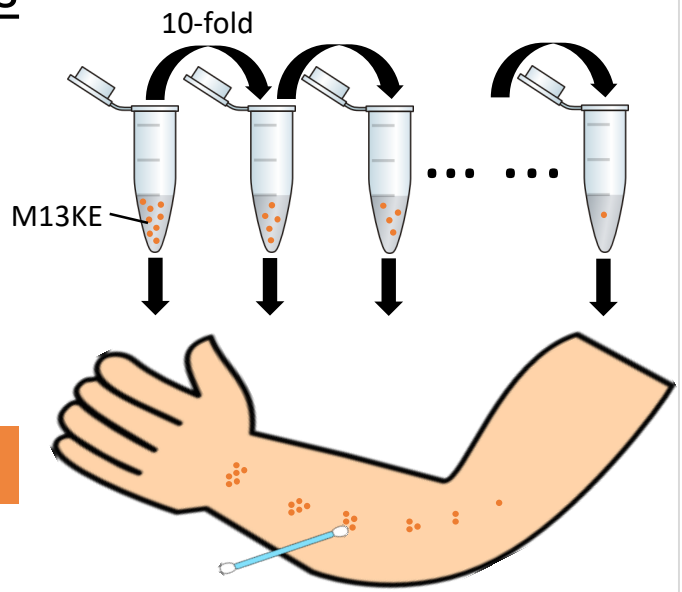
Samples were assayed in triplicate

Identifying qPCR limit of detection for phage M13 DNA

Samples

Control

Skin Swab



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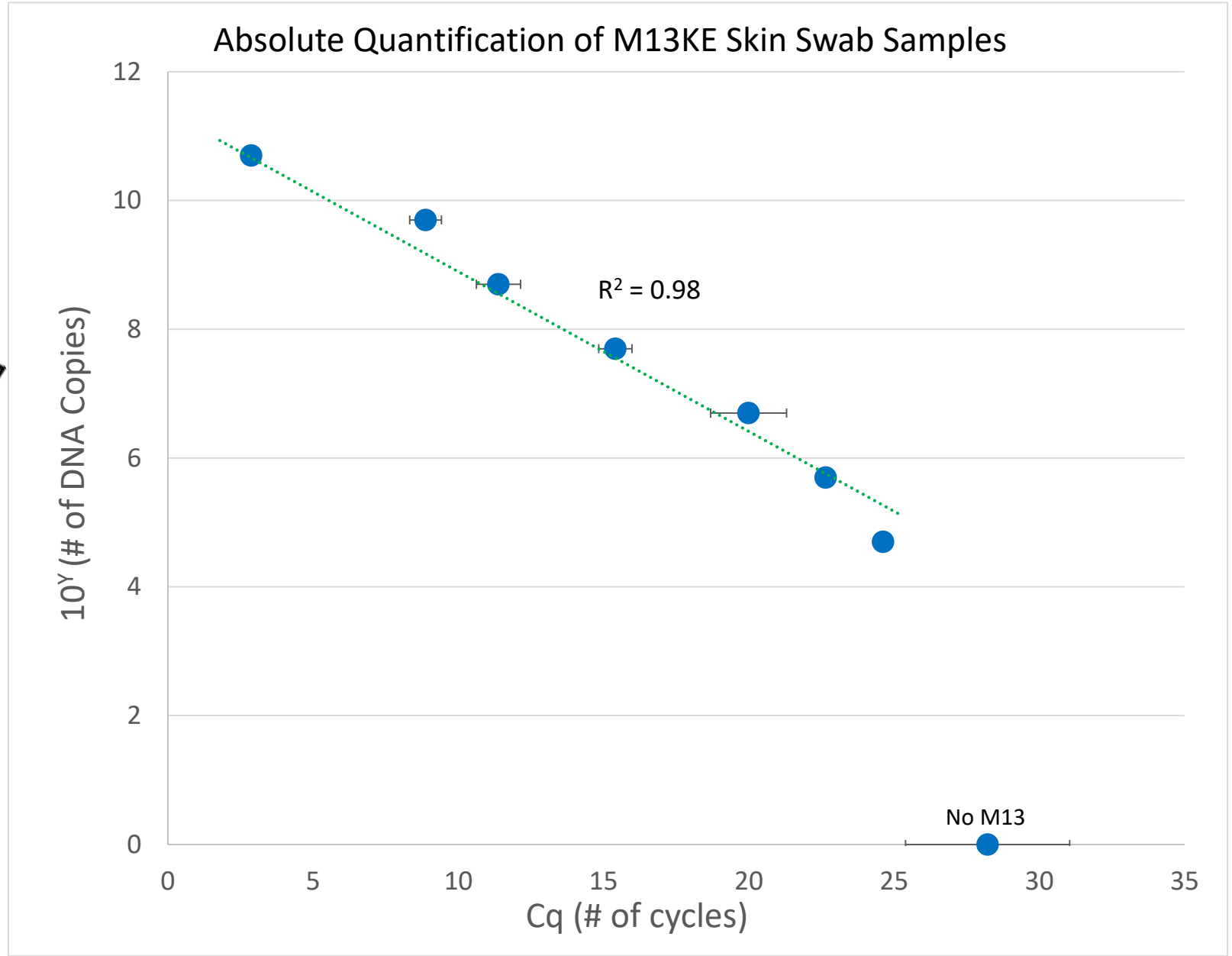
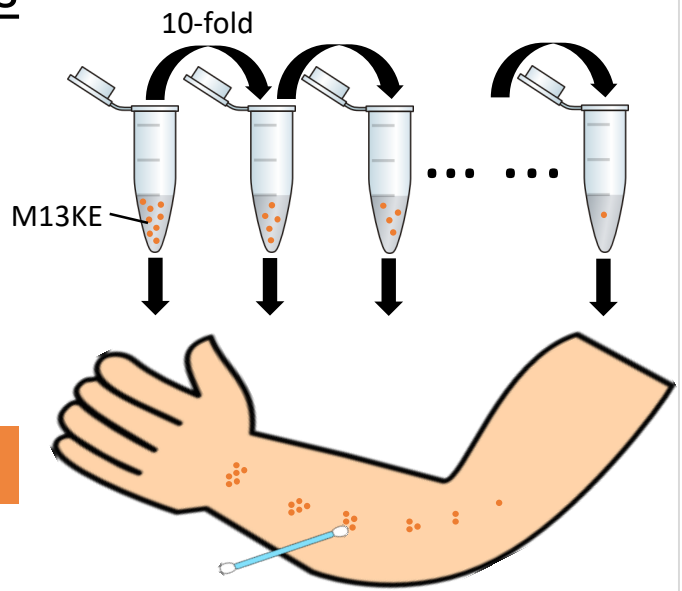


Identifying qPCR limit of detection for phage M13 DNA

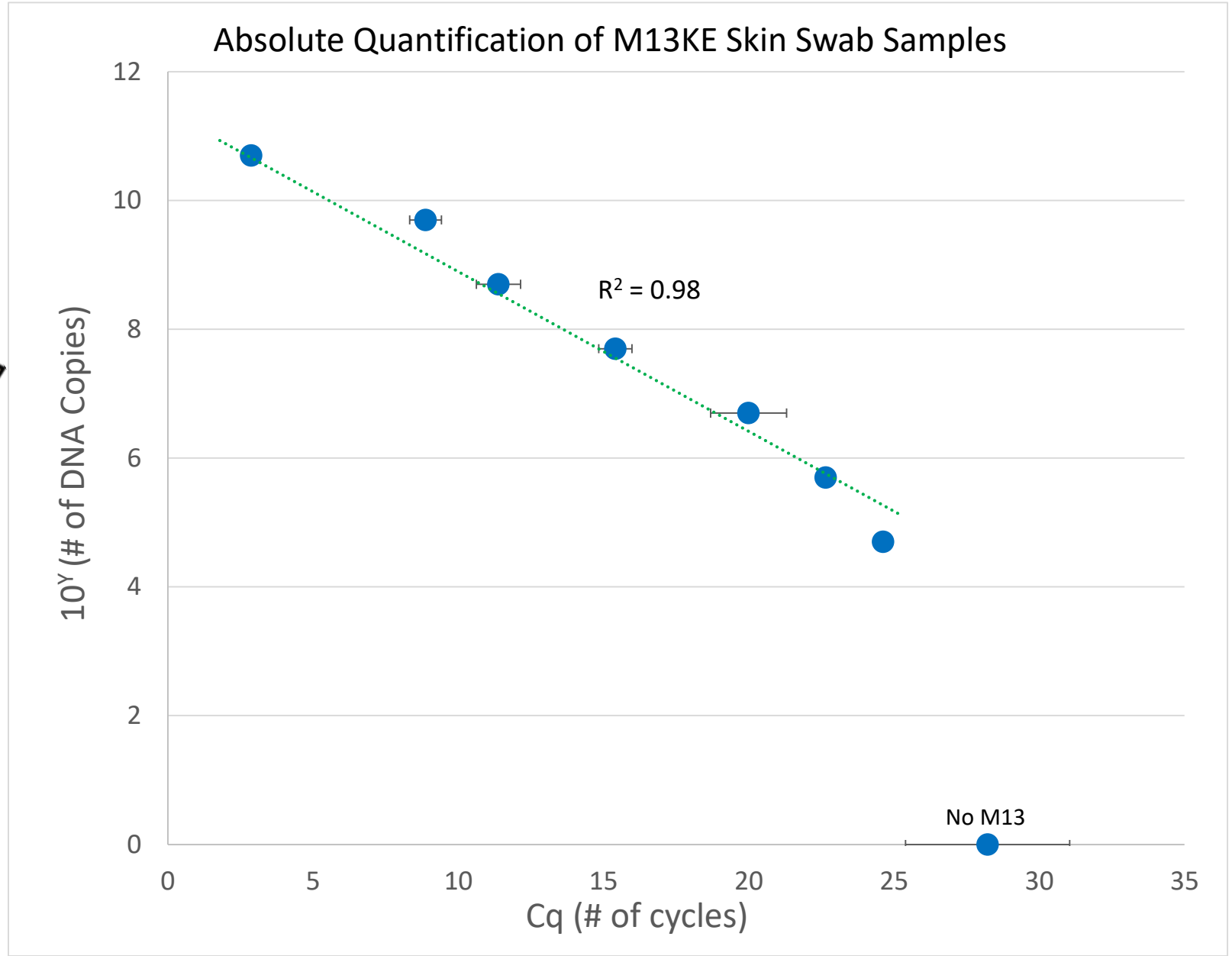
Samples

Control

Skin Swab



- qPCR baseline of detection = 2.36×10^4 copies

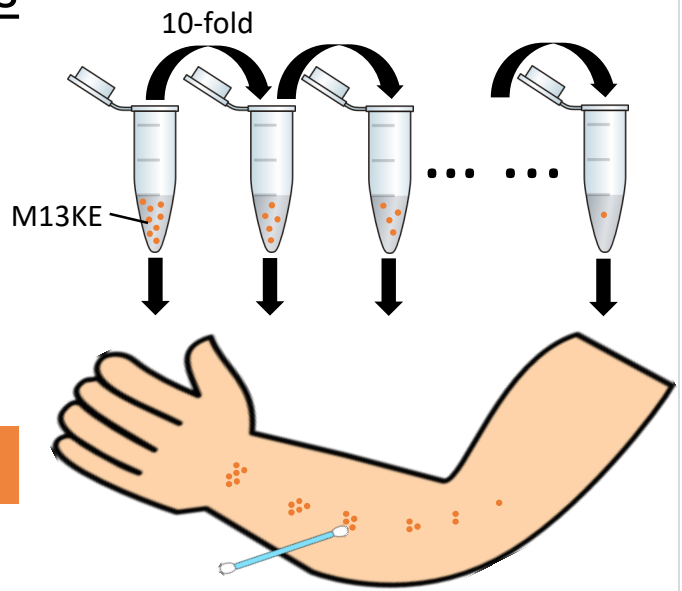


Identifying qPCR limit of detection for phage M13 DNA

Samples

Control

Skin Swab



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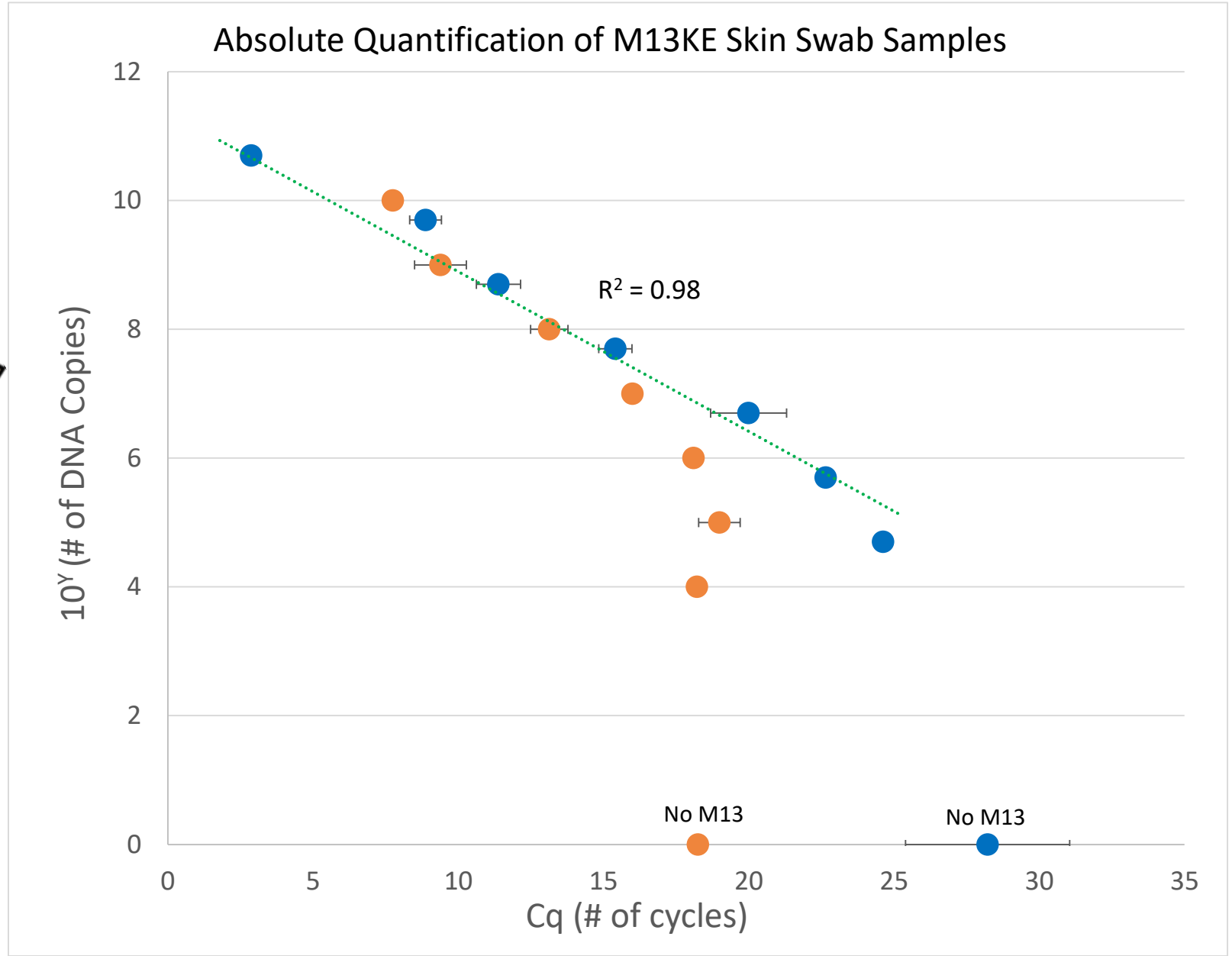
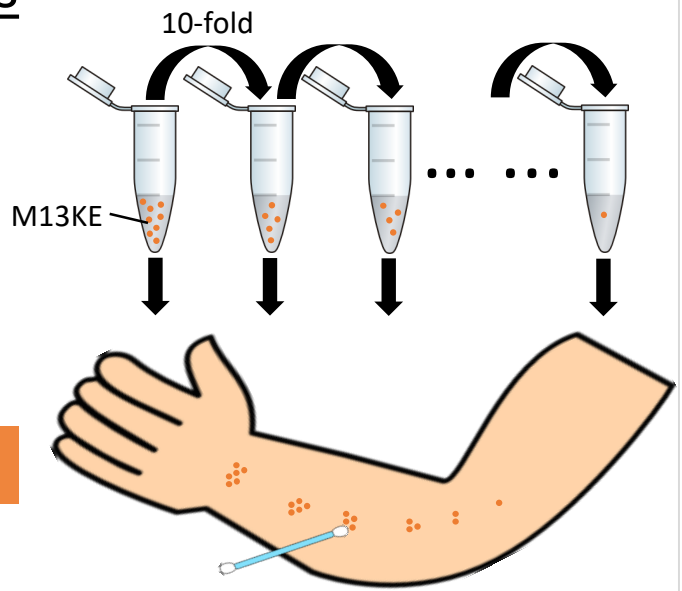
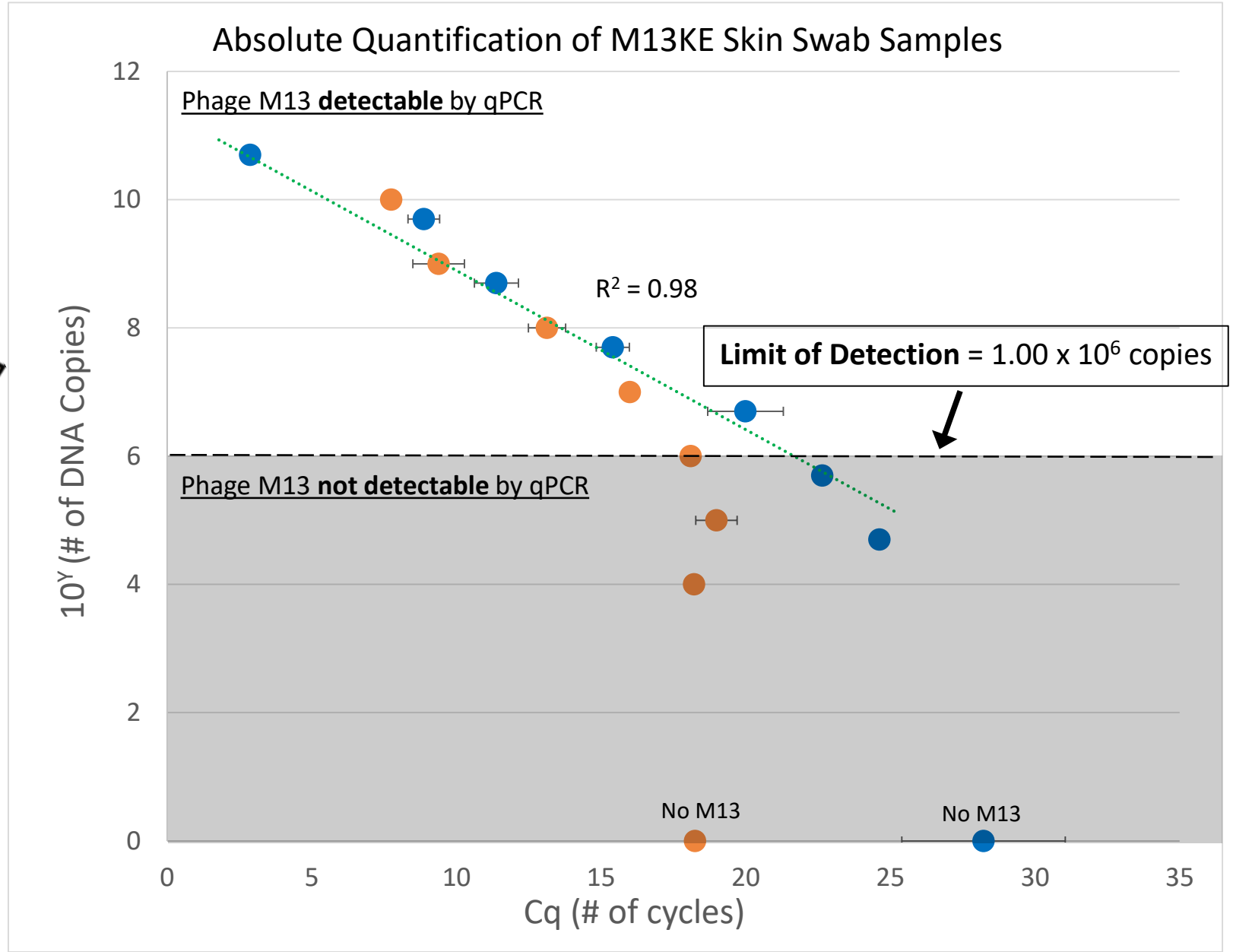


Identifying qPCR limit of detection for phage M13 DNA

Samples

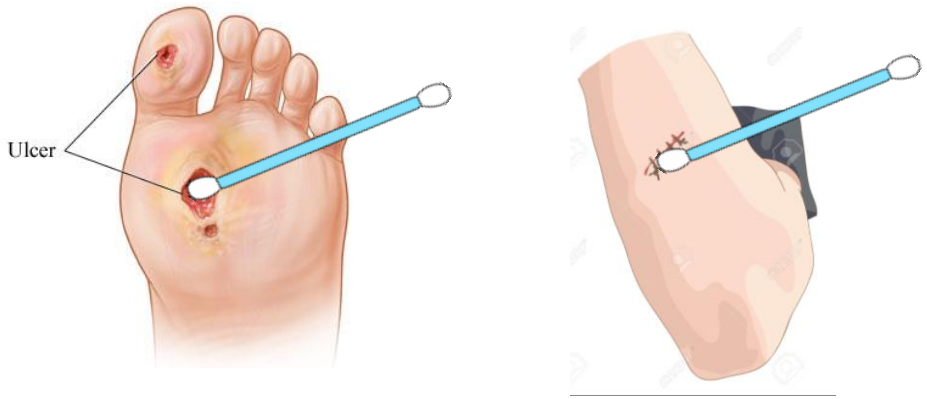


- qPCR baseline of detection = 2.36 x 10^4 copies
- Limit of detection for human skin swab = 1.00 x 10^6 copies
- Contaminating sequences on human skin



Establishing a protocol to quantify phages from wounds

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



Human skin wounds

Establishing a protocol to quantify phages from wounds

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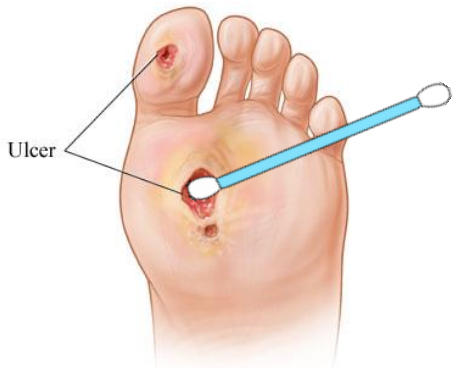

More than 10^6 copies

Human skin wounds

Establishing a protocol to quantify phages from wounds

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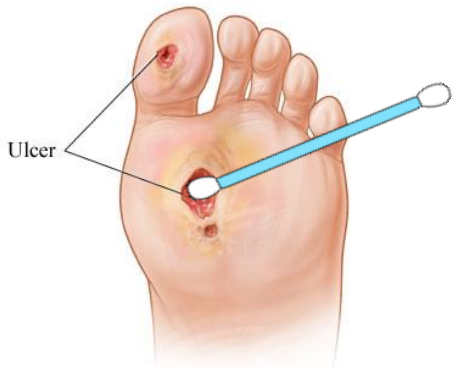
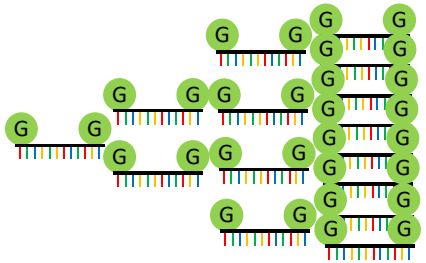


Human skin wounds

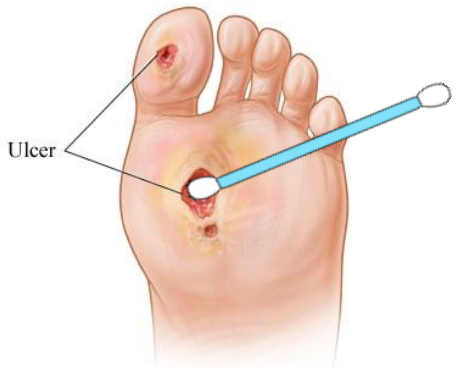
More than 10^6 copies



Can quantify using qPCR

Establishing a protocol to quantify phages from wounds

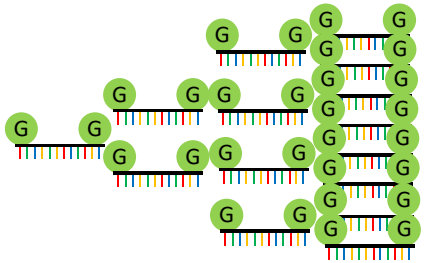
qPCR limit of detection for human skin swab = 1.00 x 10^6 copies



Human skin wounds

More than 10^6 copies

Less than 10^6 copies



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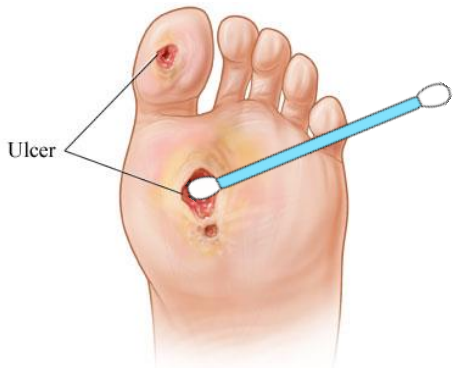
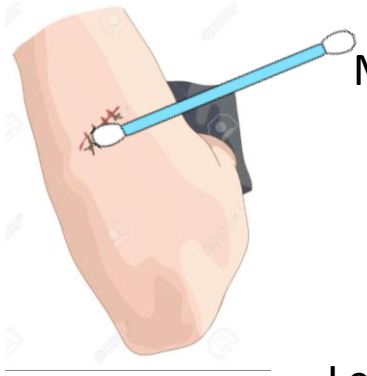
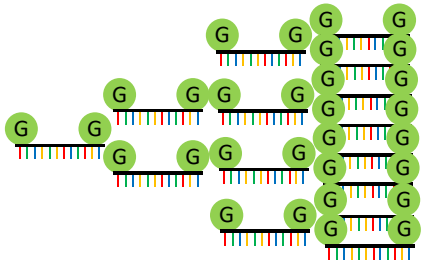




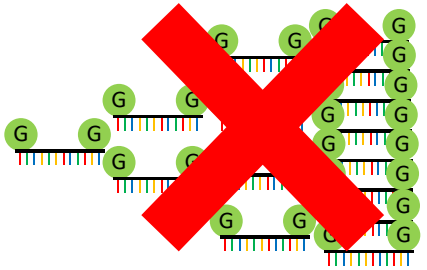
Human skin wounds

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

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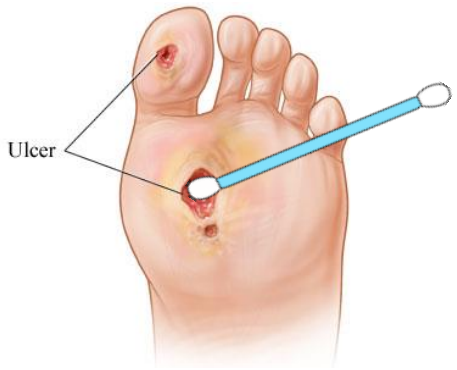
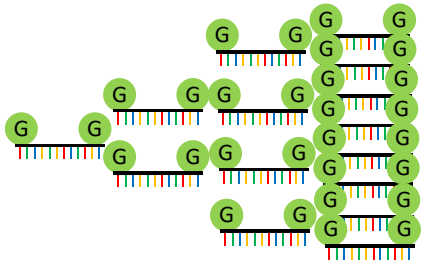


Human skin wounds

More than 10^6 copies



Can quantify using qPCR

Less than 10^6 copies

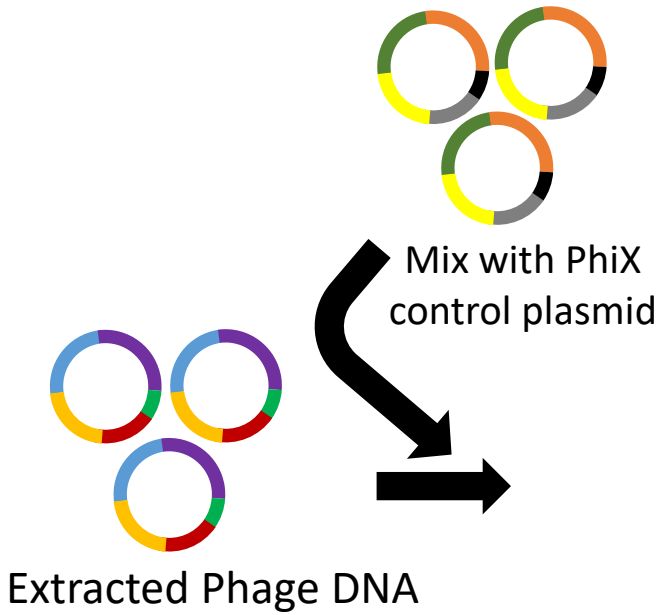
Cannot quantify using qPCR

?

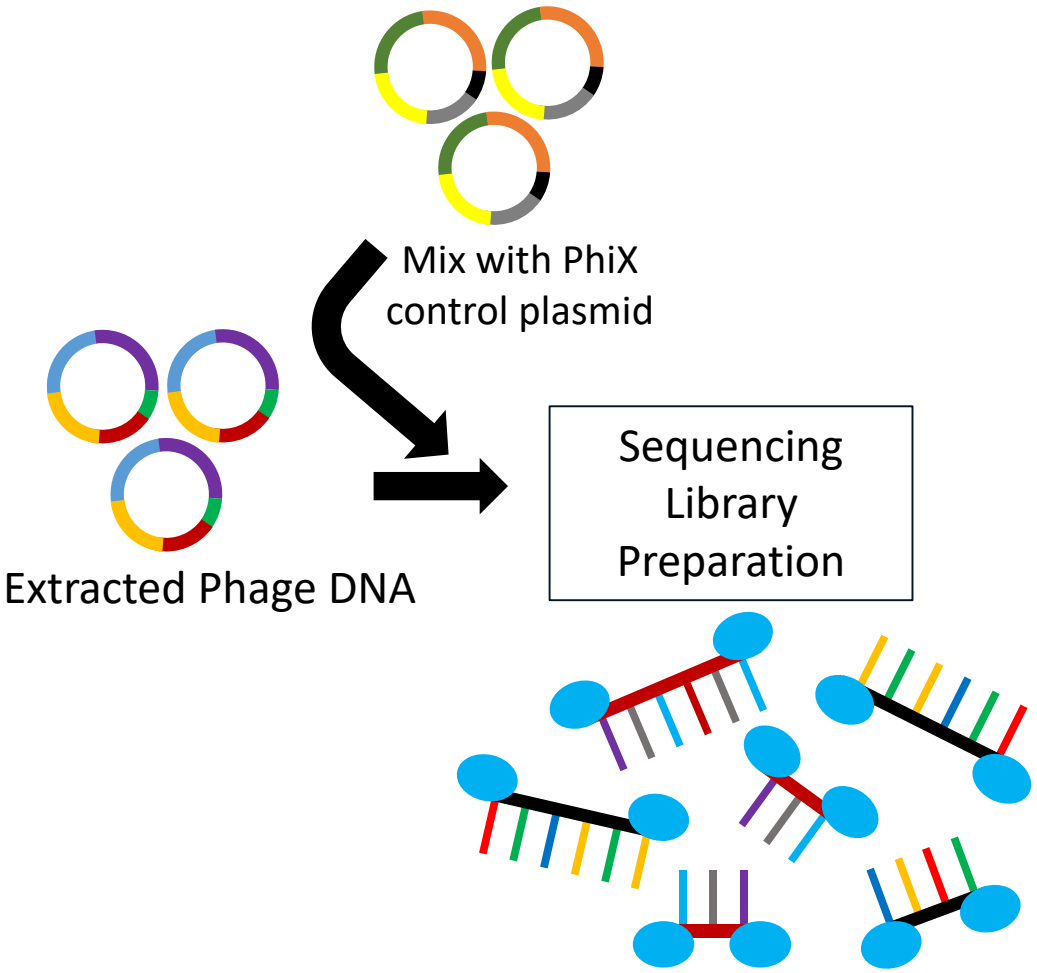
Sequencing phage M13 genome for quantification

Extracted Phage DNA

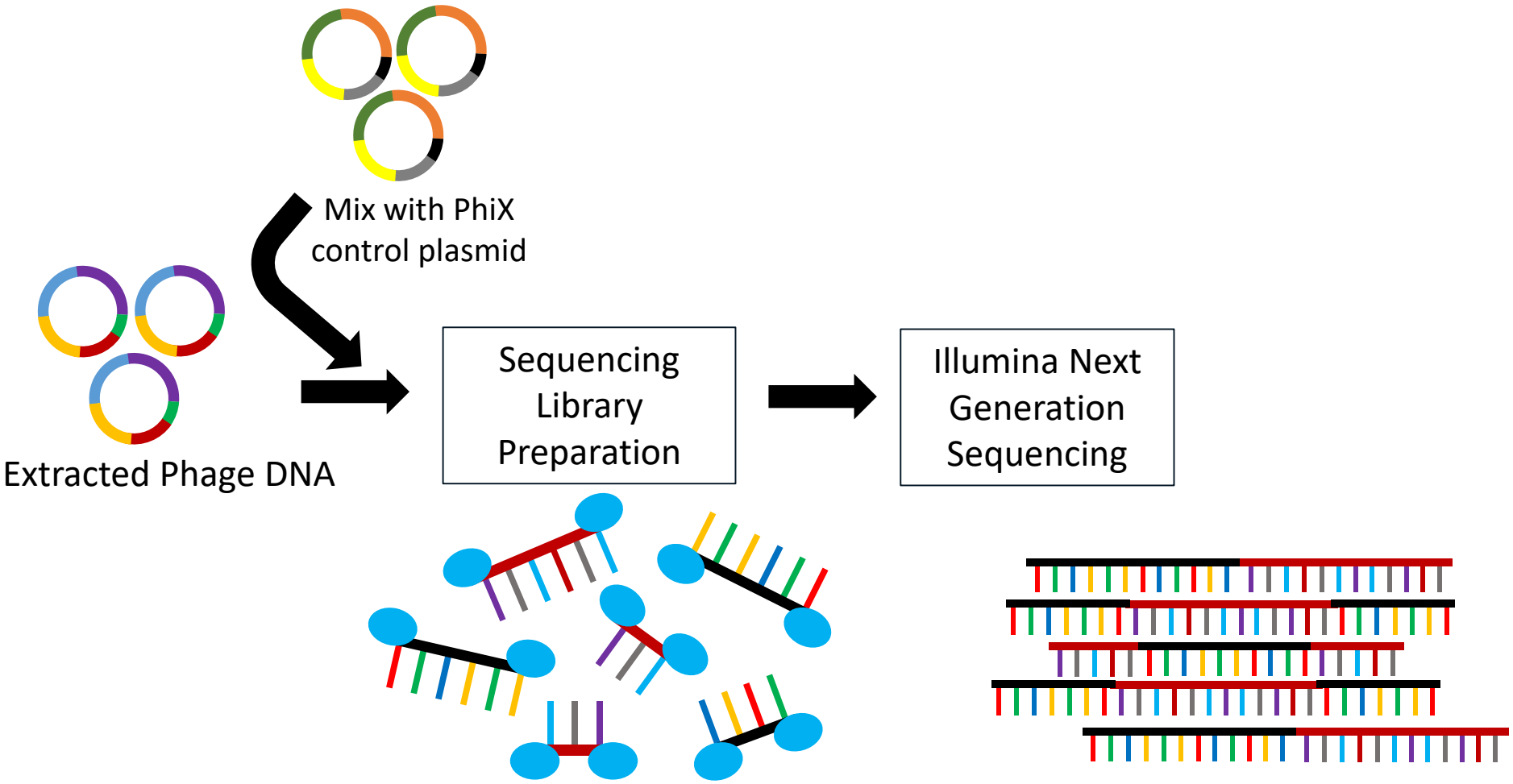
Sequencing phage M13 genome for quantification



Sequencing phage M13 genome for quantification

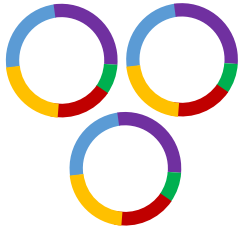


Sequencing phage M13 genome for quantification



Sequencing phage M13 genome for quantification

Mix with PhiX control plasmid

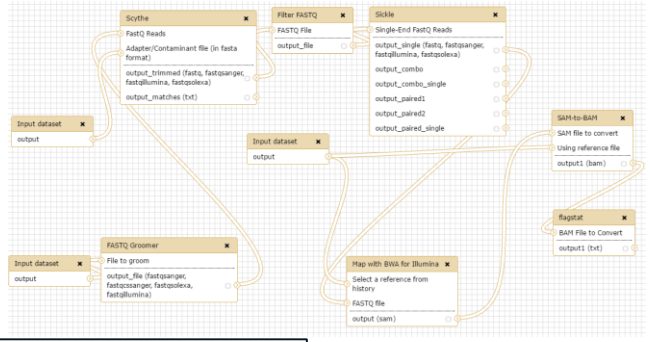


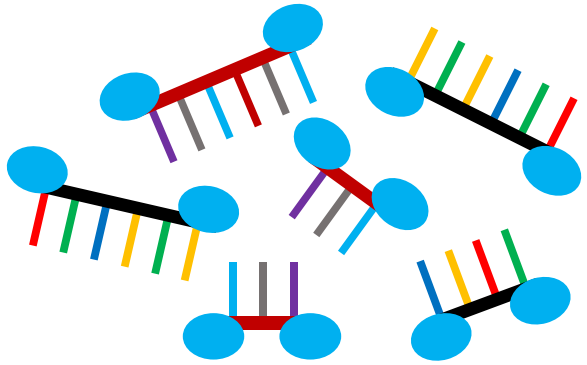
Extracted Phage DNA

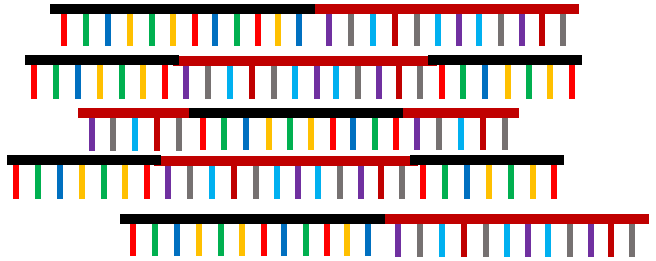
Sequencing Library Preparation

Illumina Next Generation Sequencing

Galaxy Genome Assembly & Mapping

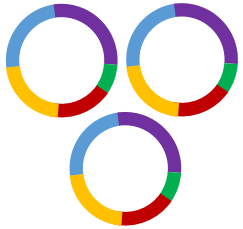






Sequencing phage M13 genome for quantification

Mix with PhiX control plasmid



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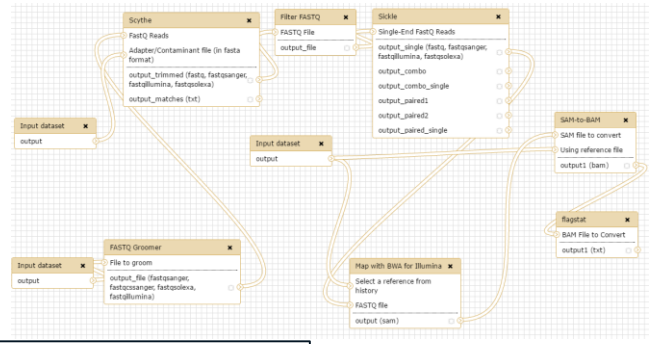
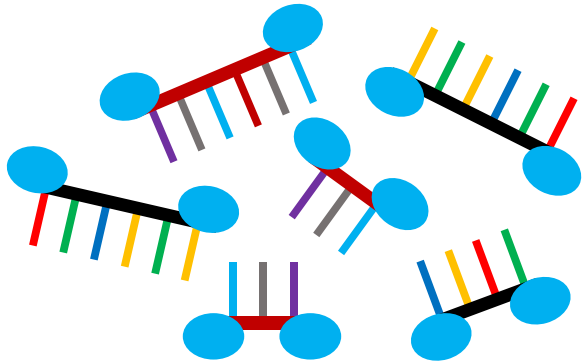
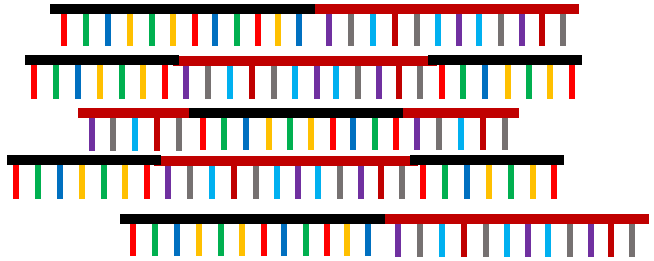


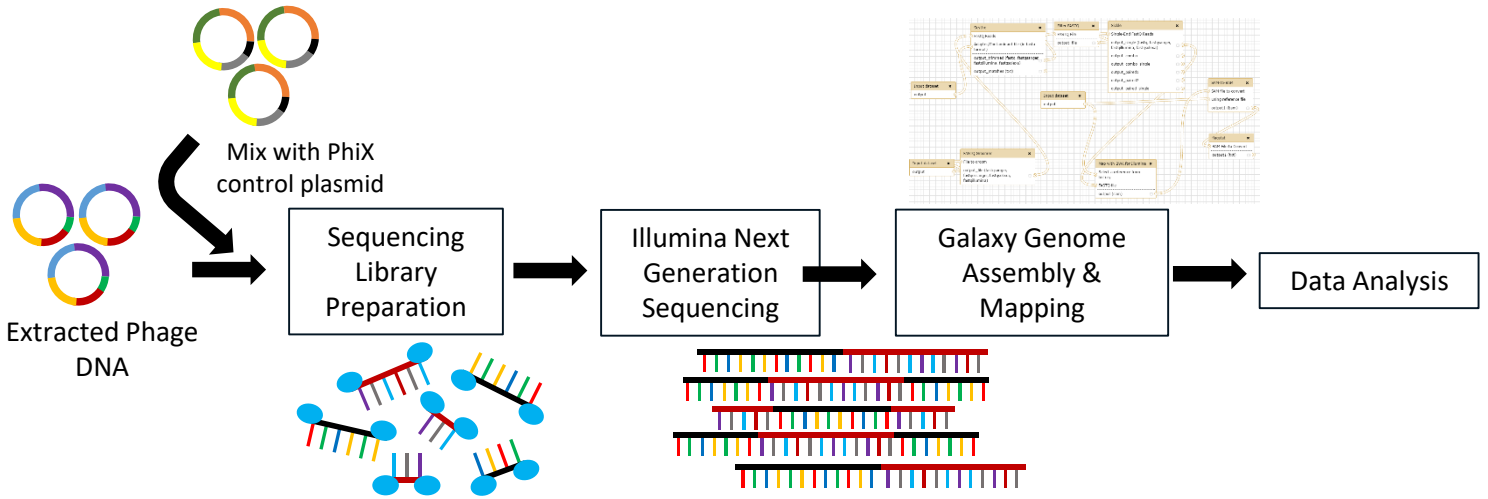
Galaxy Genome Assembly & Mapping

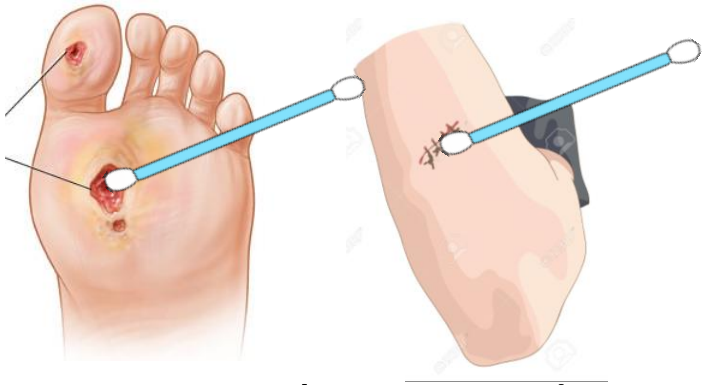
Data Analysis





Applying the protocol to analyze human skin wounds





Human skin wounds

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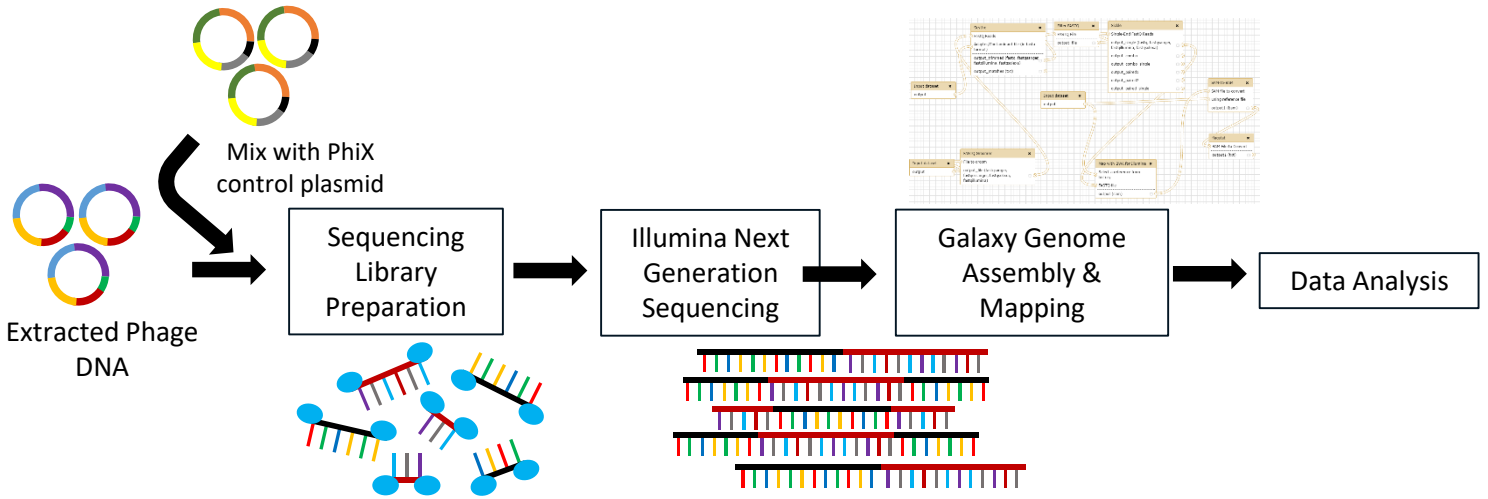
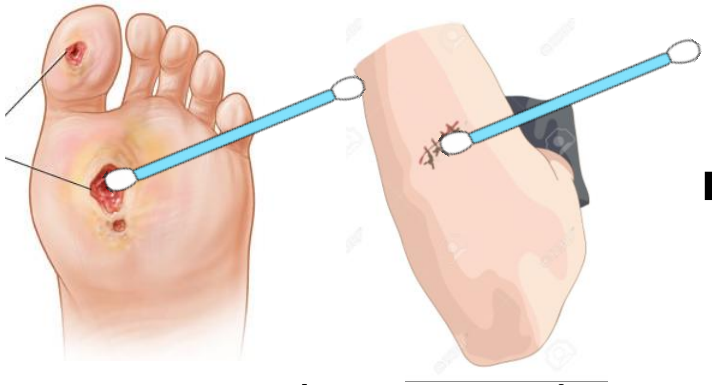
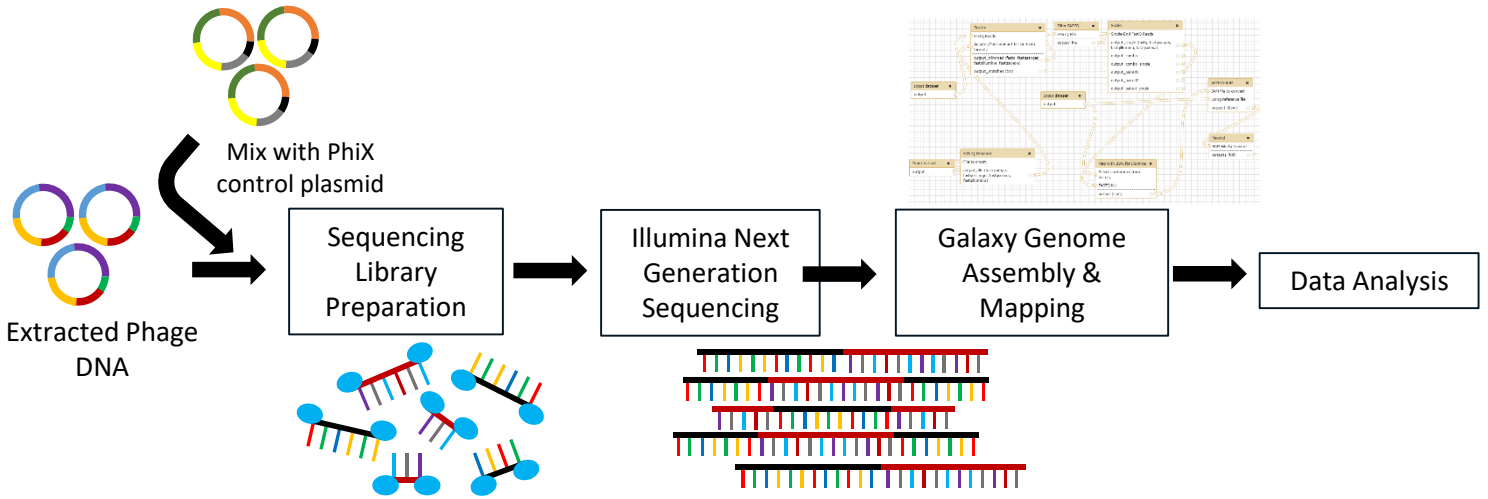


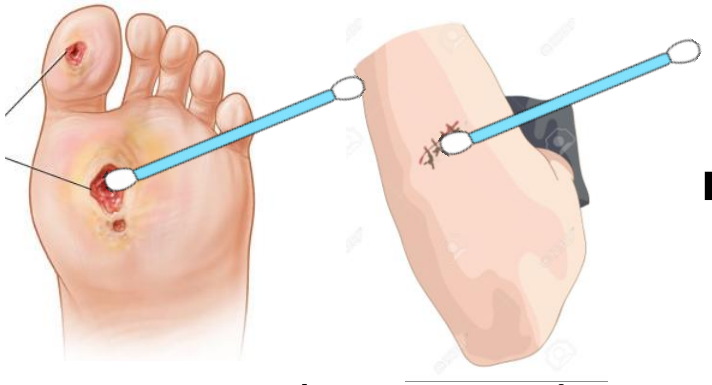


Human skin wounds

Apply the phage detection protocol

Applying the protocol to analyze human skin wounds

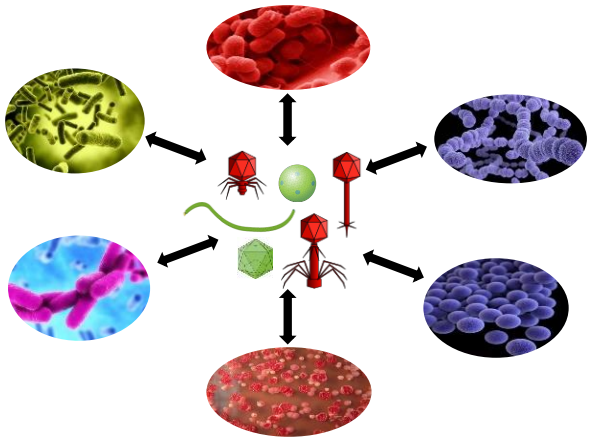




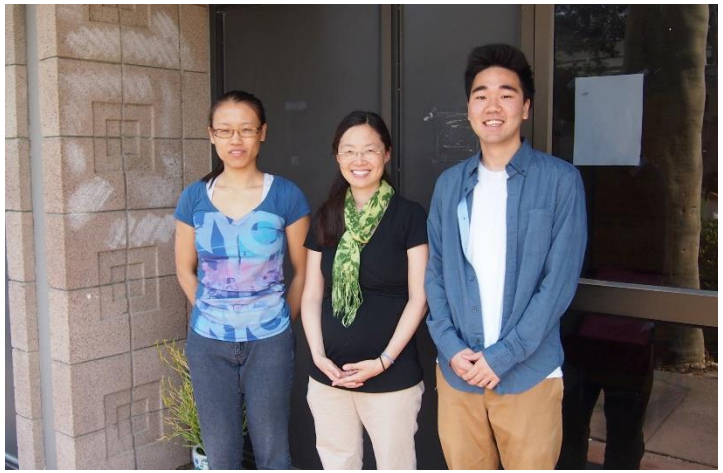
Human skin wounds

Apply the phage detection protocol

Analyze the metagenomics data in the microbiome



Acknowledgments



Chen Group:
Celia Blanco
Gregory Campbell
Irene Chen
Michael Devano
Evan Janzen
Coulter Keeler
Thomas Nguyen
Abe Pressman
Ranajay Saha
Allison Tam
Claire Tran
Samuel Verbanic
Baoqing Zhou

MARC U-STAR:
Arica Lubin
Stephanie Mendes
Joel Rothman

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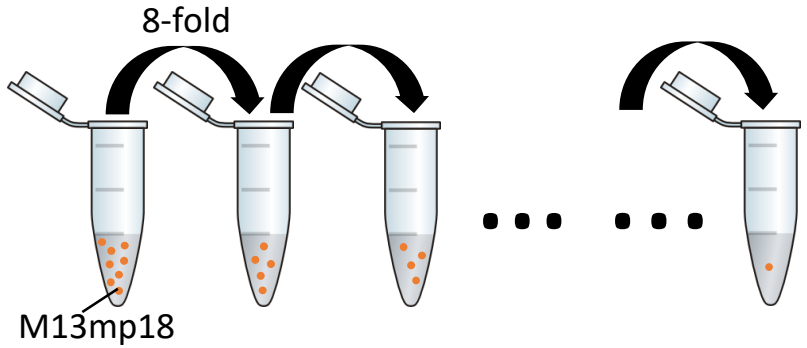
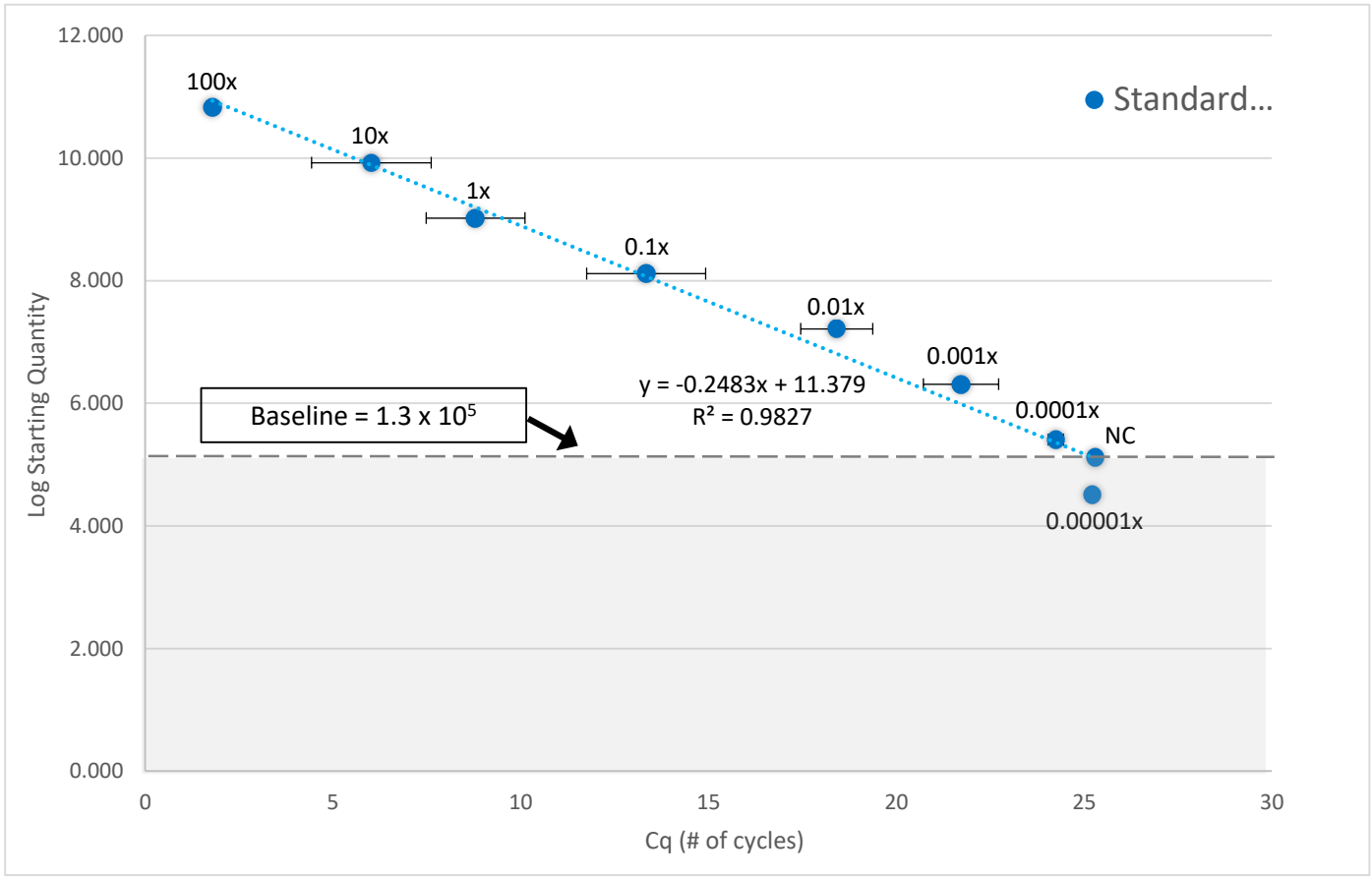
Quantifying phage M13 DNA by qPCR – Standard Curve

Serial dilution of M13mp18



Sample	*Expected* Total # of DNA	*Observed* Total # of DNA	Mean Cq	Standard Error
100x	6.7×10^{10}	6.7×10^{10}	1.79	0
10x	8.4×10^{9}	8.4×10^{9}	6.02	1.59
1x	1.0×10^{9}	1.0×10^{9}	8.80	1.32
0.1x	1.3×10^{8}	1.3×10^{8}	13.33	1.59
0.01x	1.6×10^{7}	1.6×10^{7}	18.41	0.96
0.001x	2.0×10^{6}	2.0×10^{6}	21.72	1.00
0.0001x	2.6×10^{5}	2.6×10^{5}	24.25	0.20
0.00001x	3.2×10^{4}	3.2×10^{4}	25.22	0.05
Negative Control (dH_2O)	0	1.3×10^{5}	25.29	0.155

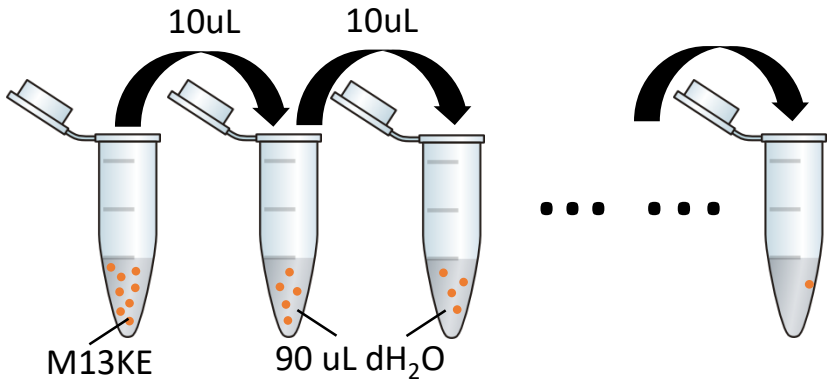


- Baseline of detection = 1.3×10^5 copies/uL
- Linear equation, $R^2 = 0.98271$

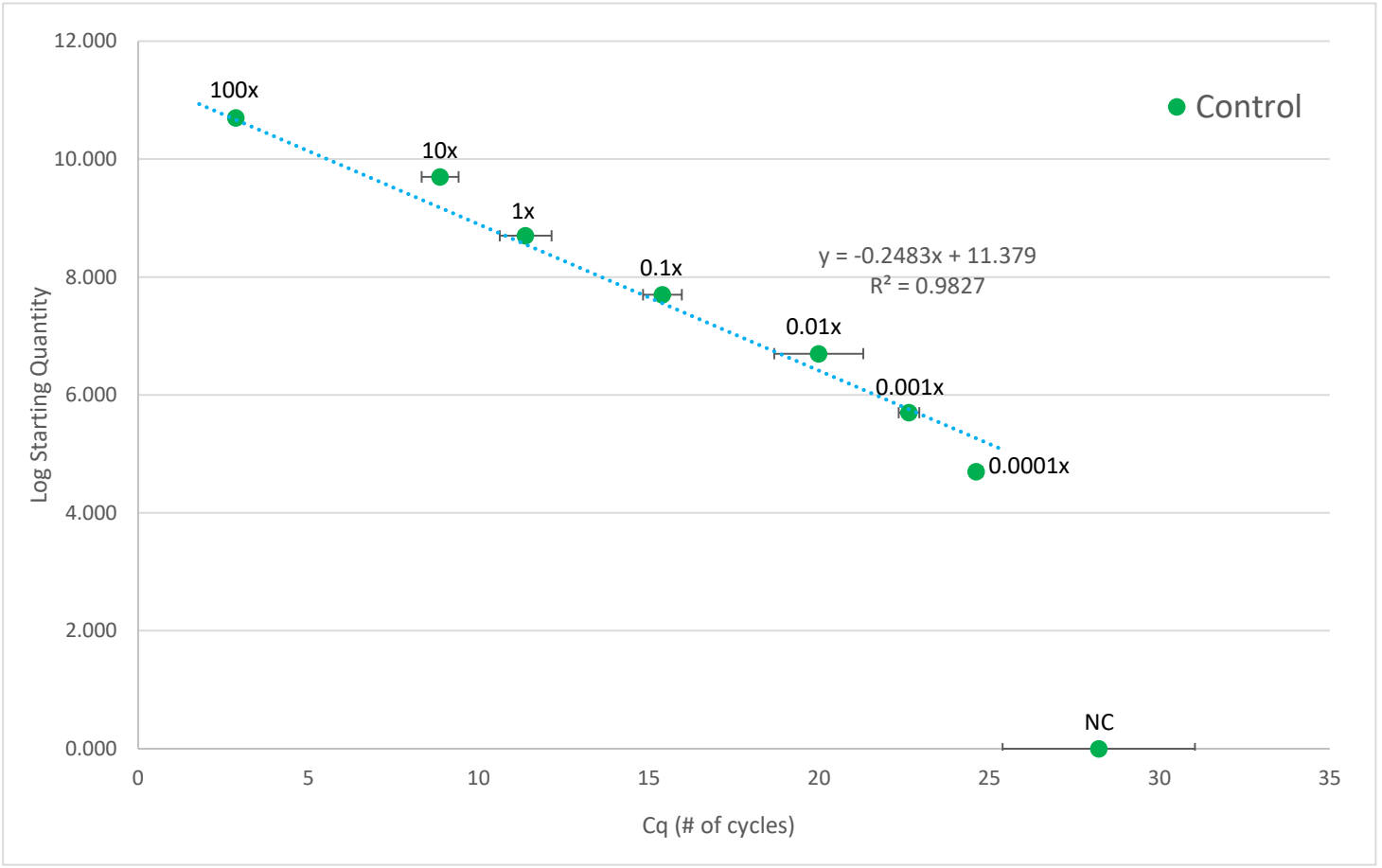
This standard curve was used in the absolute quantification of our samples.

Quantifying phage M13 DNA by qPCR – Control Samples

10-fold serial dilution of M13KE



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

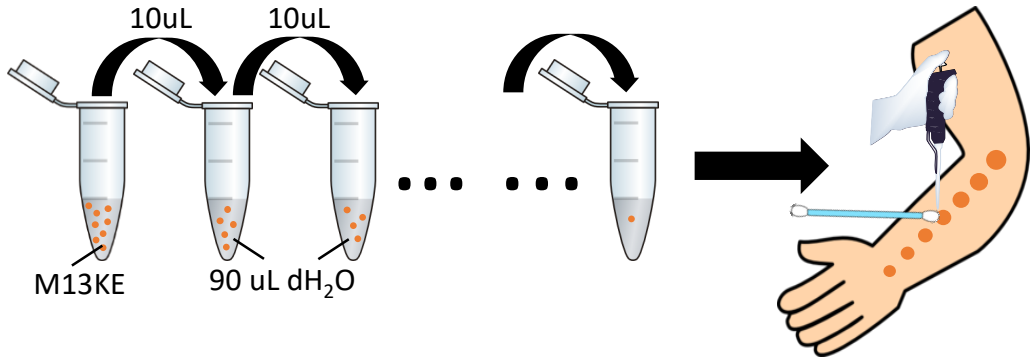


- Baseline of detection for M13KE = 2.36×10^{4} copies

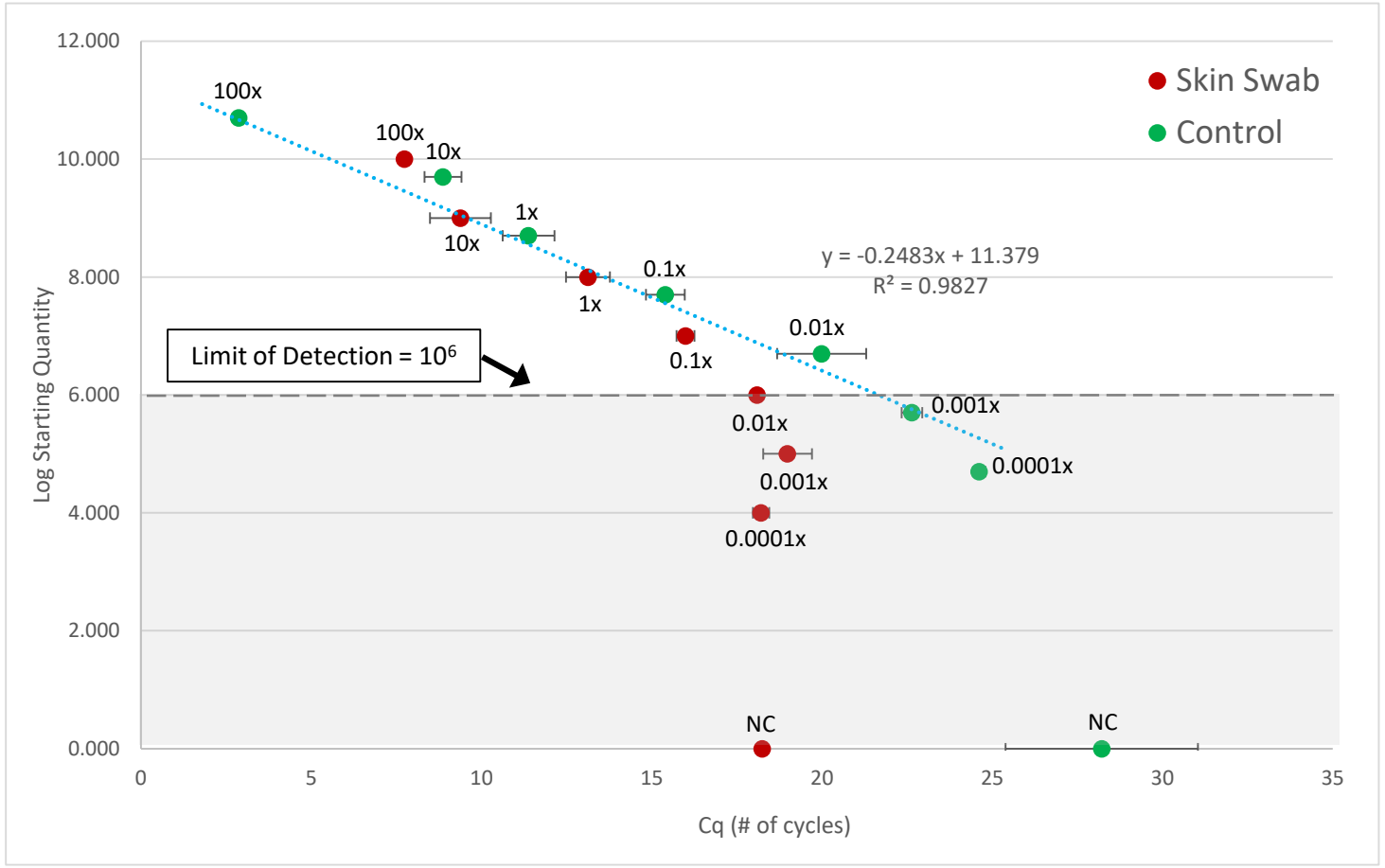
Control samples were compared to skin swab samples

Quantifying phage M13 DNA by qPCR – Swab Samples

10-fold serial dilution of M13KE



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



- Limit of detection for human skin swab = 1.0×10^{6} copies
- Contaminating sequences on human skin