The Role of *nhaP1, nhaP2,* and *nhaP3* Antiporters in the Acid Tolerance of *Vibrio cholerae.*

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Cholera

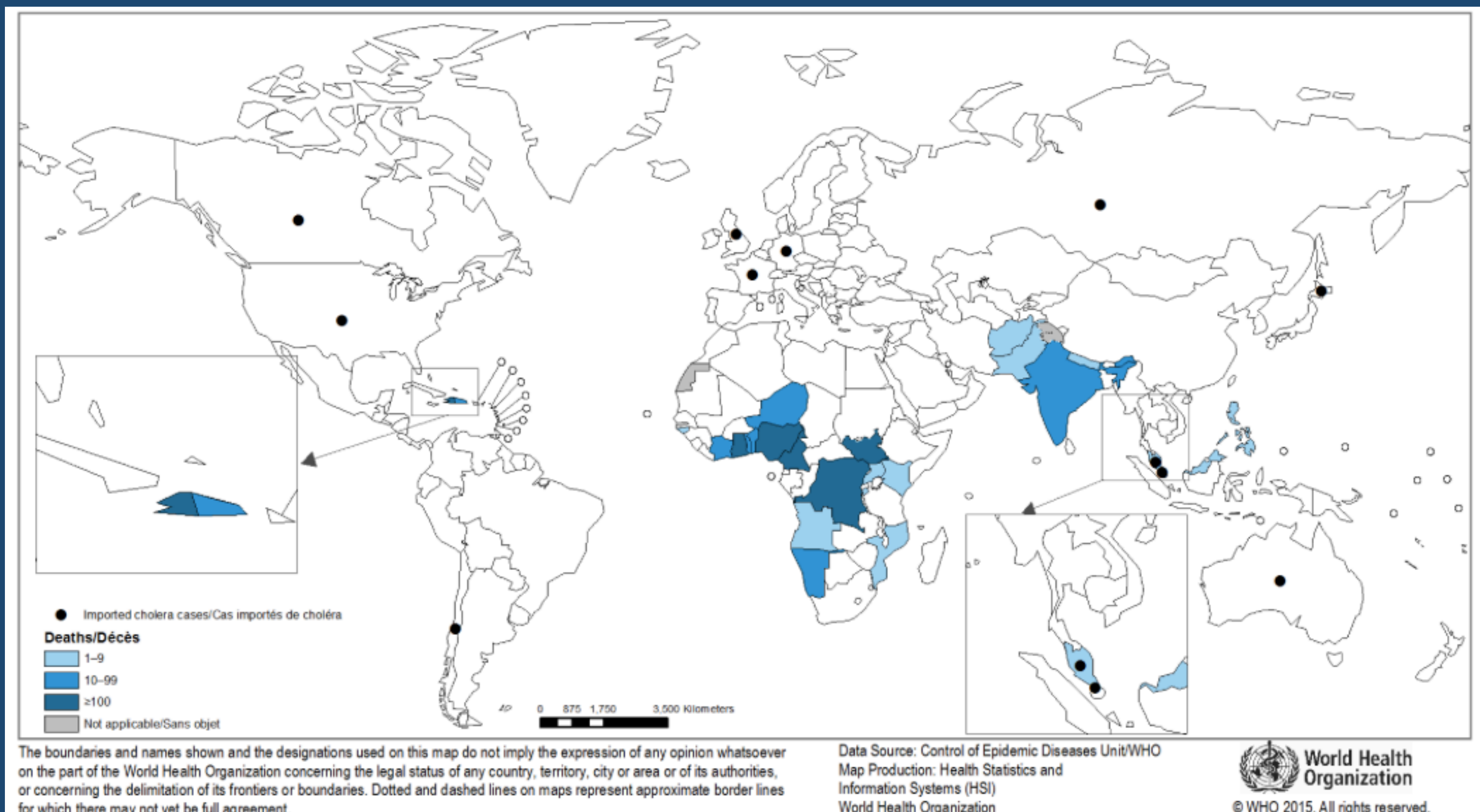
- Extreme diarrhea
- Rehydration therapy
- Vaccines

(Aljazeera, 2008)

(Knobil, 2015)

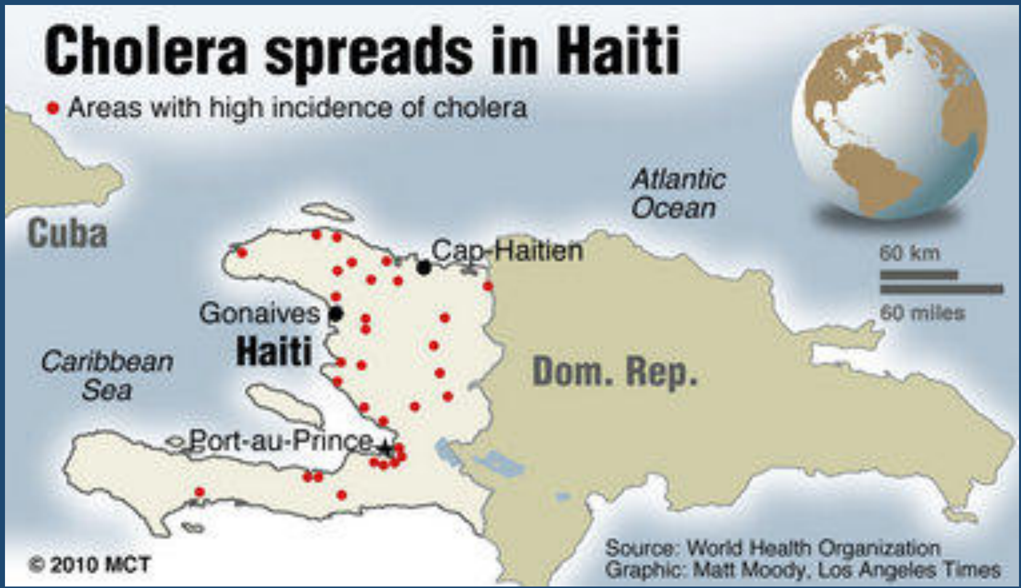
Cholera

- 1.4 – 4.3 million cases per year
  - 28,000 to 142,000 deaths



Cholera in Haiti

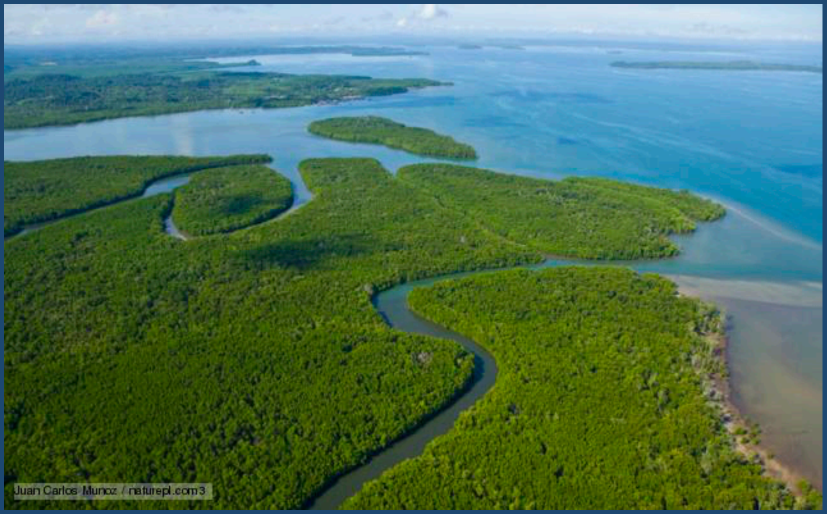
- 2010 Earthquake
  - >6% of Haitians have been infected
  - First two years: >8, 231 deaths
  - Up until 2015, >700,000 cases



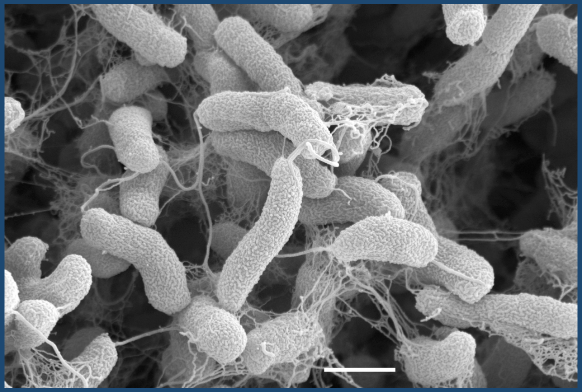
Vibrio cholerae

- *Vibrio cholerae* O395N1 (Vc)
  - – Gram negative rod
  - – Marine/Brackish waters

(WHO, 2014)



(Munoz, 2014)

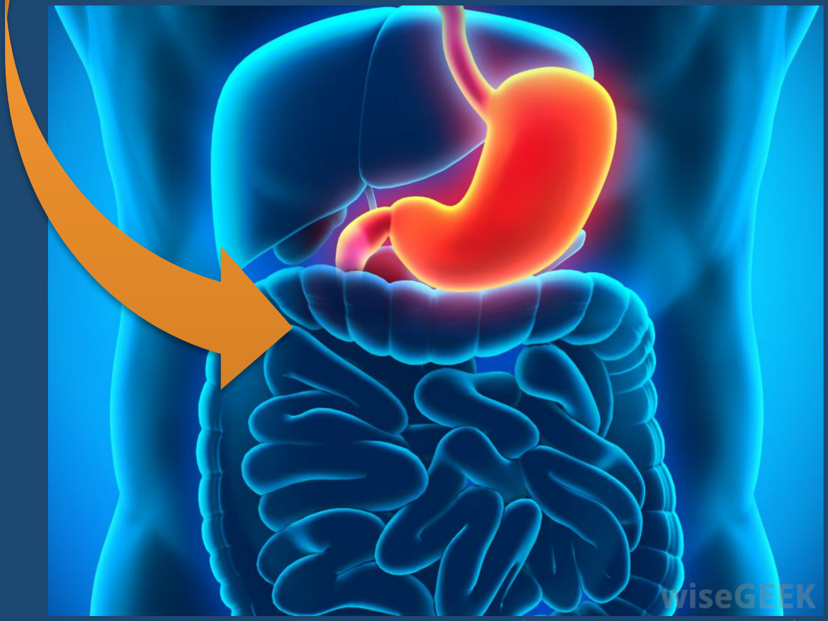


(Robert Koch Institute, 2014)

The Traveling Organism

(J. Silva/Reuters, 2011)

The Traveling Organism

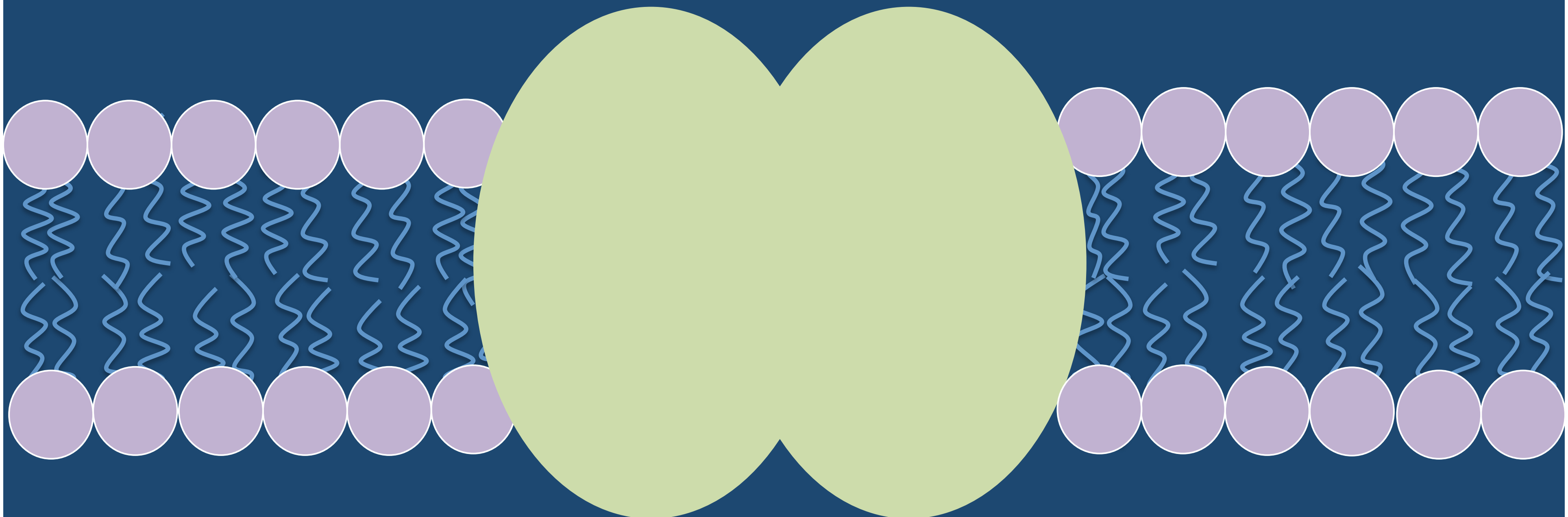


(wisegeek.com, 2015)

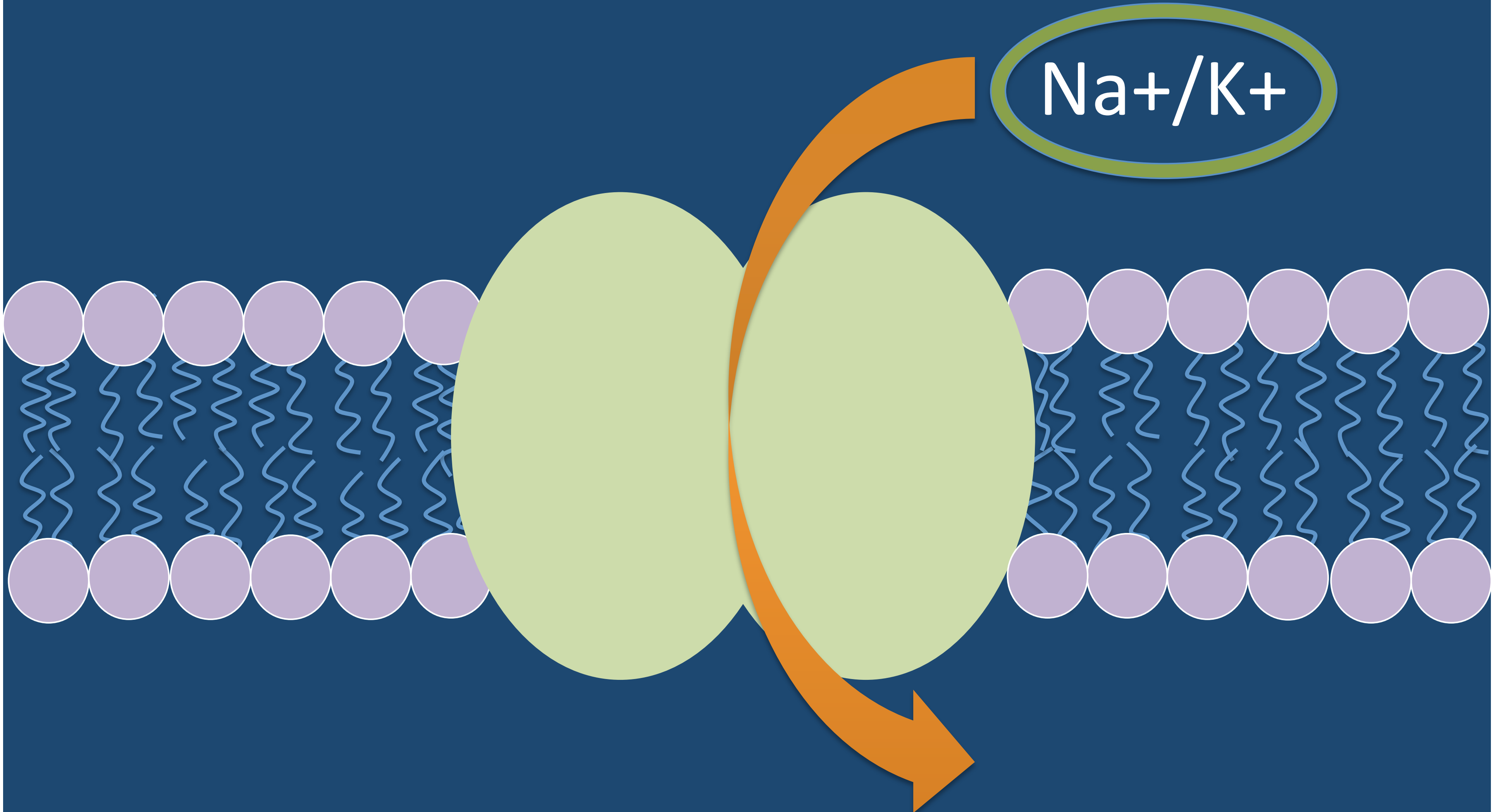
The Traveling Organism

(Kaulitzki, 2015)

Ion Homeostasis & Antiporters



The Mighty Antiporter



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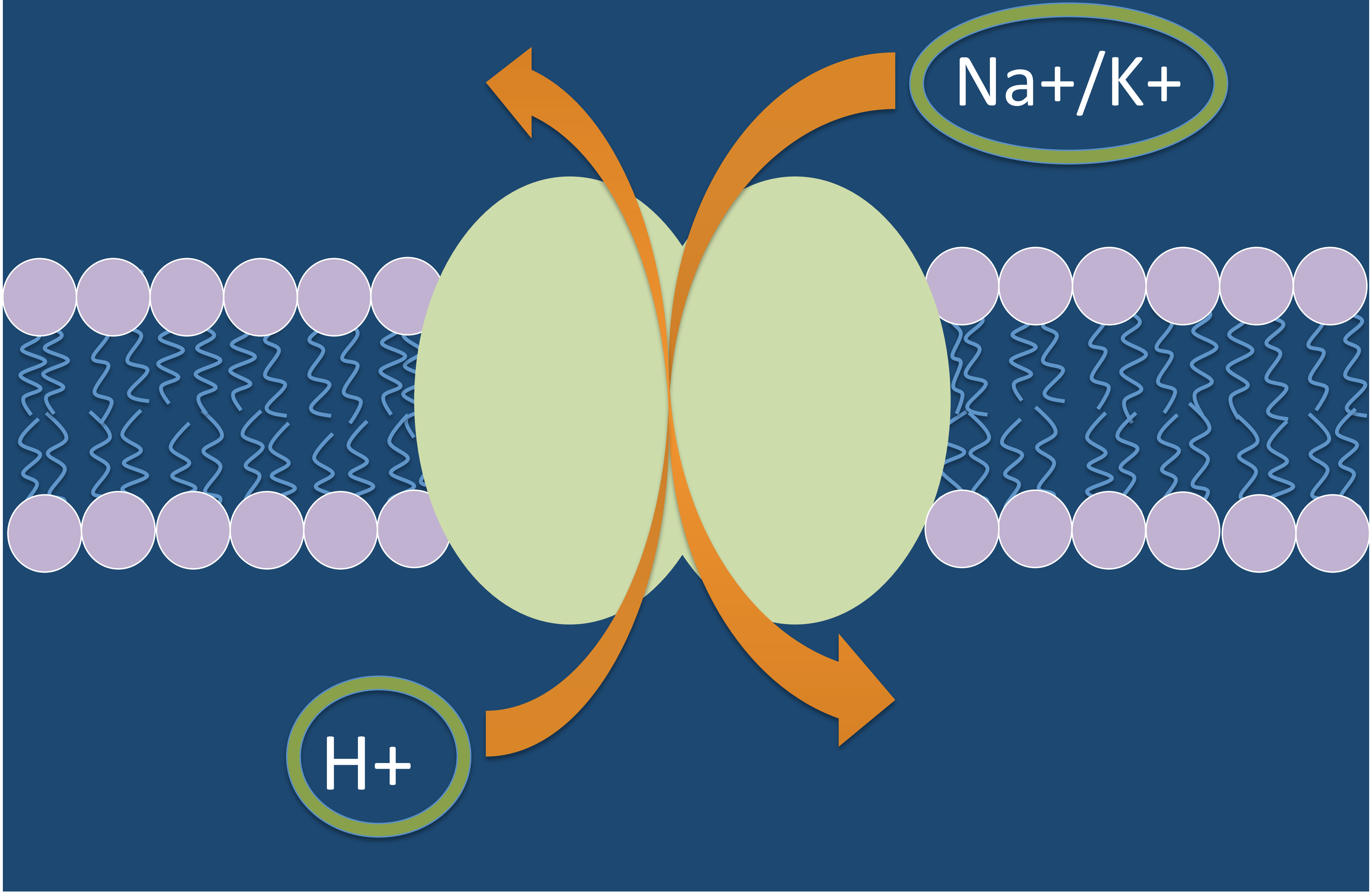
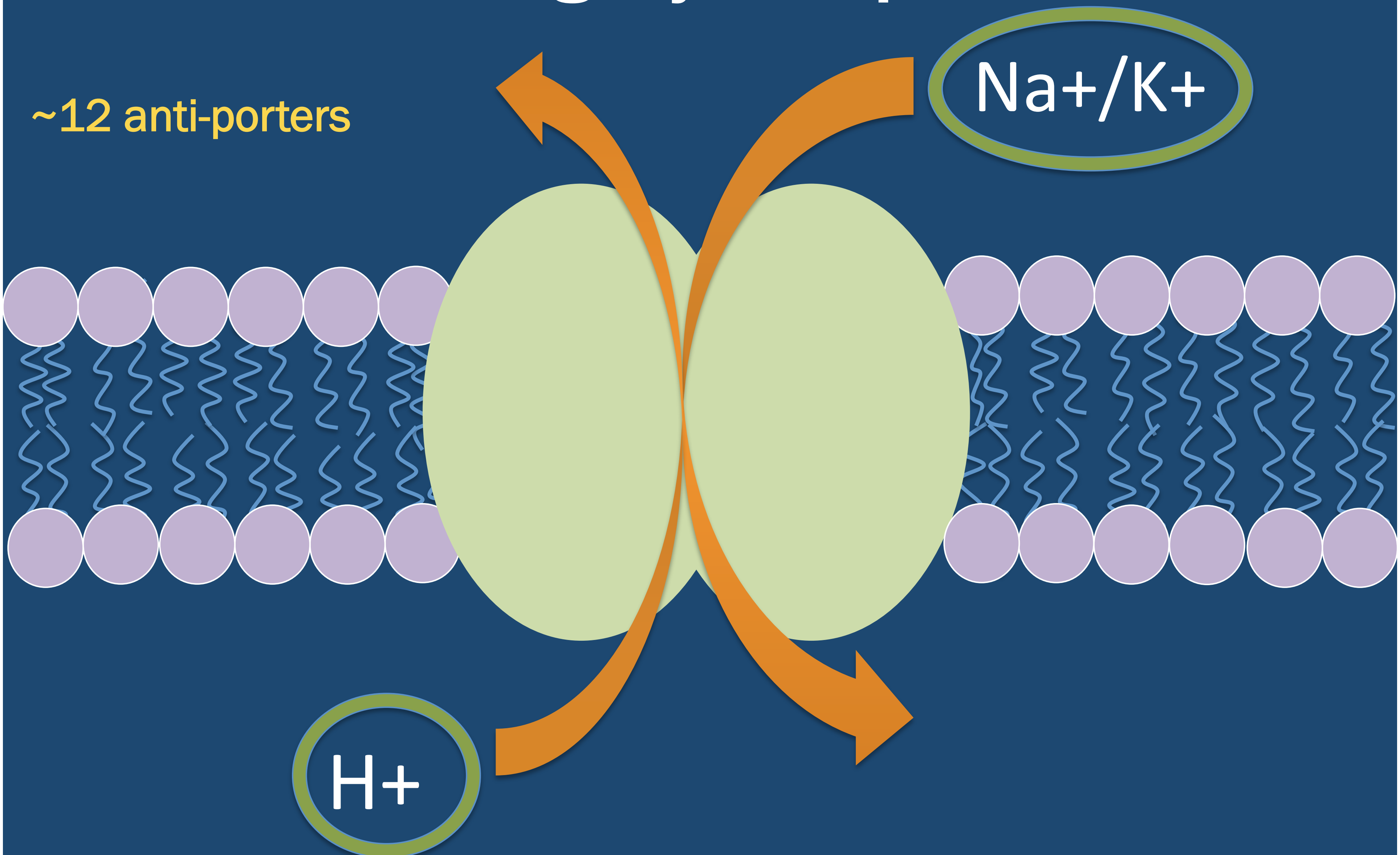


The Mighty Antiporter

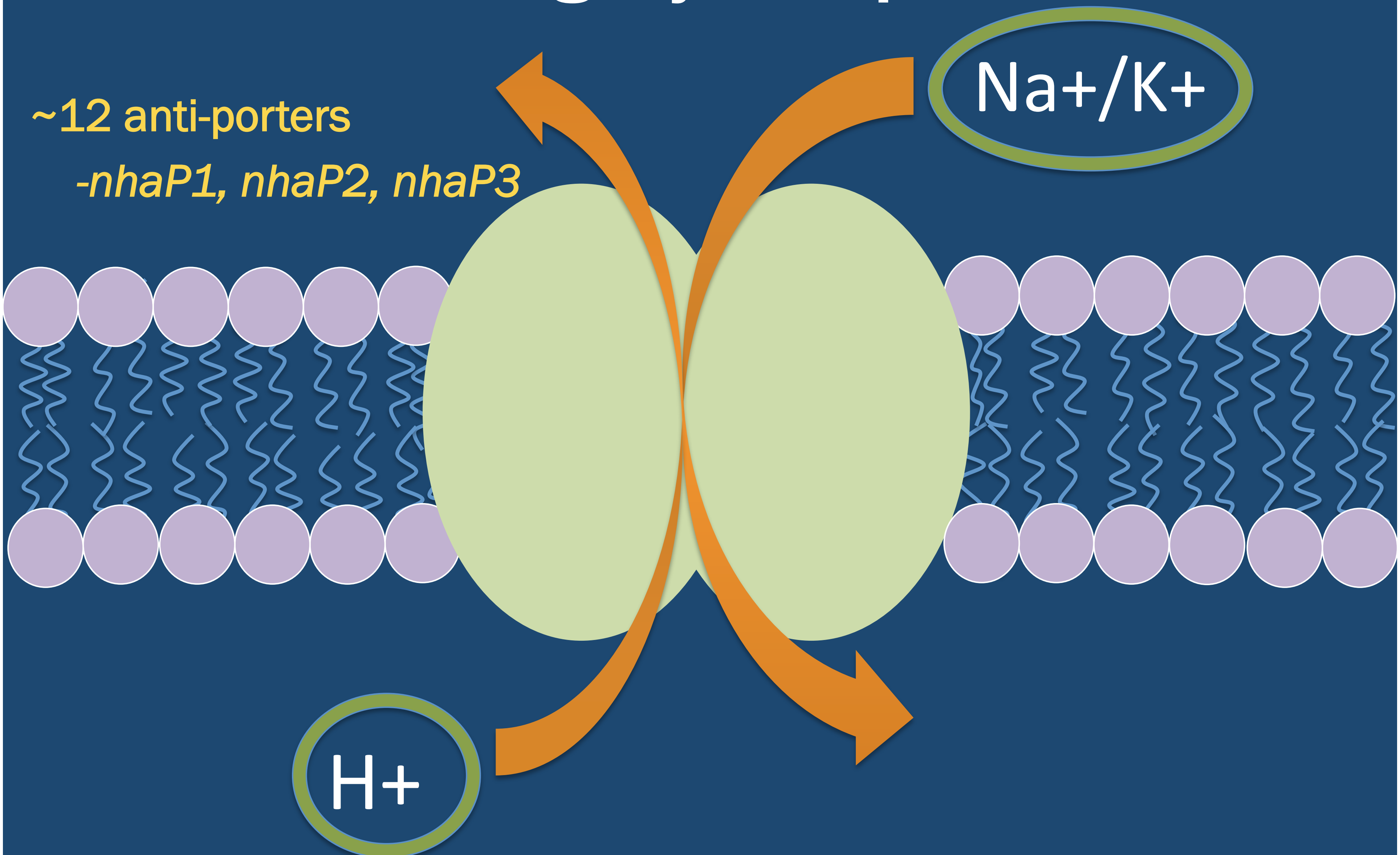
~12 anti-porters



The Mighty Antiporter

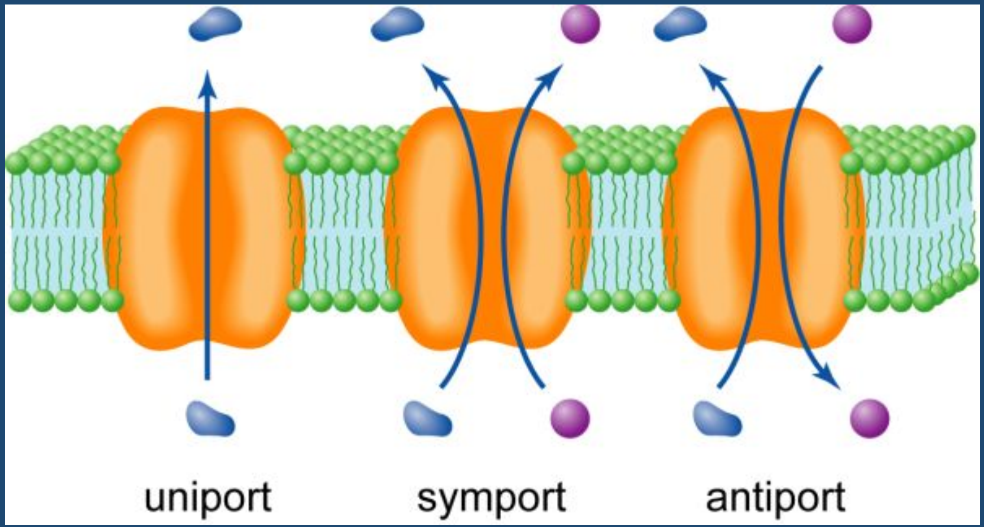
~12 anti-porters

-nhaP1, nhaP2, nhaP3



Expected Outcomes and Impact

- Understanding
- Found in *Yersinia pestis* and *Pseudomonas aeruginosa*
- Novel intervention therapies



Question & Hypothesis

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- Does the *nhaP* genes have an effect on the ability of *Vibrio cholerae* to survive in acidic broths for an extended period of time?

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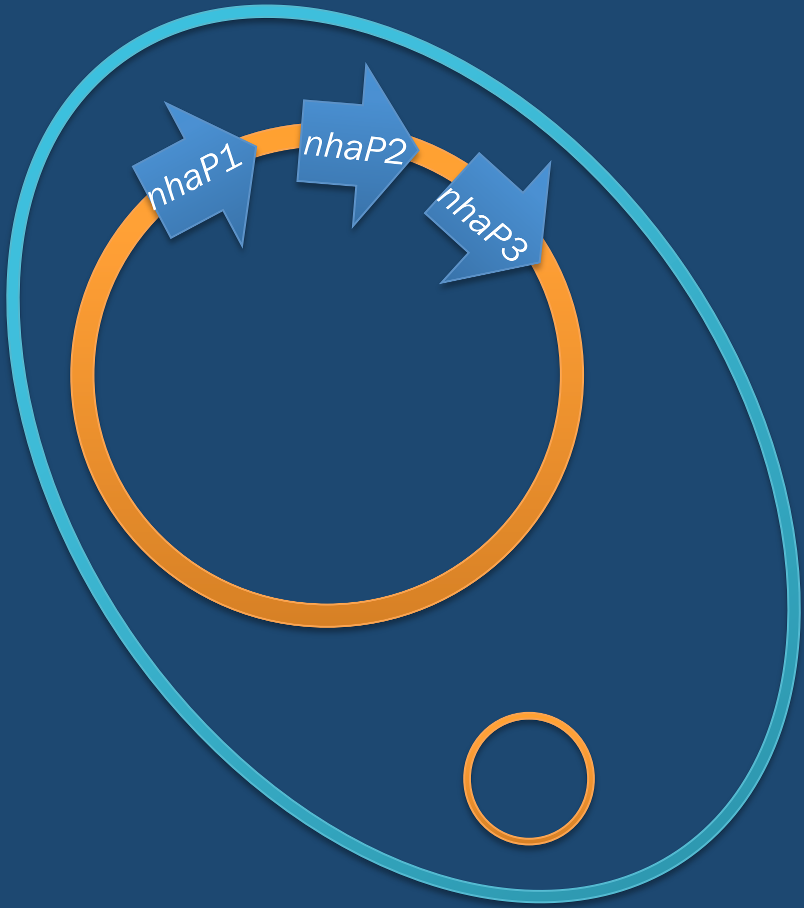
- We hypothesized that the three genes, *nhaP1, nhaP2,* and *nhaP3*, in combination, are required for the survival of *Vibrio cholerae* through the extreme acidity of the human stomach.

Strains

Strains

- Wild type
  - *Vibrio cholerae* 0395 N1



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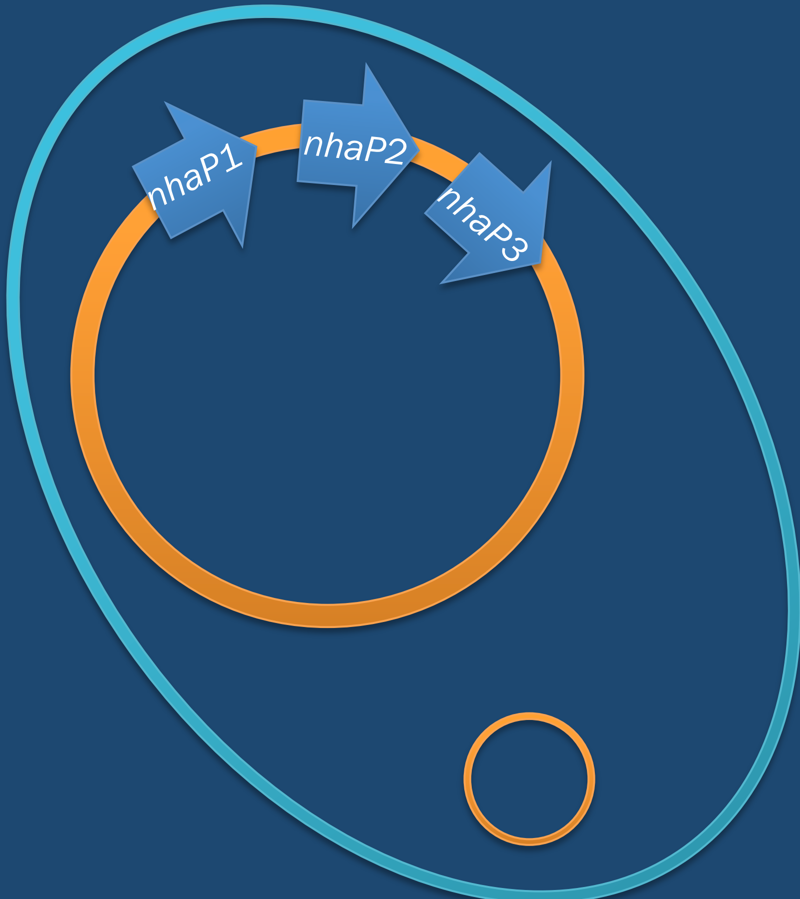

- Triple mutant
  - *Vibrio cholerae* 0395 N1 Δ*nhaP1*Δ*nhaP2*ΔnhaP3

Strains

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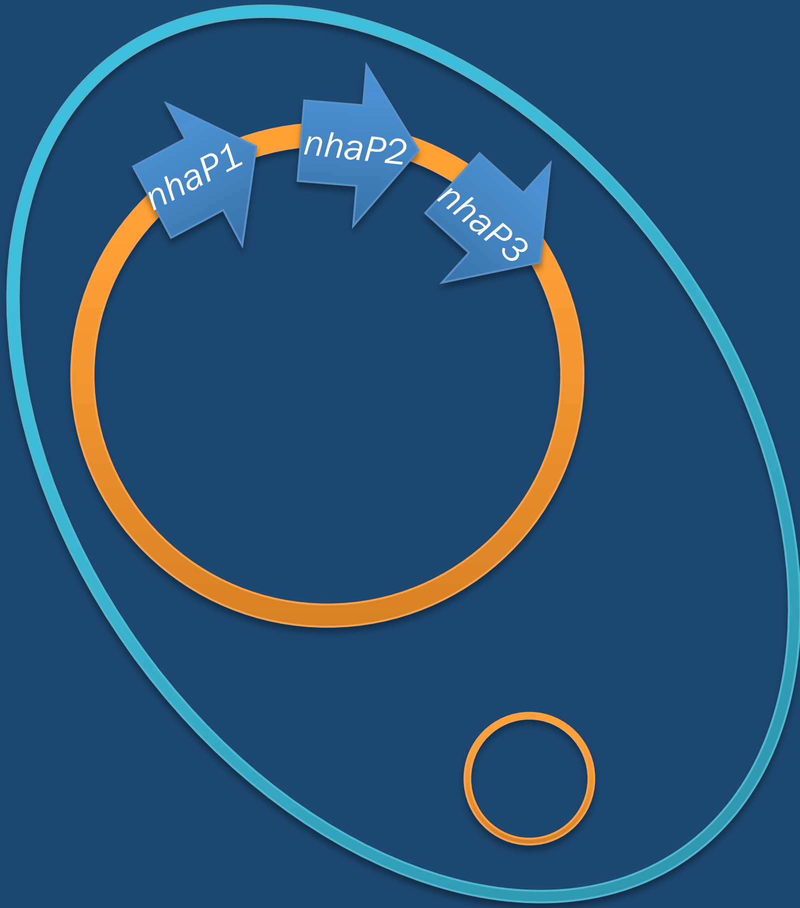
  - *Vibrio cholerae* 0395 N1



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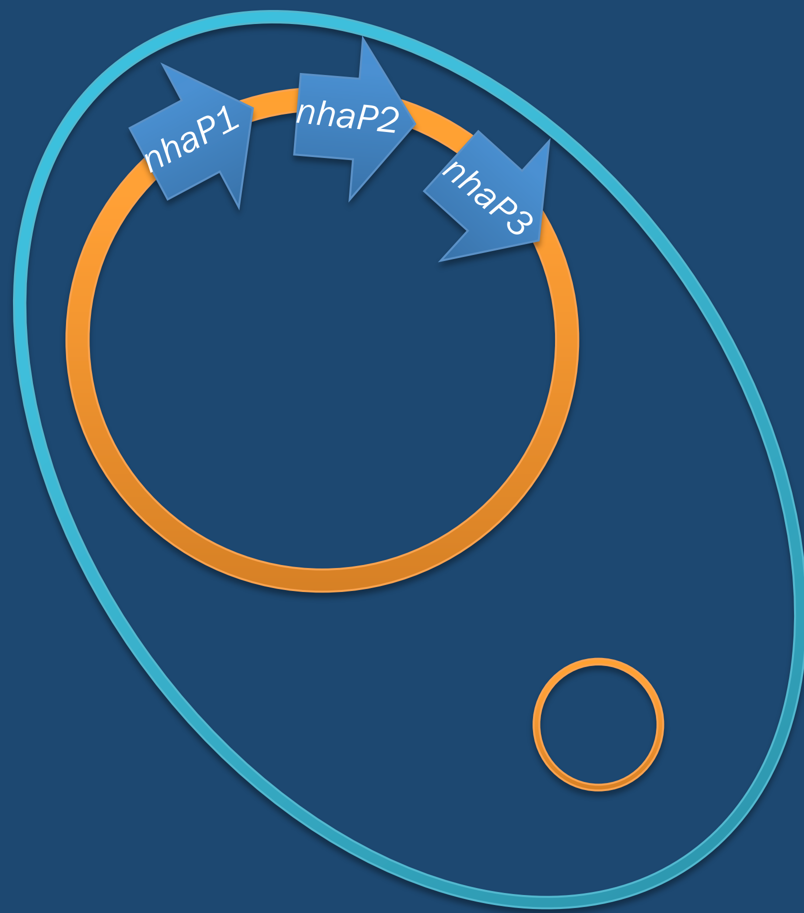

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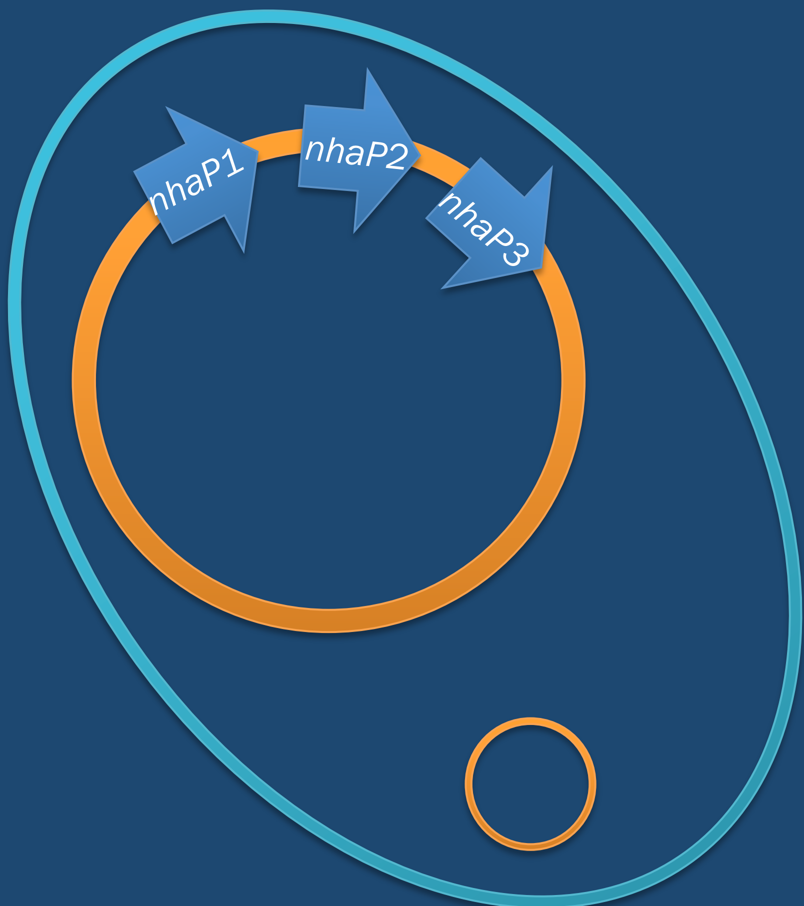

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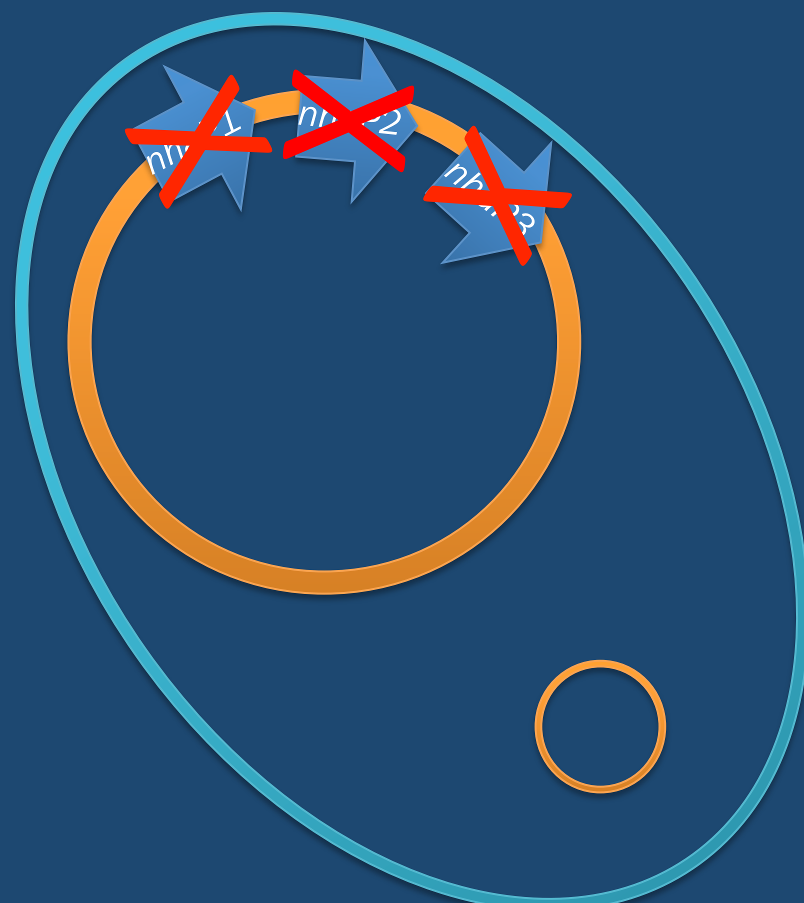

Strains

- Complement P1, Complement P2, Complement P3 & Wild type with empty plasmid
  1) Vc Δ*nhaP1*Δ*nhaP2*Δ*nhaP3* <pBAD24::nhaP1>
  2) Vc Δ*nhaP1*Δ*nhaP2*Δ*nhaP3* <pBAD24::nhaP2>
  3) Vc Δ*nhaP1*Δ*nhaP2*Δ*nhaP3* <pBAD24::nhaP3>
  4) Vc pBAD24

1)

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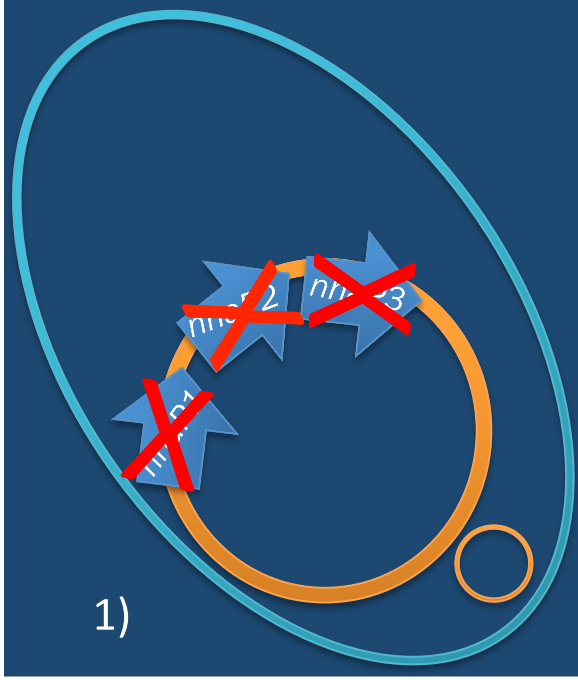

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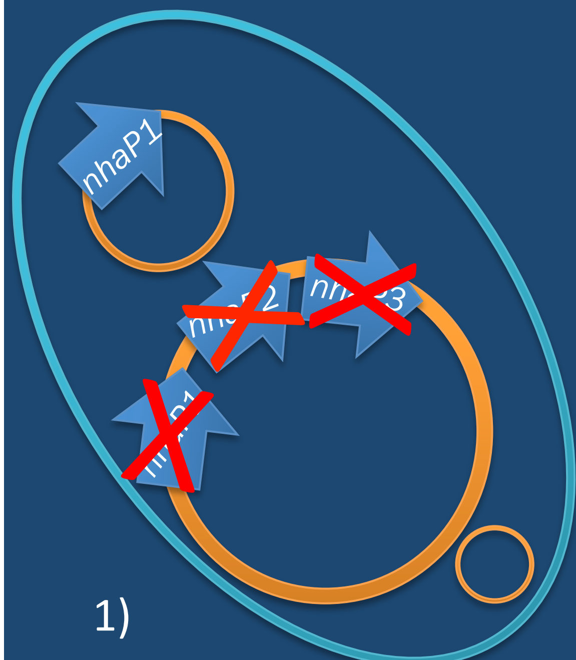

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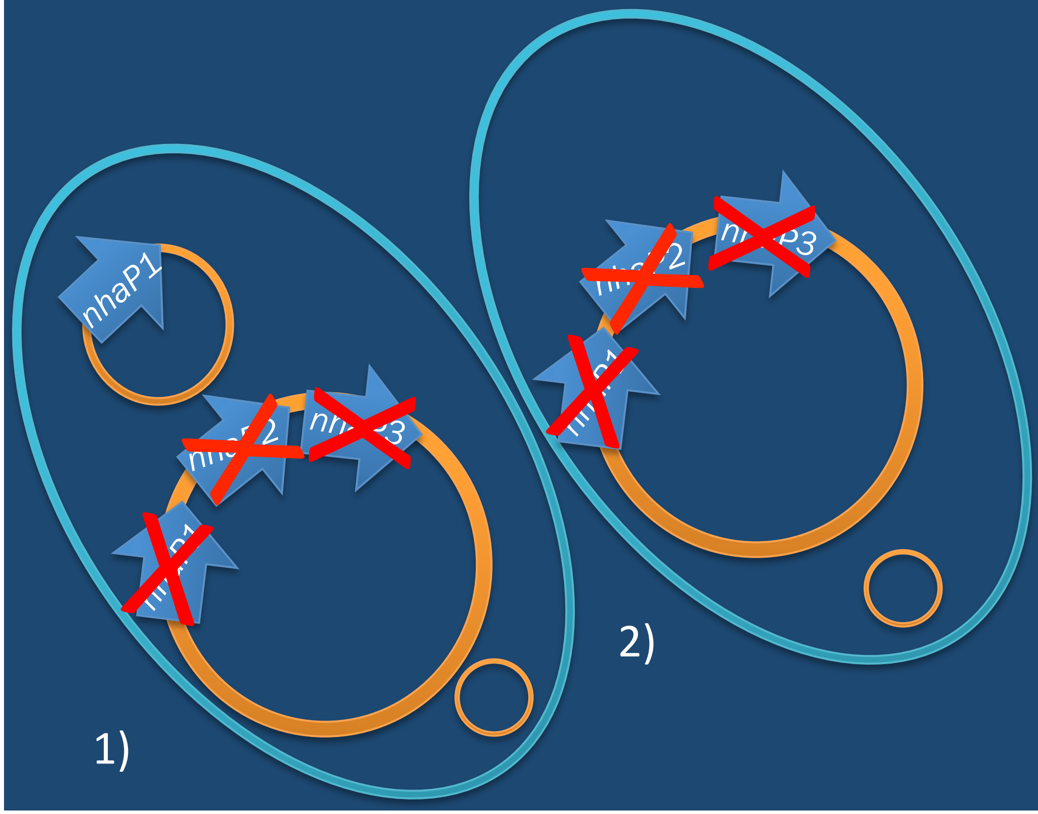
    4) Vc pBAD24



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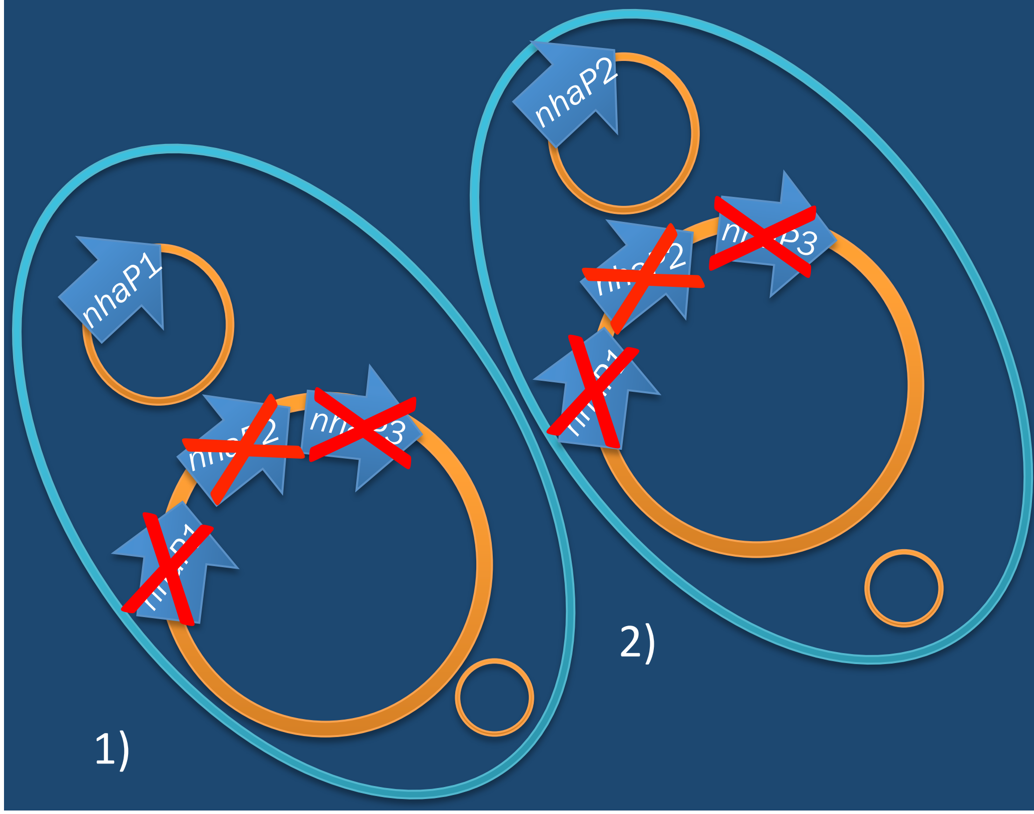

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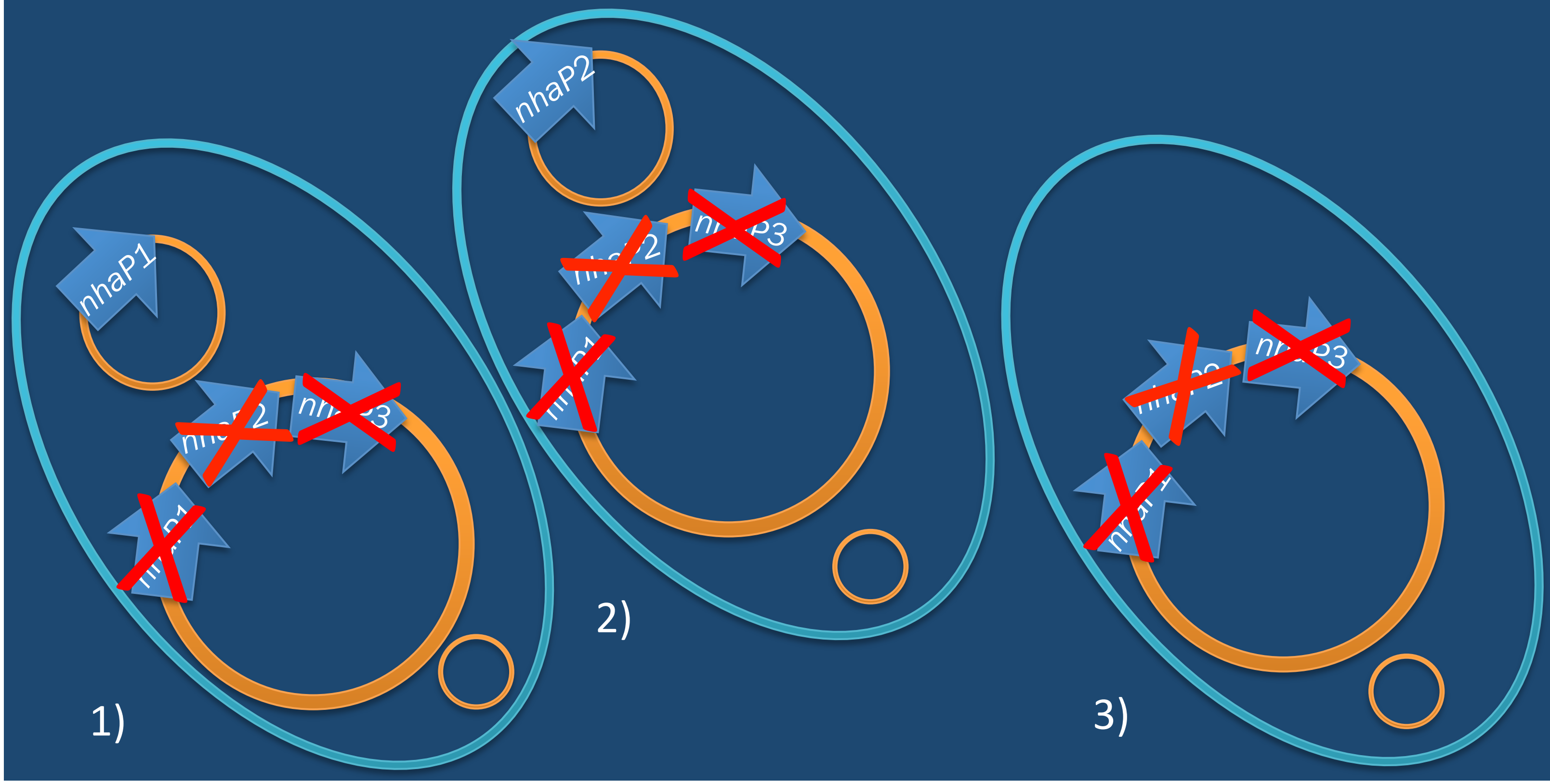
  3) Vc *ΔnhaP1ΔnhaP2ΔnhaP3* <pBAD24::nhaP3>
  4) Vc pBAD24



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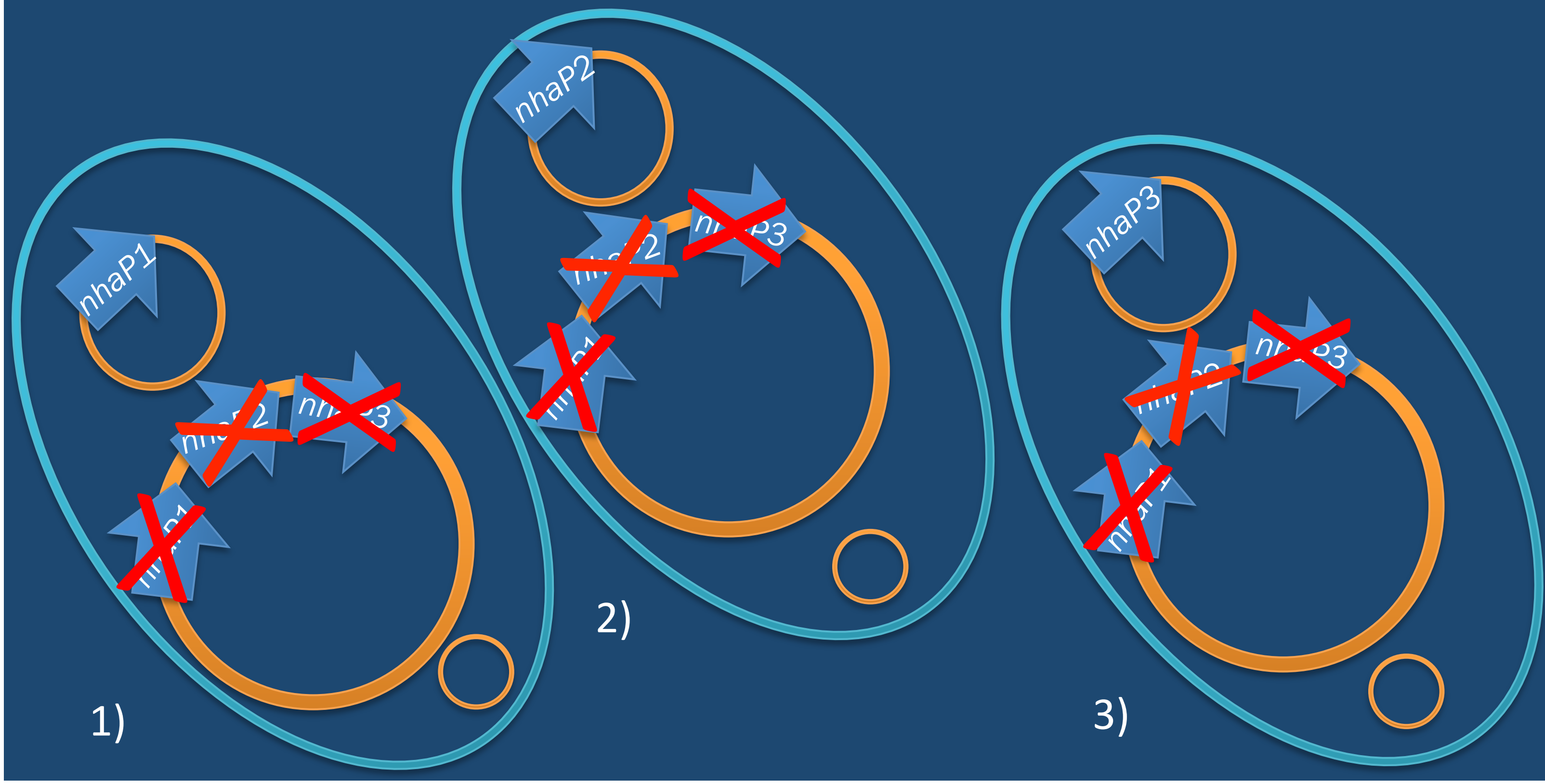

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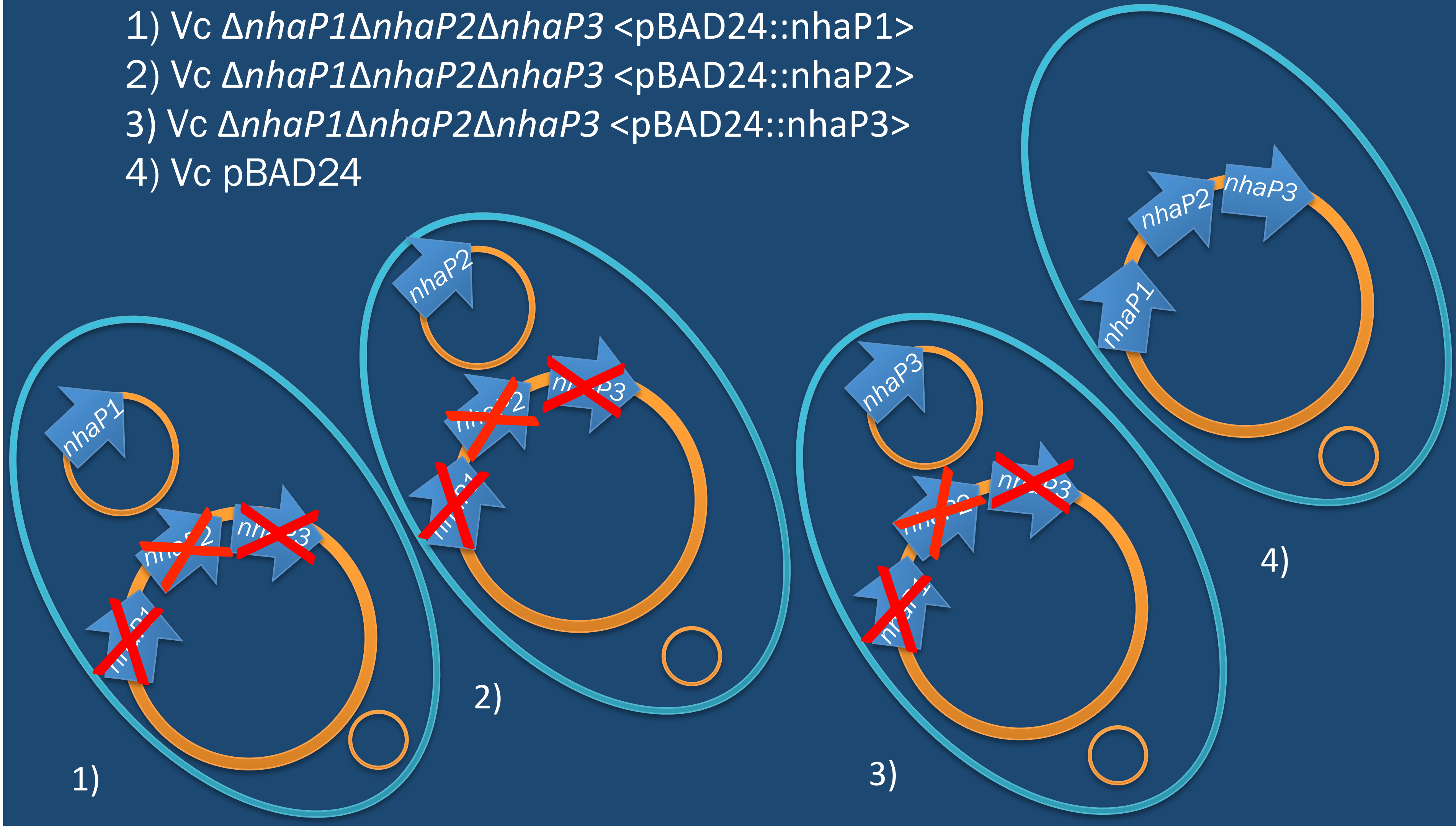

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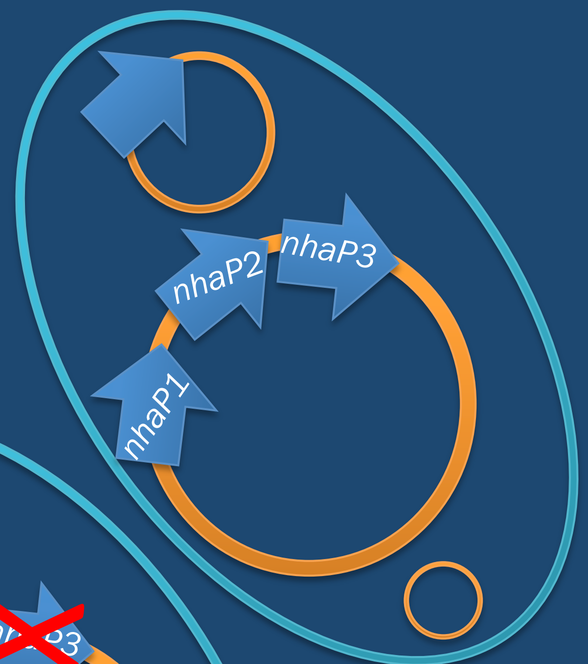
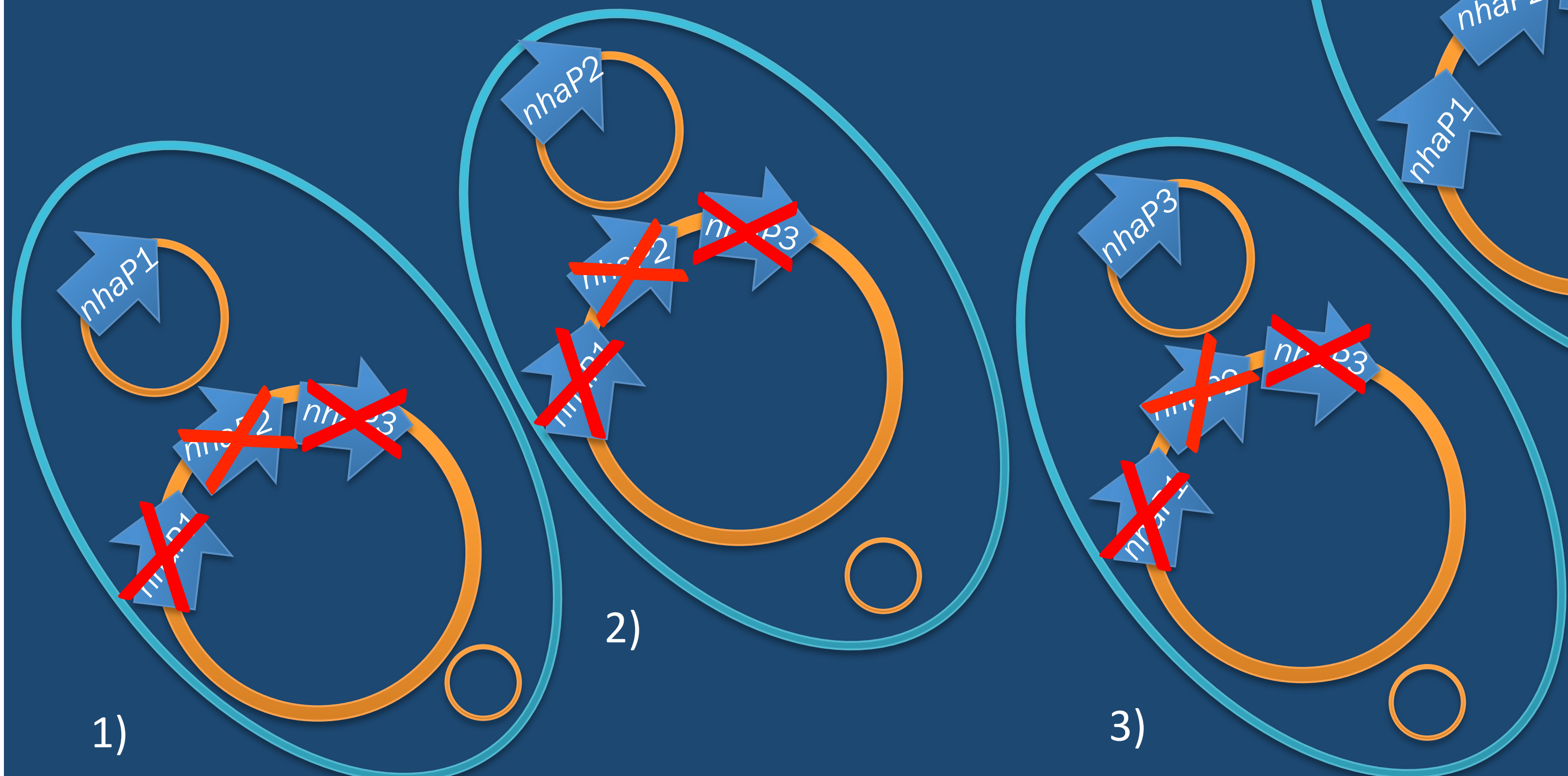
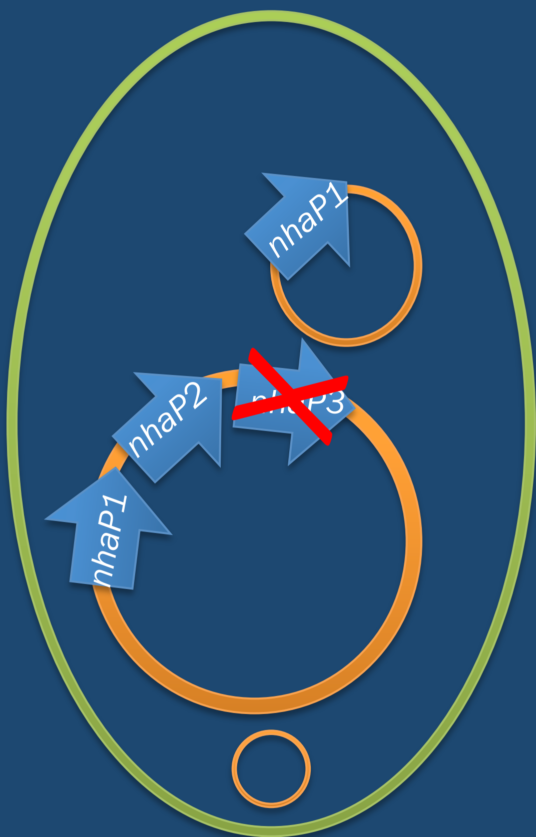




Construction of Compliment P1



Vibrio cholerae 0395 N1
ΔnhaP3<pBAD24::nhaP1>

Construction of Compliment P1

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Construction of Compliment P1

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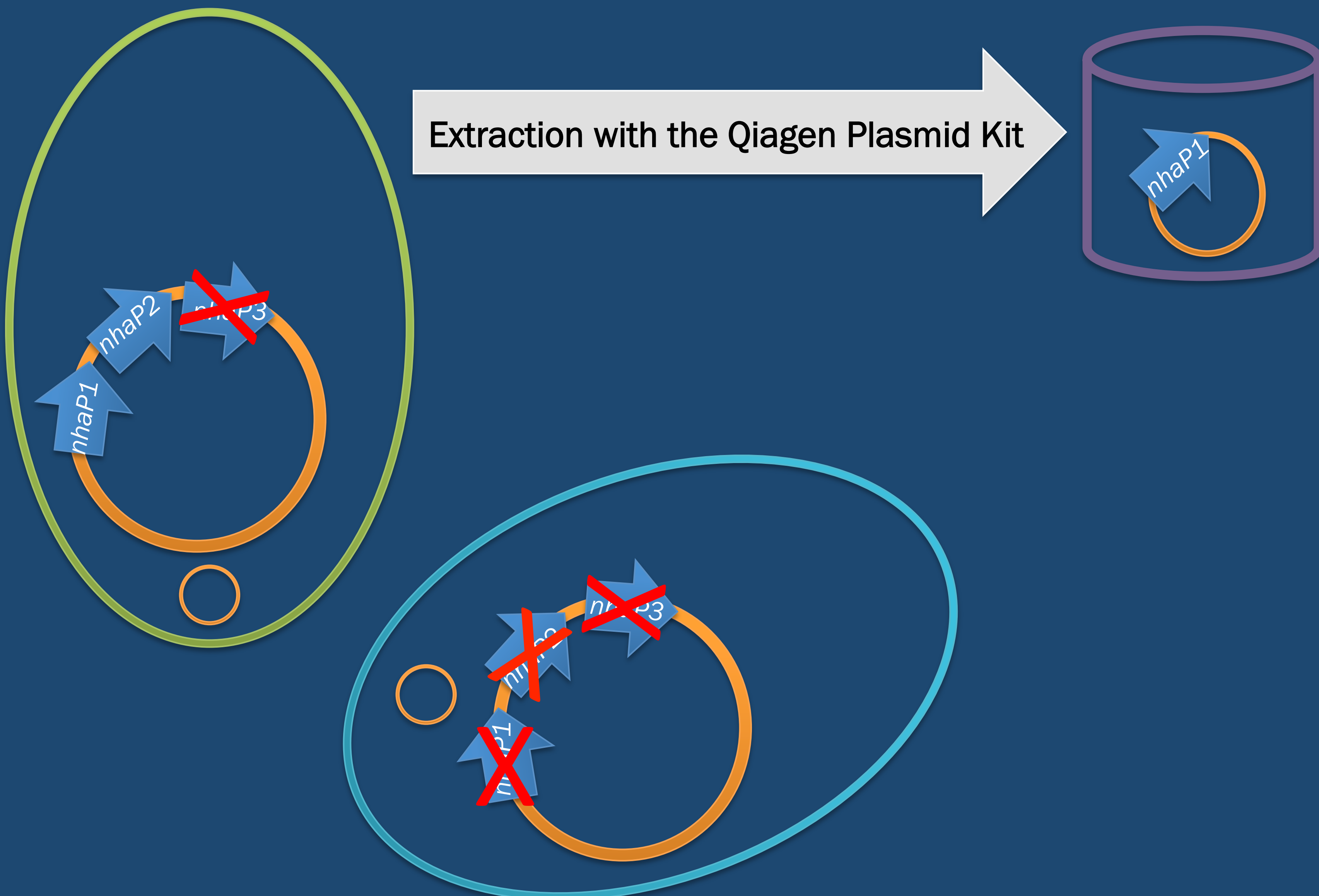

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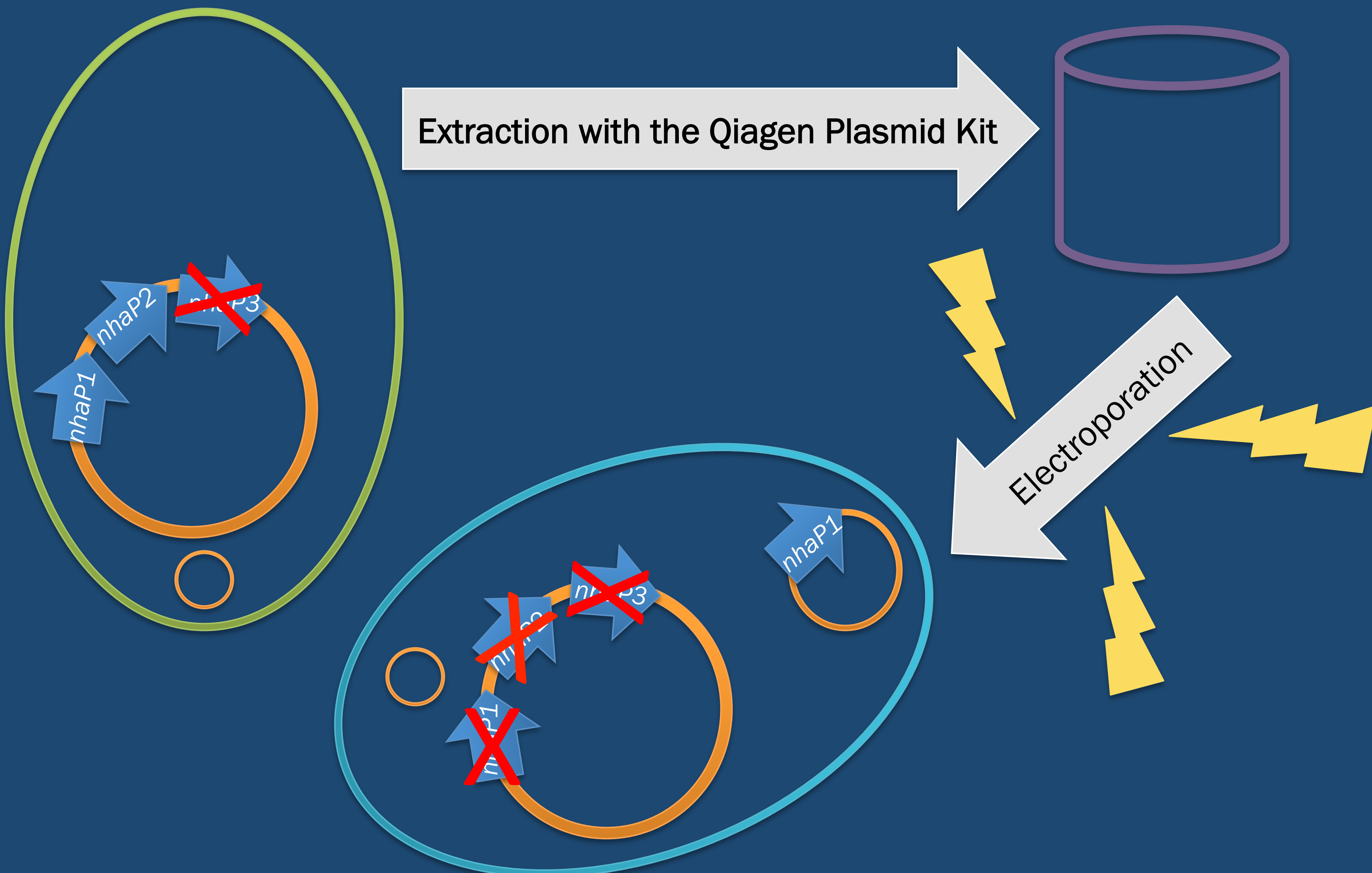
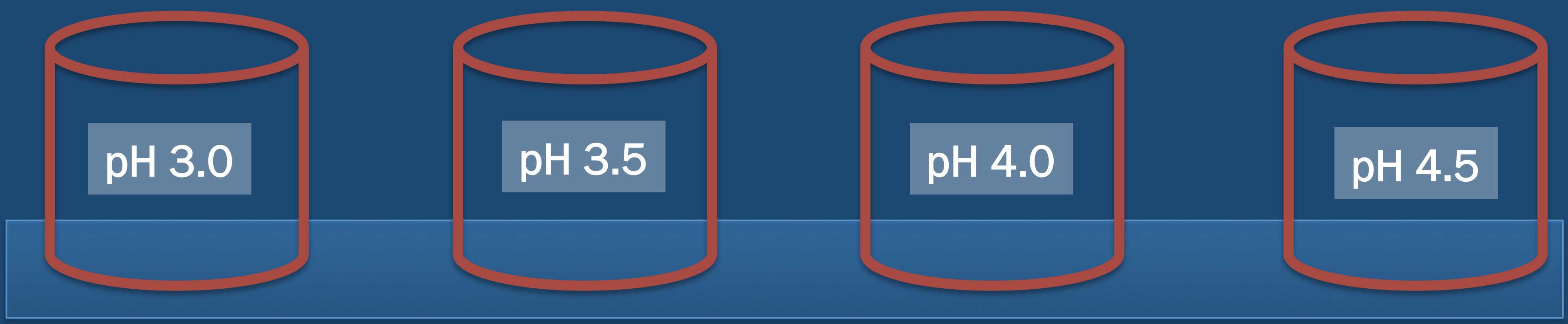


Methods

- Acid Tolerance Assays
  - Conditions:
    - pH 3.0, 3.5, 4.0 & 4.5 LBK
    - 0, 15, 60, 90 minutes



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Note

A 6×6 drop plate method for simultaneous colony counting and MPN enumeration of *Campylobacter jejuni*, *Listeria monocytogenes*, and *Escherichia coli*

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Serial Dilutions

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												
I	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}

10-fold serial dilutions with mixing from left to right (transferring 20uL of previous column into 180uL of LBK).

Serial Dilutions

(Biomart, 2007)

	1	2	3	4	5	6	7	8	9			
A												
B												
C												
D												
E												
F												
G												
H												
I	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}

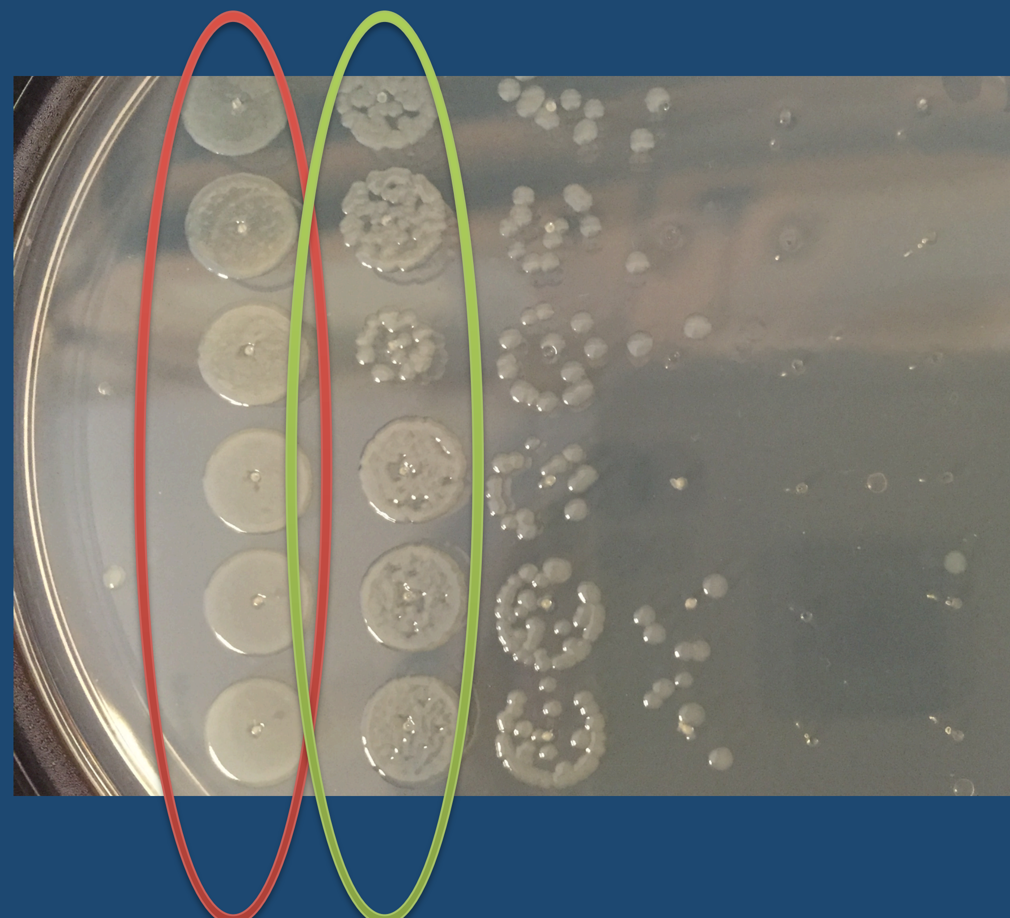
10-fold serial dilutions with mixing from left to right (transferring 20uL of previous column into 180uL of LBK).

Plating

	1	2	3	4	5	6
A						
B						
C						
D						
E						
F						
G						
H						
I	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}

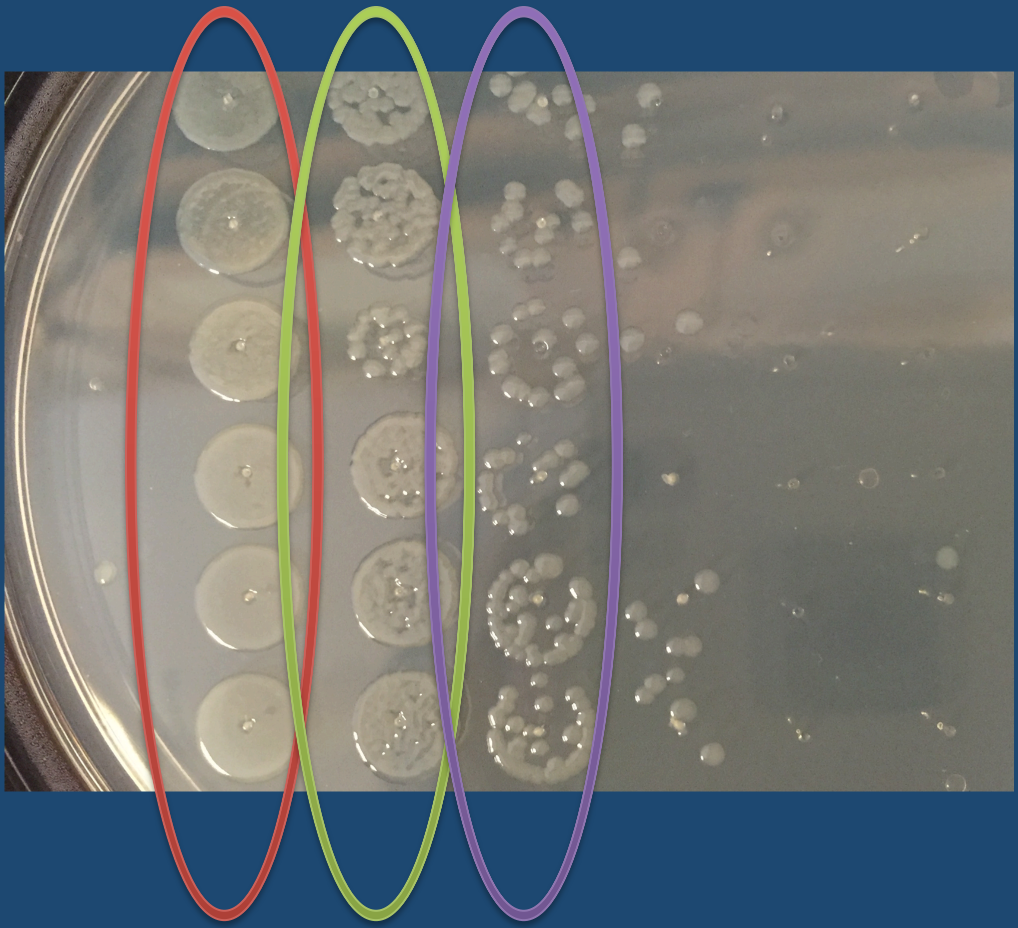
Plating

	1	2	3	4	5	6
A						
B						
C						
D						
E						
F						
G						
H						
I	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}

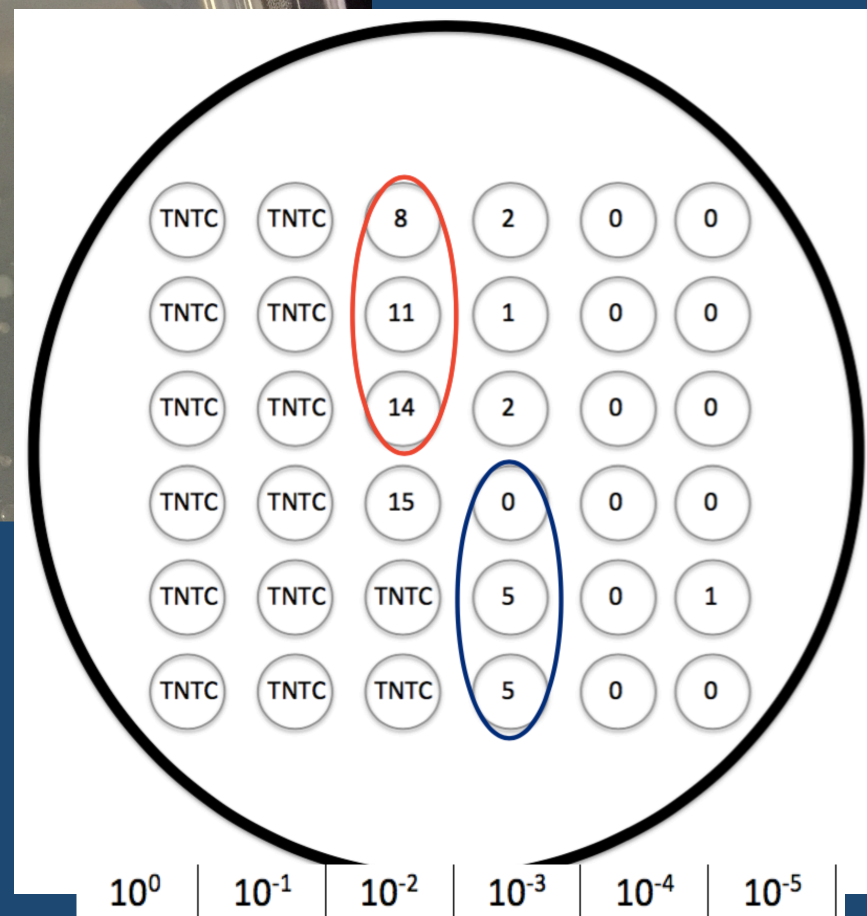


Plating

	1	2	3	4	5	6
A						
B						
C						
D						
E						
F						
G						
H						
I	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}



Calculation of Percentage of Cell Survival



$$\frac{(\#of\ colonies\ at\ t = x) \times (well\ DF\ at\ t = x) \times (plating\ DF)}{(\#of\ colonies\ at\ t = 0) \times (well\ DF\ at\ t = 0) \times (plating\ DF)}$$

$$= \_\_CFU/mL$$

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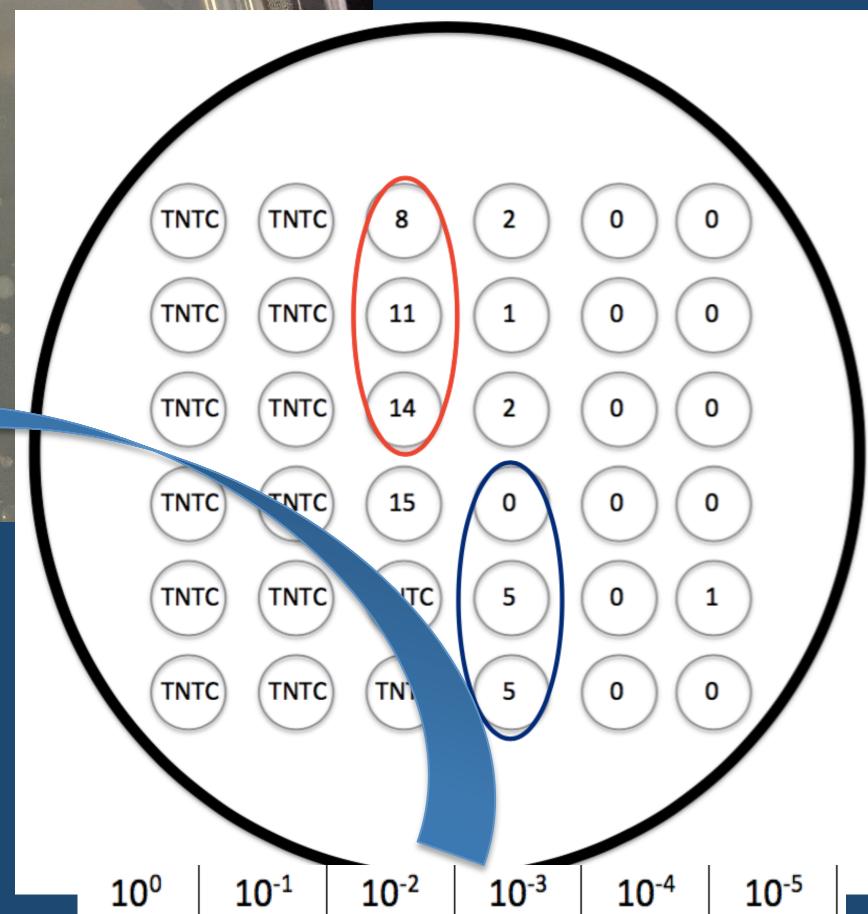

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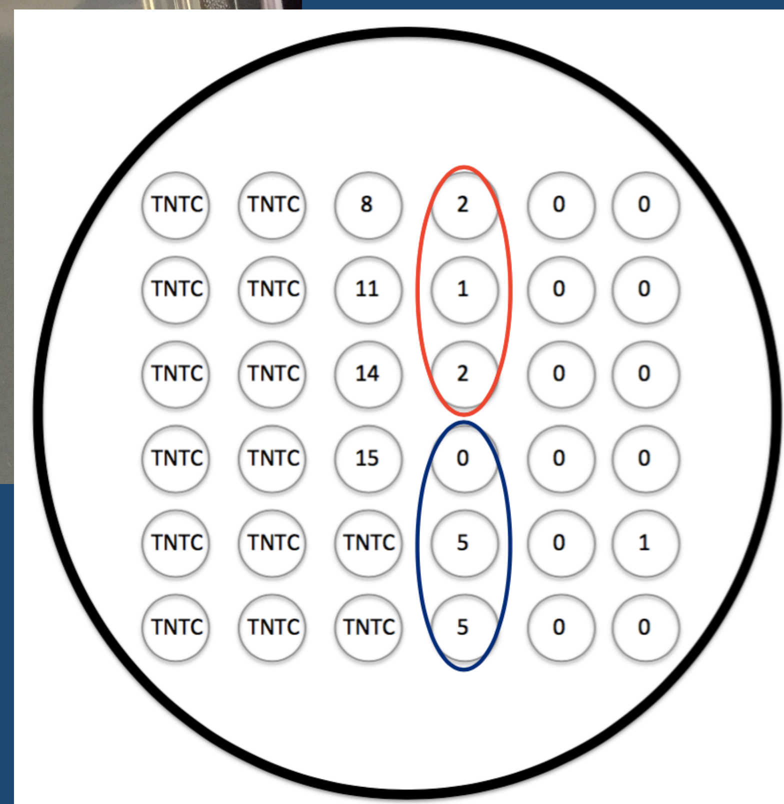
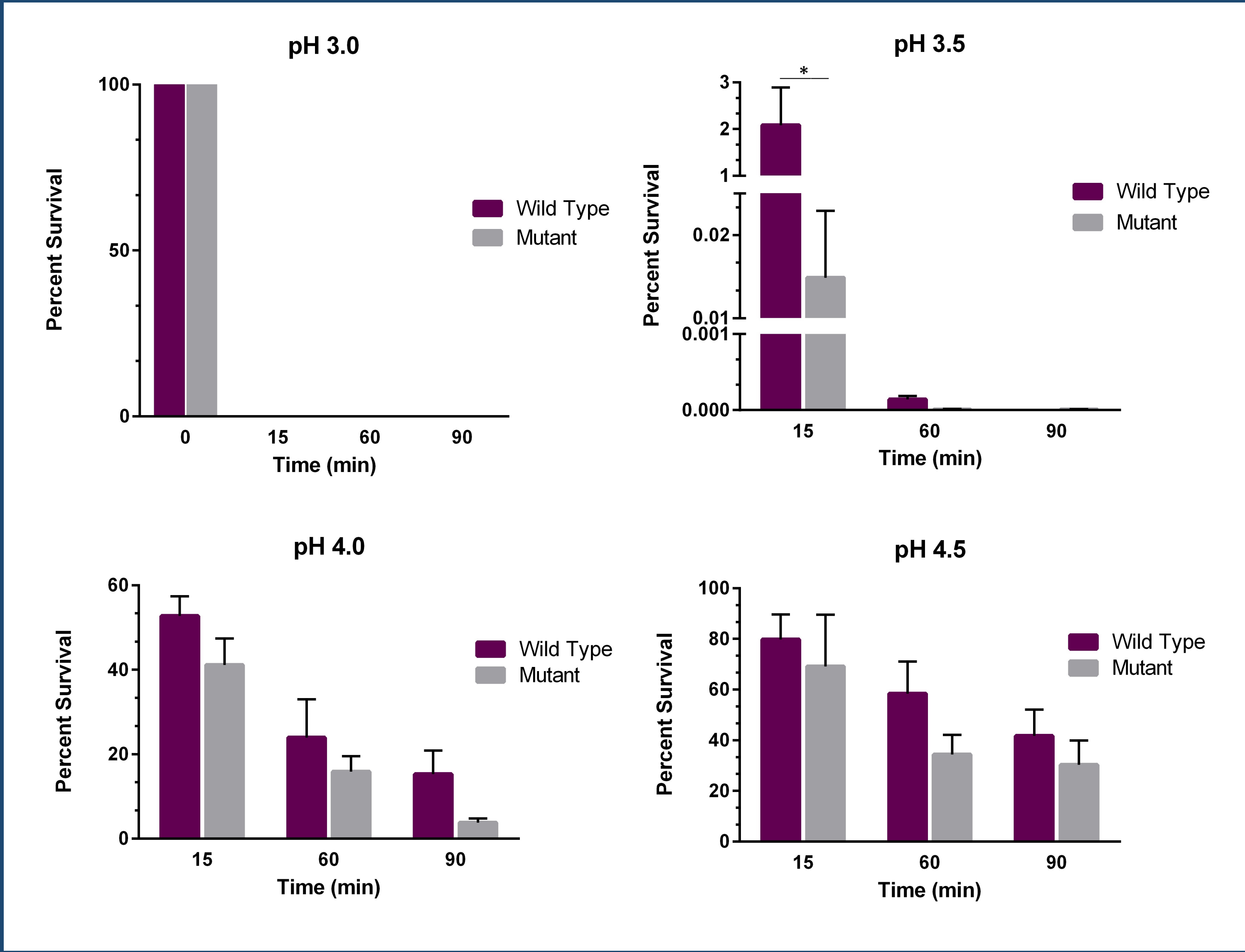
$$= \_\_\ CFU/mL$$

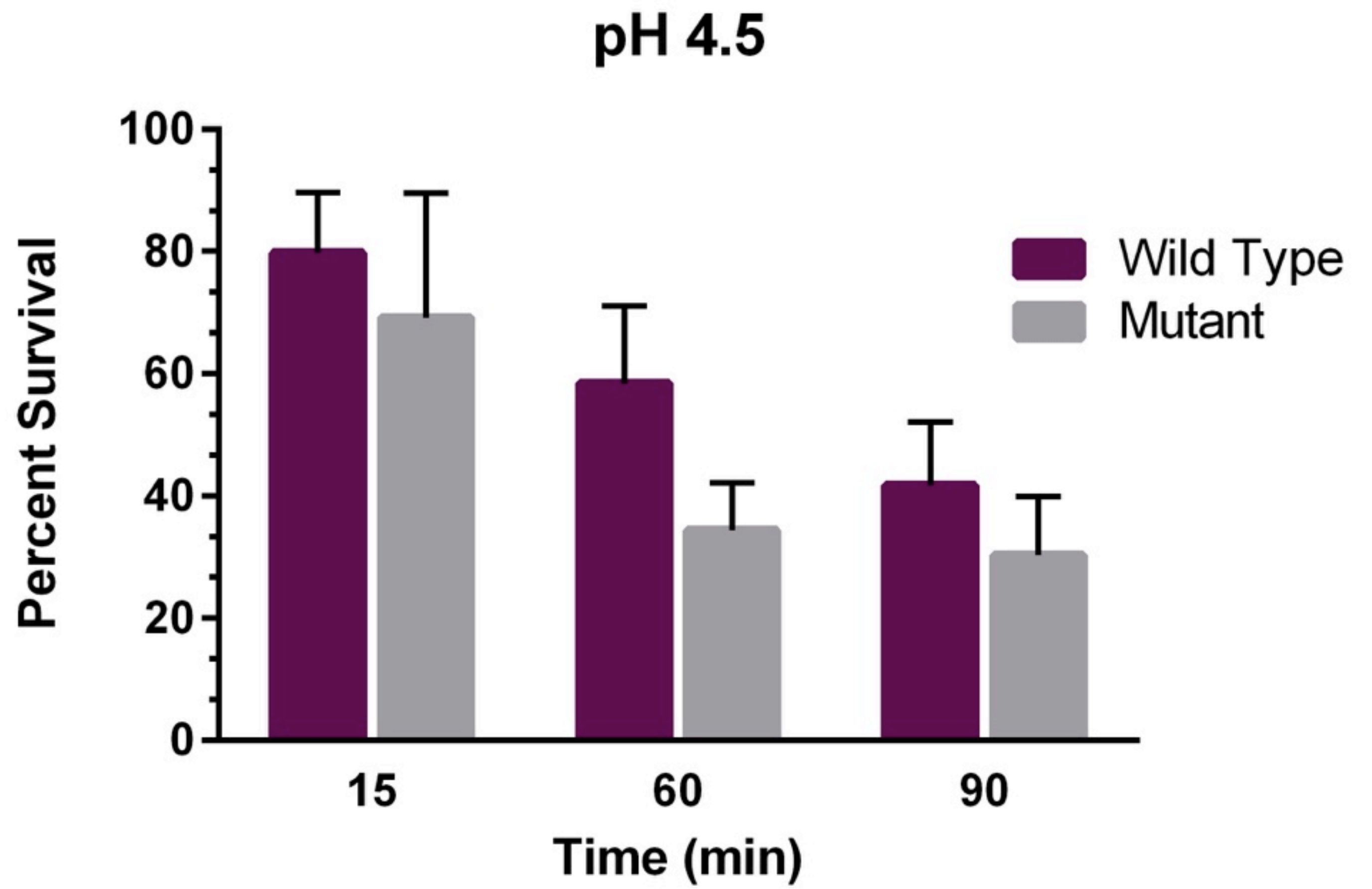


Results

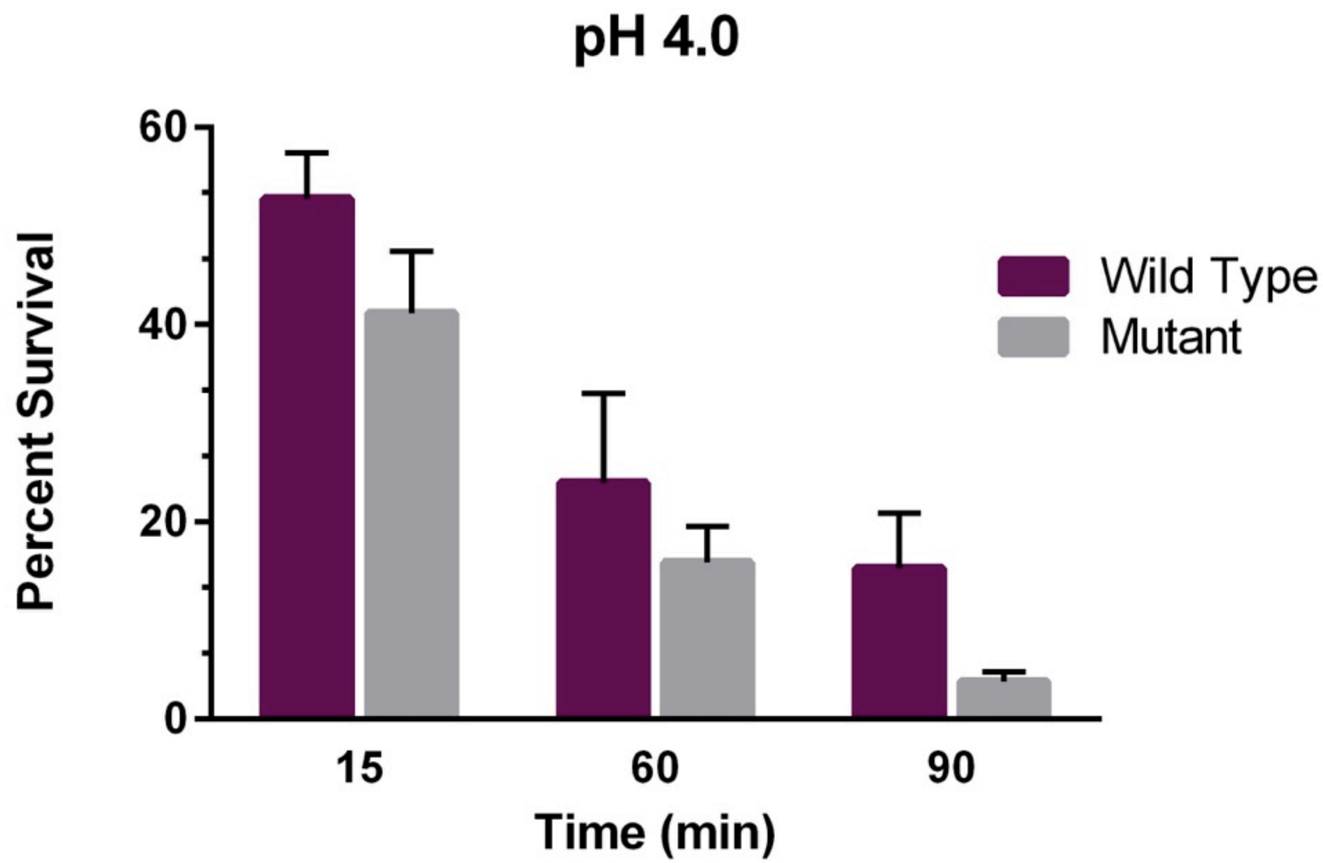


pH 3.0
100
50
0
Percent Survival
0
15
60
90
Time (min)
Wild Type
Mutant
pH 3.5
*
3
2
1
0.02
0.01
0.001
0.000
Percent Survival
15
60
90
Time (min)
Wild Type
Mutant
pH 4.0
60
40
20
0
Percent Survival
15
60
90
Time (min)
Wild Type
Mutant
pH 4.5
100
80
60
40
20
0
Percent Survival
15
60
90
Time (min)
Wild Type
Mutant

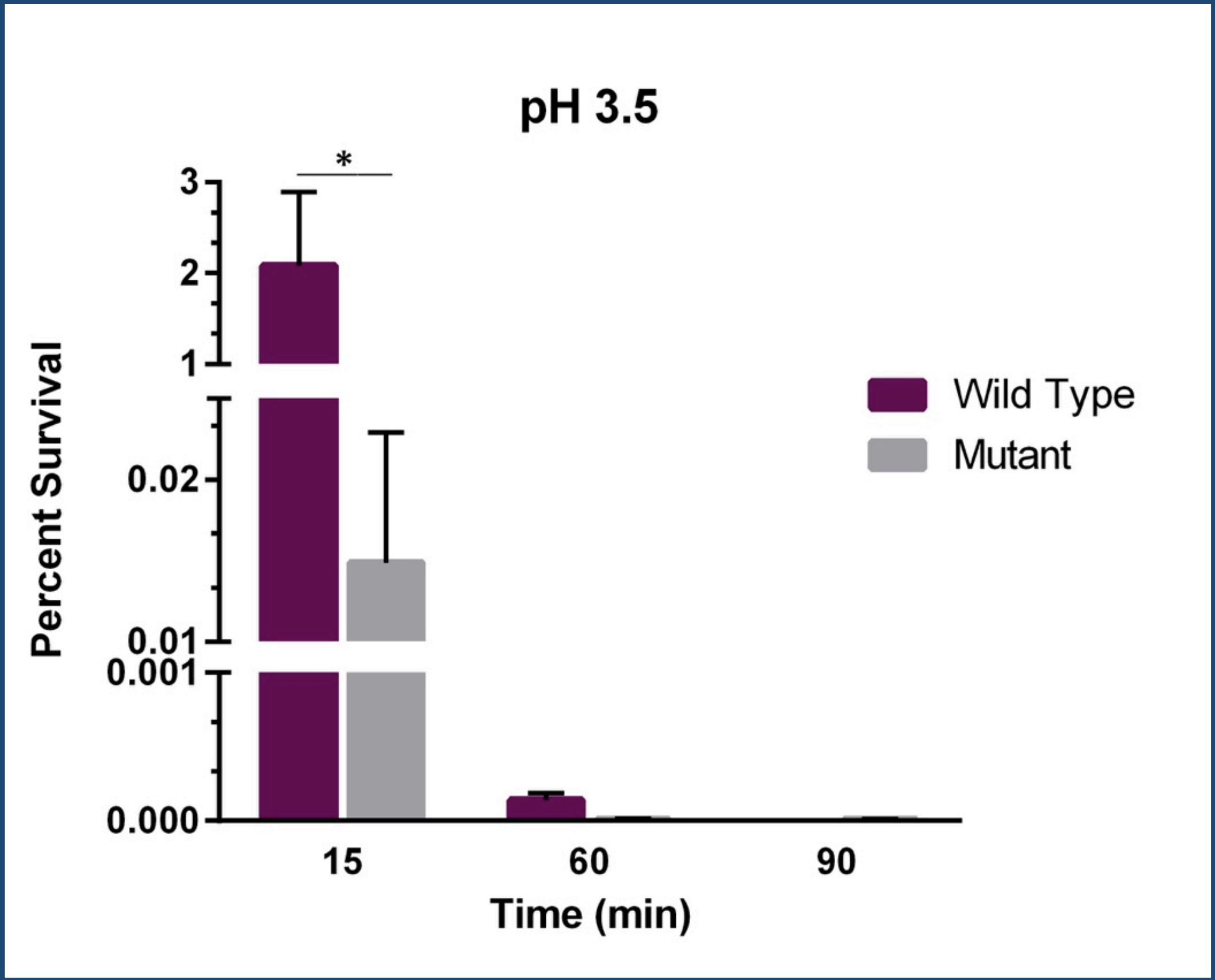
Average Percentage of Survival



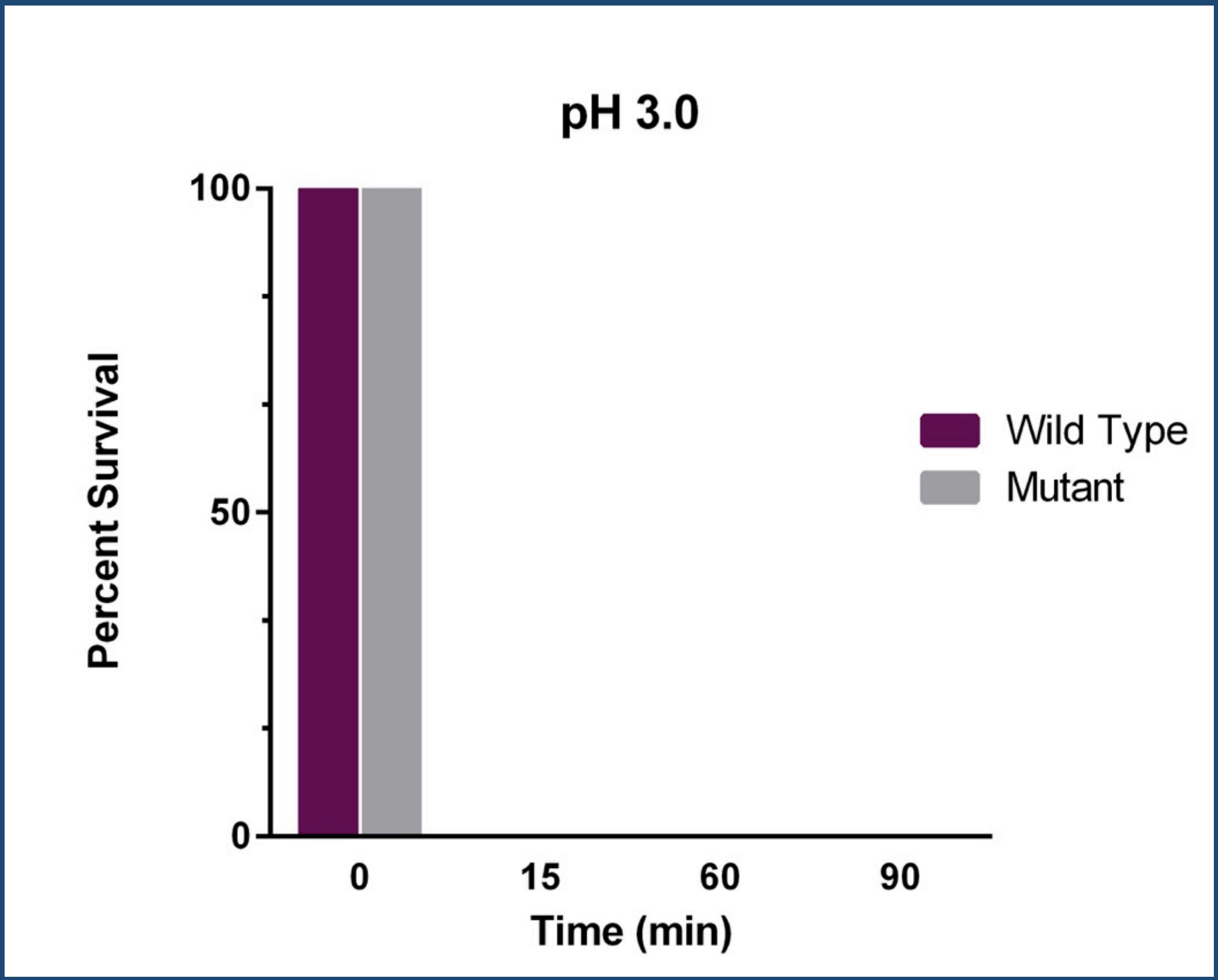
Average Percentage of Survival



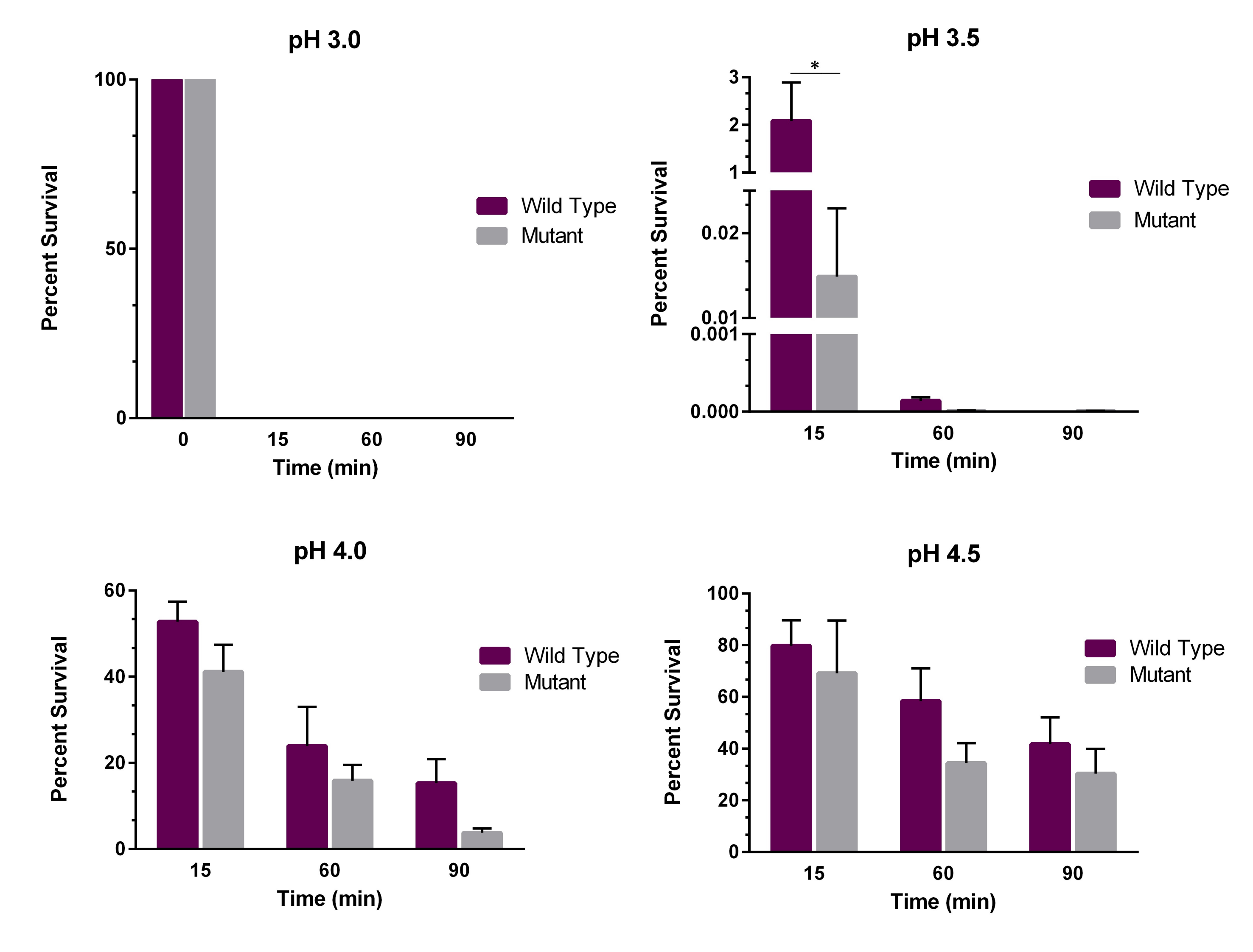
Average Percentage of Survival



Average Percentage of Survival

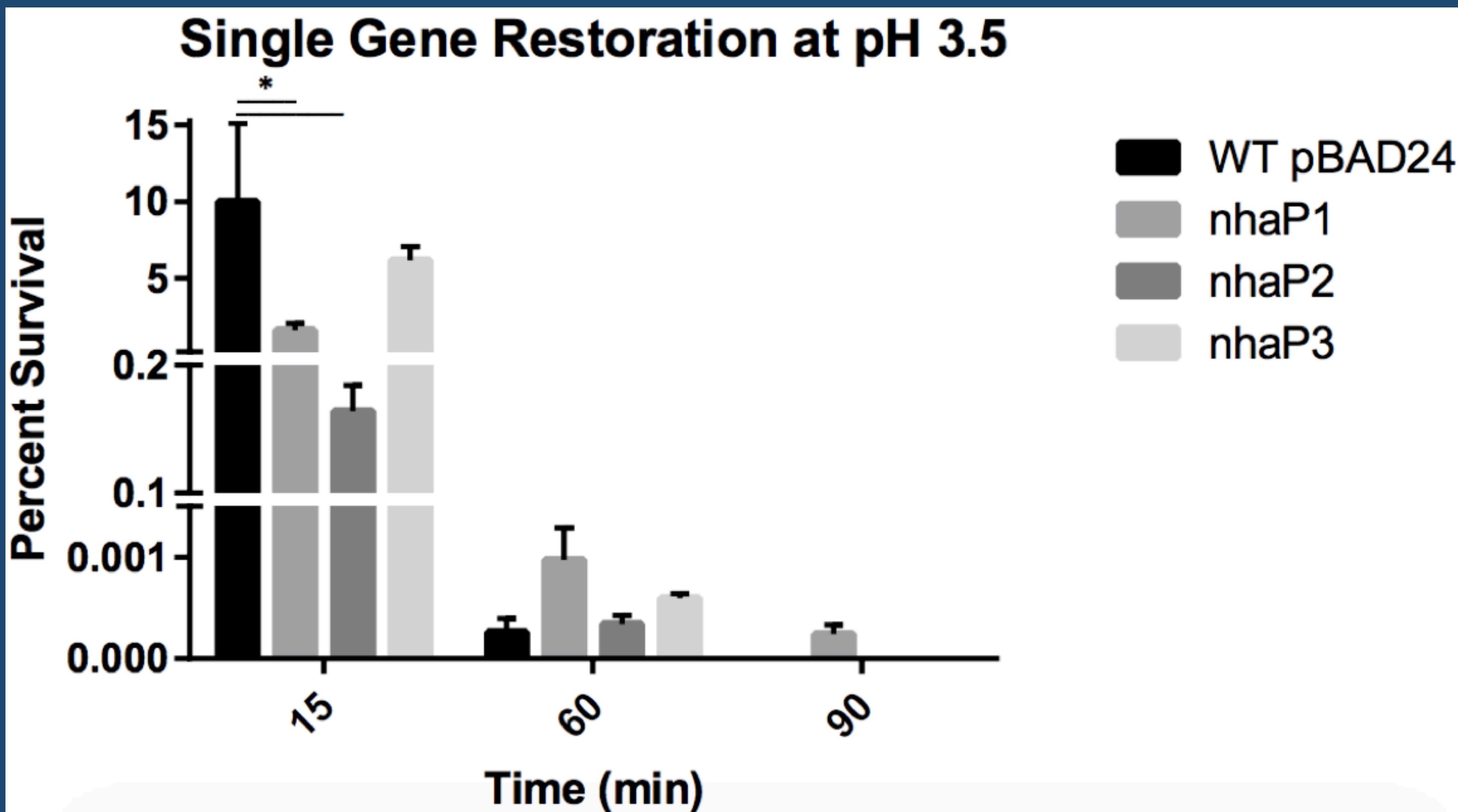


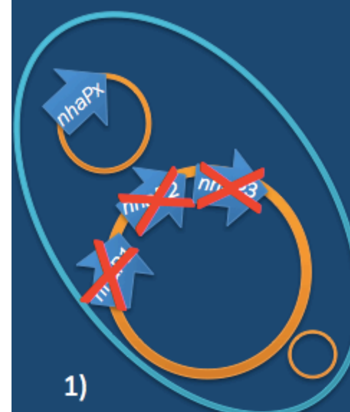
Results



pH 3.0
Percent Survival
100
50
0
0
15
60
90
Time (min)
Wild Type
Mutant
pH 3.5
Percent Survival
*
3
2
1
0.02
0.01
0.001
0.000
15
60
90
Time (min)
Wild Type
Mutant
pH 4.0
Percent Survival
60
40
20
0
15
60
90
Time (min)
Wild Type
Mutant
pH 4.5
Percent Survival
100
80
60
40
20
0
15
60
90
Time (min)
Wild Type
Mutant

Average Percentage of Survival: Compliment Testing





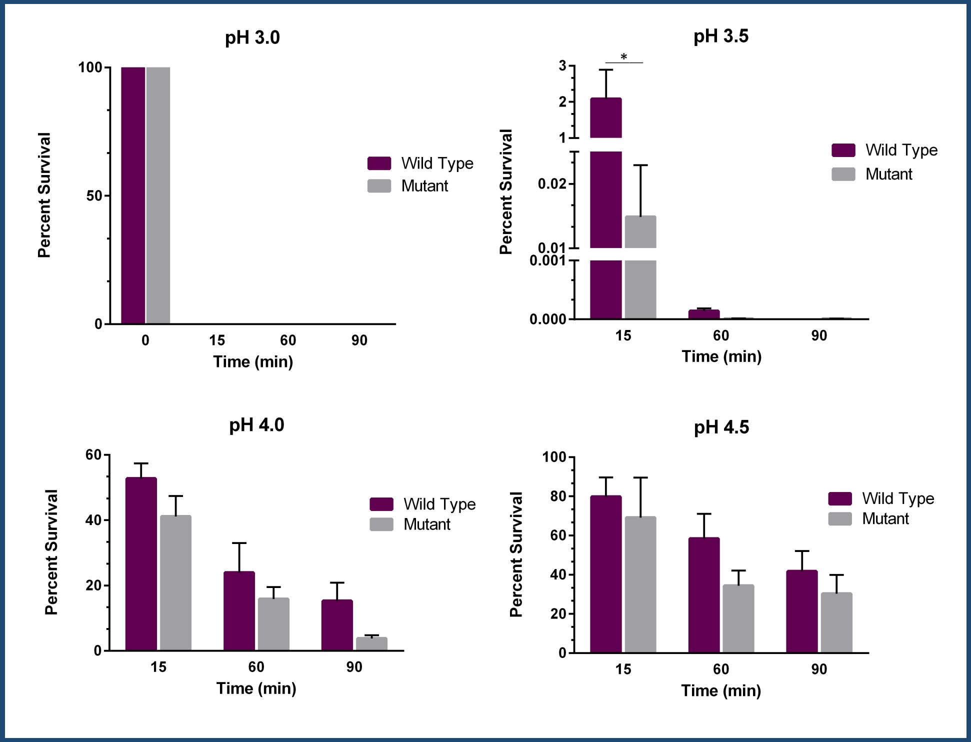
Question & Hypothesis Revisited

- Does the *nhaP* genes have an effect on the ability of *Vibrio cholerae* to survive in acidic broths for an extended period of time?

- We hypothesize that the three genes, *nhaP1, nhaP2* and *nhaP3*, in combination, are required for the survival of *Vibrio cholerae* through the extreme acidity of the human gut.

Wild Type versus Triple mutant Conclusion

Having *nhaP1, nhaP2* and *nhaP3* intact is beneficial for the organism under extremely acidic conditions but is not necessary for survival.

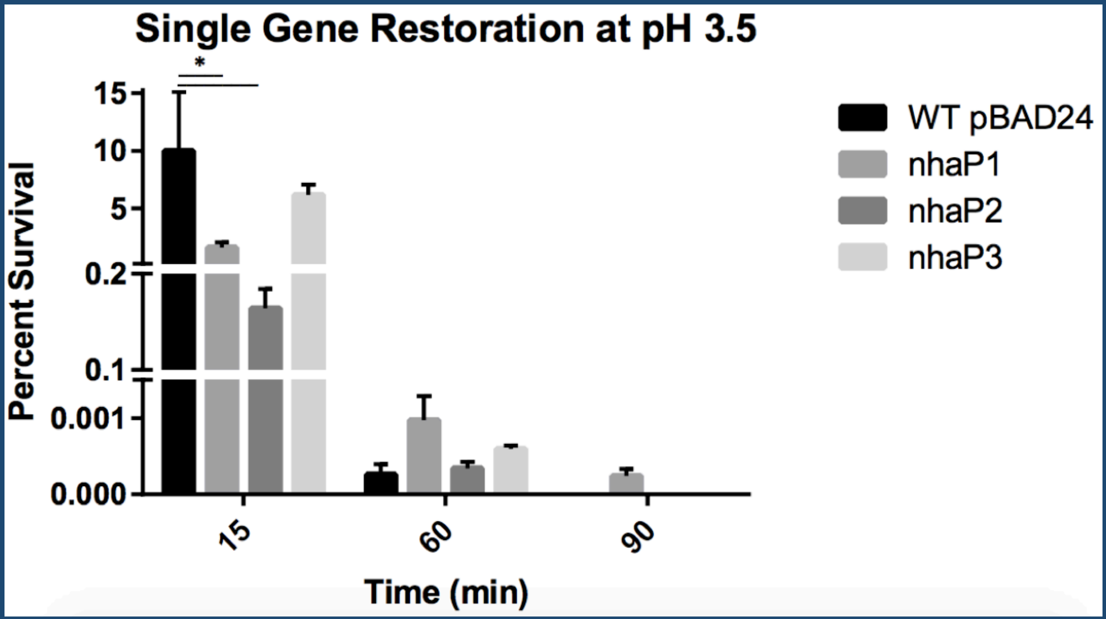


Discussion

- 6x6 Drop Plate Method
  - Saves materials & time
  - Sweet spot
  - Potential error

Complement Strain Conclusions

- Lacking two of the antiporters lead to reduced survival compared to the wild type.
- The strain having *nhaP3* restored survived at the highest rate compared to *nhaP1* and *nhaP2* at 15 minutes and acted very similar to the wild type.



Future Research

- Comparison of results w/ Standard Plate Counting
- Increasing replicates
- Combo of *nhaPx* antiporters
- Studying other antiporters in Vc to see if one has a larger effect or whether it's a combo of all antiporters that allow for survival

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Questions?