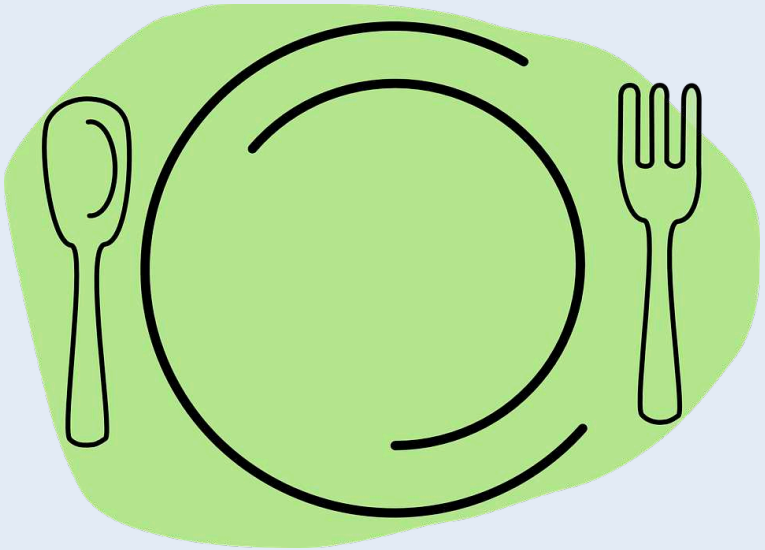
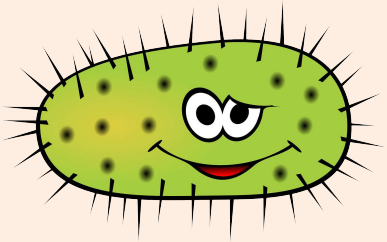
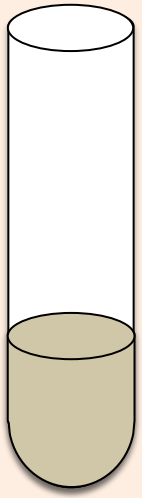
Haemophilus influenzae metabolic requirements during lung infection

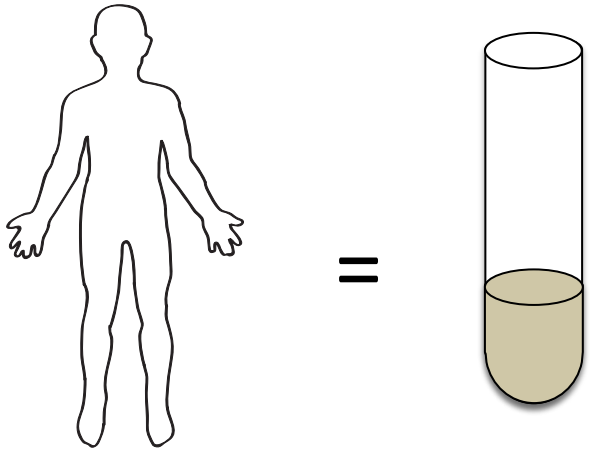
What did you have for dinner last night?







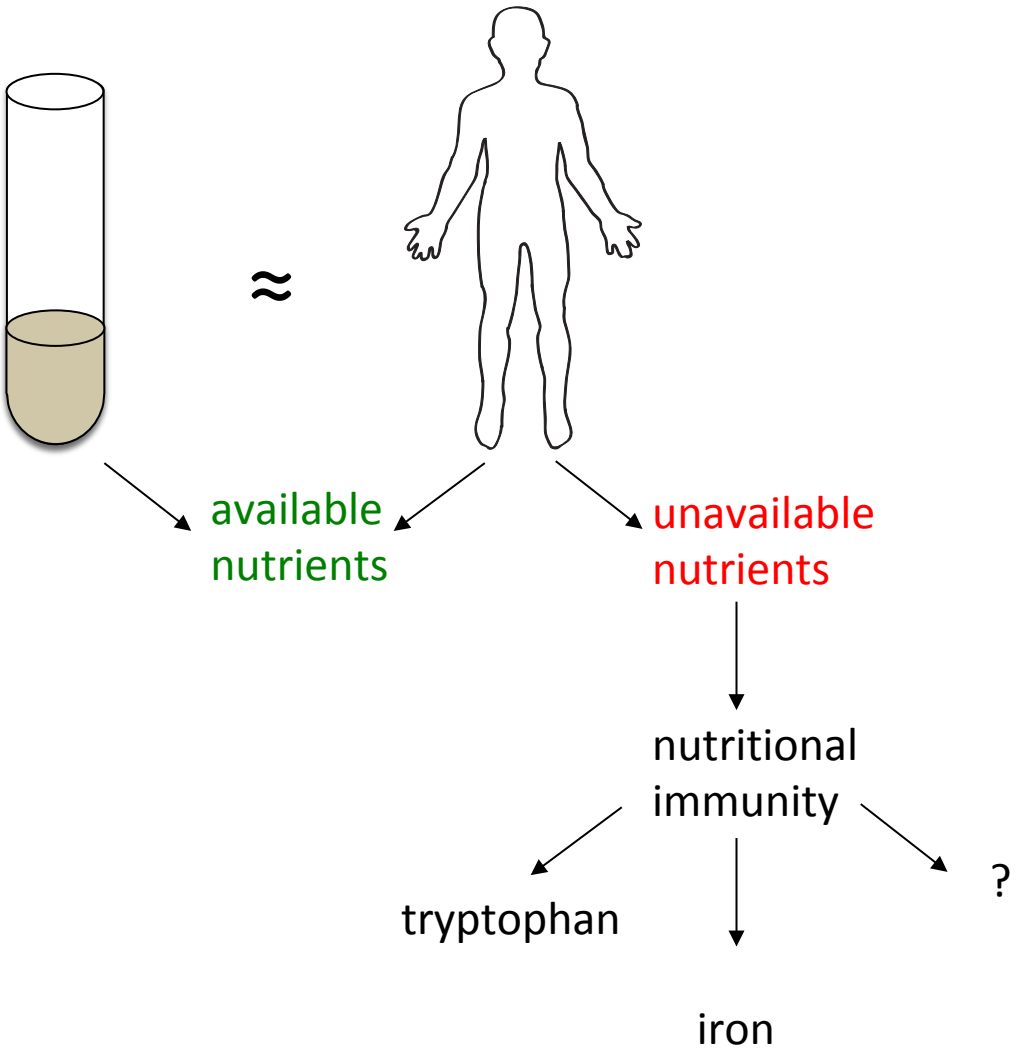
“The host as a growth medium”



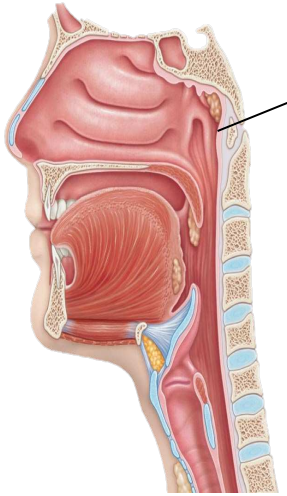
- 1870 − Louis Pasteur − body is culture vessel

- 1960 − E. D. Garber − “The host as a growth medium”
  - “The nutritional requirements of the pathogen must be satisfied at the site of inoculation or of localization”
  - We must understand bacterial physiology within the host during infection

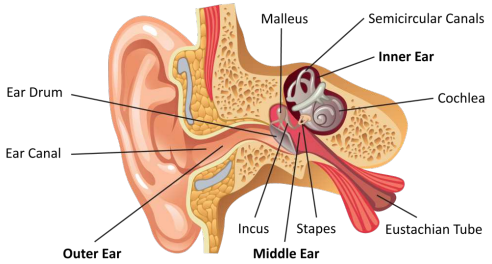
The host is a dynamic growth medium



Haemophilus influenzae

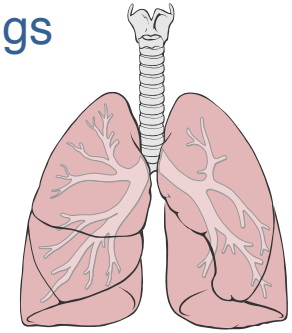


Nasopharynx → Middle ear



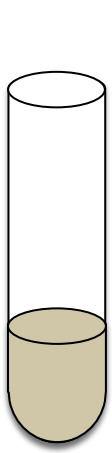
Nasopharynx → Bloodstream (rare)

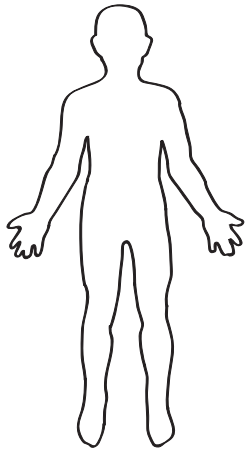
Nasopharynx → Lungs



- Obligate human pathogen
- Normally colonizes the human nasopharynx
- Causes infections including otitis media, sinusitis, bronchitis, and pneumonia

The host is a dynamic growth medium





available nutrients

unavailable nutrients

Which nutrients are available within the host for *H. influenzae* to consume?

Which nutrients are unavailable within the host for *H. influenzae* to consume?

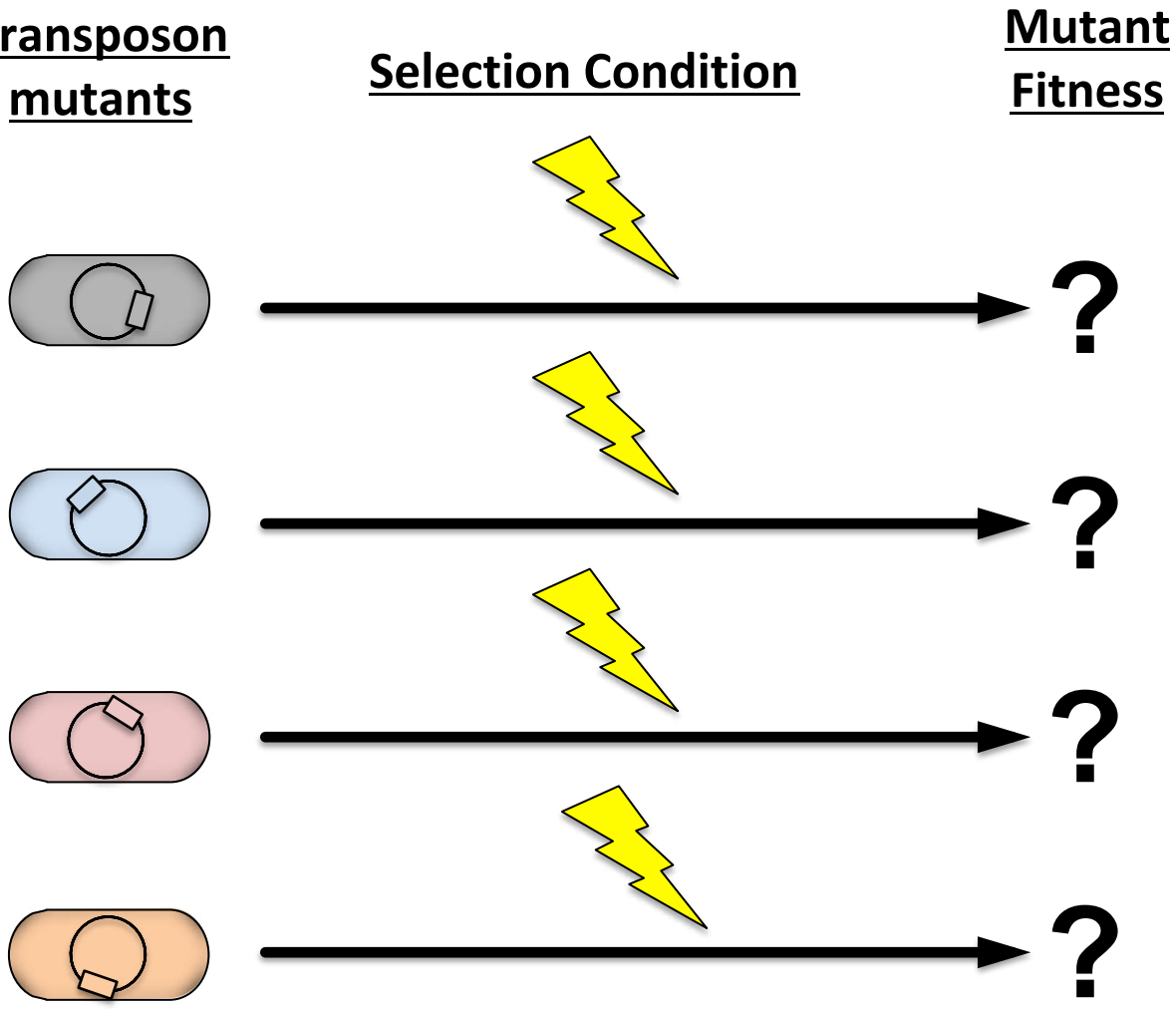
nutritional immunity

tryptophan

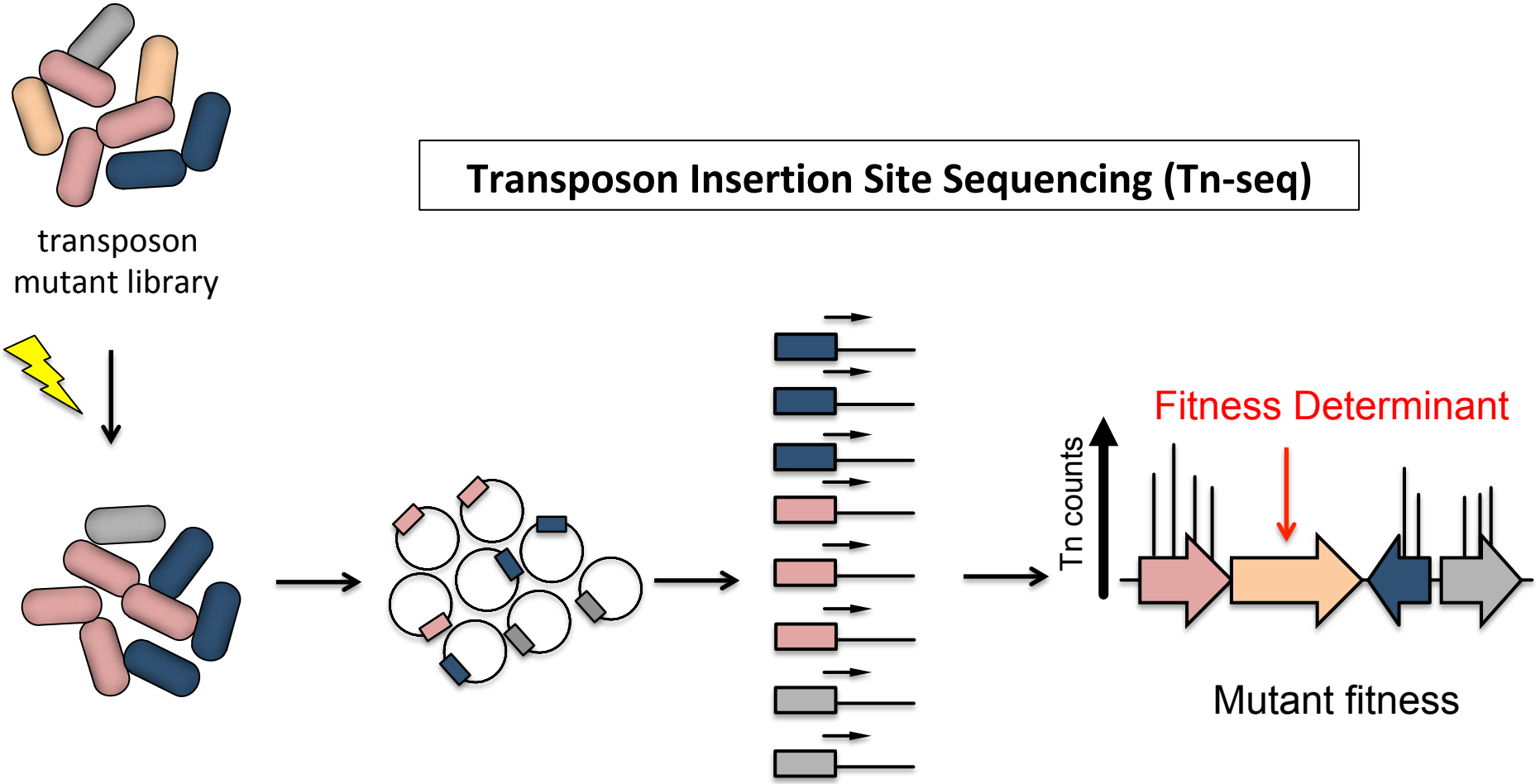
iron

?

Using transposon mutagenesis to understand bacterial fitness



Tn-seq: high-throughput method to understand bacterial fitness

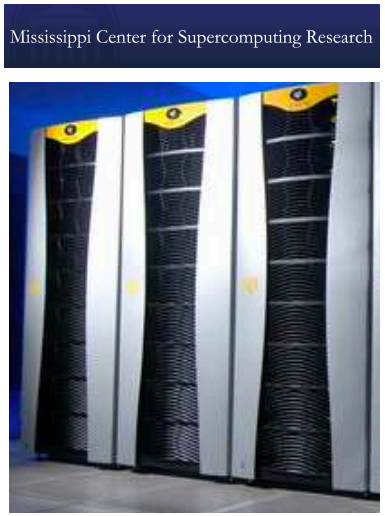


Tn-seq Data Analysis

Fastq files (reads)

↓

map reads to genome



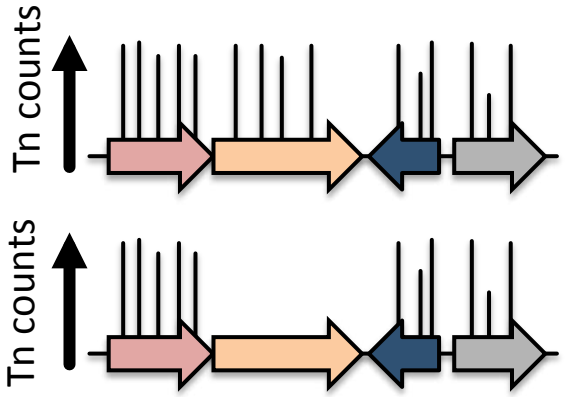
↓

Tn counts

↓

Compare conditions

Tn counts Input vs. Tn counts Output

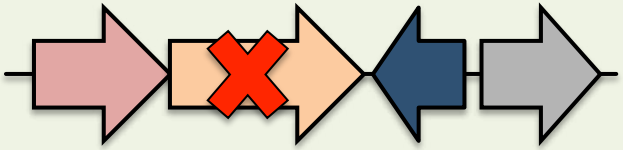


Survival ratio for each gene = $\frac{\text{\# output reads}}{\text{\# input reads}}$

Tn-seq

bacterial mutant fitness

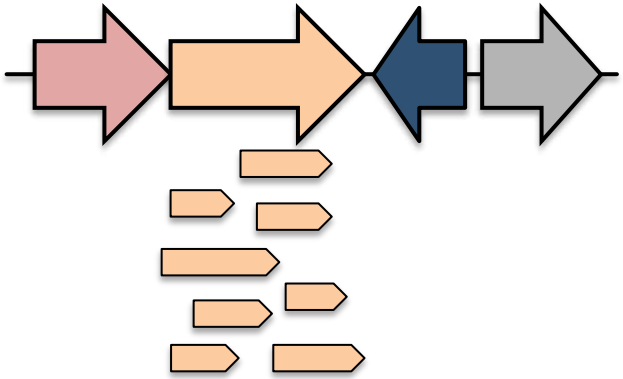
How does a mutation affect fitness?



Microarrays/Gene Expression

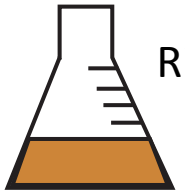
bacterial gene expression

Which genes are being transcribed?



How to determine which metabolites are available to *H. influenzae* in lung

Input Mutant Library



Rich Media

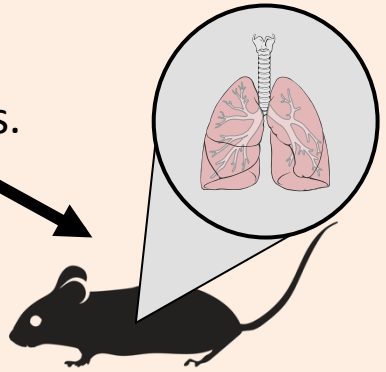
KNOWN: Chemically Defined Medium

vs.

inoculated with 10^7 cfu
grown in CDM ~20 hr

UNKNOWN: Murine Lung

vs.



Pulmonary infection model

10^7 cfu – 40 µl
inoculated intranasally

24 hr infection, homogenize lungs
Plate/harvest colonies

Chemically Defined Medium (CDM)

- *H. influenzae* is fastidious
- Herriott et al. 1970
  - MI_c
  - enhances competence
- Klein et al. 1979 - minimal

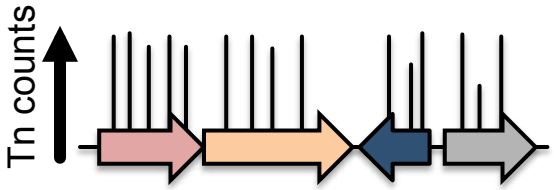
Metabolites	Minimal Requirements
aspartate	
glutamate	✔
arginine	✔
glycine	
lysine	
methionine	
serine	
leucine	
tyrosine	
histidine	
cystine	✔
thiamine	✔
pantothenate	✔
NAD	✔
heme	✔
hypoxanthine	
inosine	✔
uracil	✔

amino acids
vitamins
cofactors
iron source
nucleotides
nucleotides

Tn-seq: a method to determine the metabolic landscape of the host

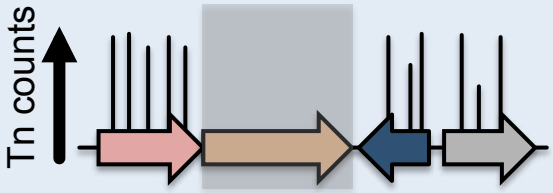
Input Mutant Library

Rich Media



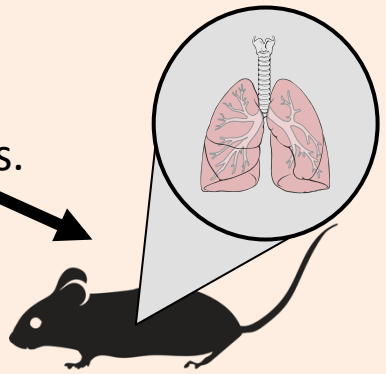
KNOWN:
Chemically
Defined Medium

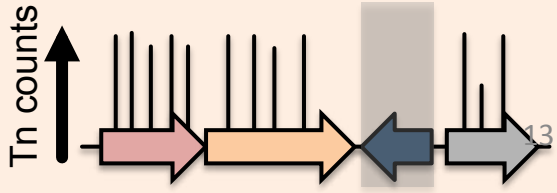
vs.



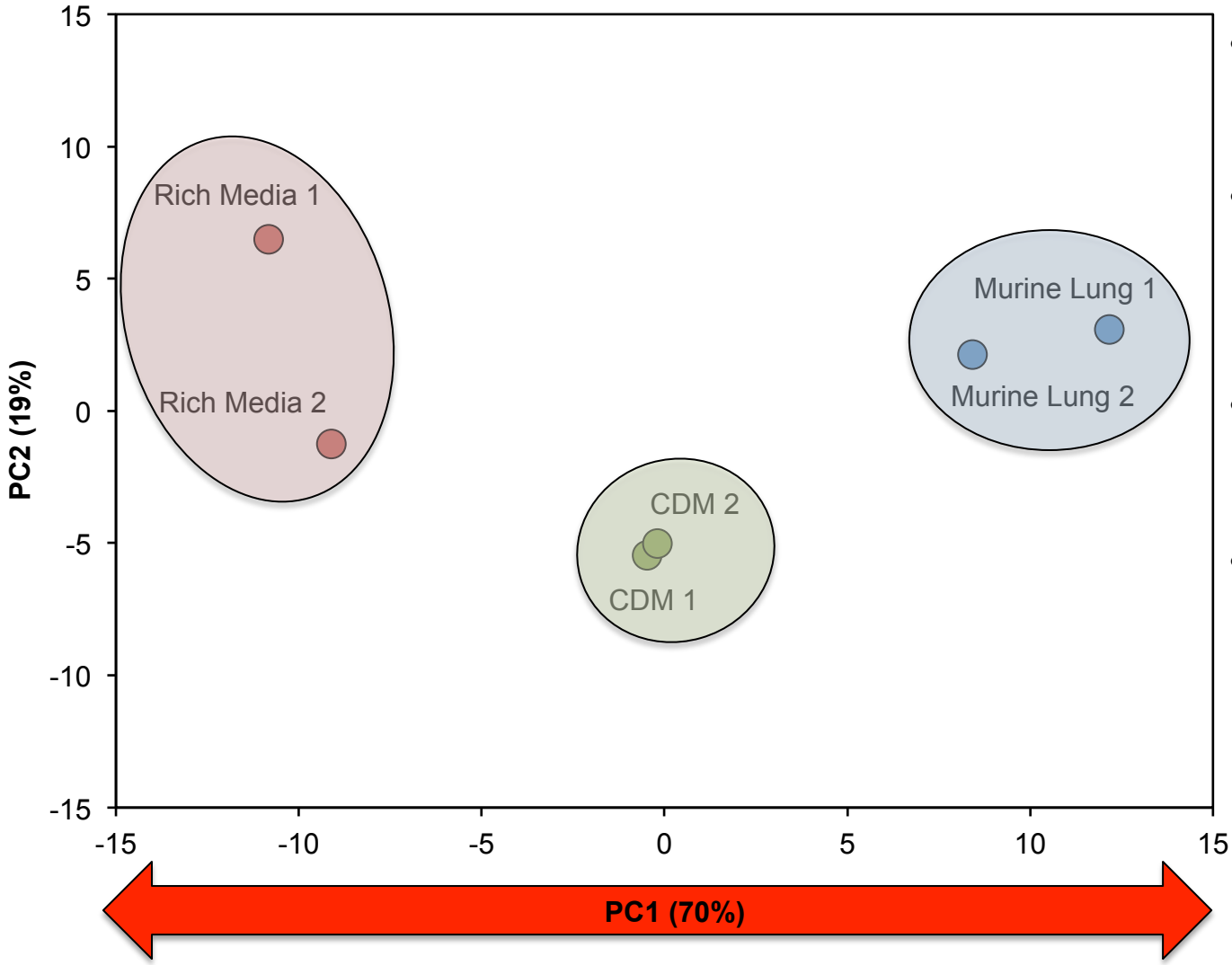
UNKNOWN:
Murine Lung

vs.



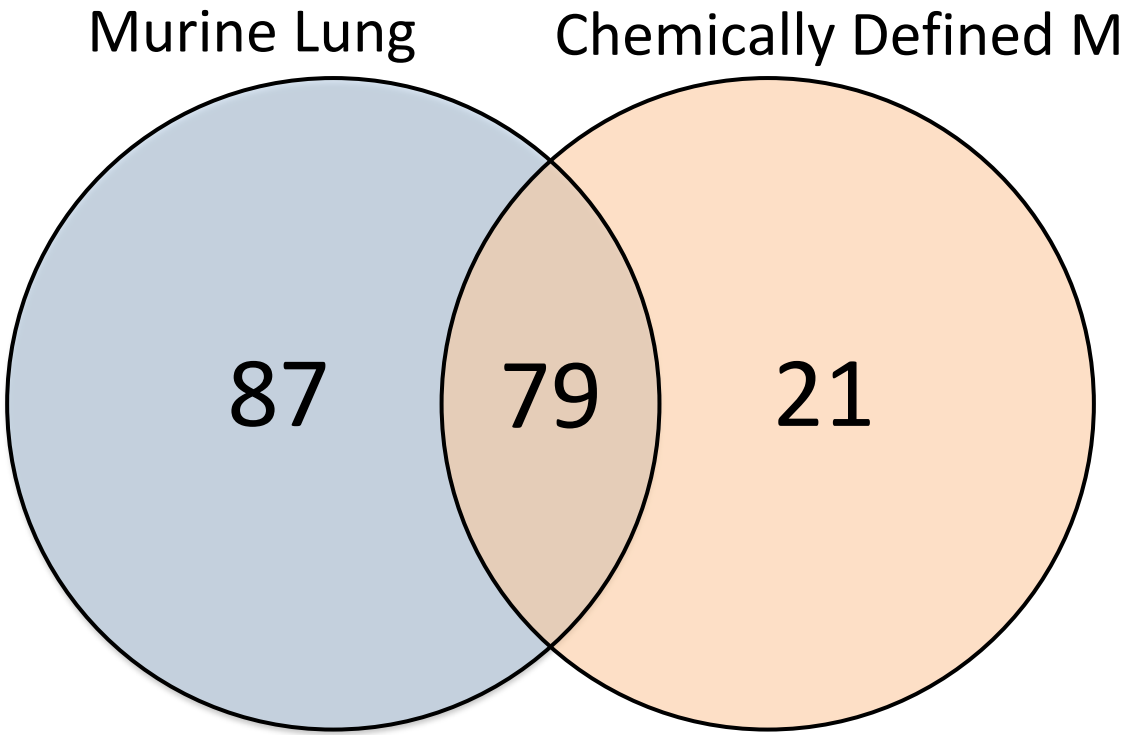


Chemically defined medium is more similar to the lung than rich media



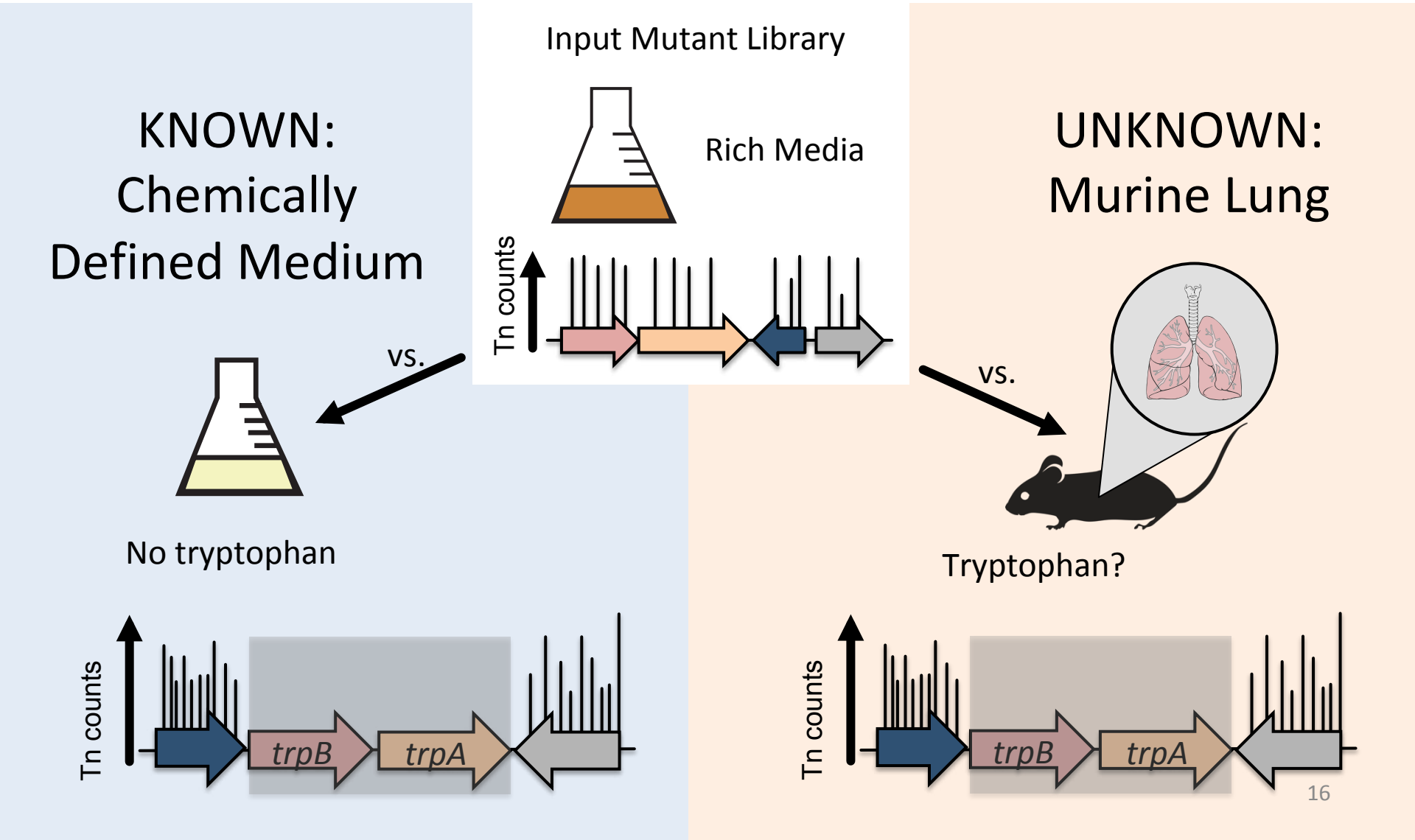
- PCA: Principal Component Analysis
- PCA transforms data into principal components
- data = Tn counts across genome
- most of the differences between the conditions is explained by PC1 (70% of the variance)

How many genes does *H. influenzae* require for fitness?



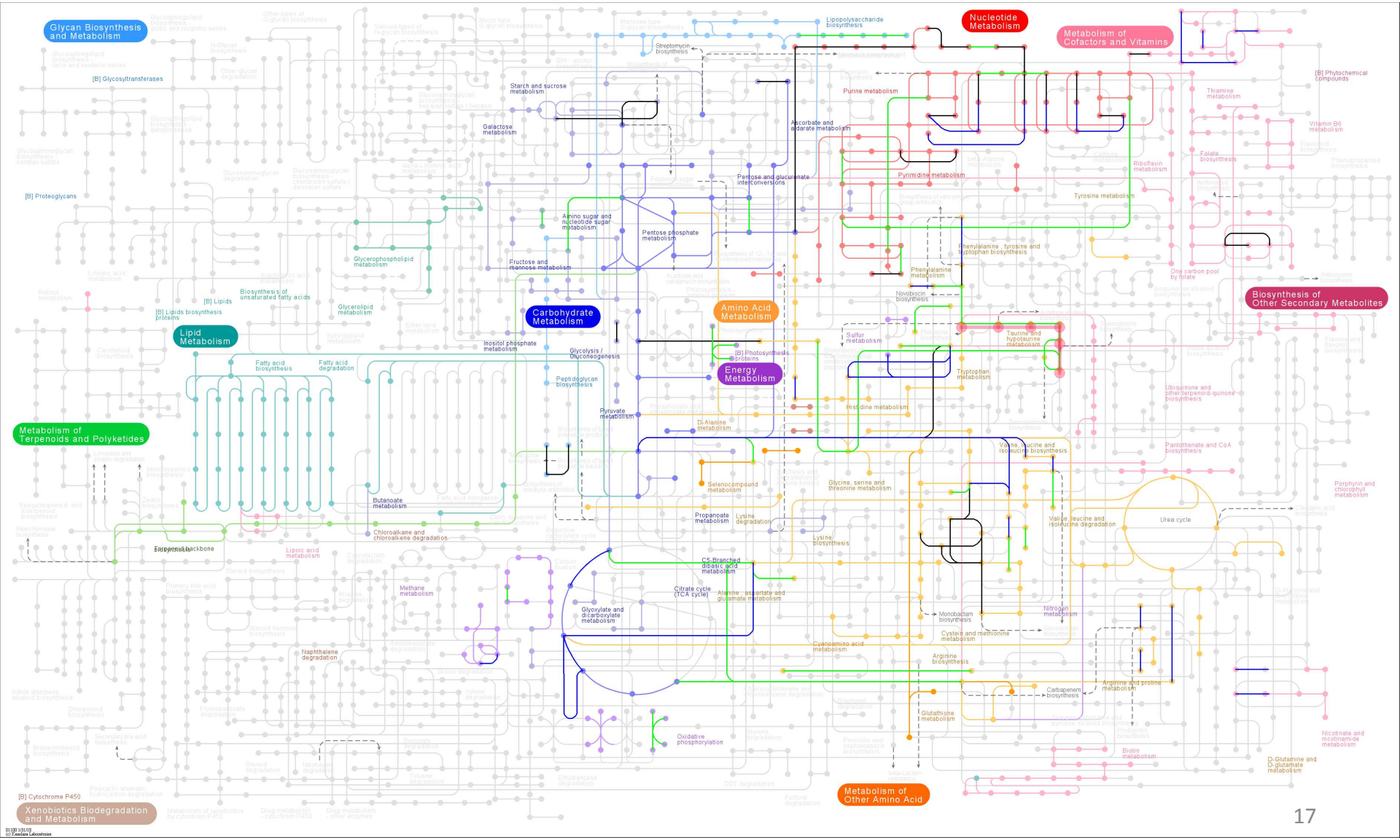
~48% (79/166) of the genes *H. influenzae* requires during lung infection, it also requires during growth in the chemically defined medium

How to determine which metabolites are available to *H. influenzae* in lung



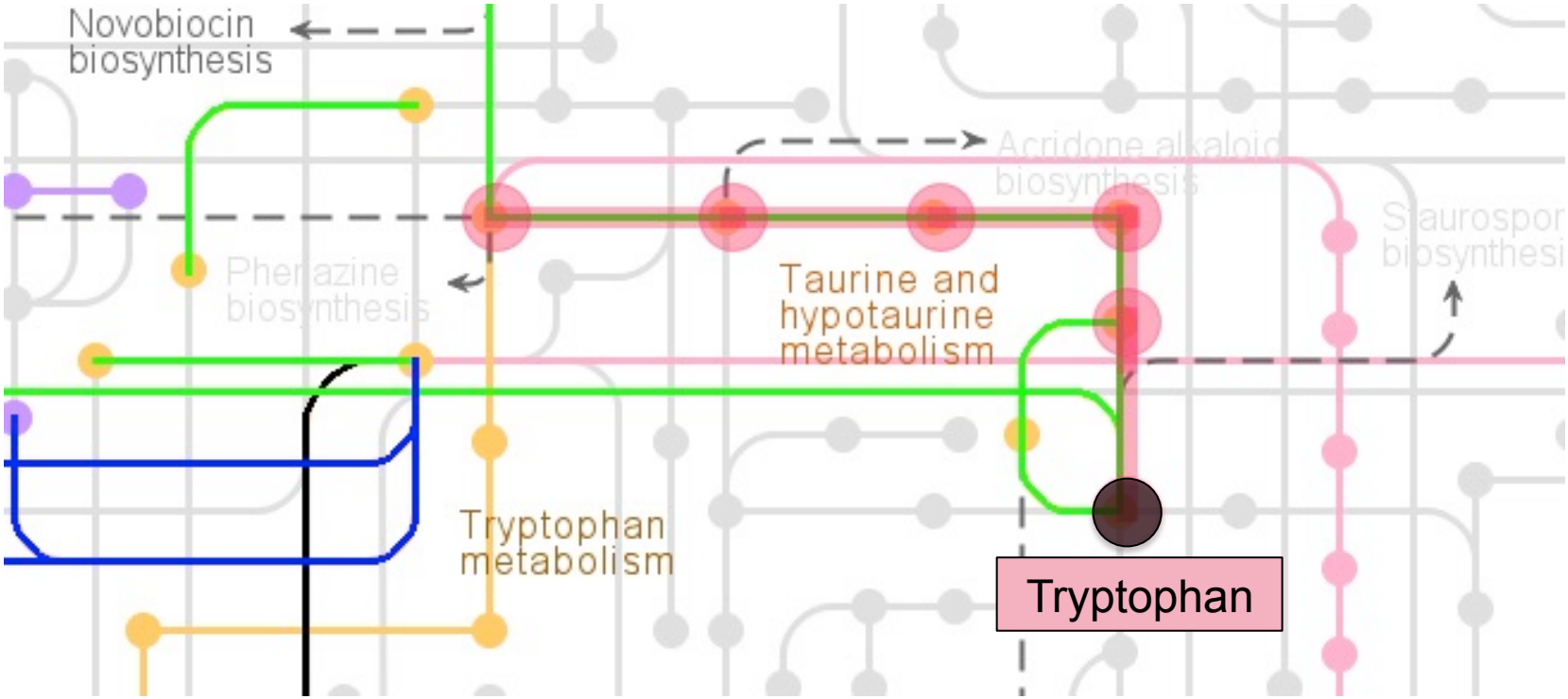
H. influenzae metabolism

CDM
Lung
Both



Tryptophan biosynthesis

CDM
Lung
Both



Metabolite availability

Genes Required	Genes Not Required
metabolite unavailable	metabolite available
higher demand for metabolite	lower demand for metabolite
stress conditions	lack of stress conditions

Which metabolites does the host provide for *H. influenzae*?

amino acids
vitamins
cofactors
iron source
nucleotides
nucleotides

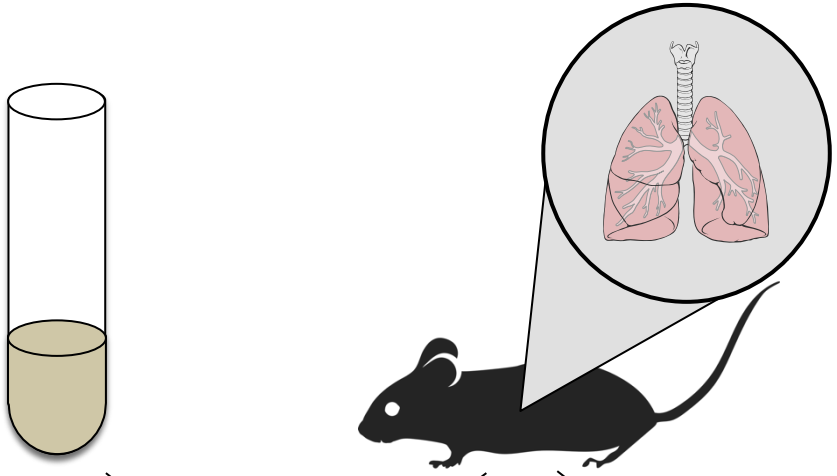
Metabolites	Available in CDM?	Available in lung?
aspartate	yes	yes
glutamate		
arginine		
lysine		
histidine		
thiamine		
pantothenate		
NAD		
heme		
methionine		no
serine		
leucine		
tyrosine		
cysteine	no	yes
proline		
pyridoxal		
purines (A, G)		no
pyrimidines (T, C)		
alanine		
asparagine		
tryptophan		
valine		
isoleucine		
phenylalanine		

Future directions: make CDM more “lung-like”

Metabolites	Available in CDM?	Available in lung?	How to modify CDM?
aspartate, glutamate, arginine, lysine, histidine	yes	yes	**Keep**
thiamine, pantothenate	yes	yes	**Keep**
NAD	yes	yes	**Keep**
heme	yes	yes	**Keep**
methionine, serine, leucine, tyrosine	yes	no	**Remove**
cysteine, proline	no	yes	**Add**
pyridoxal	no	yes	**Add**
purines (A, G)	no	no	inosine
pyrimidines (T, C)	no	no	uracil
alanine, asparagine, tryptophan, valine, isoleucine, phenylalanine	no	no	**Do not add**

amino acids
vitamins
cofactors
iron source
nucleotides
nucleotides

Future Directions: study nutritional immunity



Which nutrients are unavailable within the host for *H. influenzae* to consume?

Which nutrients are available within the host for *H. influenzae* to consume?

available nutrients

unavailable nutrients

nutritional immunity

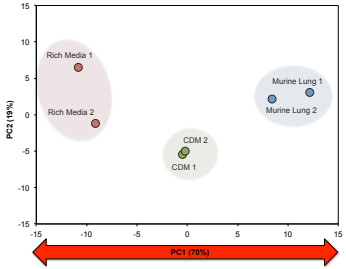
tryptophan

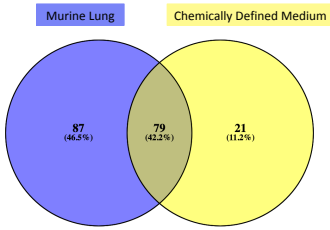
iron

Sequestered?
methionine
serine
leucine
tyrosine
purines (A, G)
pyrimidines (T, C)
alanine
asparagine
valine
isoleucine
phenylalanine

Conclusions

- Our chemically defined medium is more similar to the murine lung than rich media
- Almost half (~48%, 79/166 genes) of the genes *H. influenzae* requires during lung infection, are required in chemically defined medium
- Fitness data from a chemically defined medium aids in
  - in vivo metabolite prediction
  - improving in vitro systems





Metabolites	Available in CDM?	Available in lung?	How to modify CDM?
aspartate glutamate arginine lysine histidine	yes	yes	Keep
thiamine pantothenate NAD heme			
methionine serine leucine tyrosine cysteine proline		no	Remove
pyridoxal	no	yes	Add
purines (A, G)			inosine
pyrimidines (T, C)			uracil
alanine asparagine tryptophan valine isoleucine phenylalanine		no	Do not add