DATABASE SEARCHING AND MULTIPLE ALIGNMENT: INVESTIGATING ANTIBIOTIC RESISTANCE
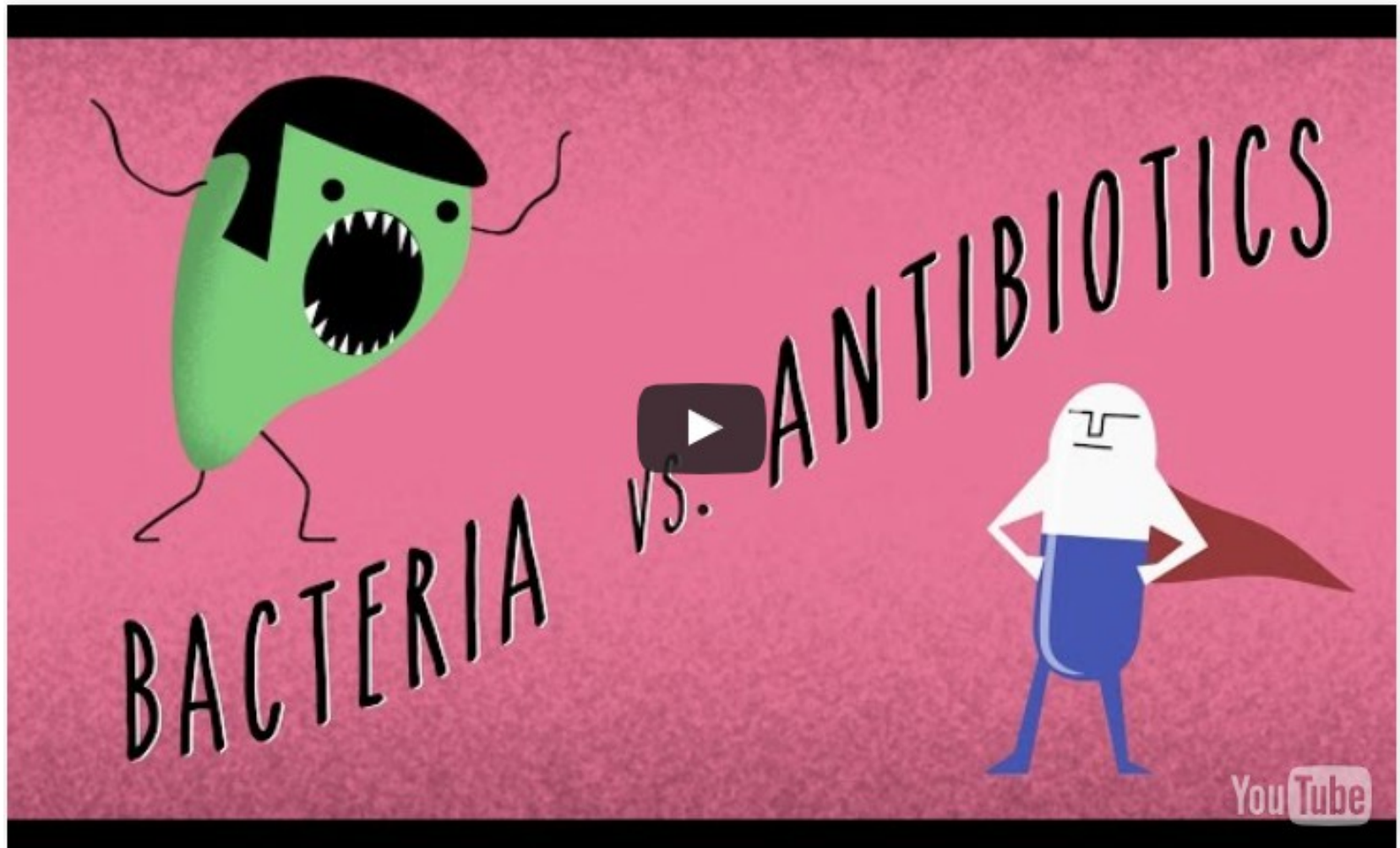
BIO 300/CMPSC 300

Dr. Kristen Webb

Spring 2016
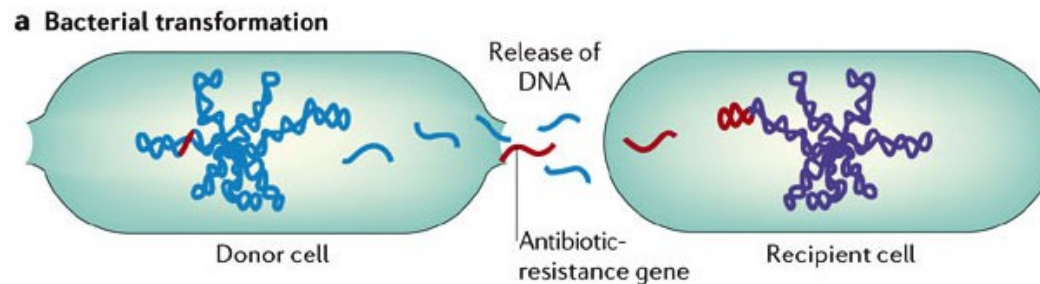
Antibiotic Resistance

http://ed.ted.com/lessons/how-antibiotics-become-resistant-over-time-kevin-wu

Horizontal Gene Transfer

Horizontal gene transfer (HGT) refers to the **transfer** of **genes** between organisms in a manner other than traditional reproduction.

a Bacterial transformation

Release of DNA

Donor cell

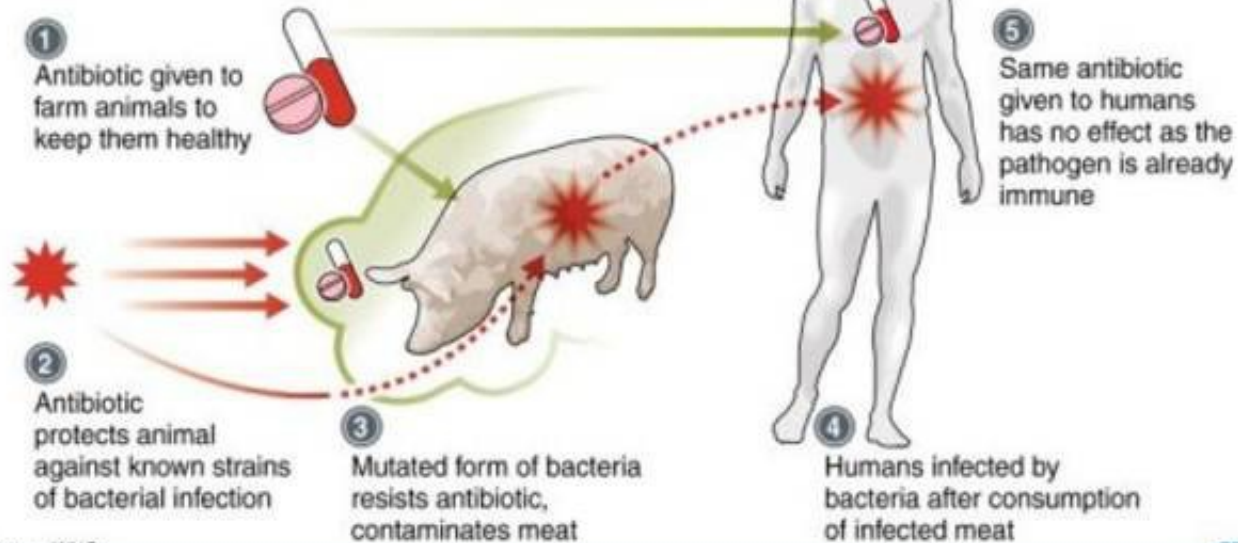Antibiotic-resistance gene

Recipient cell

Farm-to-Fork Spread of Antibiotic Resistance

Antibiotic drug abuse

Overuse of antibiotics in agriculture is widening the spread of superbugs that are immune to common drugs

Path of resistance

① Antibiotic given to farm animals to keep them healthy

② Antibiotic protects animal against known strains of bacterial infection

③ Mutated form of bacteria resists antibiotic, contaminates meat

④ Humans infected by bacteria after consumption of infected meat

⑤ Same antibiotic given to humans has no effect as the pathogen is already immune

Source: WHO

AFP

Farm-to-Fork Spread of Antibiotic Resistance Salyers et al 2004

Table 1.

Prevalence of *tetQ*, *ermF*, *ermG* and *ermB* genes in colonic *Bacteroides* spp.

Isolates	*ermB* (%)	*ermG* (%)	*ermF* (%)	*tetQ* (%)
Community (pre-1970)[a]	0	0	0	32
Clinical (pre-1970)[b]	0	0	9	22
Community (1996–1997)[a]	3	8	15	81
Clinical (1980–1995)[b]	3	18	30	86

a   *Bacteroides* isolates from the colon of people who were healthy and did not have a history of recent antibiotic use.

b   Isolates from people with *Bacteroides* infections.

Table options ▼

BLAST: A Heuristic Approach to Database Searching

- <u>B</u>asic <u>L</u>ocal <u>A</u>lignment <u>S</u>earch <u>T</u>ool
  - NCBI – DNA and protein sequence
  - Compares one sequence to database of > 100 million
  - Finds best hits (optimal alignments) in a matter of seconds
  - (would take >3 years using Needlman-Wunsch algorithm)

BLAST: A Heuristic Approach to Database Searching

query sequence: foreviltoflourishitonlyrequiresgoodmentodonothing

k-tuples ("words"):

35	...
36	men
37	ent
38	nto
39	...

threshold = 15

database sequence A: allthatisnecessaryforthetriumphofevilisthatgood
                      mendonothing

no match

initial match

database sequence B: allthatisnecessaryforeviltosucceedisforgoodm**ent**o
                                  ito---nl--yrequires---goodm**ent**o
                      donothing
                      donothing

extend

score: 13

database sequence C: allthatsnecessaryfortheforcesofeviltowinintheworld

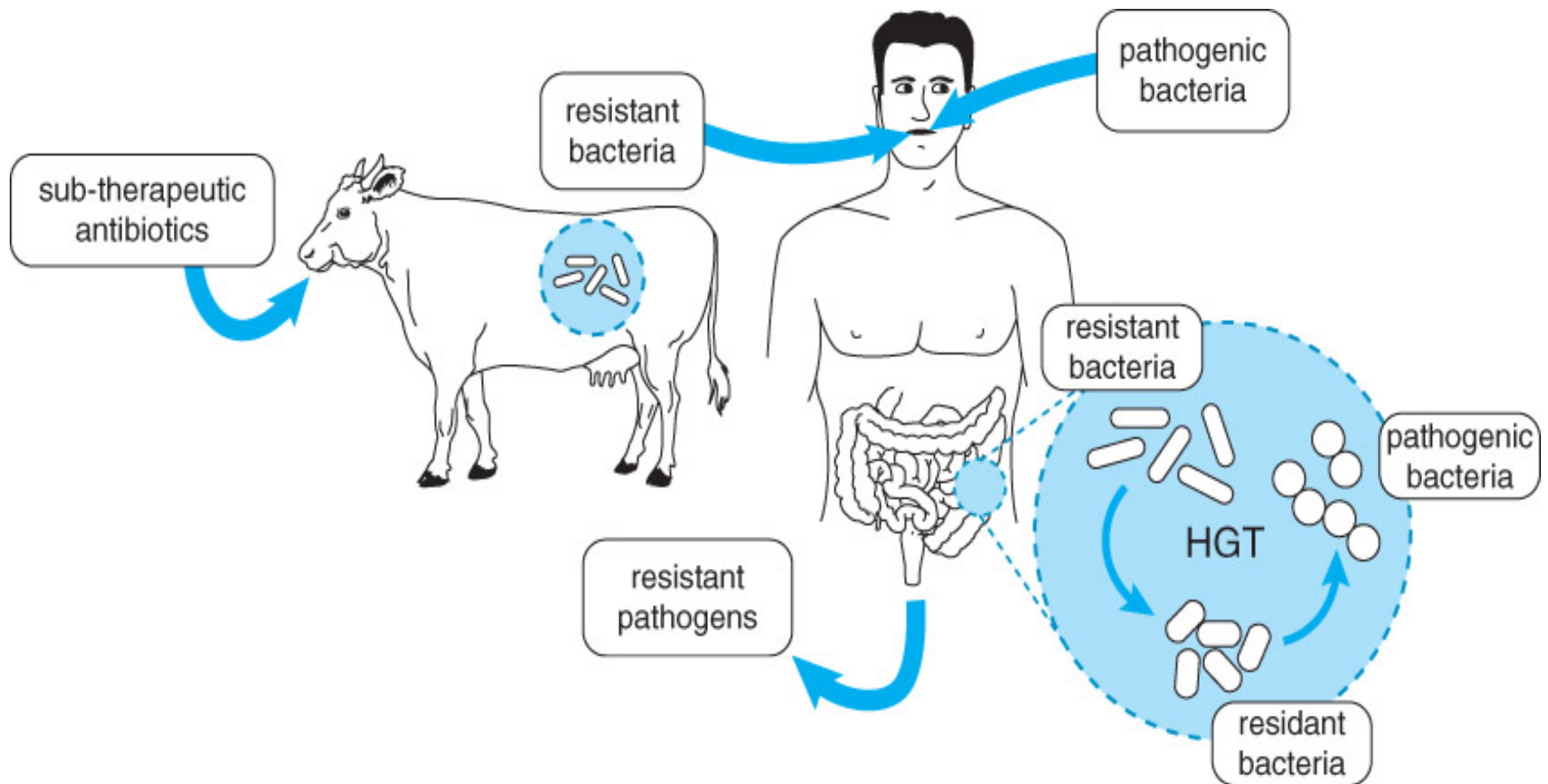isfo---renough-goodm**ent**odonothing
it-onlyrequiresgoodm**ent**odonothing

score: 17
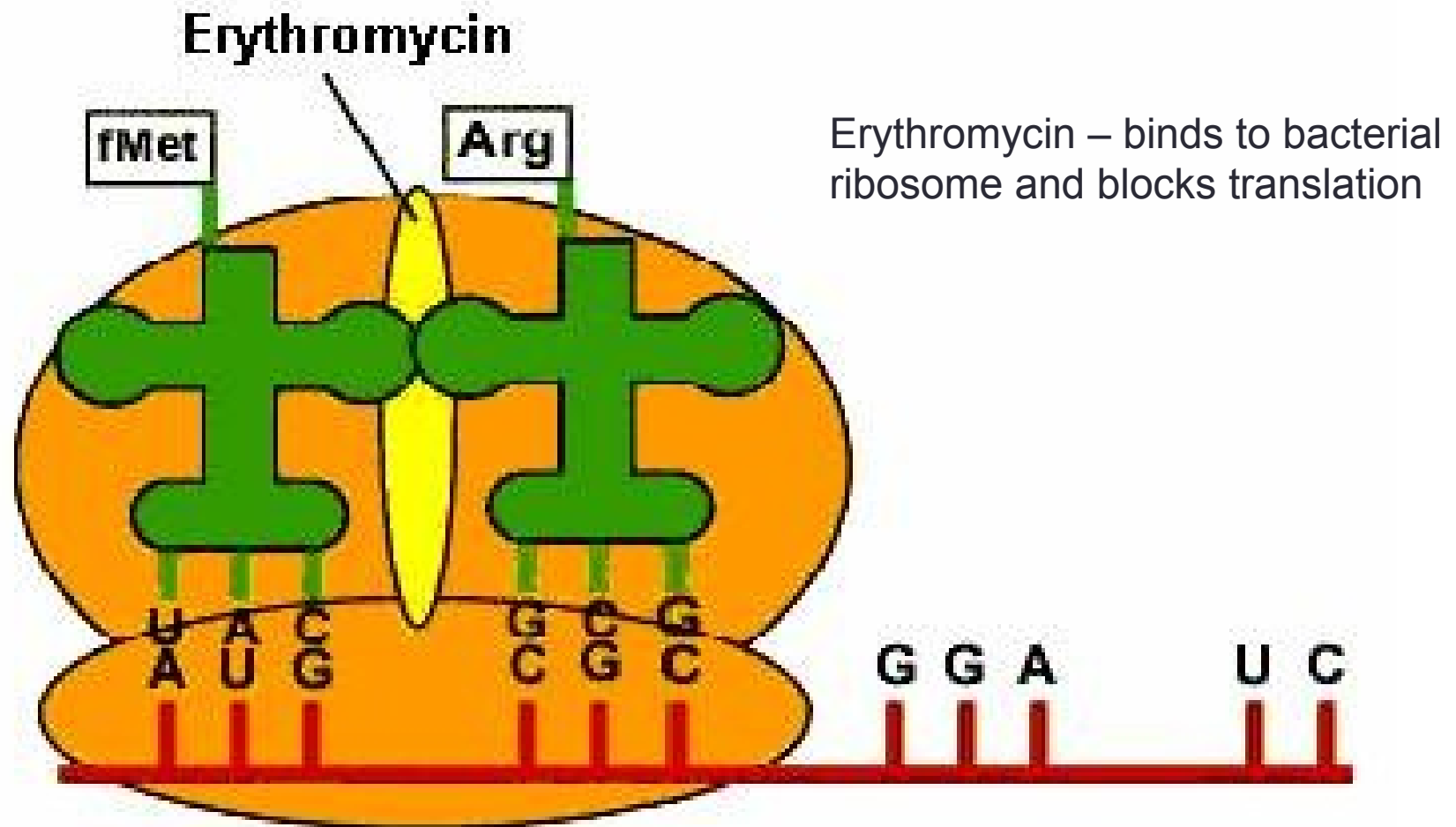
extend   initial match

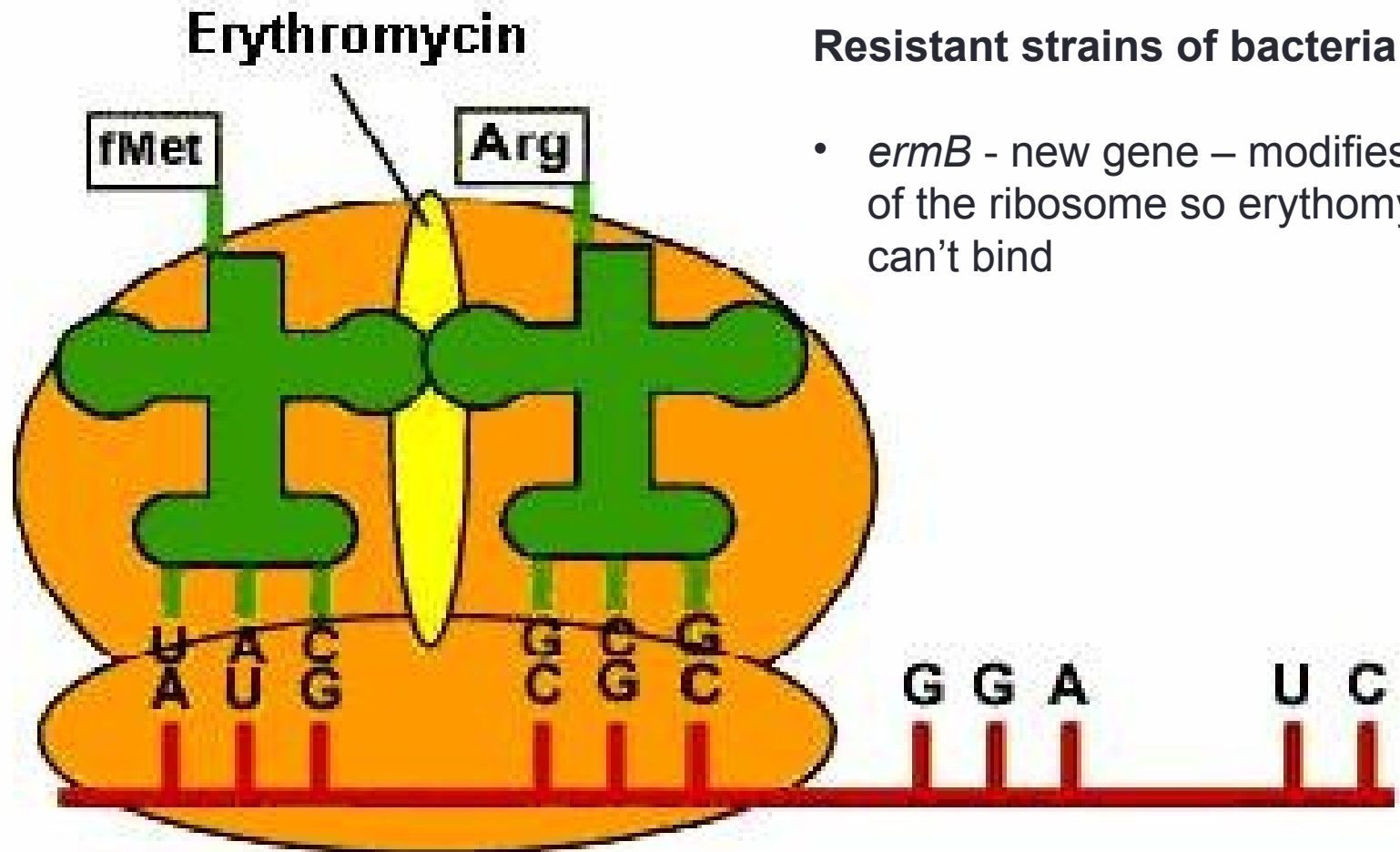# Horizontal Gene Transfer of Antibiotic Resistance

What is the likelihood that agricultural use of antibiotics is resulting in resistant human gut bacteria and/or resistant human pathogens?

- *ermB* gene

Searching for Erythromycin Resistance Genes Using BLAST

Erythromycin – binds to bacterial ribosome and blocks translation

Searching for Erythromycin Resistance Genes Using BLAST

Resistant strains of bacteria

- *ermB* - new gene – modifies part of the ribosome so erythomycin can't bind

What is the likelihood that agricultural use of antibiotics is resulting in resistant human gut bacteria and/or resistant human pathogens?

- *ermB* gene – codes for a protein that methylates rRNA so erythromycin can't bind – antibiotic resistance gene
  - Generate FASTA-formatted file of diverse *ermB* genes using BLAST
    - Diverse species

Multiple Sequence Alignment: Clustal and MUSCLE

- Align a set of sequences (2 – ~2,000)
  - May find distinctly identifiable groups - evolution
  - Can create a consensus sequence: the nucleotides (or amino acids) that appear the most frequently at each position in a set of sequences

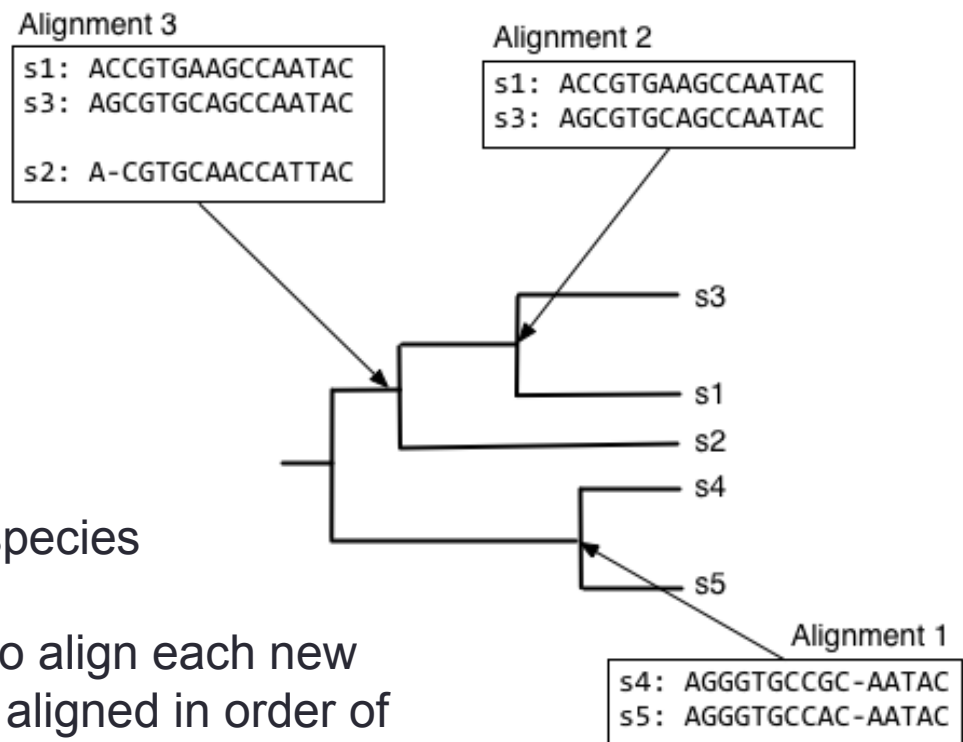# Multiple Sequence Alignment: Clustal

- Order matters

A
```
GTCT
G-GT
```

B
```
GTCT
G-GT
CT--
```

C
```
GTCT
--CT
-GGT
```

Solutions?
- Needleman-Wunsch L x M x N matrix
  - Impractical for long sequences
- Heuristic
  - Progressive alignment via a guide tree

Multiple Sequence Alignment: Clustal

- Heuristic
  - Progressive alignment via a guide tree

    - Pairwise alignments, group sequences together based on similarity

    - Start with most closely related species

      - Global, pairwise alignments to align each new sequence with those already aligned in order of decreasing relatedness

Alignment 3

s1: ACCGTGAAGCCAATAC
s3: AGCGTGCAGCCAATAC

s2: A-CGTGCAACCATTAC

Alignment 2

s1: ACCGTGAAGCCAATAC
s3: AGCGTGCAGCCAATAC

s3
s1
s2
s4
s5

Alignment 1

s4: AGGGTGCCGC-AATAC
s5: AGGGTGCCAC-AATAC

Multiple Sequence Alignment: MUSCLE

What is the likelihood that agricultural use of antibiotics is resulting in resistant human gut bacteria and/or resistant human pathogens?

- *ermB* gene – codes for a protein that methylates rRNA so erythromycin can't bind – antibiotic resistance gene
  - Generate FASTA-formatted file of diverse *ermB* genes using BLAST
    - Diverse species
  - Generate multiple alignment and tree

Summarize your findings regarding the likelihood that agricultural use of antibiotics can result in resistant human gut bacteria and/or resistant human pathogens

- Preliminary evidence to support or reject hypothesis of Horizontal Gene Transfer of *ermB*
  - Bacterial species/hosts used – why chosen/rationale?
  - Screenshot of alignment and tree
  - Evidence for or against HGT?

- Next steps to continue investigation…