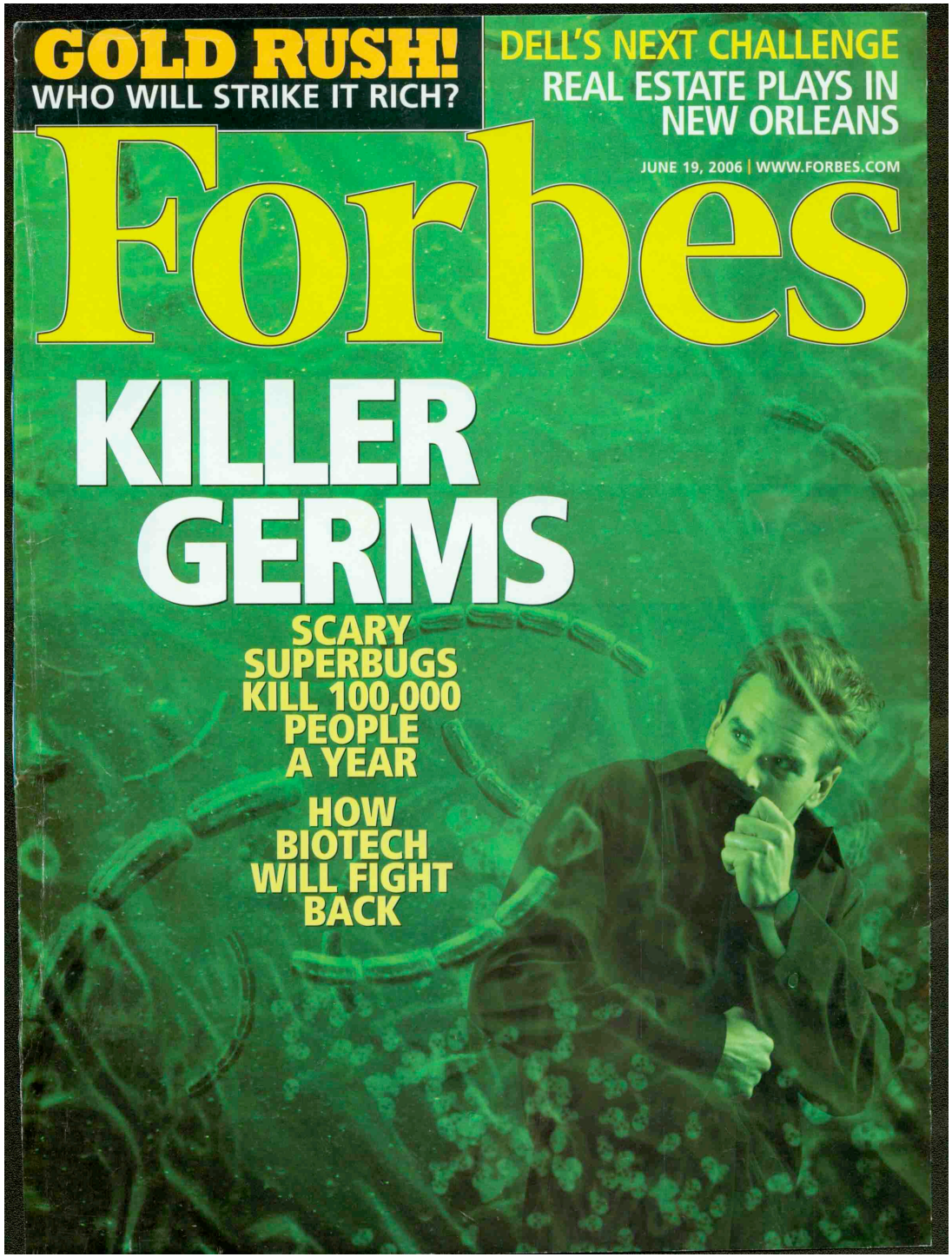
TODO

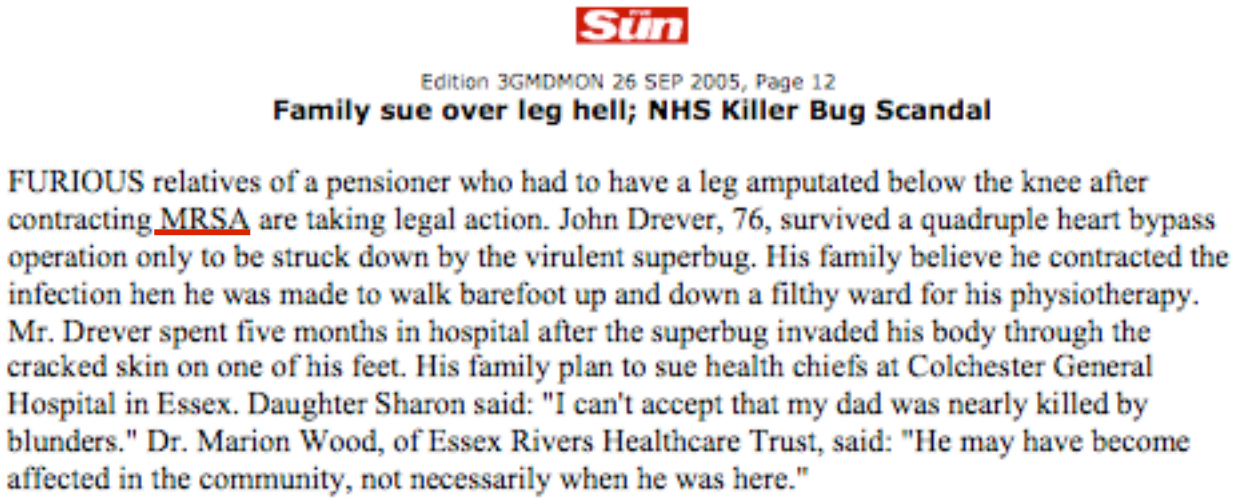
Intro: general antibiotic resistsance, need hetero resistance slide, aux genes, mecA, VRSA vs VISA, multi gene vs single gene table of drugs, targets and resistances.

get tomasz science PR on soil products

Forbes


GOLD RUSH!
WHO WILL STRIKE IT RICH?
DELL'S NEXT CHALLENGE
REAL ESTATE PLAYS IN NEW ORLEANS
JUNE 19, 2006 | WWW.FORBES.COM
Forbes
KILLER GERMS
SCARY SUPERBUGS KILL 100,000 PEOPLE A YEAR
HOW BIOTECH WILL FIGHT BACK

Staphylococcus aureus and British elections I



The Sun

Edition 3GMDMON 26 SEP 2005, Page 12

Family sue over leg hell; NHS Killer Bug Scandal

FURIOUS relatives of a pensioner who had to have a leg amputated below the knee after contracting MRSA are taking legal action. John Drever, 76, survived a quadruple heart bypass operation only to be struck down by the virulent superbug. His family believe he contracted the infection hen he was made to walk barefoot up and down a filthy ward for his physiotherapy. Mr. Drever spent five months in hospital after the superbug invaded his body through the cracked skin on one of his feet. His family plan to sue health chiefs at Colchester General Hospital in Essex. Daughter Sharon said: "I can't accept that my dad was nearly killed by blunders." Dr. Marion Wood, of Essex Rivers Healthcare Trust, said: "He may have become affected in the community, not necessarily when he was here."

Methacillin Resistant S.aureus (MRSA) enters the popular press

S.aureus and British elections II

Sun

Edition 3GMDMON 26 SEP 2005, Page 12

I caught MRSA 9 times in the same hospital; NHS Killer Bug Scandal

EMMA MORTON, HEALTH REPORTER

AN EX-trucker told last night how he lost his right knee after catching the MRSA superbug NINE TIMES in hospital.

Ken Sutton, 60, was repeatedly hit by the deadly infection after an op to rebuild his leg following a motorbike smash.

The bug -which he blames on hygiene blunders -crippled him for life, wrecked his marriage and left him unable to work again.

Ken said last night: "My life is ruined. I had a good job, a loving wife and house of my own and I have lost everything."

"Now I live in a disabled bungalow on Pounds 80 benefits a week and cannot walk properly."

He added: "Having MRSA once is a nightmare -imagine nine times over two years."

"I'd wake up at night screaming in pain. Passers-by in the street would hear and bang on the door."

"I've had a raw deal. To catch it over and over is an appalling indictment of the NHS."

S.aureus and British elections III

Edition 2GMDMON 22 MAR 2004, Page 1

NHS killer bug shock;Sun investigation;Exclusive

ANTHONY FRANCE

We find 80 times danger level of MRSA in hospital

EIGHTY times the danger level of killer superbug MRSA were found at an NHS hospital in a shock investigation by The Sun.

A reporter took samples while working undercover at the North Middlesex Hospital in Edmonton, North London -named a year ago as the joint worst infected in Britain. Bosses vowed then to improve hygiene. But microbiologist Christopher Malyszewicz said of our investigation: "These are the worst results I've seen. It is frightening. The hospital is failing to tackle the problem."

Our reporter took 24 swabs from toilets, soap dispensers, lifts and banisters. An average 40 colony forming units (CFU) were found growing per square centimetre. A reading of 0.53 CFUs is regarded as "the base line" -and is still a risk to patients and visitors.

Mr Malyszewicz, whose company Chemsol tested our samples, said: "In some areas we found 80 times the level which has normally been found, which is unusually high and very dangerous.

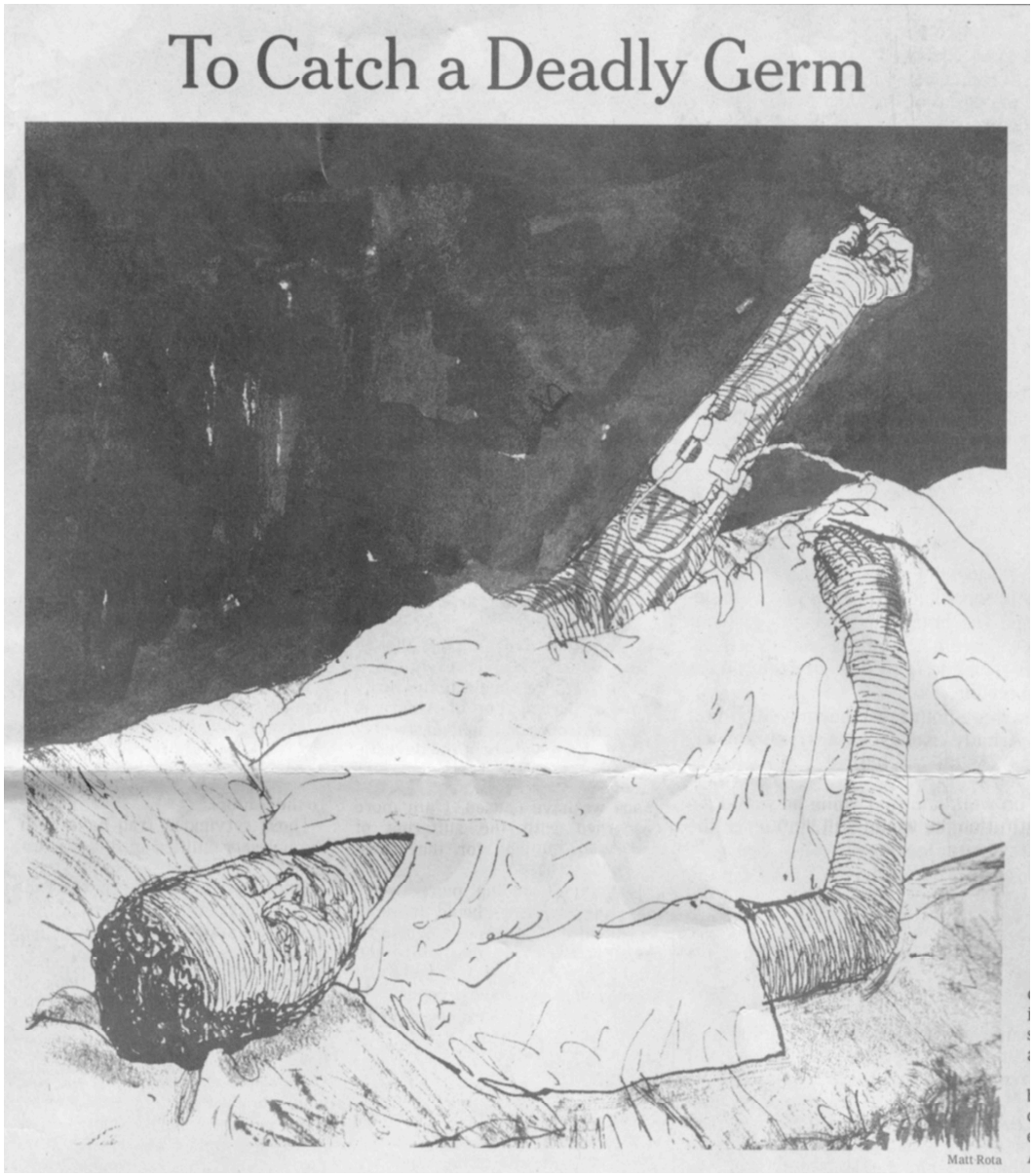
Deadly

"Almost every swab you had taken had MRSA present, which is unusual."

"This means MRSA is widespread throughout the hospital and not just in isolated patches."

Deaths from MRSA -Methicillin-resistant Staphylococcus Aureus –have risen 15-fold nationally in the past decade. In 2002, 800 people died, compared with 51 in 1993.

NYTimes 11/14/06 OP-ED



- MRSA kills 5x more americans than HIV, 60% S.aureus infections resistant, cost of hospital infections \$3 10^{10}.
- Spread via bedrails, wheelchairs, door knobs, stethoscopes, lab coats, blood pressure cuffs (77% contaminated in Fr Hospital)..
- Screen, isolate patients can reduce infections 90%. Select your hospital carefully or go to Denmark.

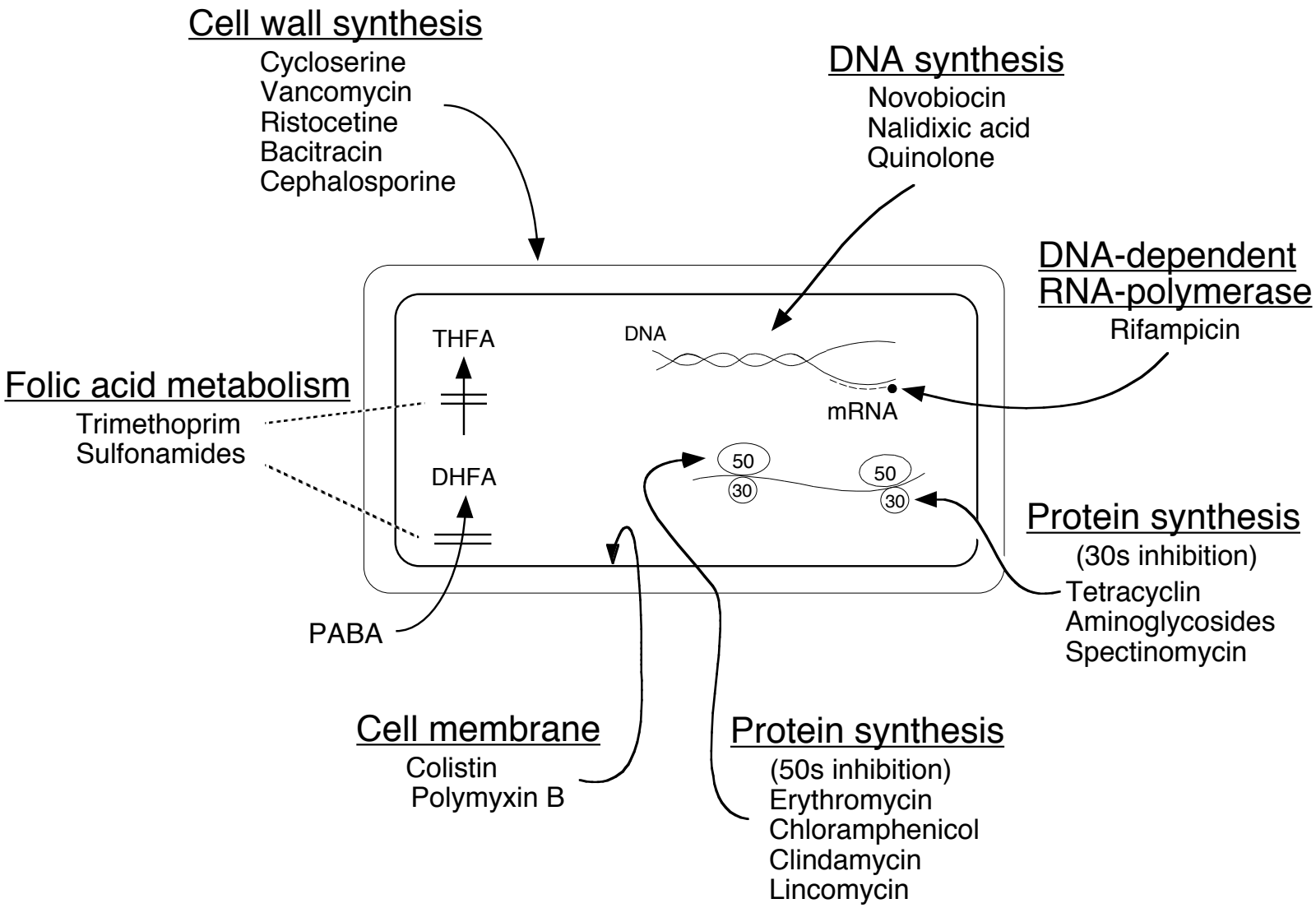
Evolution of antibiotic resistance in S.aureus by whole genome sequencing

Most antibiotics are natural products, or derivatives, hence while we prospect the biosphere for novel compounts, the bacteria do likewise for novel defense mechanisms.

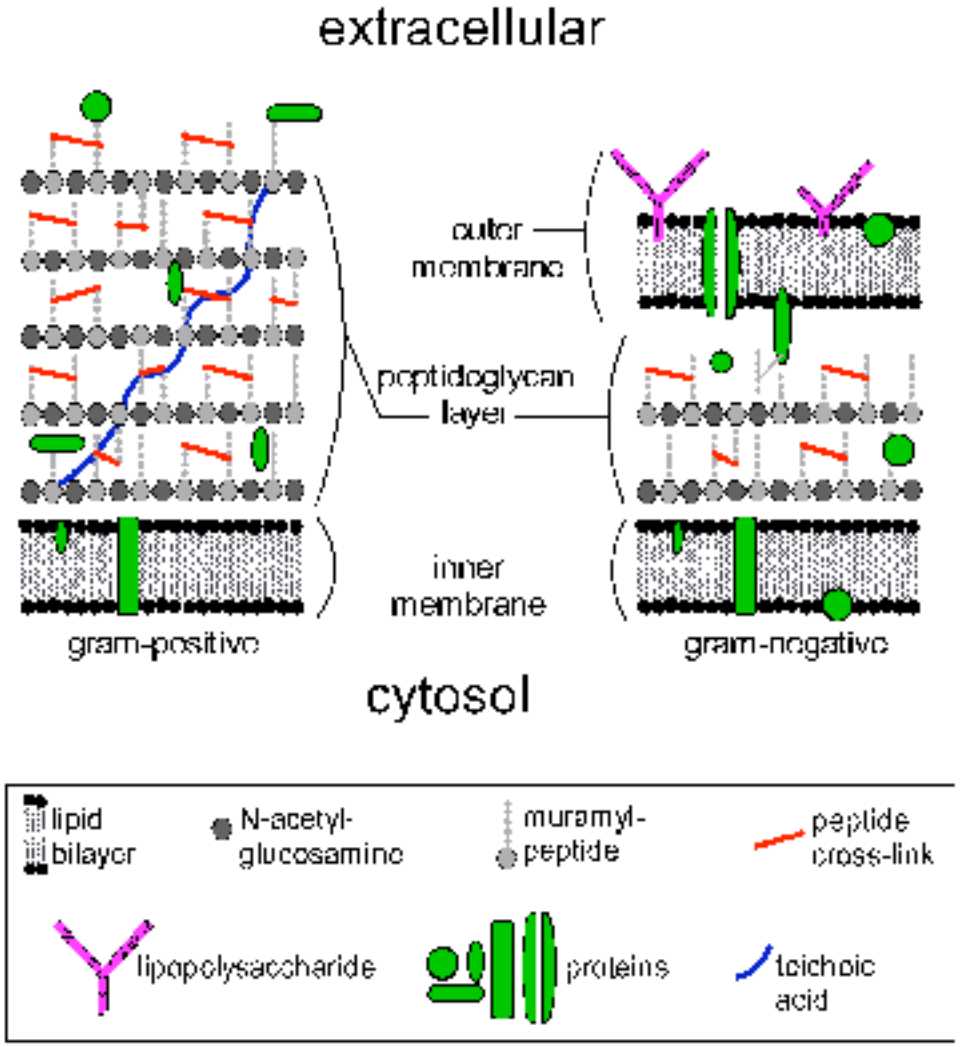
Strategies:

- pump it out or restrict intake,
- chemically inactivate it,
- modify the target by point mutations,
- use completely new genes for targeted function

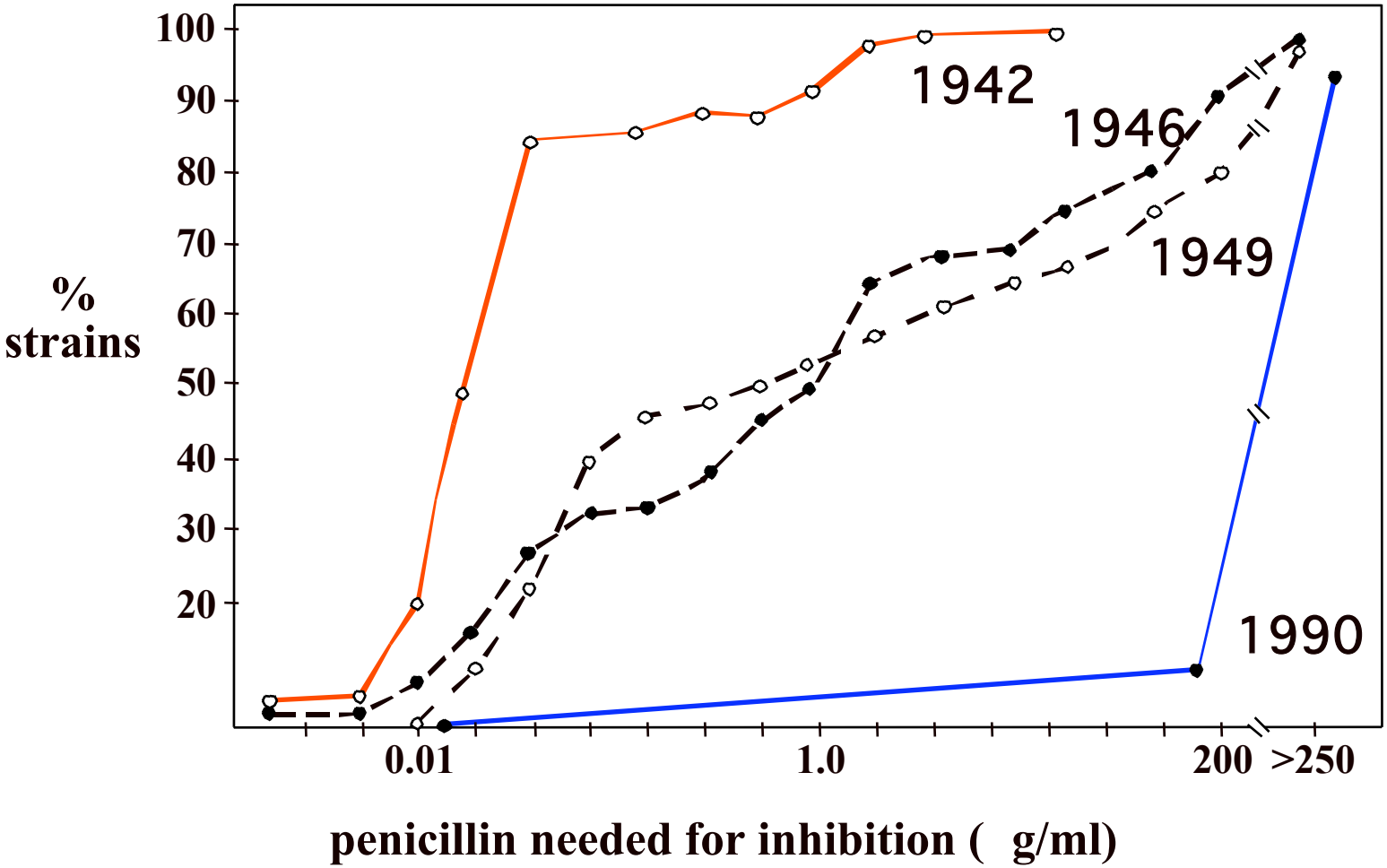
Antibiotics target many pathways



Primer on bacterial cell walls

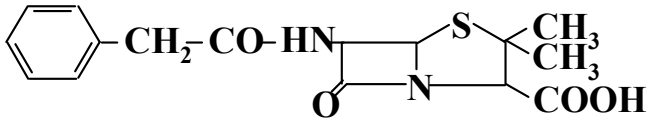


Penicillin resistance

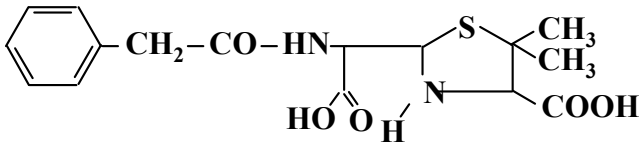


Penicillin derivatives, beta-lactams, also induced resistance

1943: Penicillin in therapy

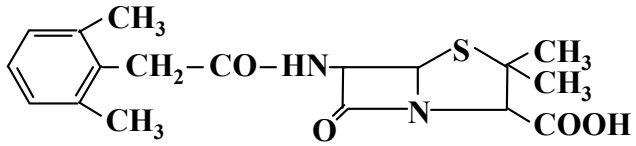


1946-1950:import Penicillinase gene



1960: Penicillin resistant staphylococci become global through plasmid epidemics

1960: Methicillin in therapy (resistant to Penicillinase)



1961: import *mecA gene*

Methicillin resistant strains become global by 1980s

Spread of antibiotic resistance

	S. aureus ATCC 6538 (1930)	MRSA Brazilian epidemic Clone (1994)	Resistance mechanism acquired (+) adaptive (A)
Penicillin	S	R	+(1945)
Streptomycin	S	R	+ (1948)
Tetracycline	S	R	+ (1950)
Methicillin	S	R	+ (1961) *mecA*
Oxacillin	S	R	
Cephalothin	S	R	
Cefotaxime	S	R	
Imipenem	S	R	
Chloramphenicol	S	R	+
Ciprofloxacin	S	R	A
Clindamycin	S	R	+
Erythromycin	S	R	+
Gentamycin	S	R	+
Rifampin	S	R	A
Vancomycin	S	S	A (1997) VISA
Vancomycin	S	S	+ (2002) *vanA*
Teicoplanin	S	S	+
Trimeth/Sulfa	S	R	A
Mupirocine (topical)	S	R	+

Cell wall specific antibiotics

beta-lactams (eg methacillin) inhibit the enzymes that link the protein and sugars in the cell wall.
Resistance via a foreign gene cassette with mecA gene that is not bound by drugs, but requires 10's of 'helper' genes in host, mechanism unclear

Vancomycin: binds to the two terminal amino acids of pentapeptide cell wall precursor, gums up export, x-linking (??)
Resistance: Intermediate (**V**anco**I**nter**S**taph**A**ureus) general 'stress response' thickened cellwall, problems dividing, slow growth
Resistance: Full (**VRSA**). Gene casssette that changes last amino acid on cell wall precursors, acquired 2002 from E. faecalis

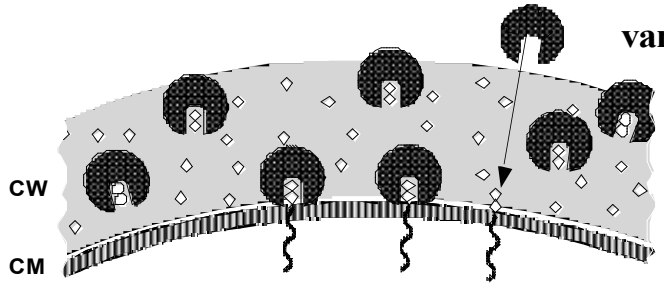
Altered cell wall & morphology in VISA strain

wt VISA

wt VISA

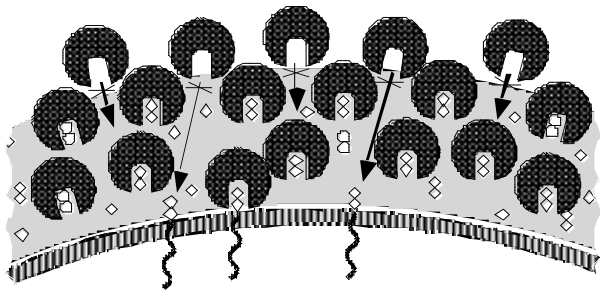
Wall chemistry changed: more monomers

Susceptible cell



Few D -ala D -ala termini in wall

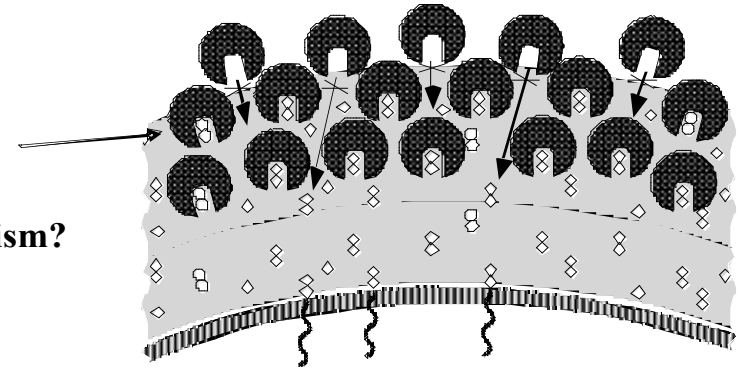
Resistant Cell Stage 1



Many free D -ala D -ala termini in wall

Resistant Cell Stage 2

Captured drug molecules: part of resistance mechanism?

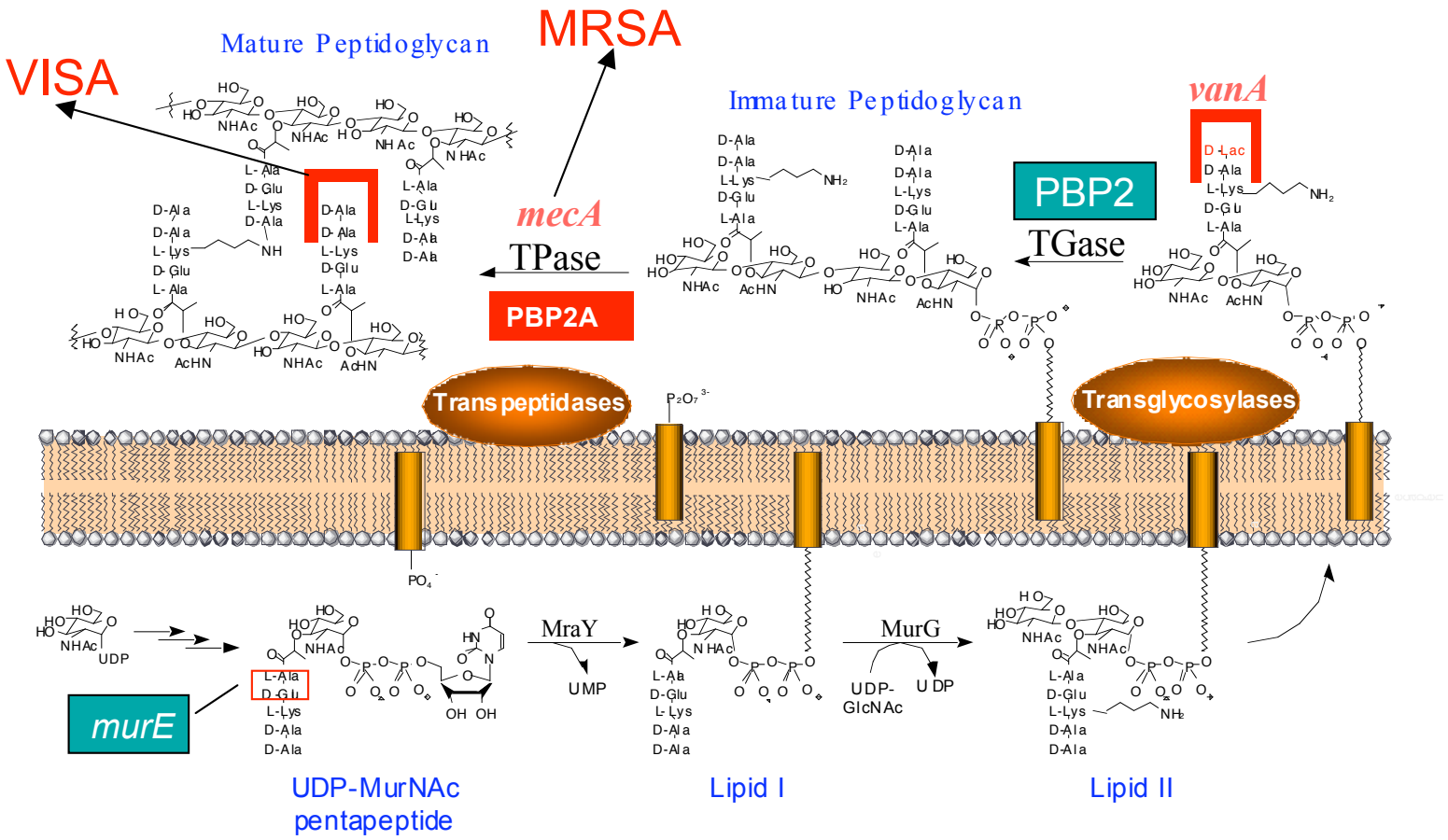


Wall turnover inhibited: thicker cell wall

Sieradzki, Pinho and Tomasz. J. Biol. Chem. 2000.

Cell Wall Biosynthesis

Peptidoglycan Biosynthesis



Origins of Methacillin Resistance S.aureas (MRSA)

***mecA*: 2.1kb determinant of low affinity penicillin binding protein PBP2A**

Foreign gene (*mecA*) embedded in foreign carrier (*mec* element)

***mec* elements: four different structural types, 20-60 kb**

Evolutionary origins unknown

First MRSA in UK: 1960

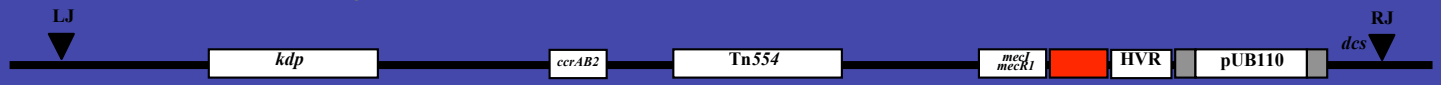
First MRSA in Denmark: 1963

mecA gene is associated with large heterologous DNA cassettes

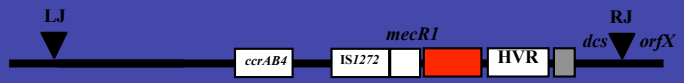
SCC*mec* type I

SCC*mec* type III

SCC*mec* type II



SCC*mec* type IV



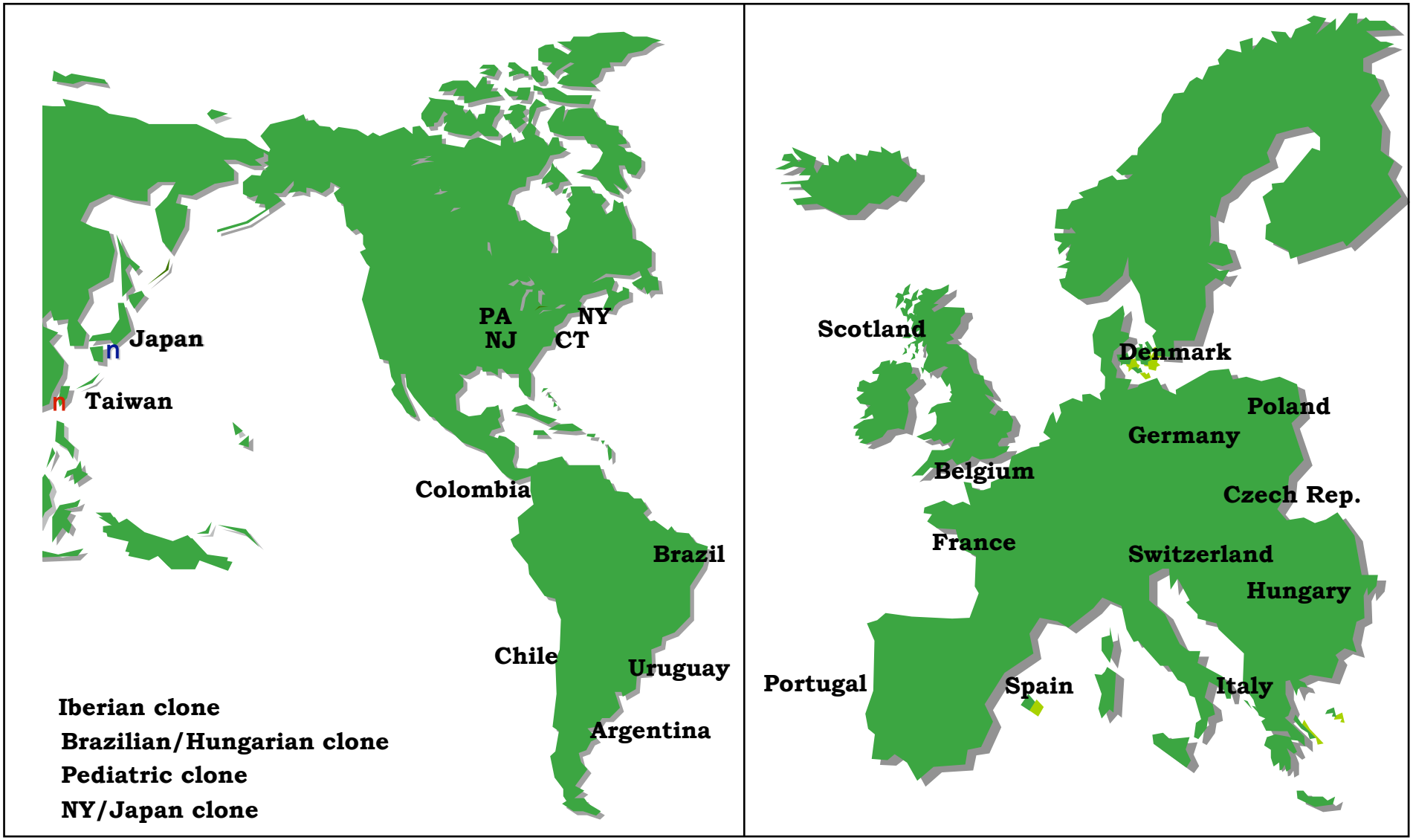
mecA

IS*431*

LJ - chromosomal left junction
RJ - chromosomal right junction

Ito et al 2001. AAC
Hiramatsu et al 2001. Trends in Microb.
Oliveira et al. 2001. Microb. Drug Resist.

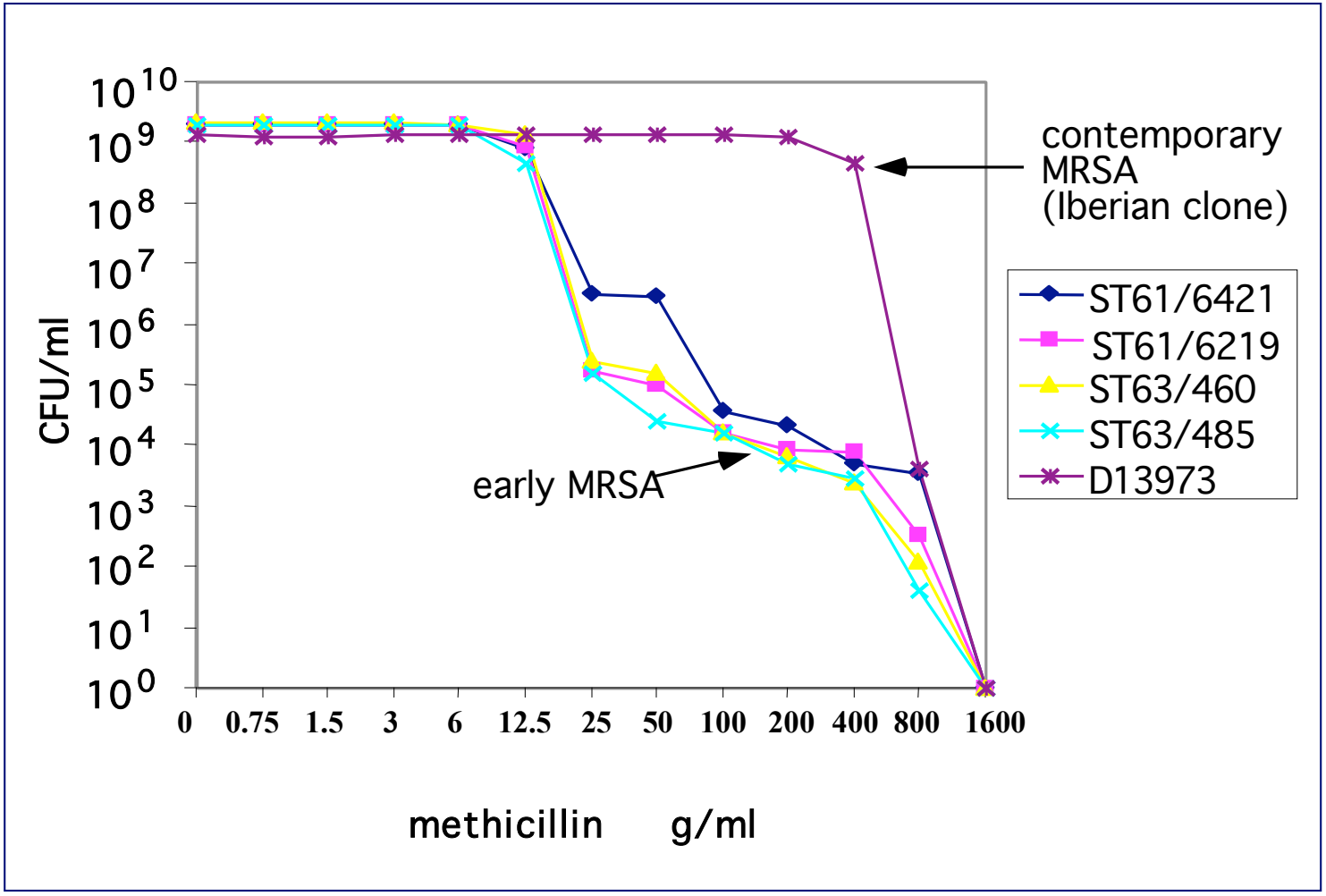
Geographic Spread of Pandemic MRSA Clones



Oliveira et al. Lancet 2002. **2**:180-189

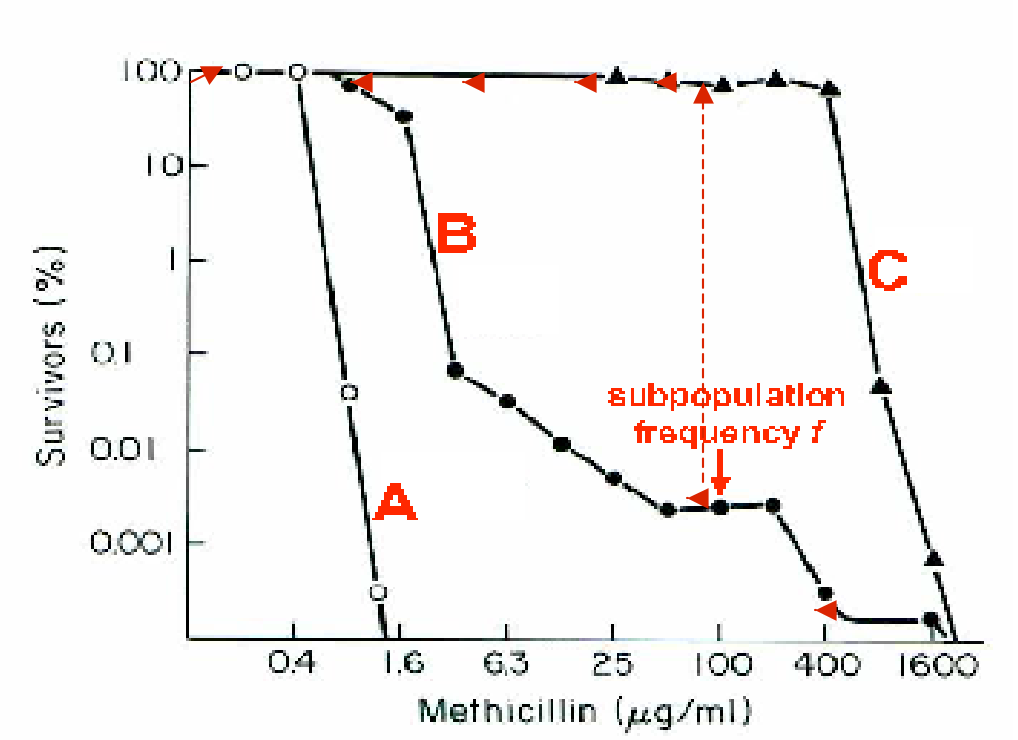
Growth characteristics of resistant bugs

Population Analysis Profile



Grow bugs permissive media, plate on media with increasing antibiotic, count colonies.

Heteroresistance



A: susceptible, B: heteroresistant, C homogeneous resistance

Pick colony from B, re-grow permissive media -> homogeneous resistant to origional level and trait genetic.

Resistance -> growth penalty, heteroresistance good evolutionary strategy.

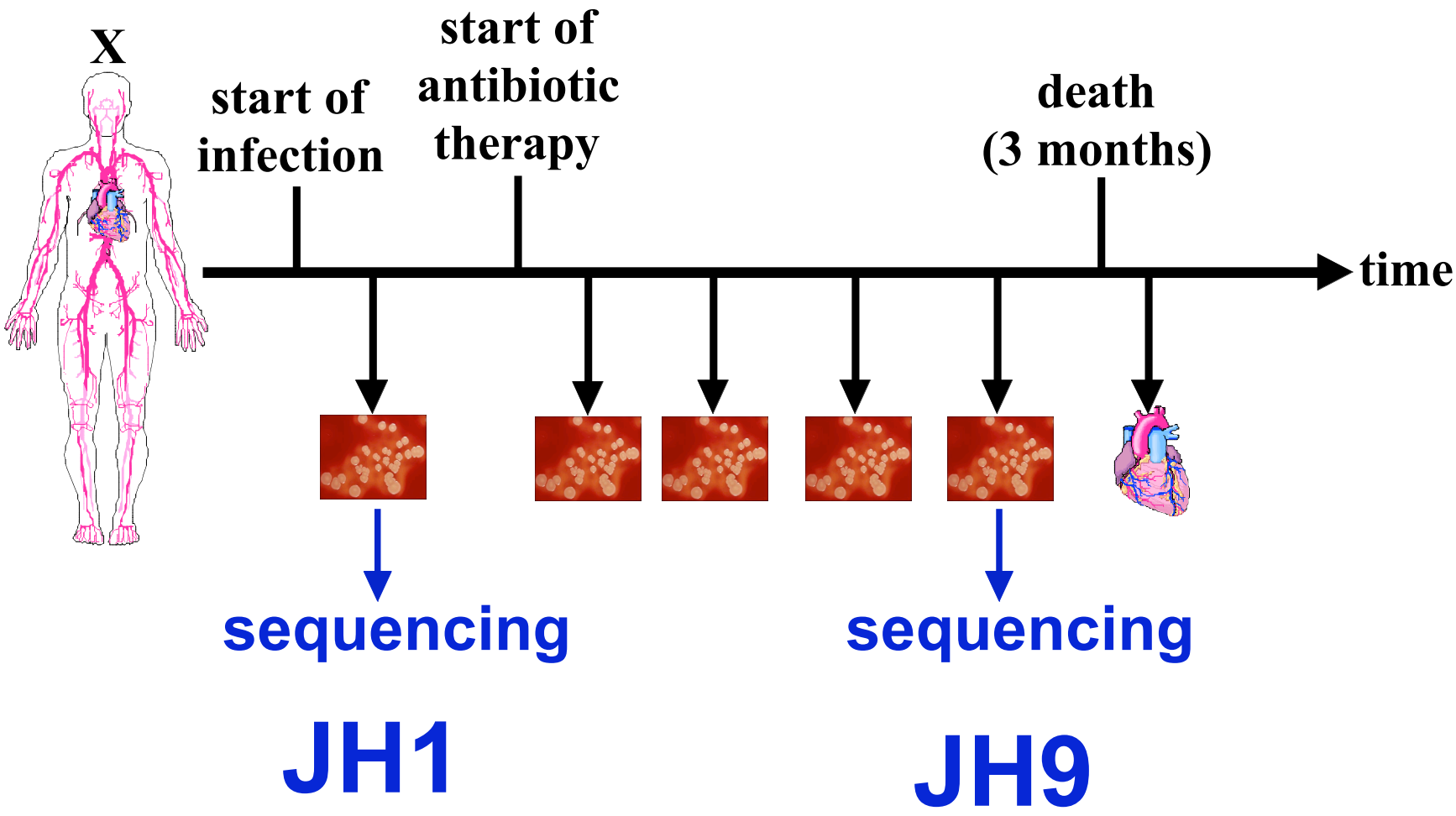
Antibiotic resistance via genome sequencing

1. Intermediate vancomycin resistance multigenetic trait and no obvious ‘resistance’ gene.

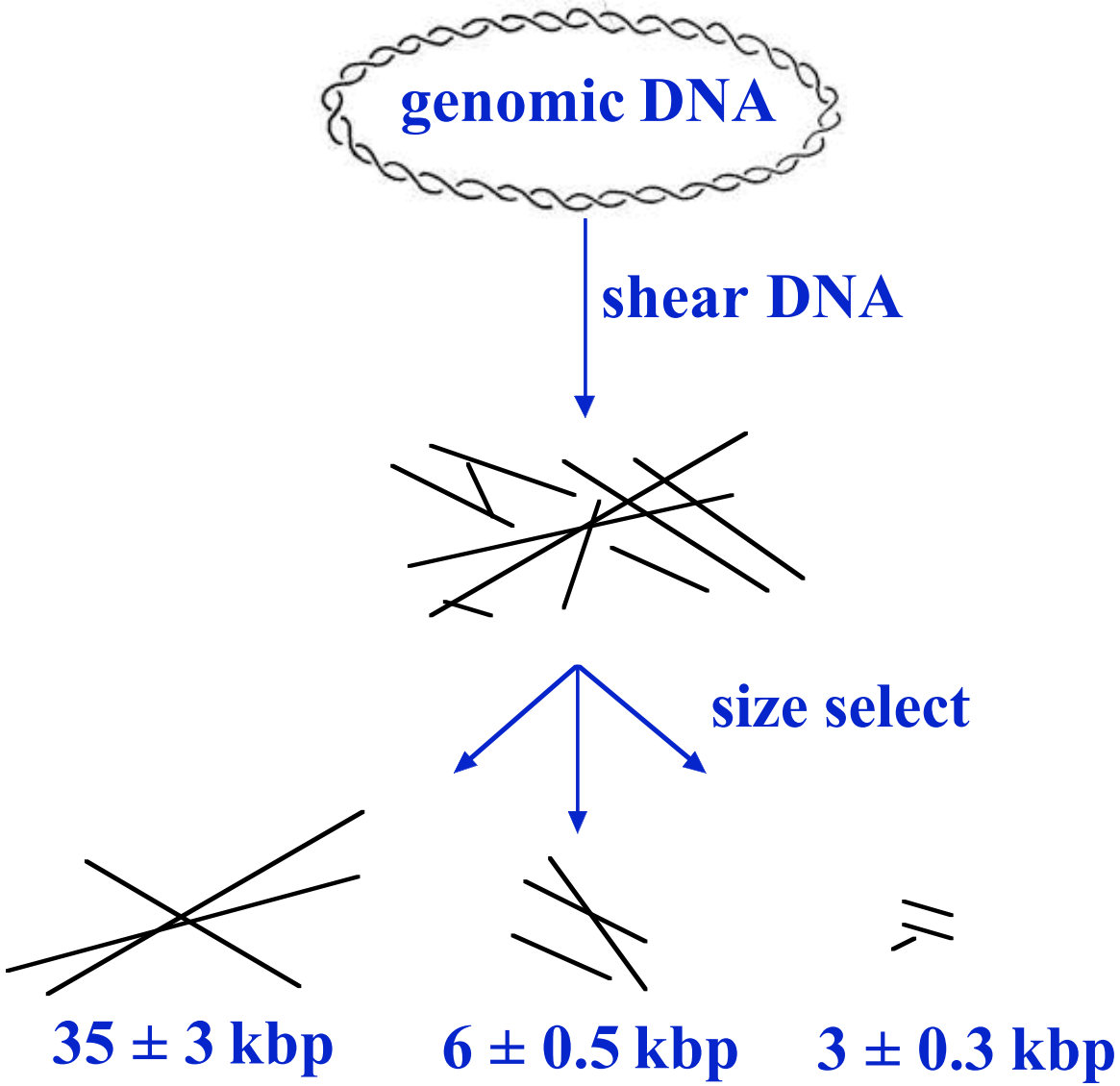
2. What mutations are involved in creating heterotypic resistance population analysis curves.

No lack of S.aureus genomes (9 to date), but none close enough (0.01% - 2% point mutation rate + 10’s kb of novel mobile elements)

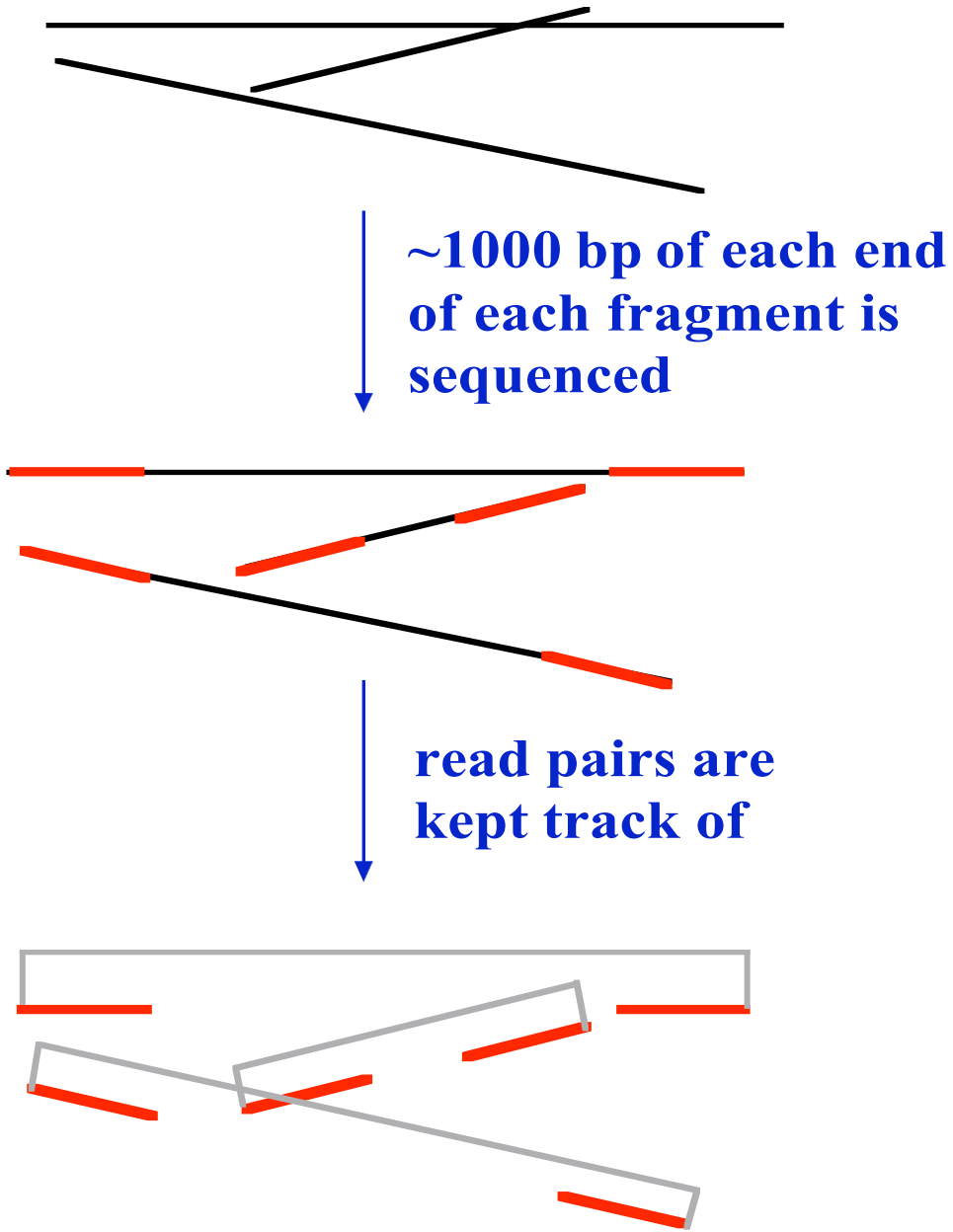
Compare isolates from one patient undergoing vanco therapy



Shotgun sequencing I

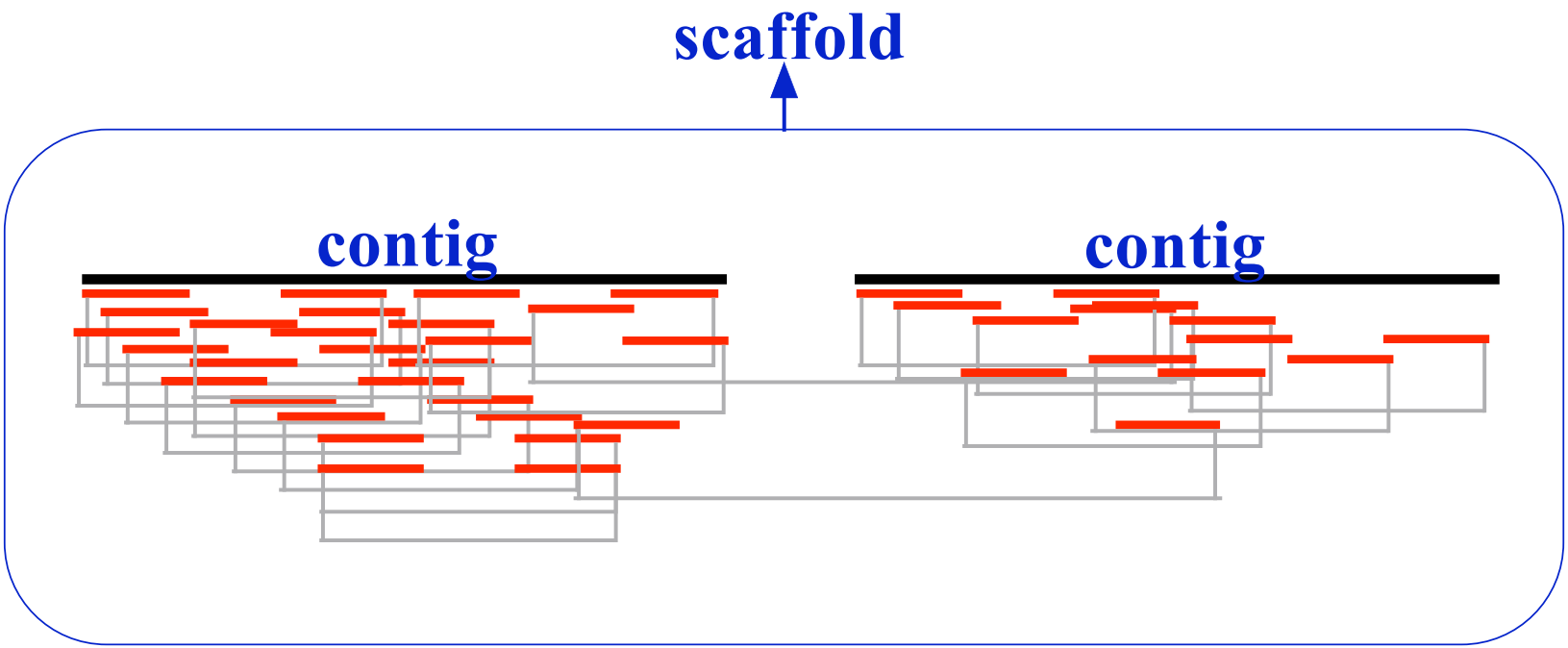


Shotgun sequencing II



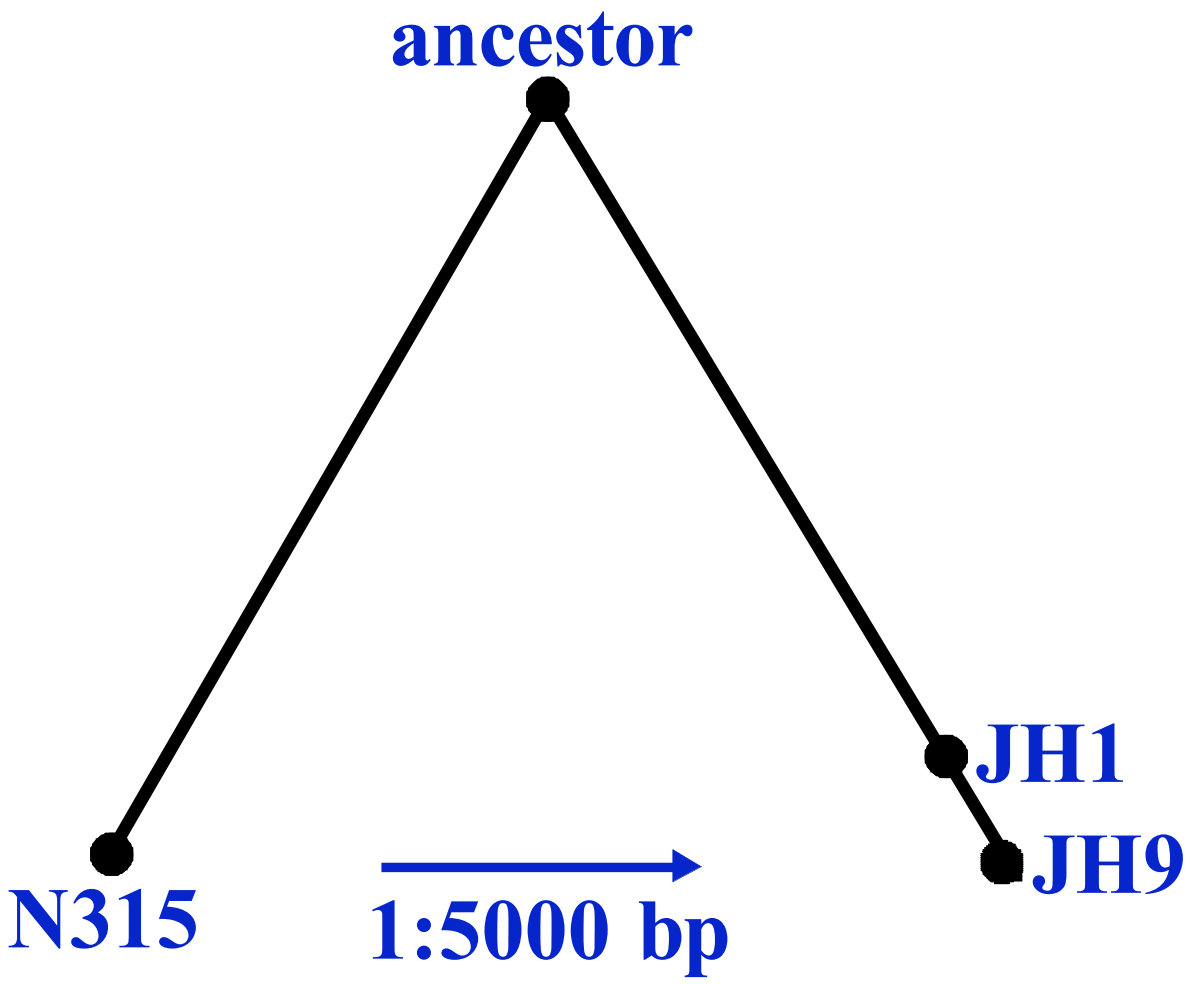
JGI

Assembly using Celera assembler

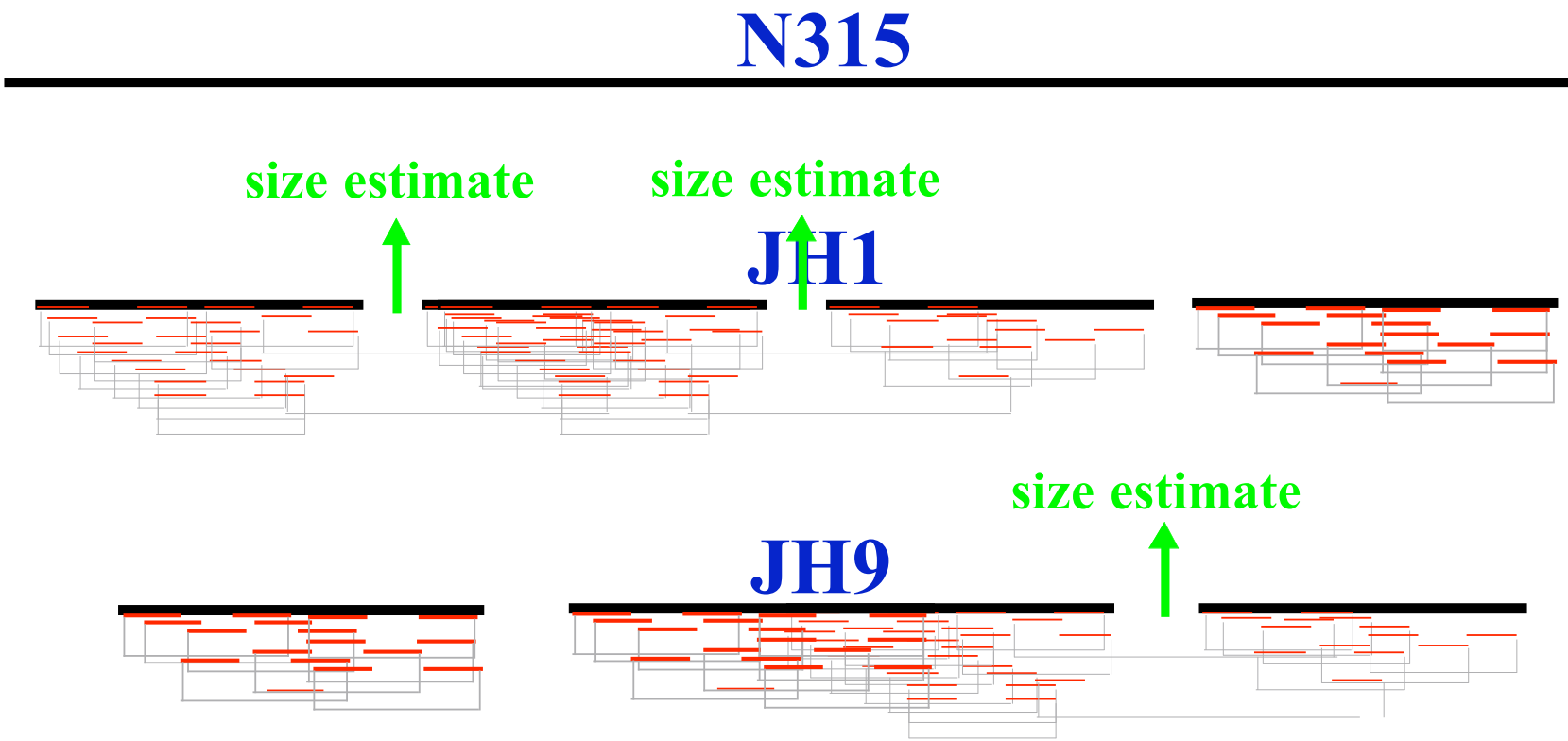


Myers

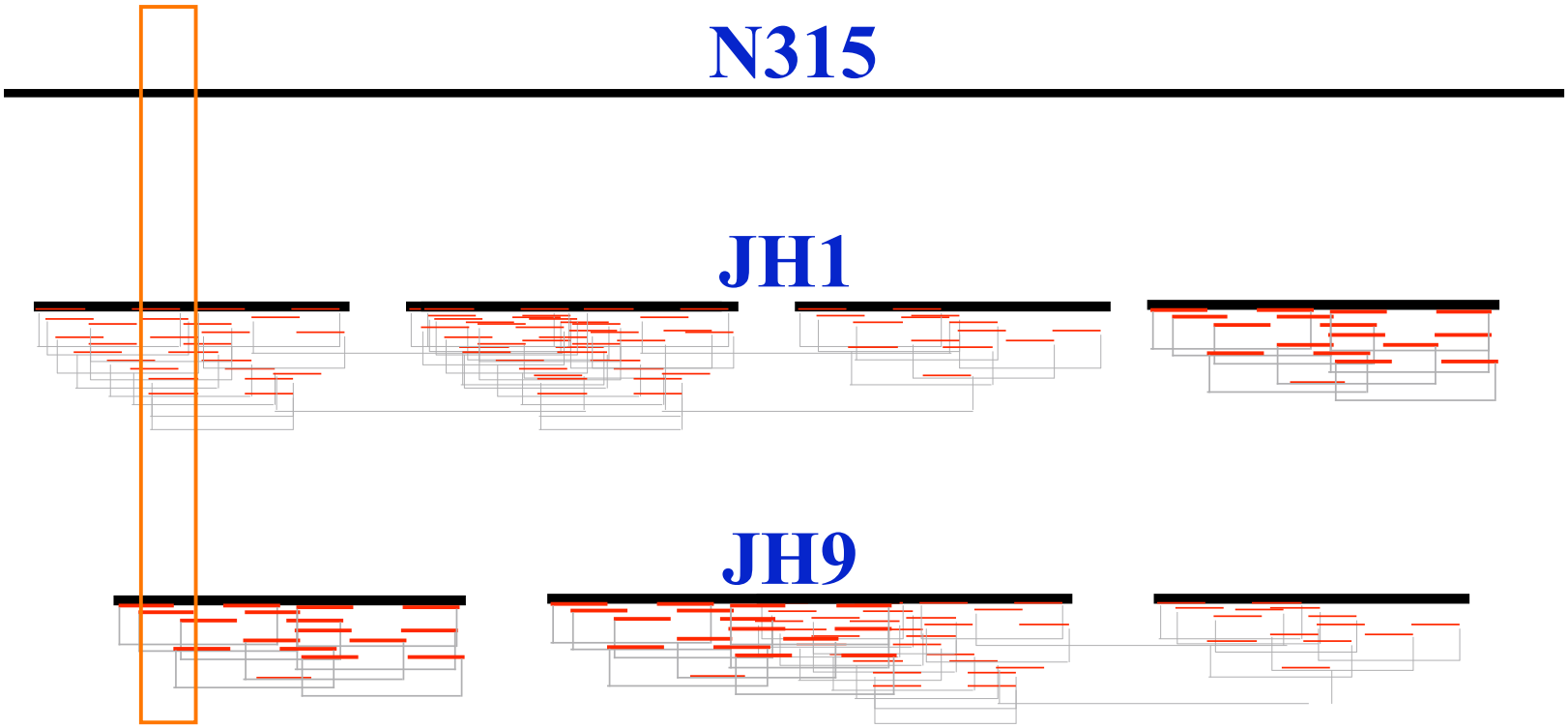
Use of previously sequenced N315



Multialignment I



Read errors and quality values

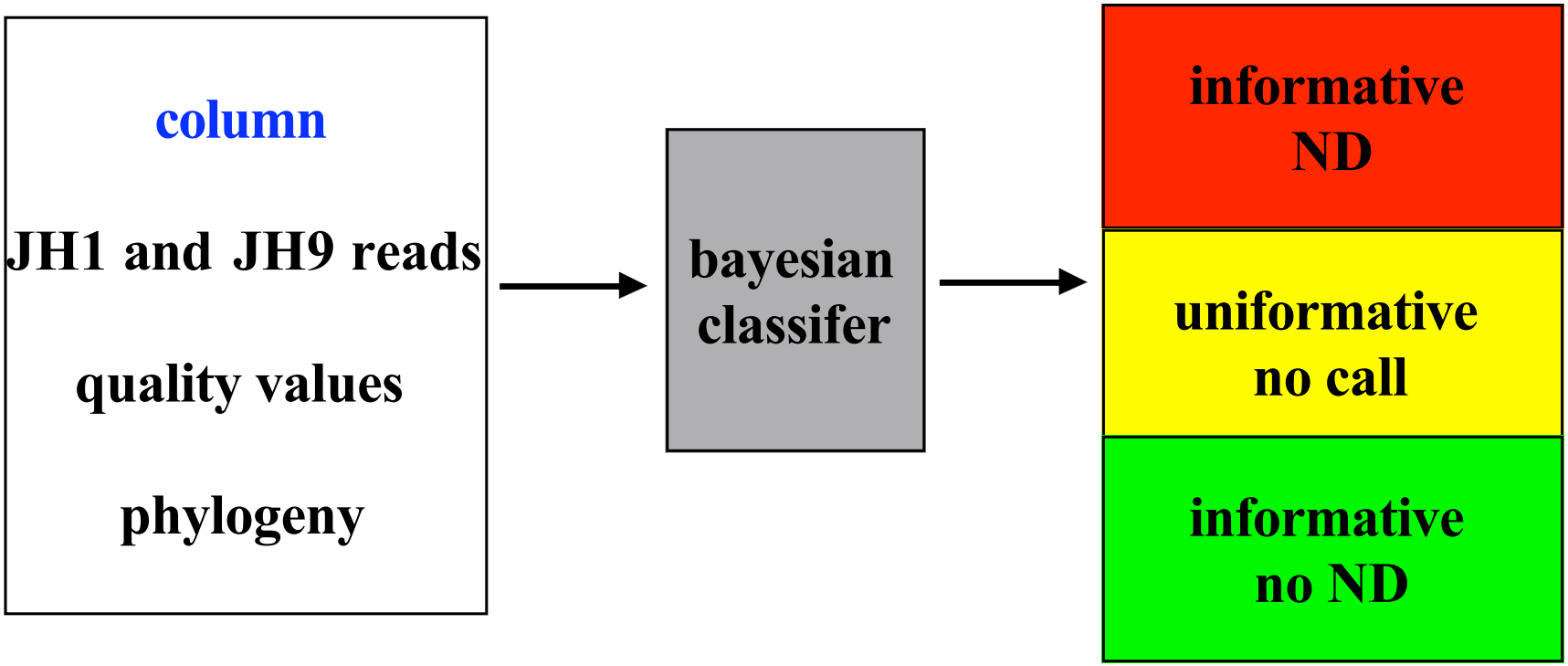


Read errors and quality values

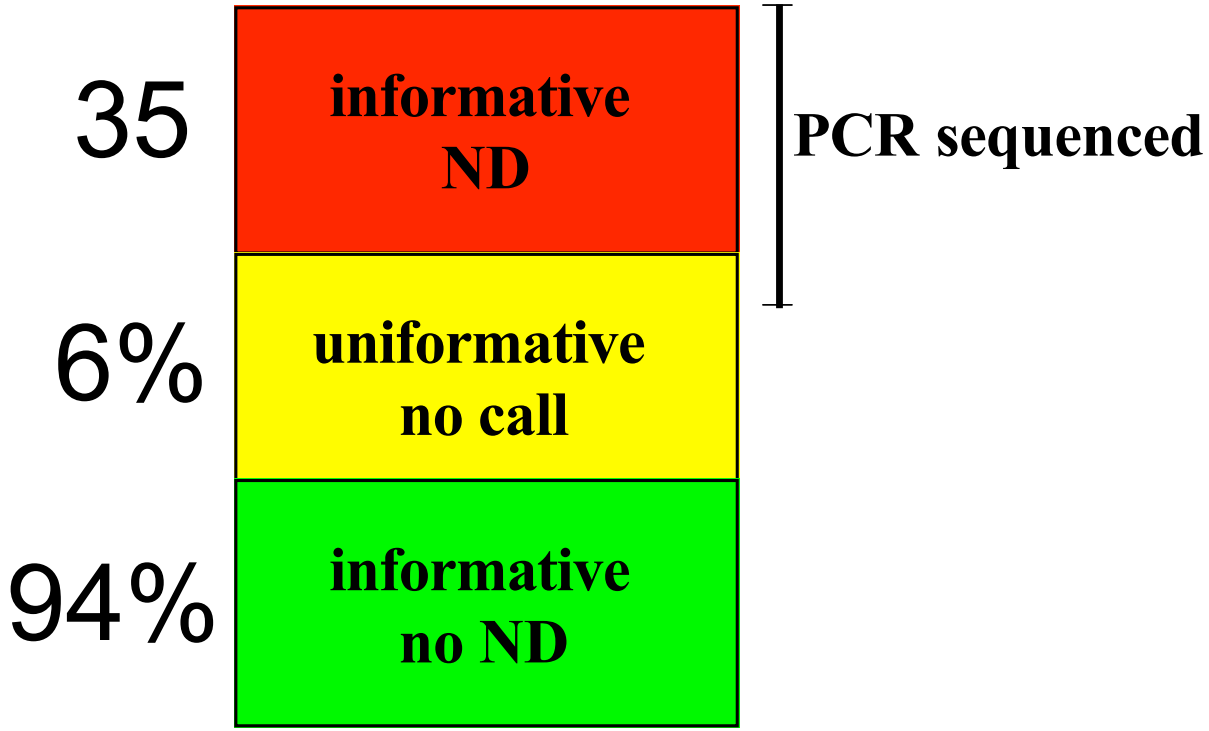
N315		**GATTCGA**
	read 1	**GATTCGA**
	read 2	**GATTCGA**
JH1	**read 3**	**GACTCGA**
	read 4	**GATTCGA**
	read 5	**GATTCGA**
	read 1	**GATTCCA**
JH9	**read 2**	**GATTCGA**
	read 3	**GATTCCA**

$$E = 10^{-Q/10}$$

Bayesian classifier



Validation of Bayesian classifier by PCR sequencing



Wu, Yanjiao

Summary of Assembly and mutations

- 2.9Mb genomes, ~8x coverage, ~60 contigs, ~2% sequence in gaps (30kb plasmid ~50x one contig)
- Changes: N315 vs JH1 about 120kb new sequence in mobile elements, 1:5000 point mutation rate
- Changes: JH1 vs JH9 Call point mutations on 94% of bases (eg 6% = 2% +2% gaps + 2% low coverage) -> 34 total. No larger elements, all gaps consistent with no change