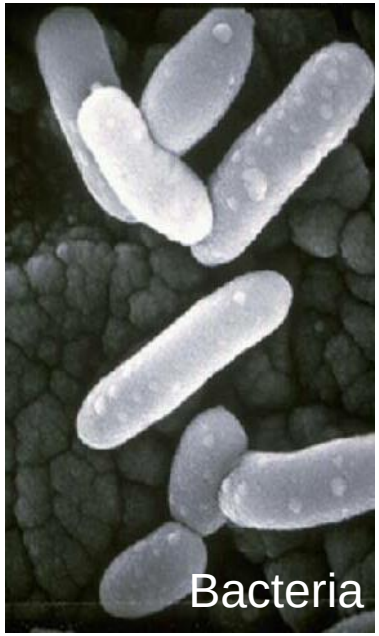
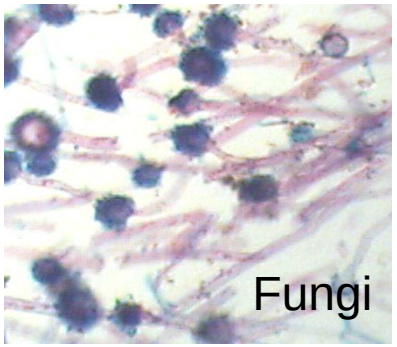
In Good Company: Our Microbial Ecosystems in Health and Disease

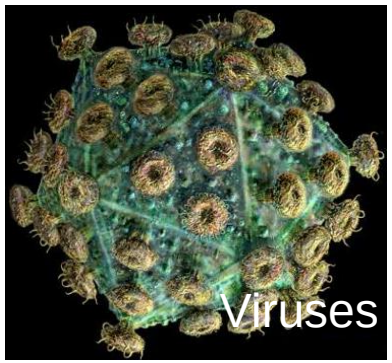
Julie Segre, PhD
Senior Investigator
National Human Genome Research Institute,
National Institutes of Health, Bethesda, MD USA

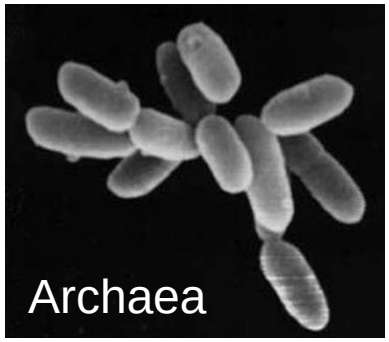
Humans as microbial ecosystems

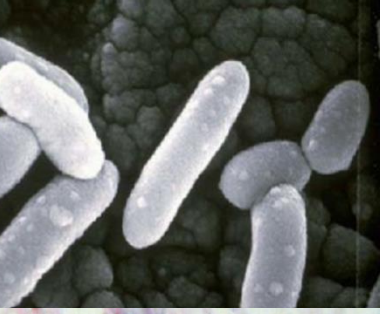


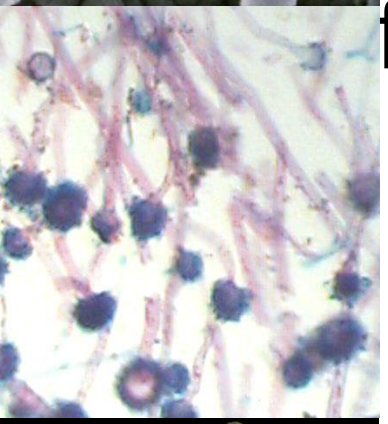
- Humans are hosts to many microbes (bacteria, fungi, viruses)
- Microbial cells outnumber human cells
- Many microbes are often considered pathogenic
  - *Staphylococcus aureus* (bacteria)
  - *Klebsiella pneumoniae* (bacteria)
  - *Aspergillus fumigatus* (fungi)
  - *Ebola virus*

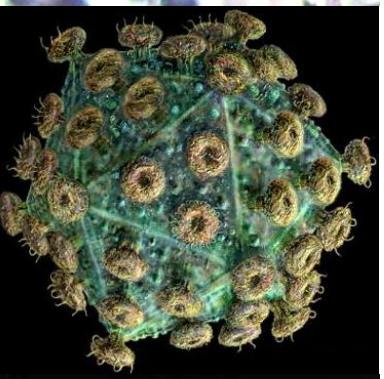


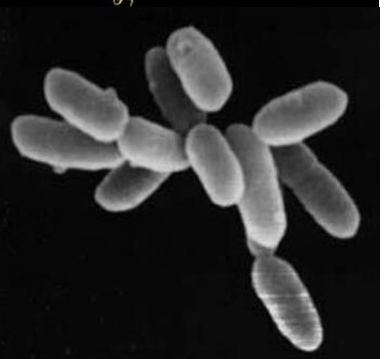










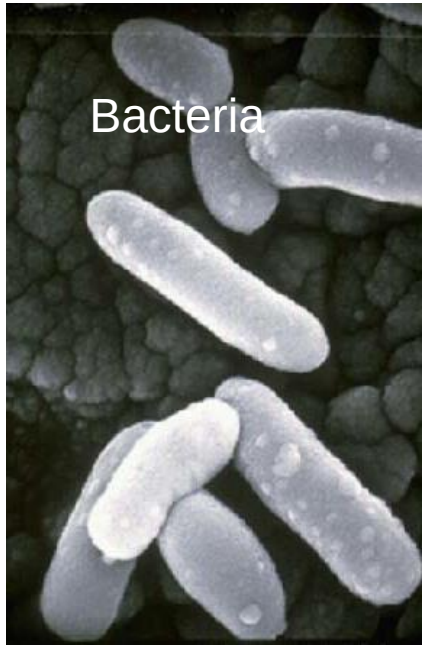


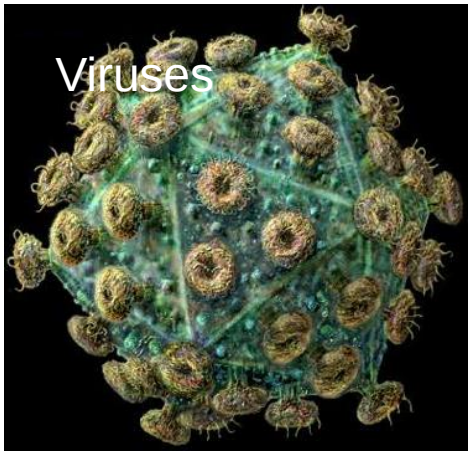
Not all microbes are bad: Beneficial microbes perform functions essential for human health

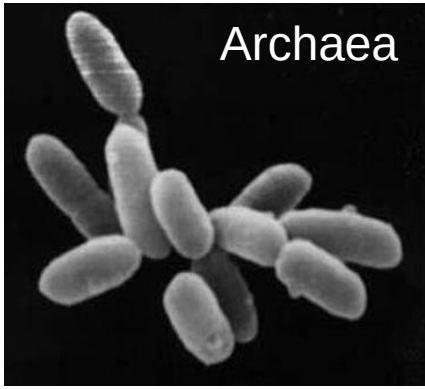
- Vitamin synthesis
- Digestion
- Education and activation of immune system
- Colonization resistance to pathogens

Many microbial-host and microbial-microbial interactions remain unknown

Why the Human Microbiome?







Each human cell has the same protein-encoding potential. Microbes are more diverse and dynamic than human genome. (Microbiome is totality of microbial community's DNA.)

Elucidating the diversity of the human microbiome

- Traditional approaches rely on isolating bacteria in pure culture
- The majority of bacterial species do not grow in culture = “the great plate count anomaly”
- Culturing favors lab weeds--not necessarily the most dominant or influential species
- Excludes microbes that rely on community interactions

Genome Sequencing Technology Continues to Evolve Driving Innovation

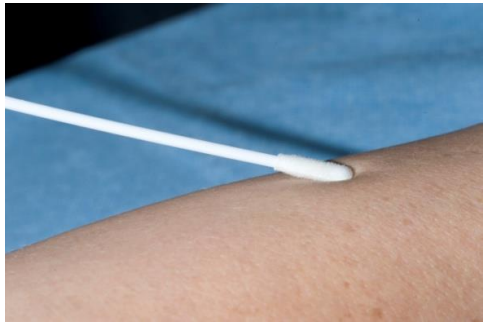
454/Roche
400 bp reads
50 kb contigs
$4,000/genome

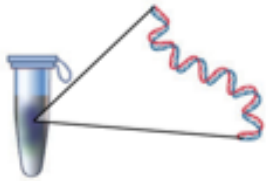
Illumina MiSeq
2x300 bp reads
250 kb contigs
$500/genome

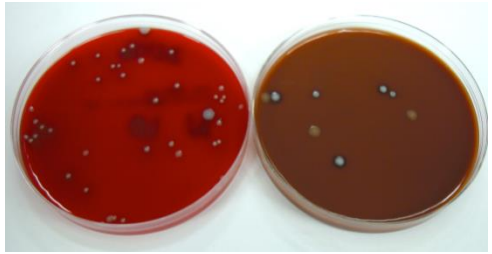
Illumina HiSeq
100 bp reads
100 kb contigs
$200/genome

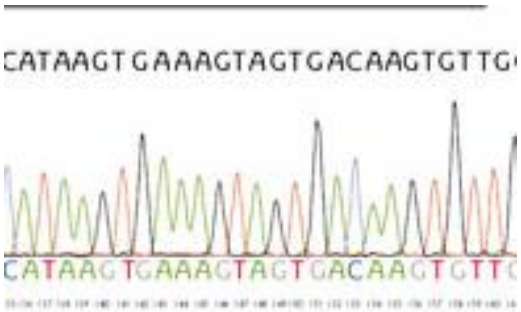
PacBio
15,000 bp reads
Fully assembled
$1,200 genome

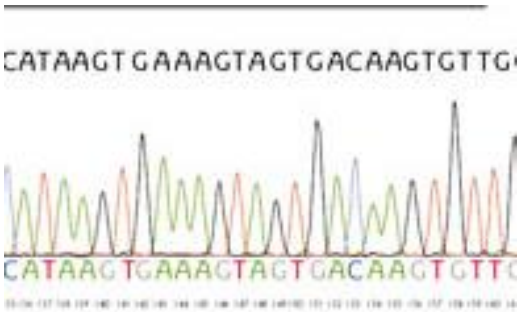
Direct sequencing vs. culture-based methods



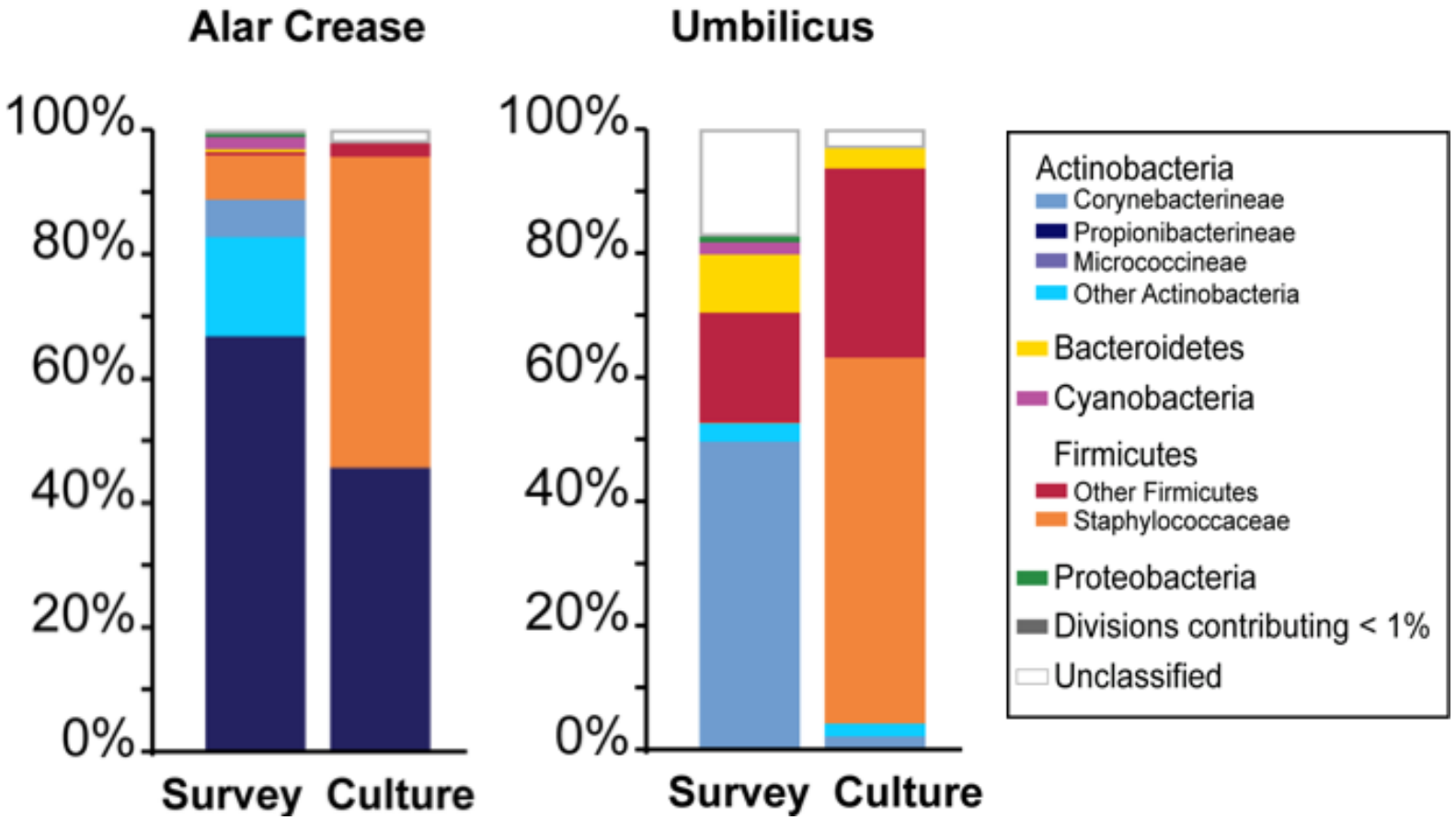








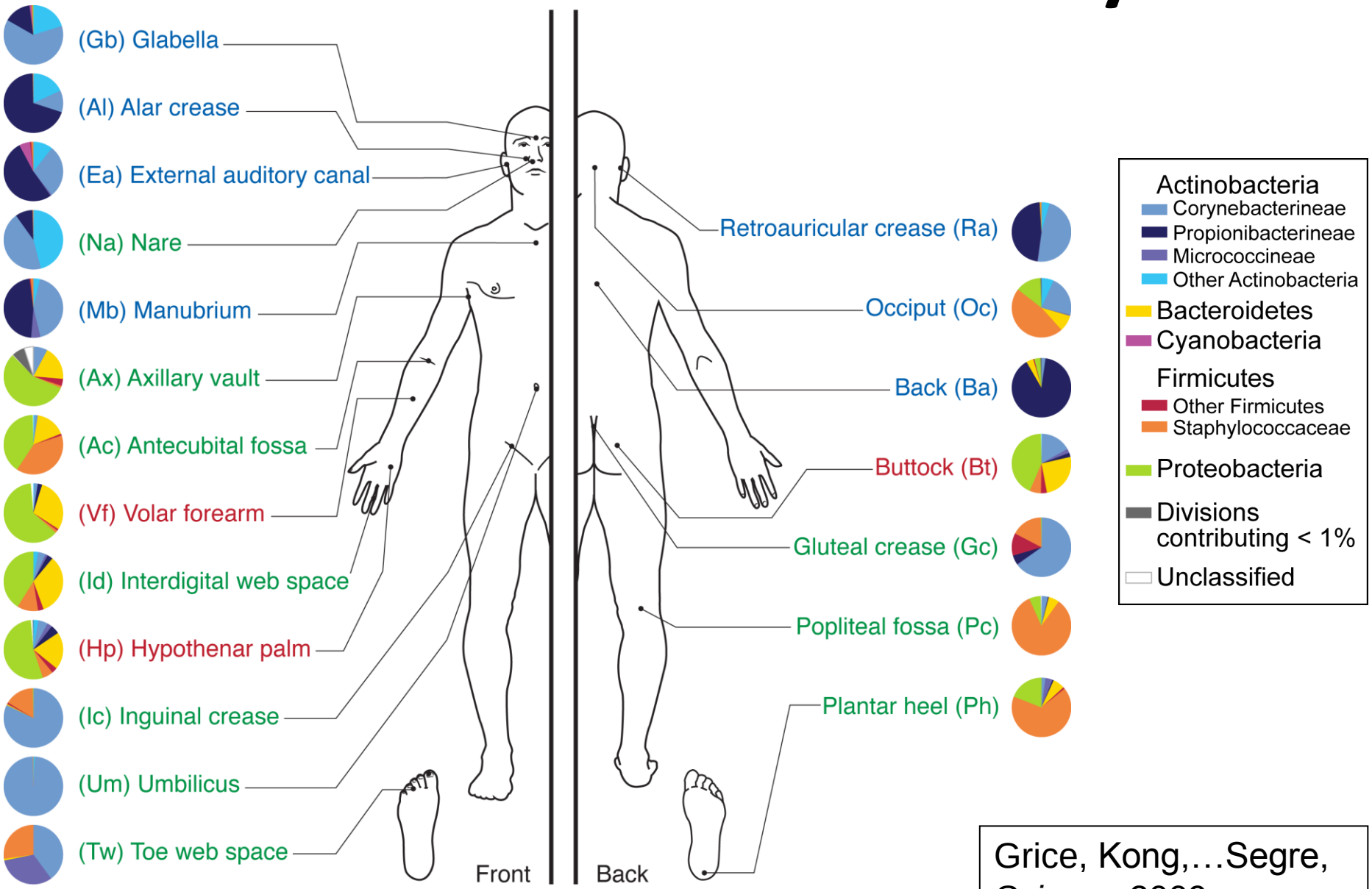
Direct sequencing vs. culture data



Topics for today's talk

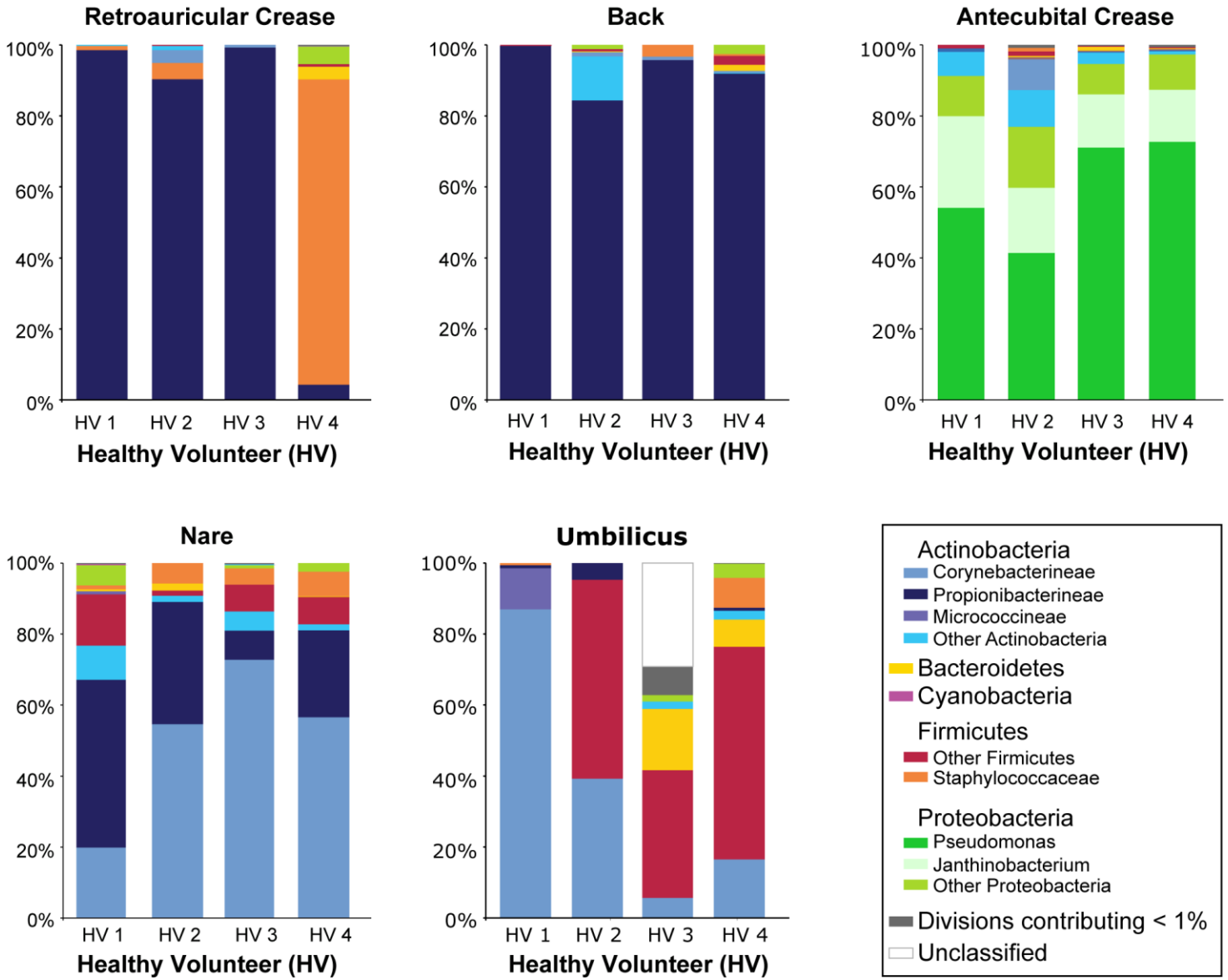
1. Bacterial diversity studies: 16S rRNA
2. Changes in bacterial populations in disease (childhood eczema)
3. Metagenomics: Bacteria, fungi, viruses, strains
4. Bad pathogens: Hospital-acquired infections

Human Skin Sites Survey



Grice, Kong,…Segre, *Science* 2009

Inter-personal site variation

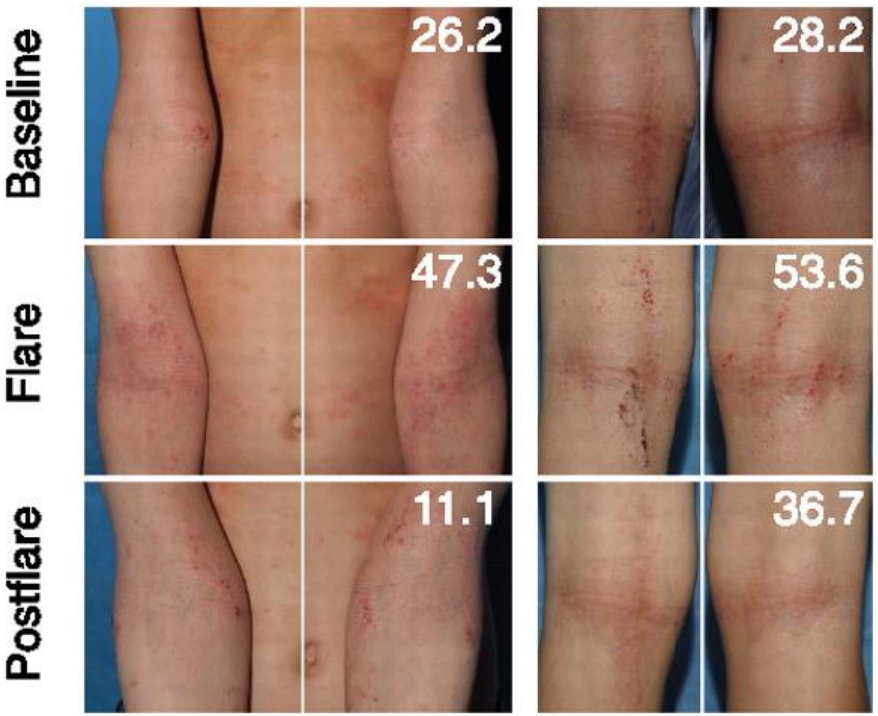


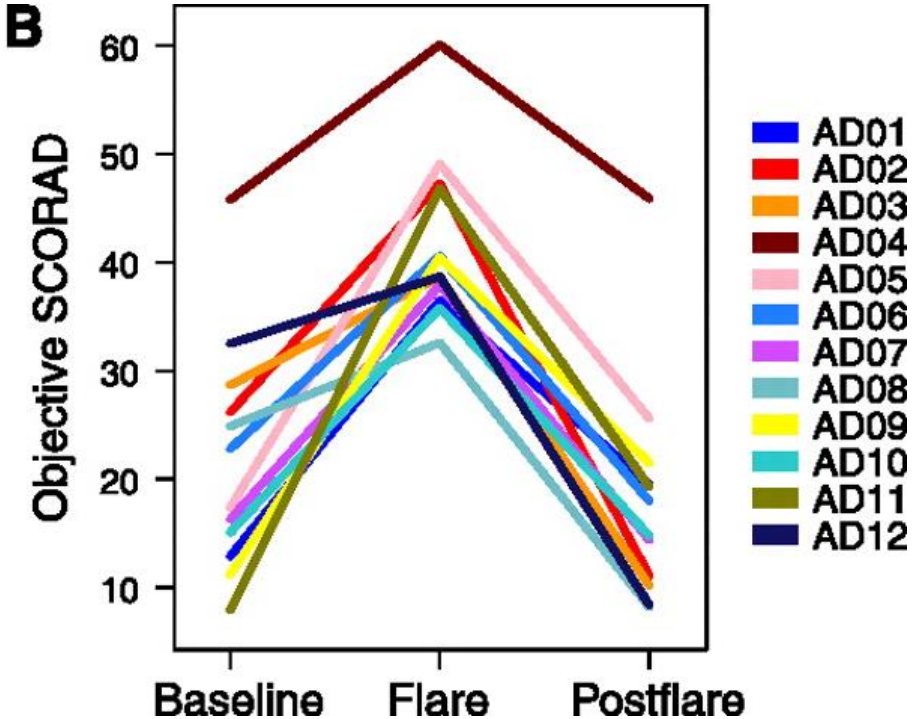
Increased Prevalence of Atopic Dermatitis

- Currently affects 15% of US Children
- 2-fold increase in last 30 years
- "Hygiene hypothesis"
  - Increased incidence of atopic disorders is related to decreased exposure to common infections in early life
- Increased prevalence of atopic disorders in westernized countries

Strachan *et al. BMJ* 1989

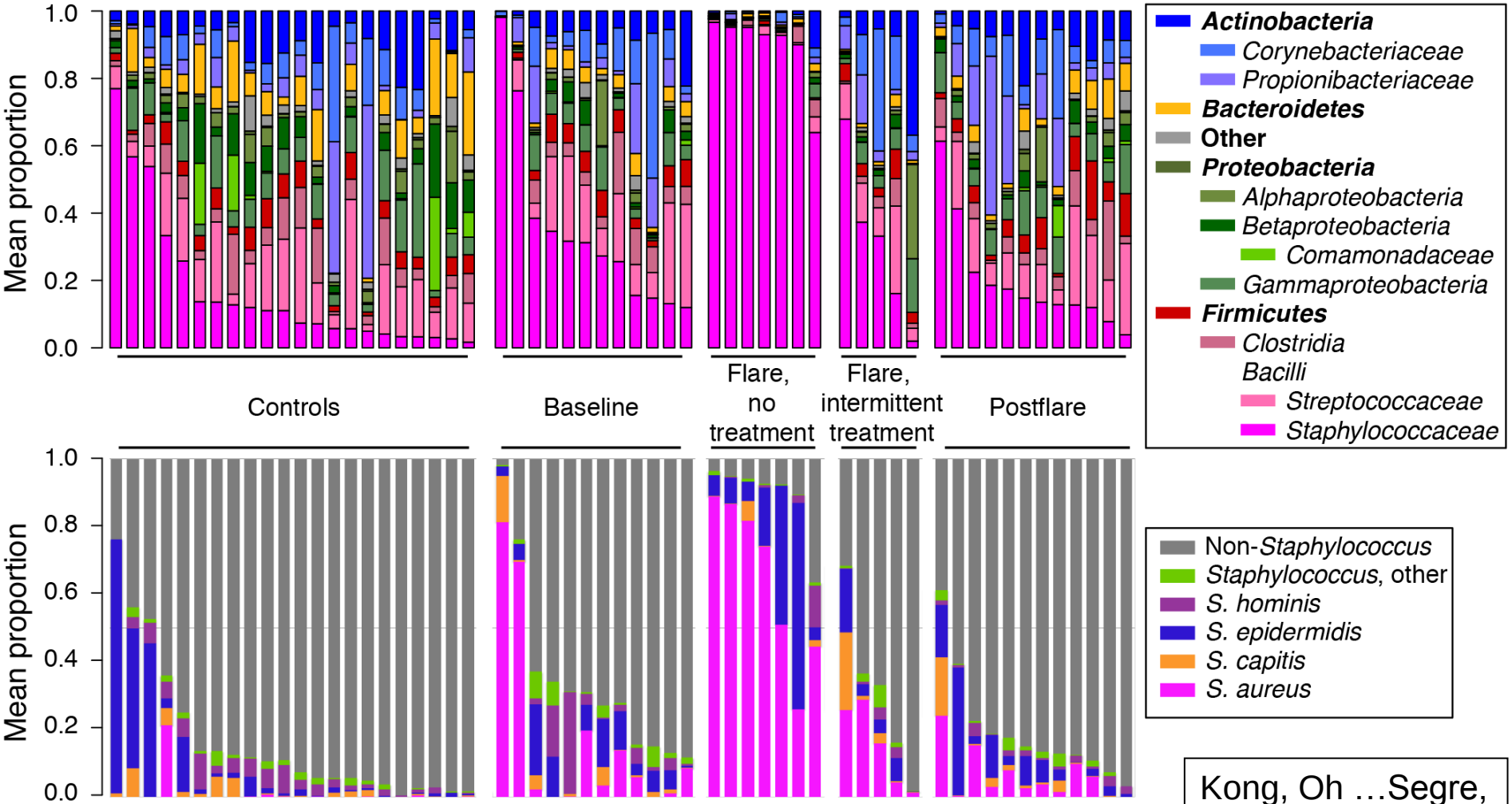
AD disease severity





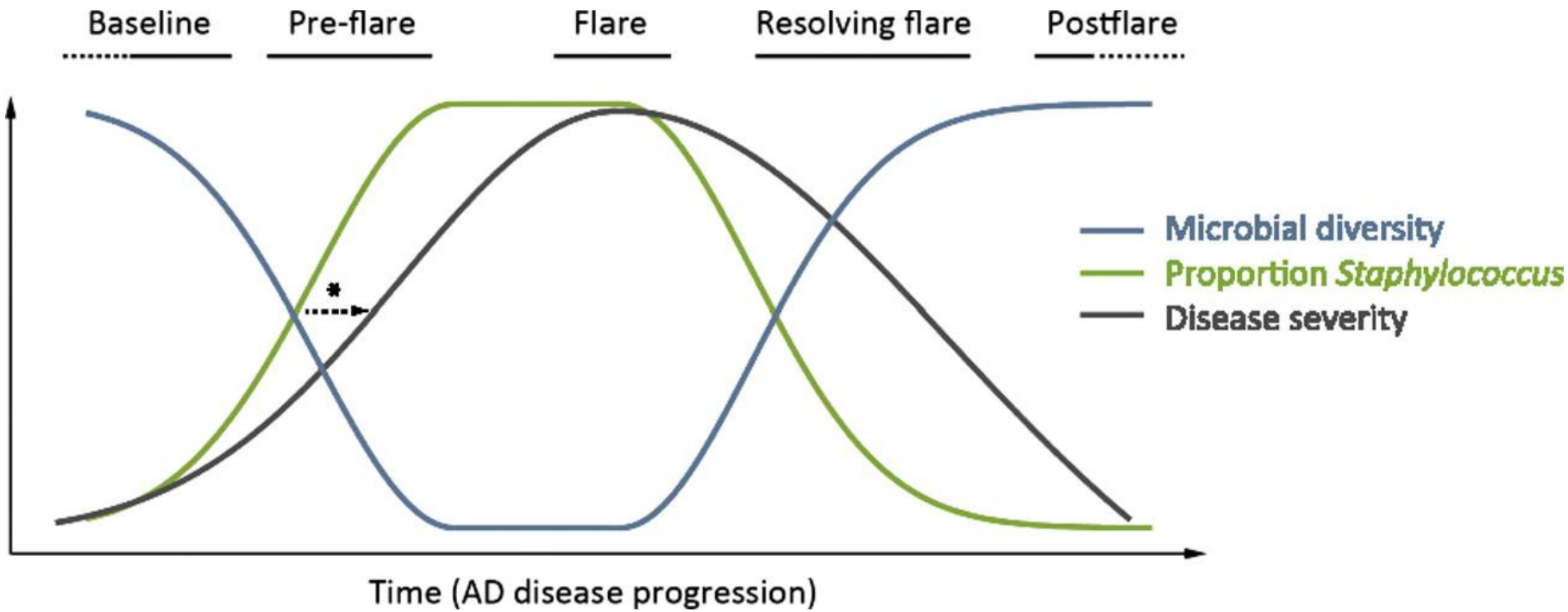
Kong, Oh, …Segre.
Genome Res. 2012

Staphylococcus aureus increases during AD flares

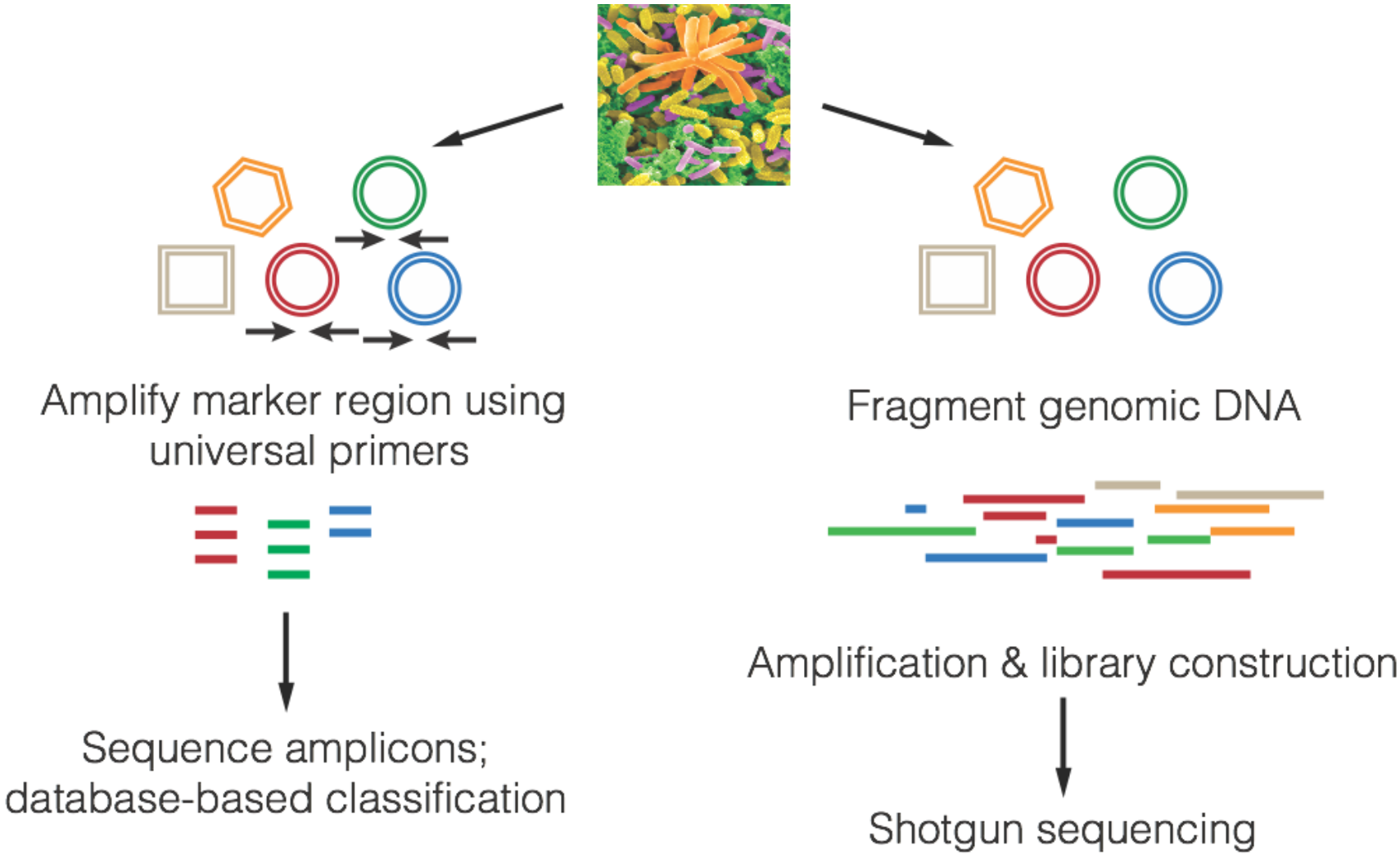


Kong, Oh …Segre, *Gen Res* 2012

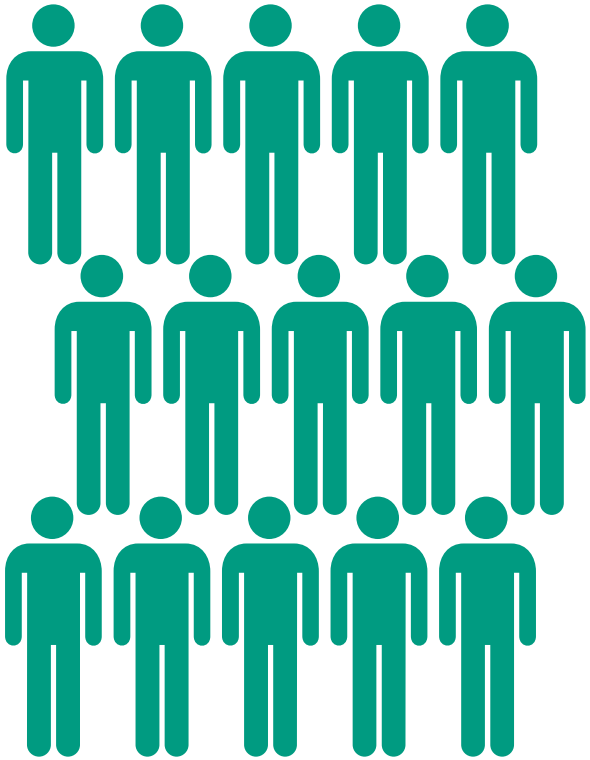
AD microbiome progression hypothesis



Whole genome shotgun vs. amplicon sequencing

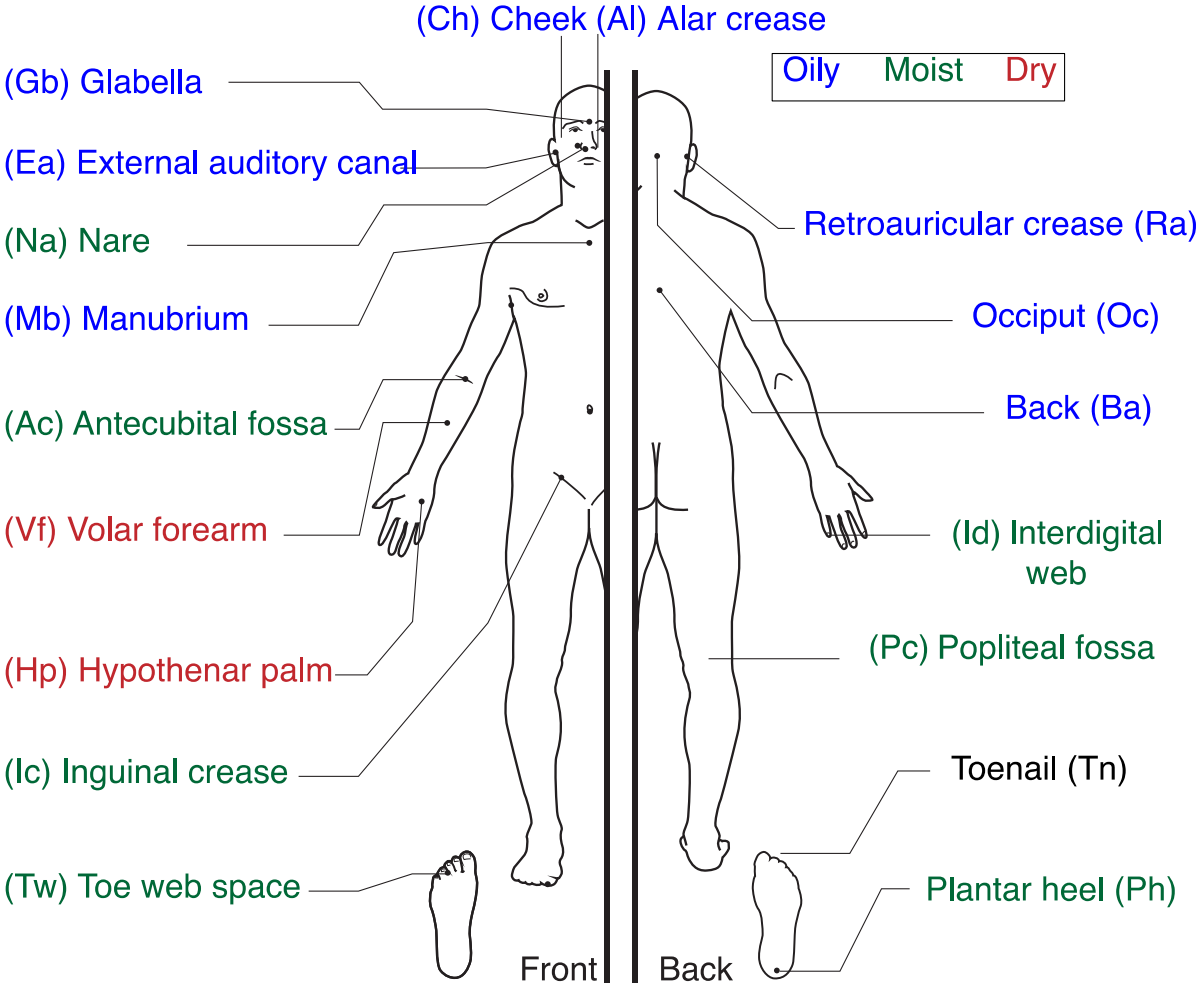


Survey of Healthy Skin Microbiome



15 Healthy Adults

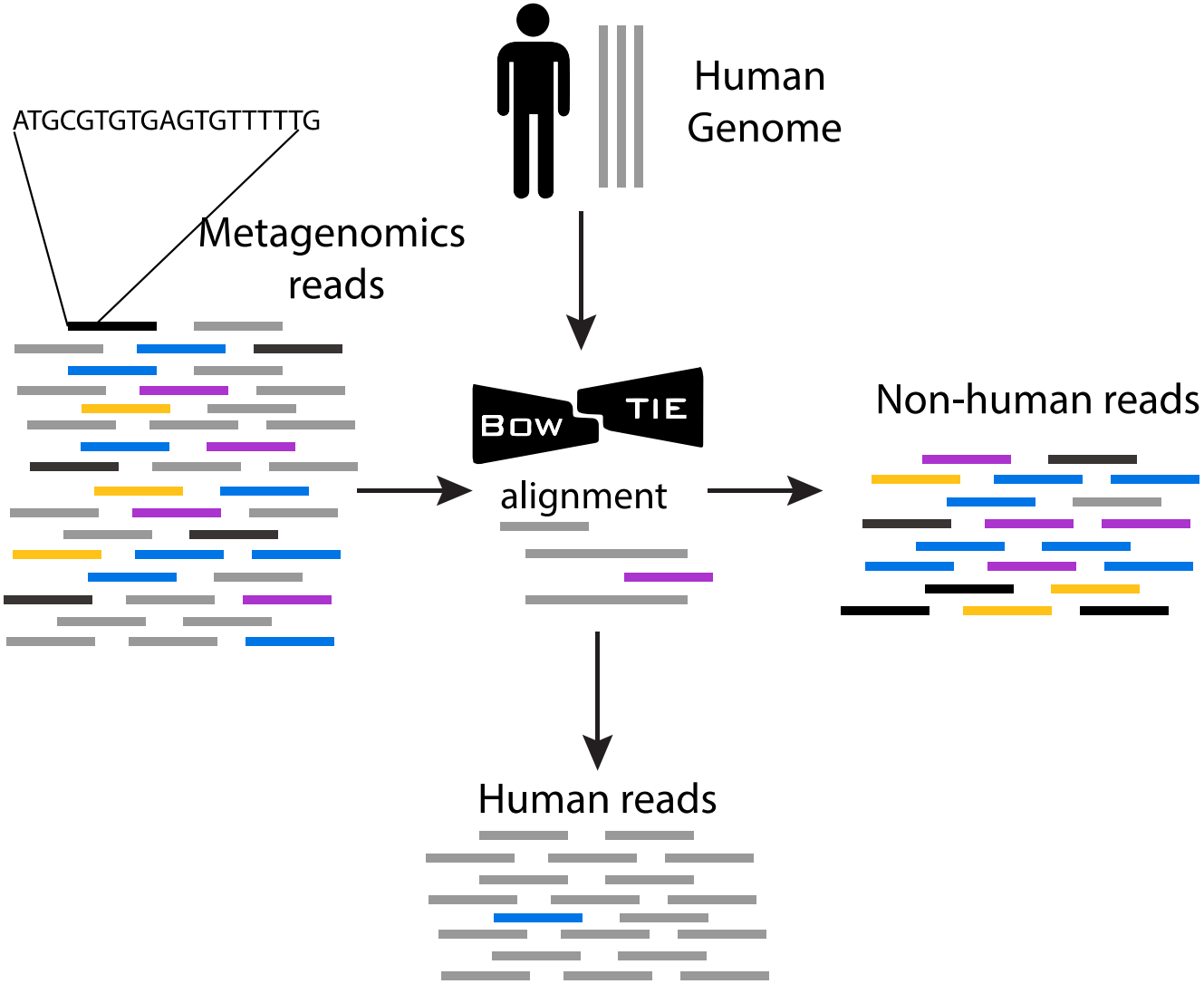
Aged 23-39



18 body sites

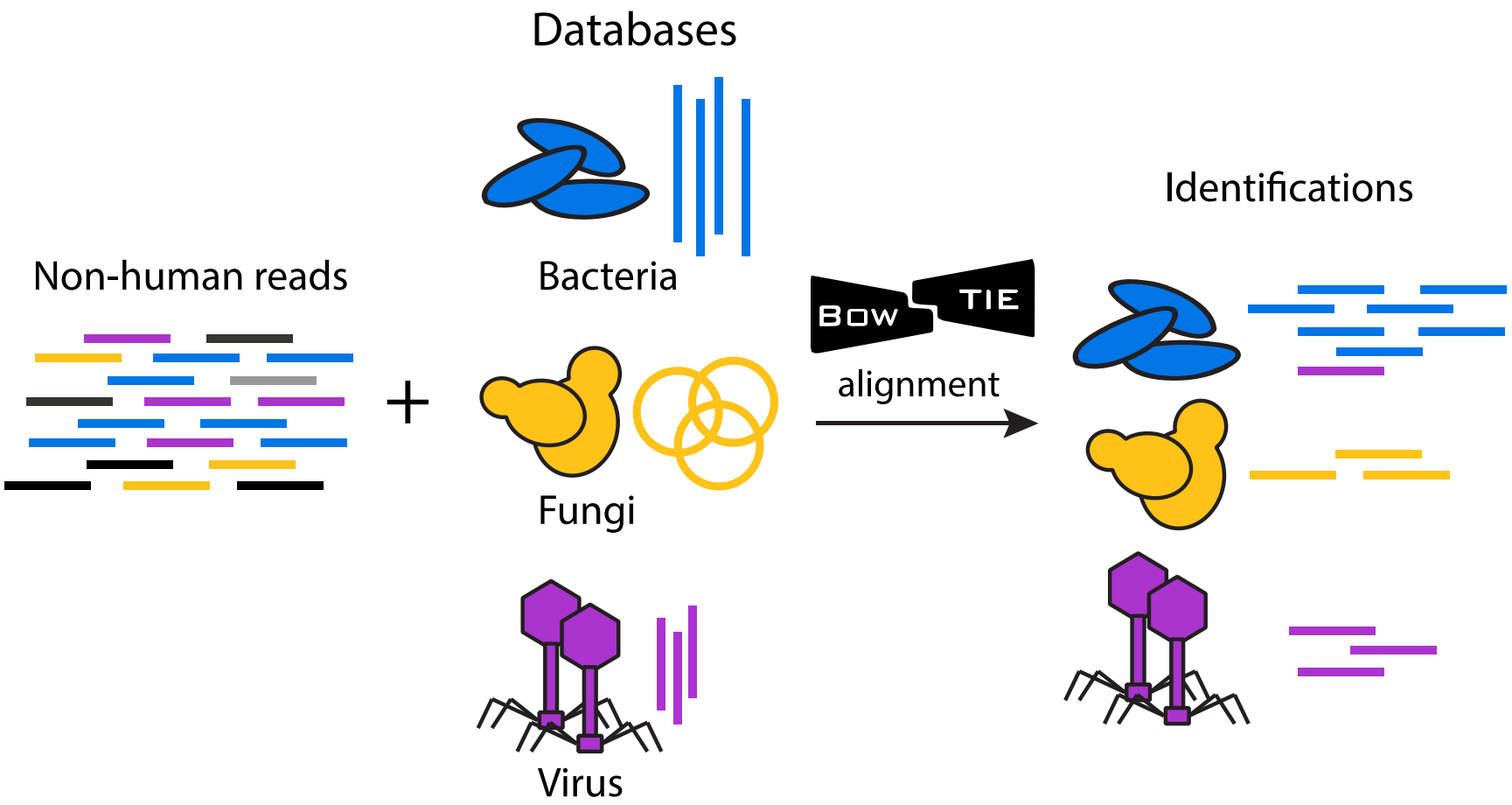
Metagenomics Pipeline

Step 1: Remove human reads

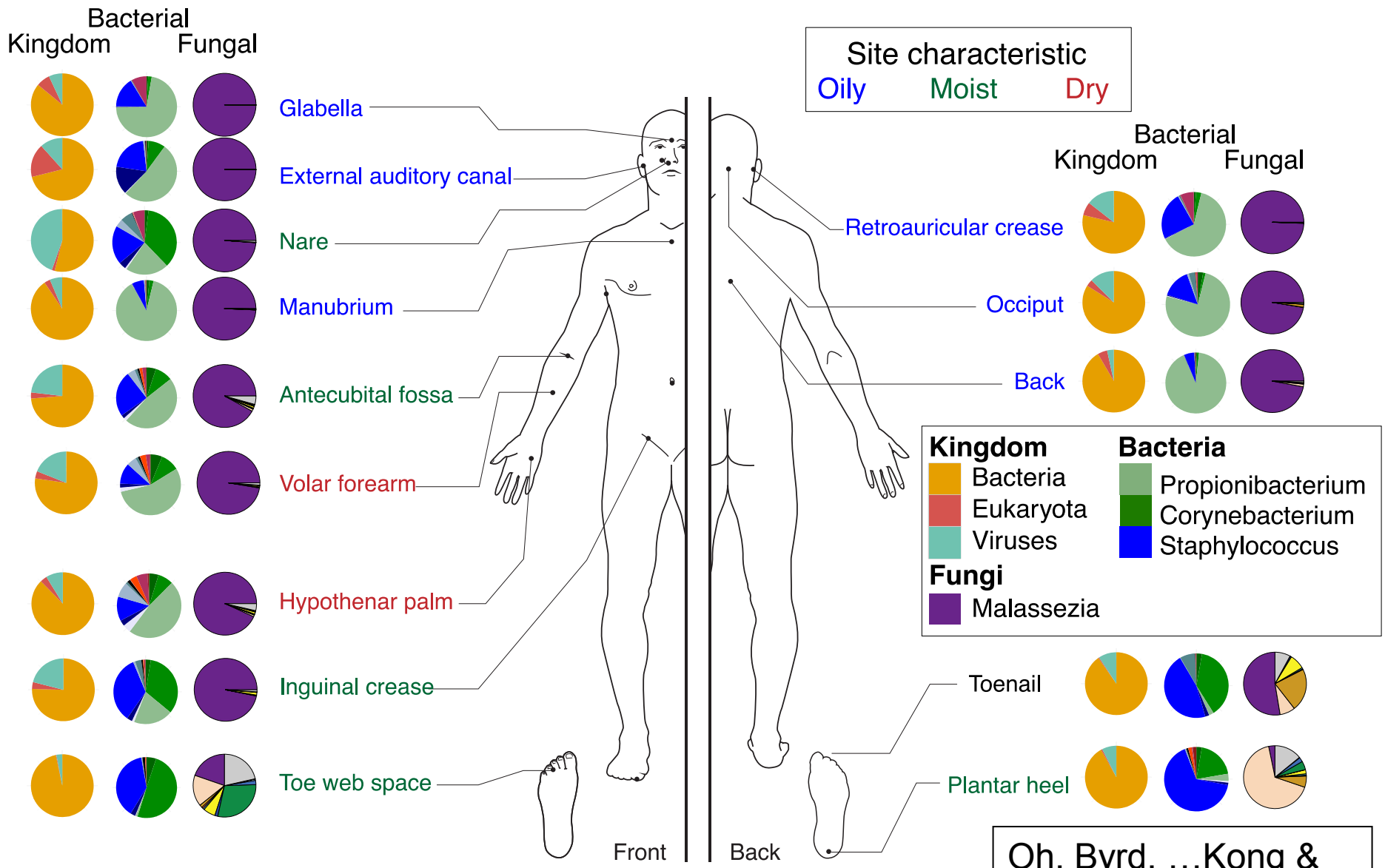


Metagenomics Pipeline

Step 2: Assign reads to kingdoms

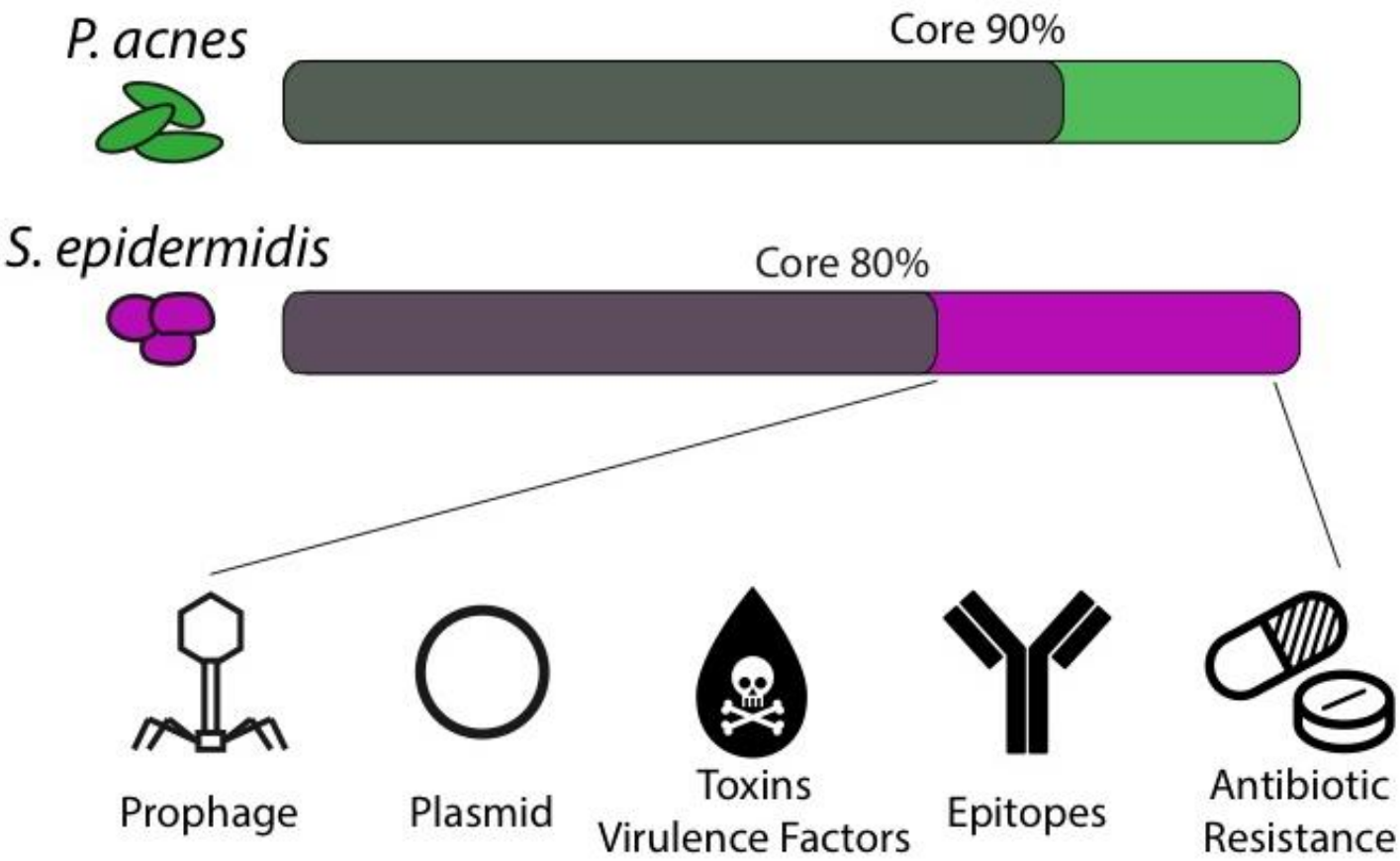


Skin Microbial Metagenomics

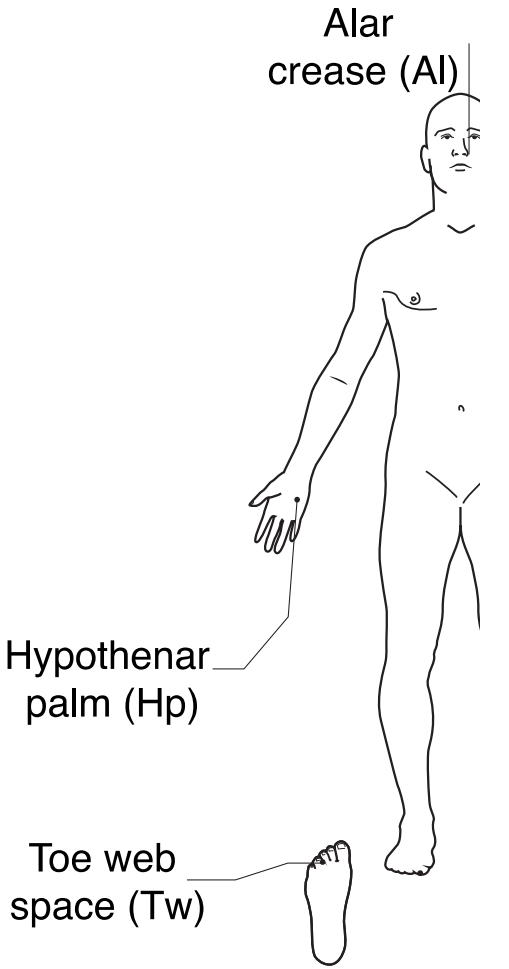


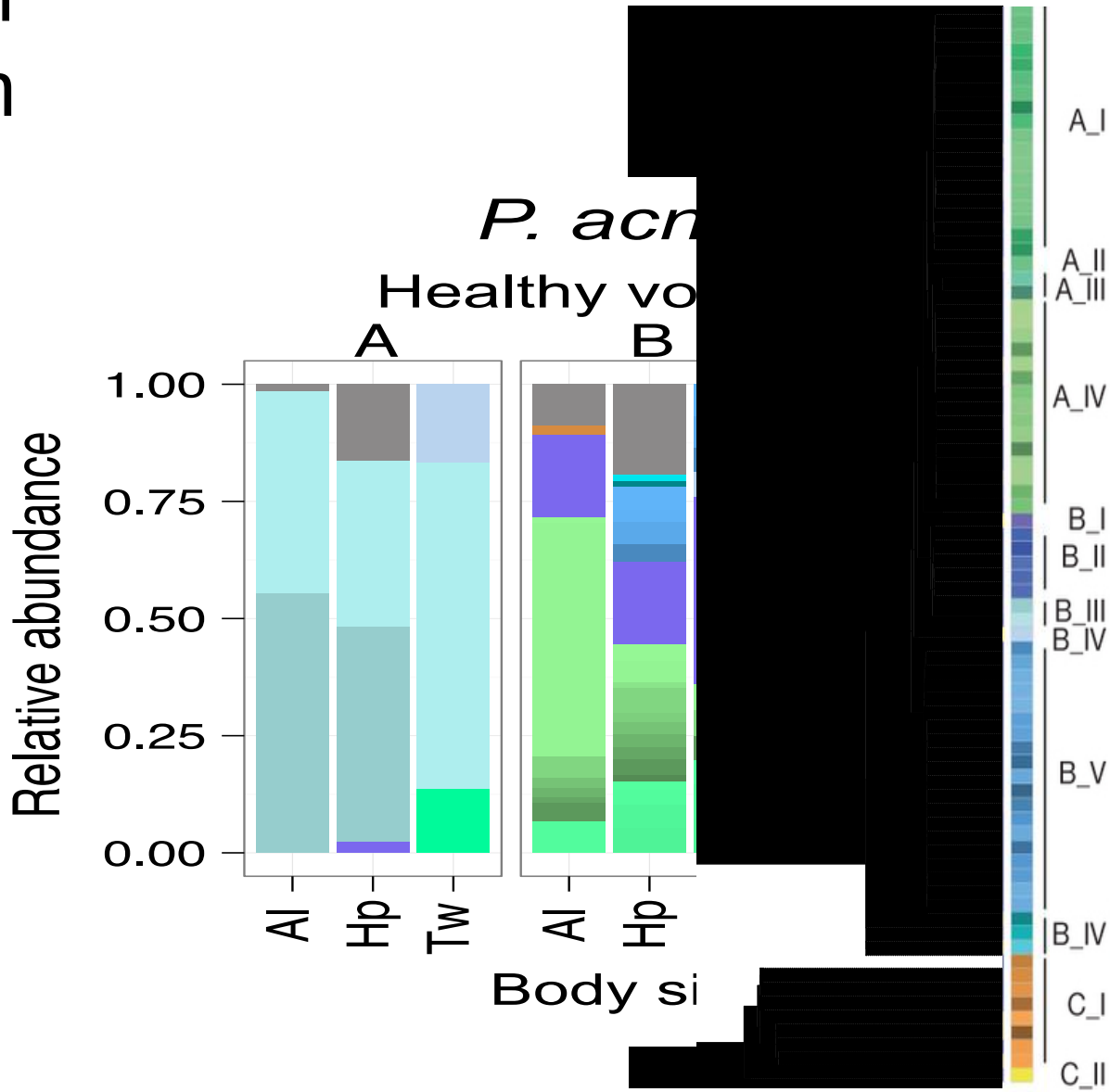
Oh, Byrd, …Kong & Segre, Nature, 2014

Strain Specificity: Noncore regions confer important functional differences

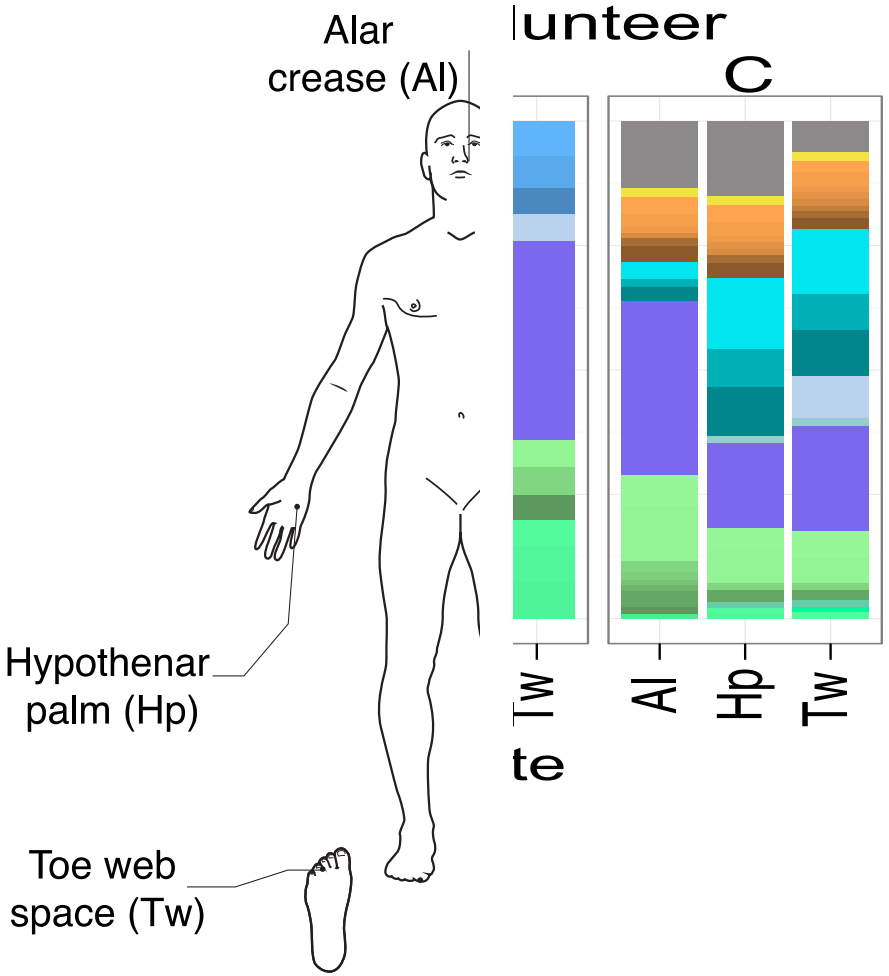


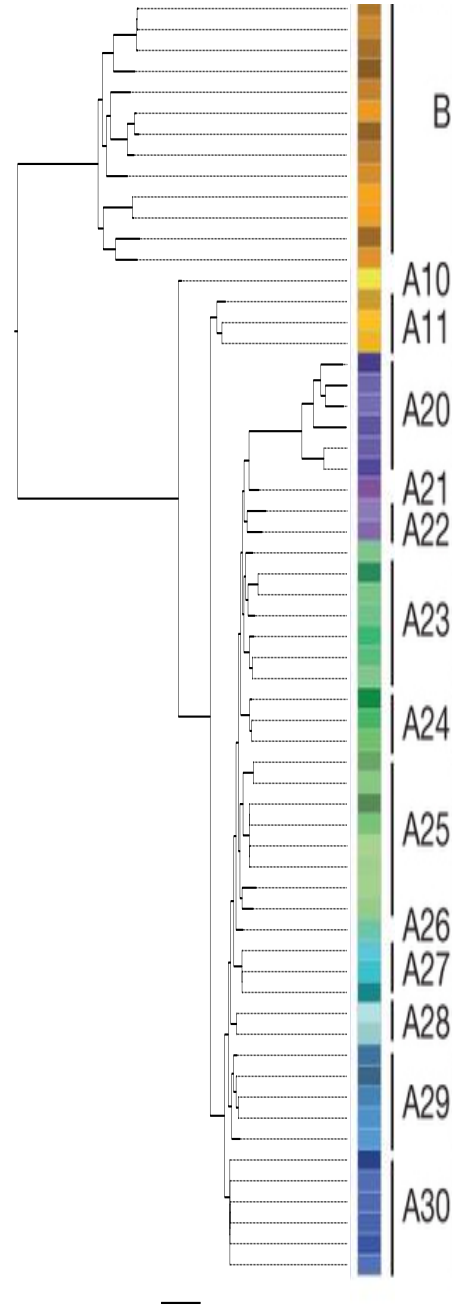
Strain variation in *P. acnes* is driven by host-differences



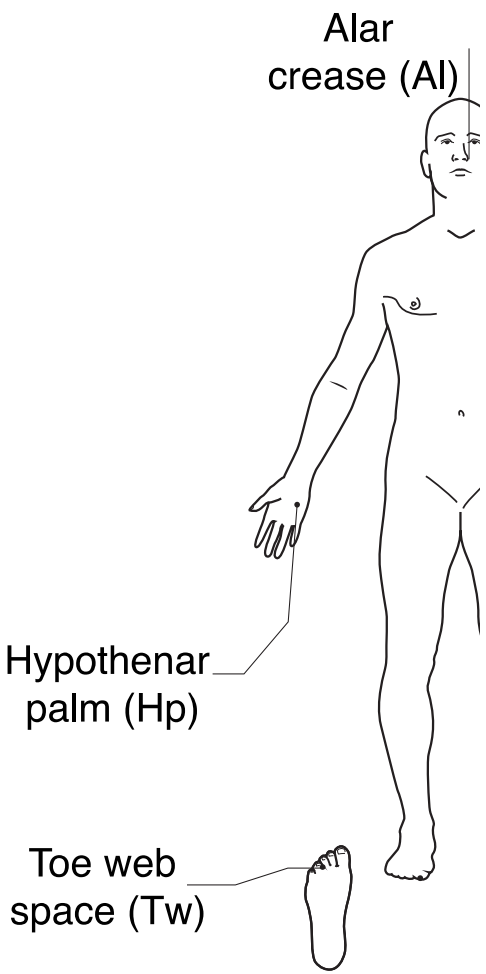


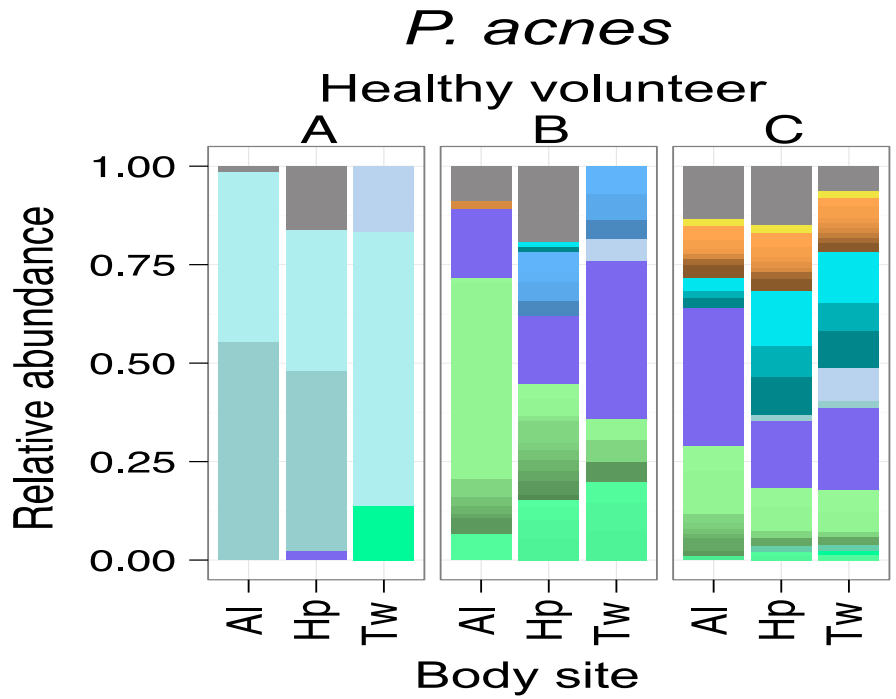
Strain variation in *S. epi* is driven by host-differences and site characteristics

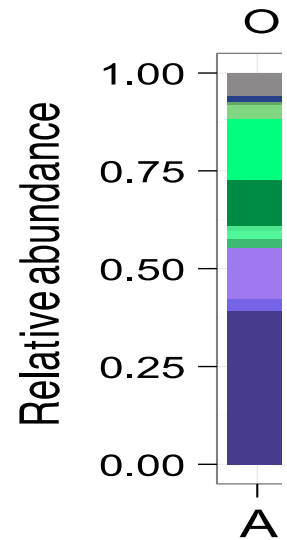


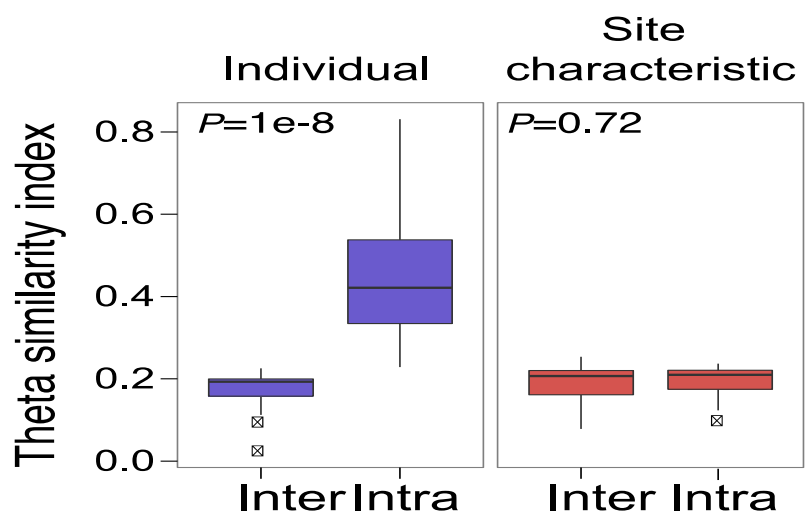


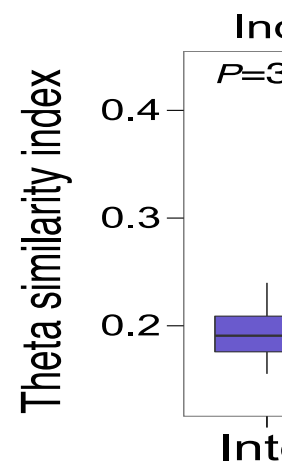
Species can be differentially shaped by host and environment



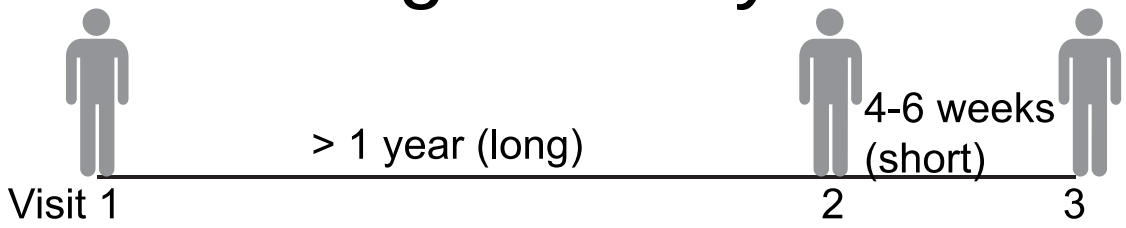


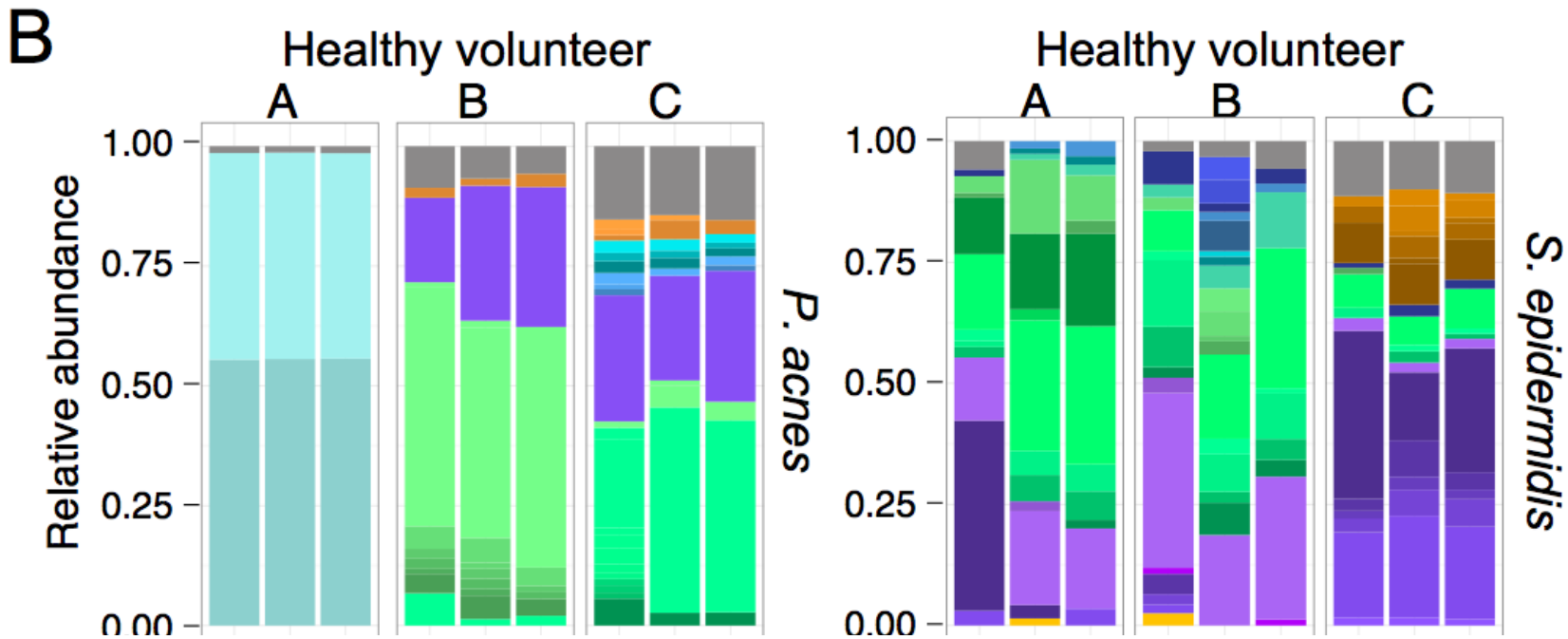




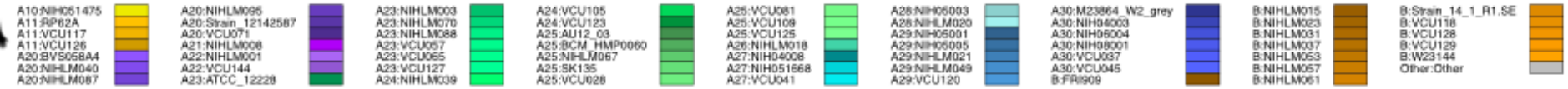


Do individual strain signatures persist longitudinally?

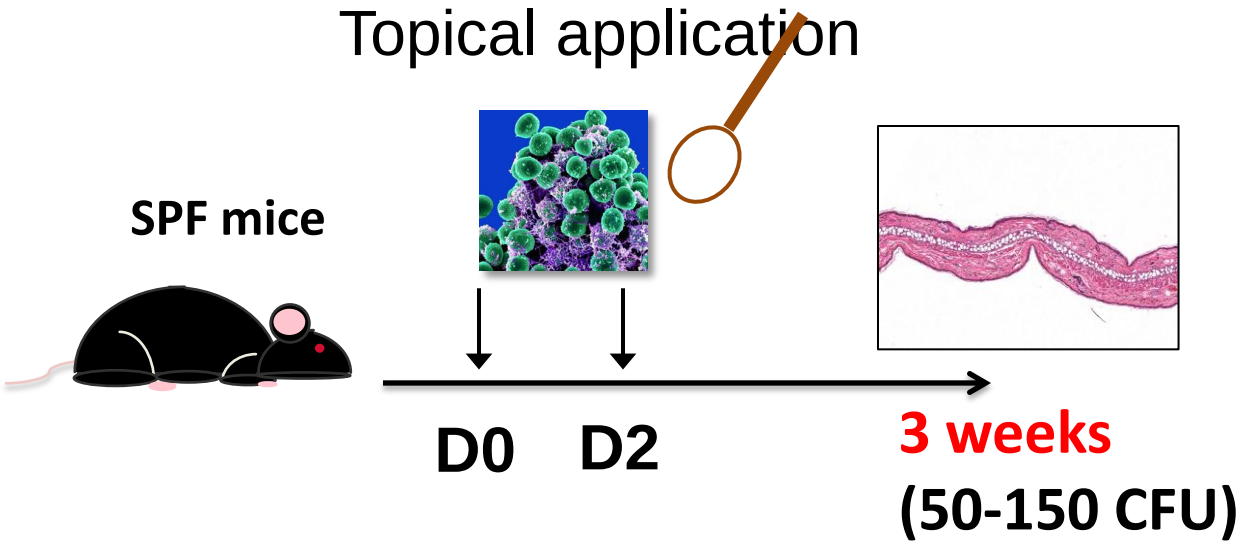


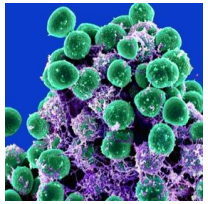


S. epidermidis



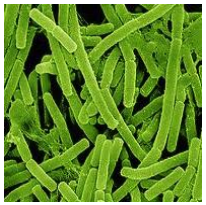
Commensal specific immune modulation ?





Staphylococcus epidermidis

Staphylococcus xylosus

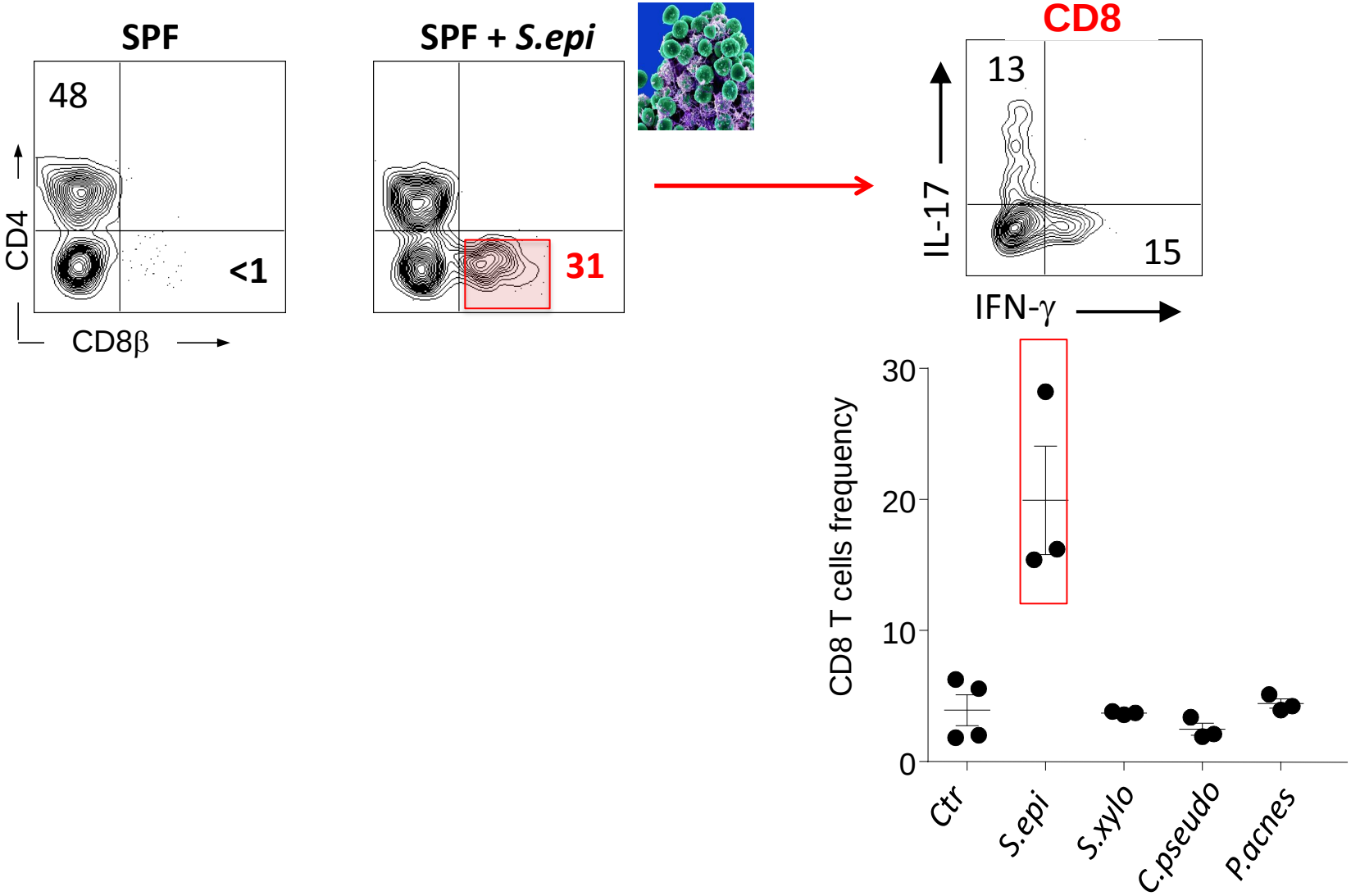


Propionibacterium acnes

Corynebacterium pseudodiphtheriticum

Naik, Boulodoux, ..Segre, Belkaid, Nature, 2015

S.epidermidis increases CD8$^+$ T cell number and activation



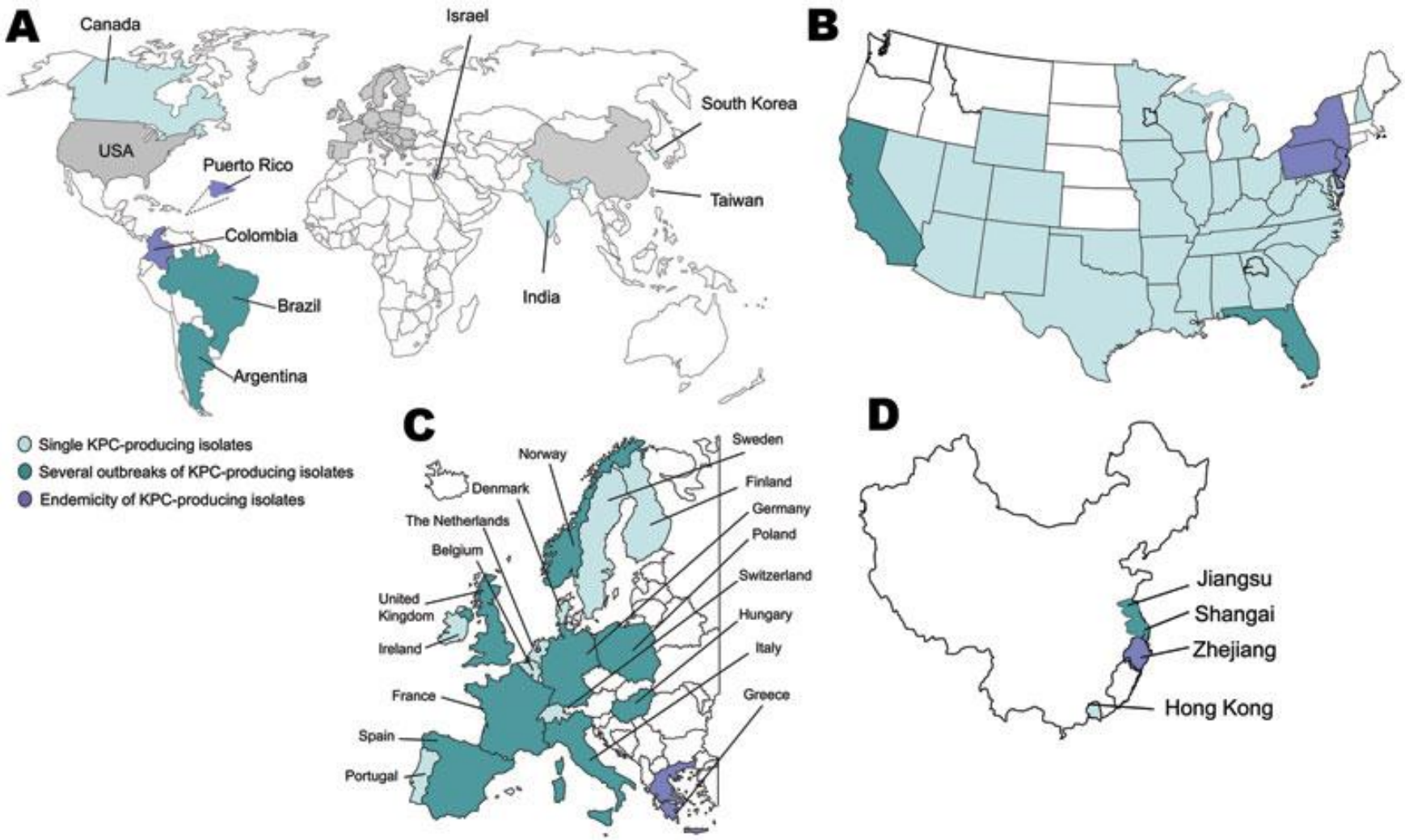
Koch's Postulates: The basis for assigning causality to an infectious disease. 1 microbe => 1 disease

- Microorganism abundant in diseased hosts and absent in healthy hosts.
- Microorganism isolated from diseased host and grown in pure culture.
- Cultured microorganism should cause disease when introduced into a healthy host.
- Microorganism must be re-isolated from diseased experimental host.

Healthcare-associated infections

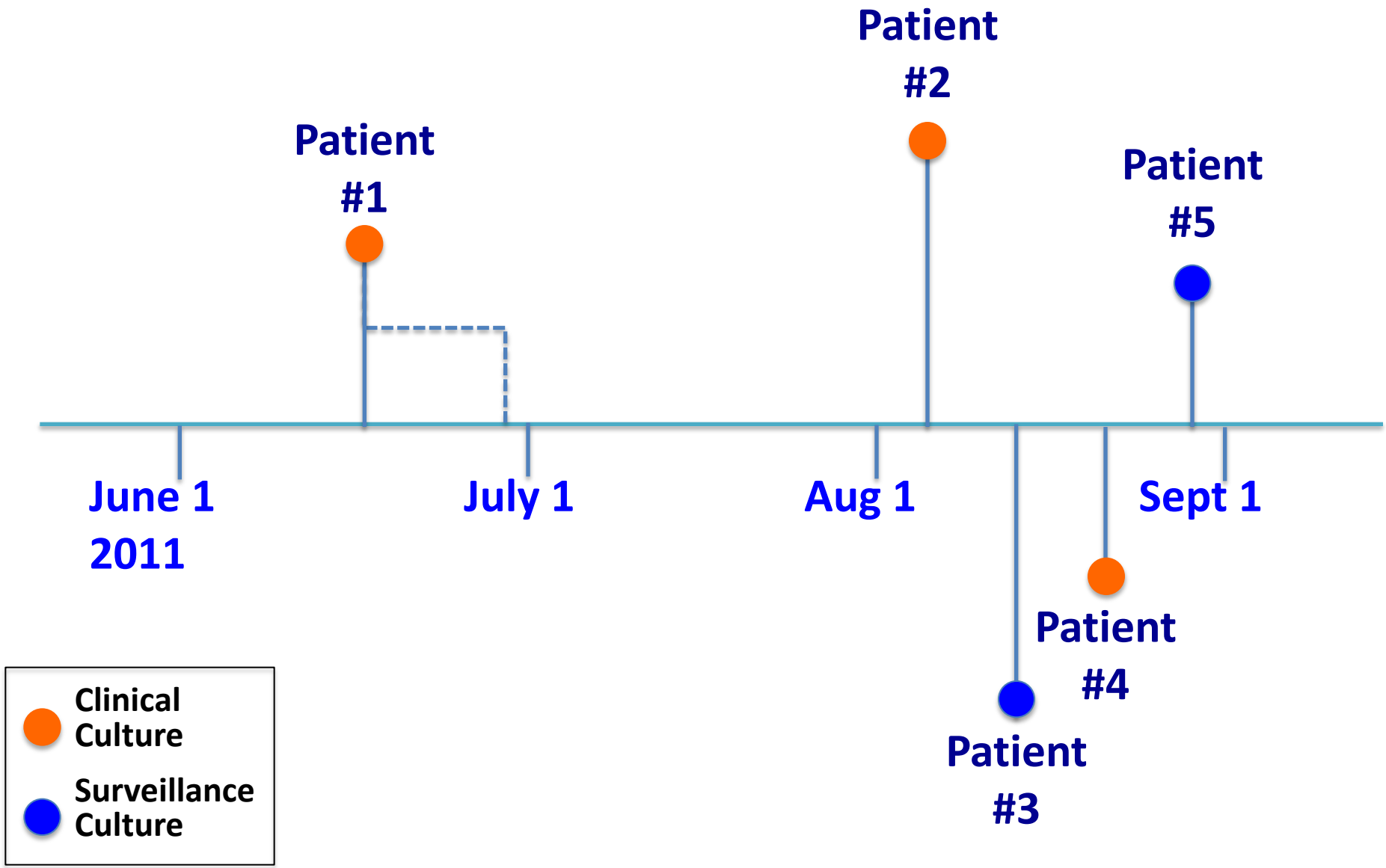
- Human toll
  - Complicate 5% of all hospital admissions
  - Afflict 2 million US people annually
  - 99,000 deaths/annually.
  - Among the 10 leading causes of death in US
  - Increased antibiotic resistance
- Economic toll: $30B/annually in US
- Societal toll: Contributes to public concern about seeking hospital care

Worldwide distribution of KPC-*Klebsiella pneumoniae*

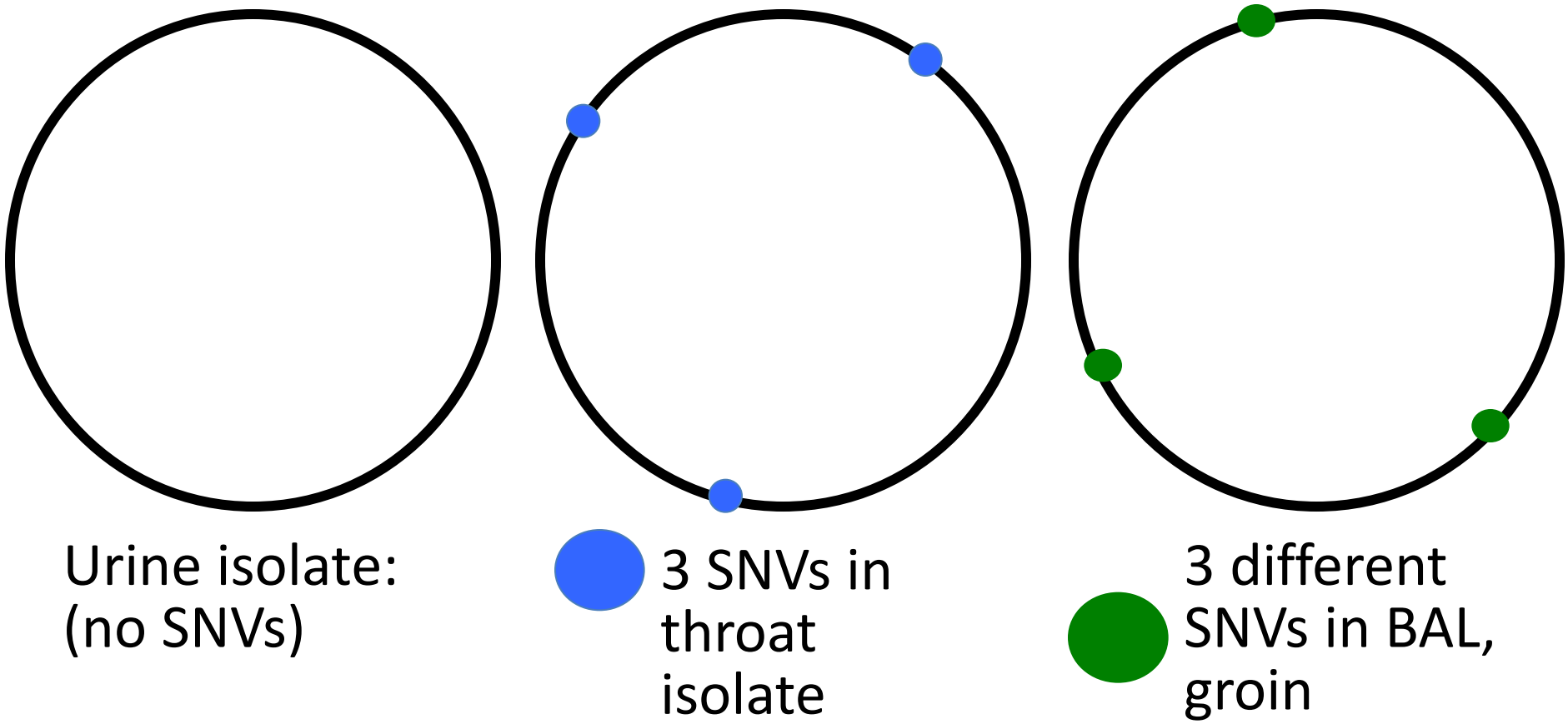


Nordmann et al. EID 2011; 17(10)

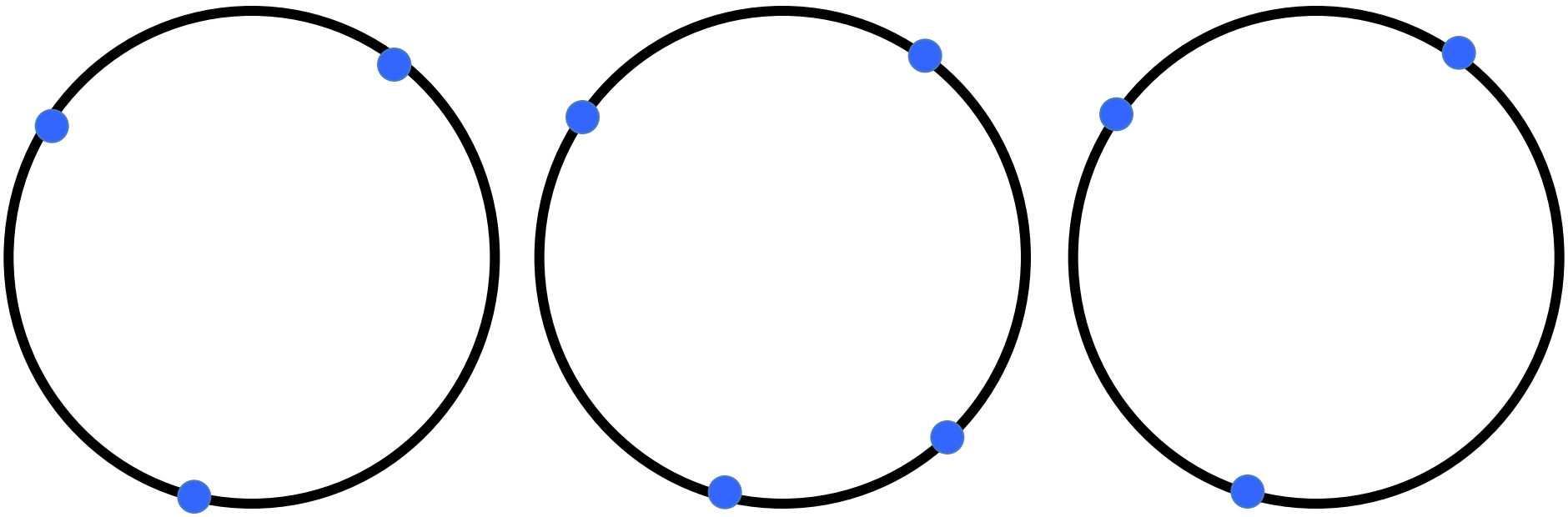
2011 NIH Clinical Center outbreak of carbapenem-resistant *Klebsiella pneumoniae*



Sequence nucleotide variants (SNV) reveal genetic heterogeneity amongst isolates of index patients' urine, throat, lung and groin



Patients 2 and 3 match index patient



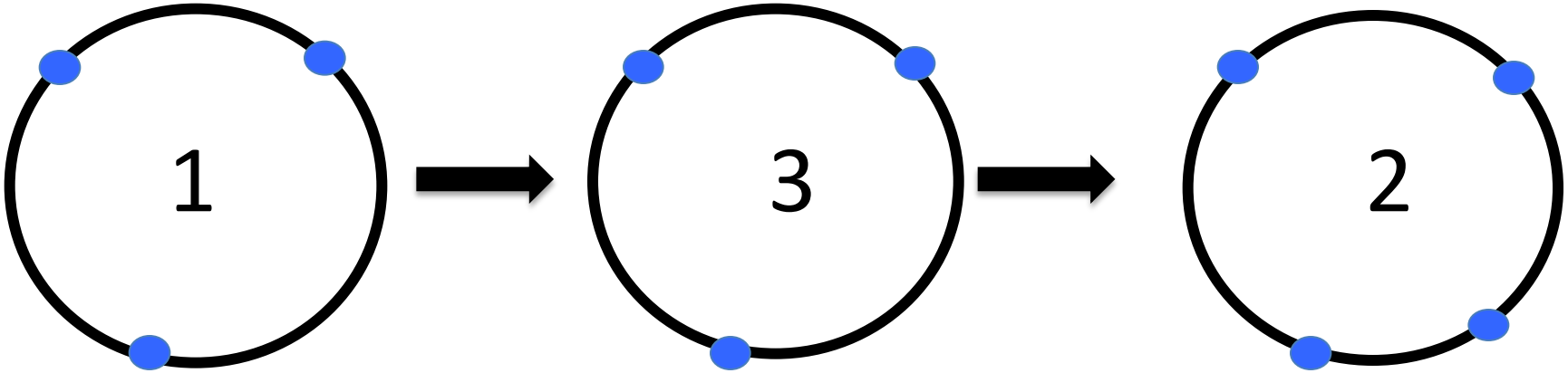
Index patient throat isolate

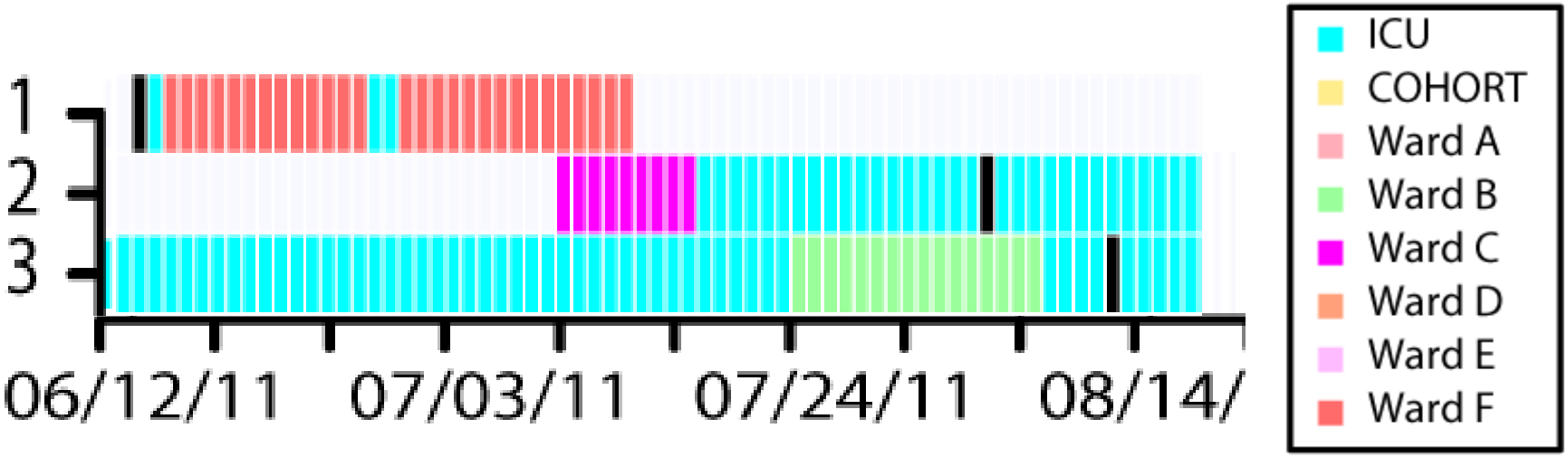
Patient 2 tracheal aspirate isolate

Patient 3 throat isolate

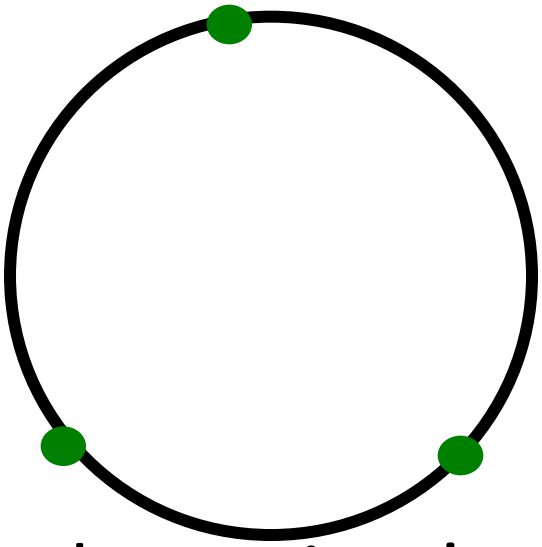
SNV relative to index patients initial isolate (urine)

Chain of transmission: Patient 1➔3➔2
Genetic and epidemiology data agree

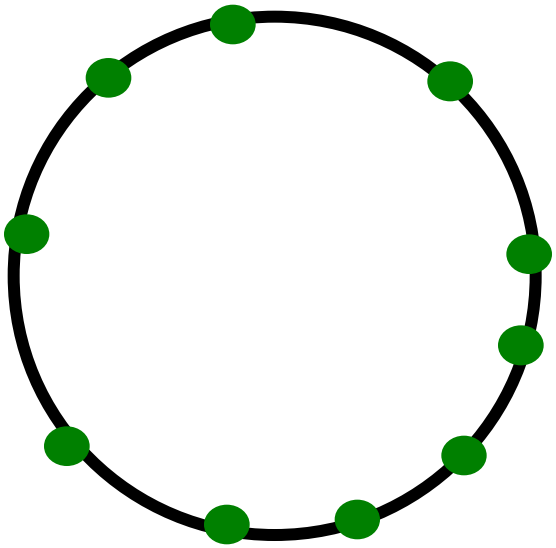




Patient 4 is independent transmission from index patient's lung (BAL) or groin



Index patient's BAL and groin isolates



Patient 4

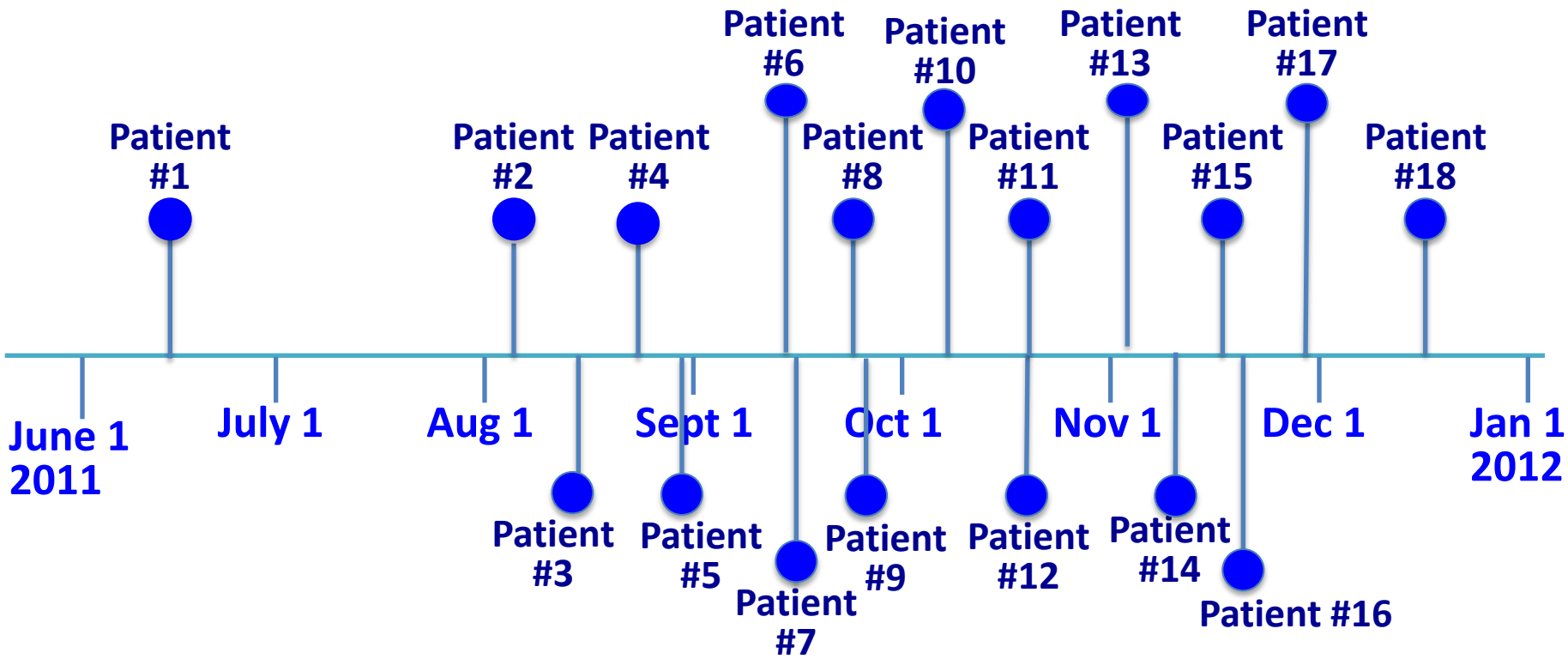
SNV relative to index patients initial isolate (urine)

2011 outbreak at NIH of carbapenem-resistant *Klebsiella pneumoniae*:

18 patients colonized

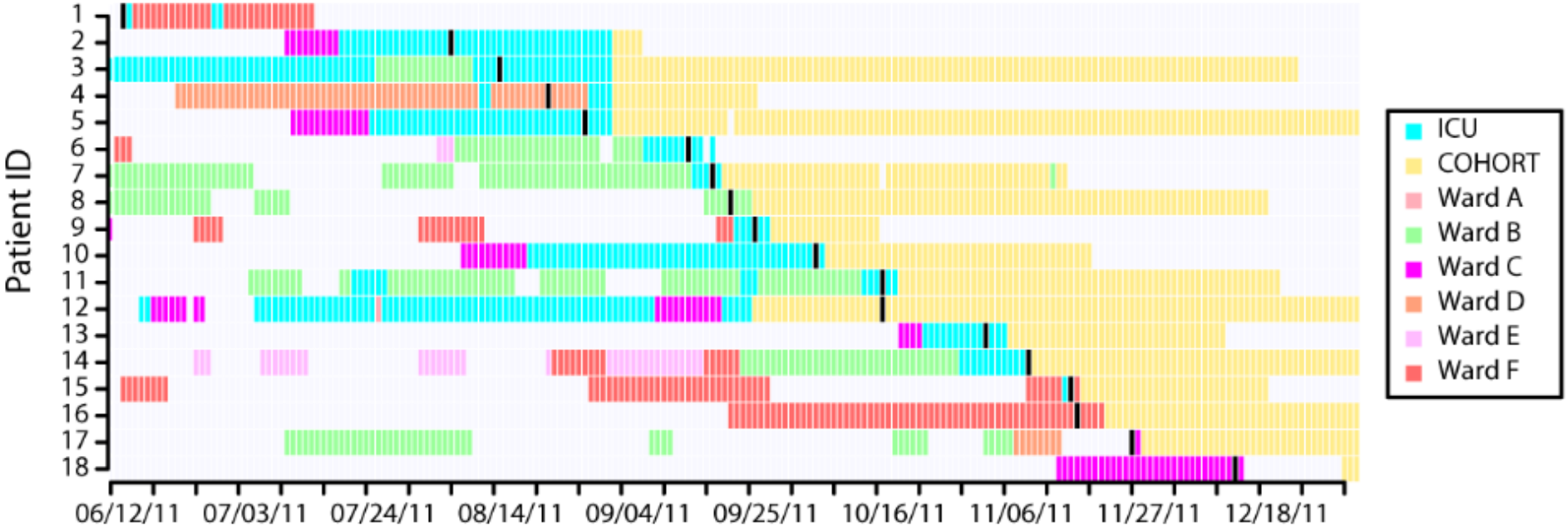
7 deaths attributed to bloodstream infections

6 deaths attributed to underlying disease

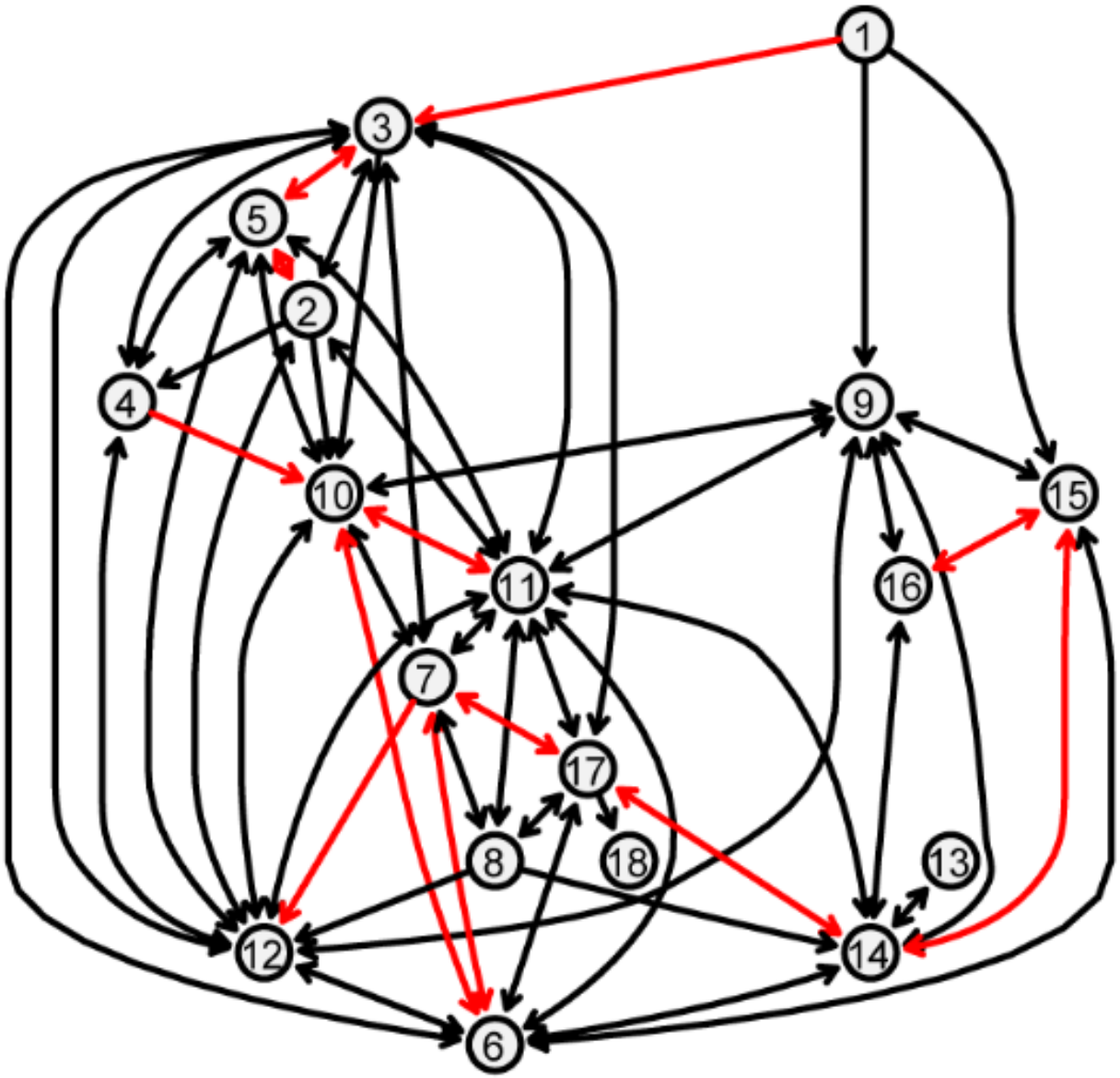


Snitkin,…Palmore, Segre
Science Translational Medicine, 2012

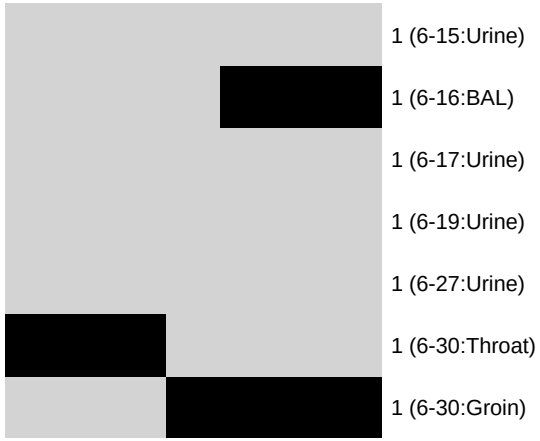
Reconstructing transmission

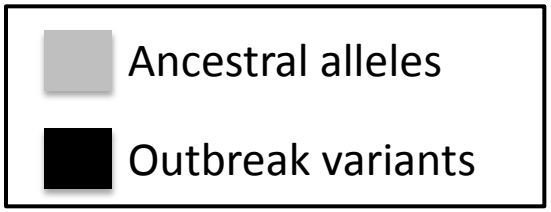


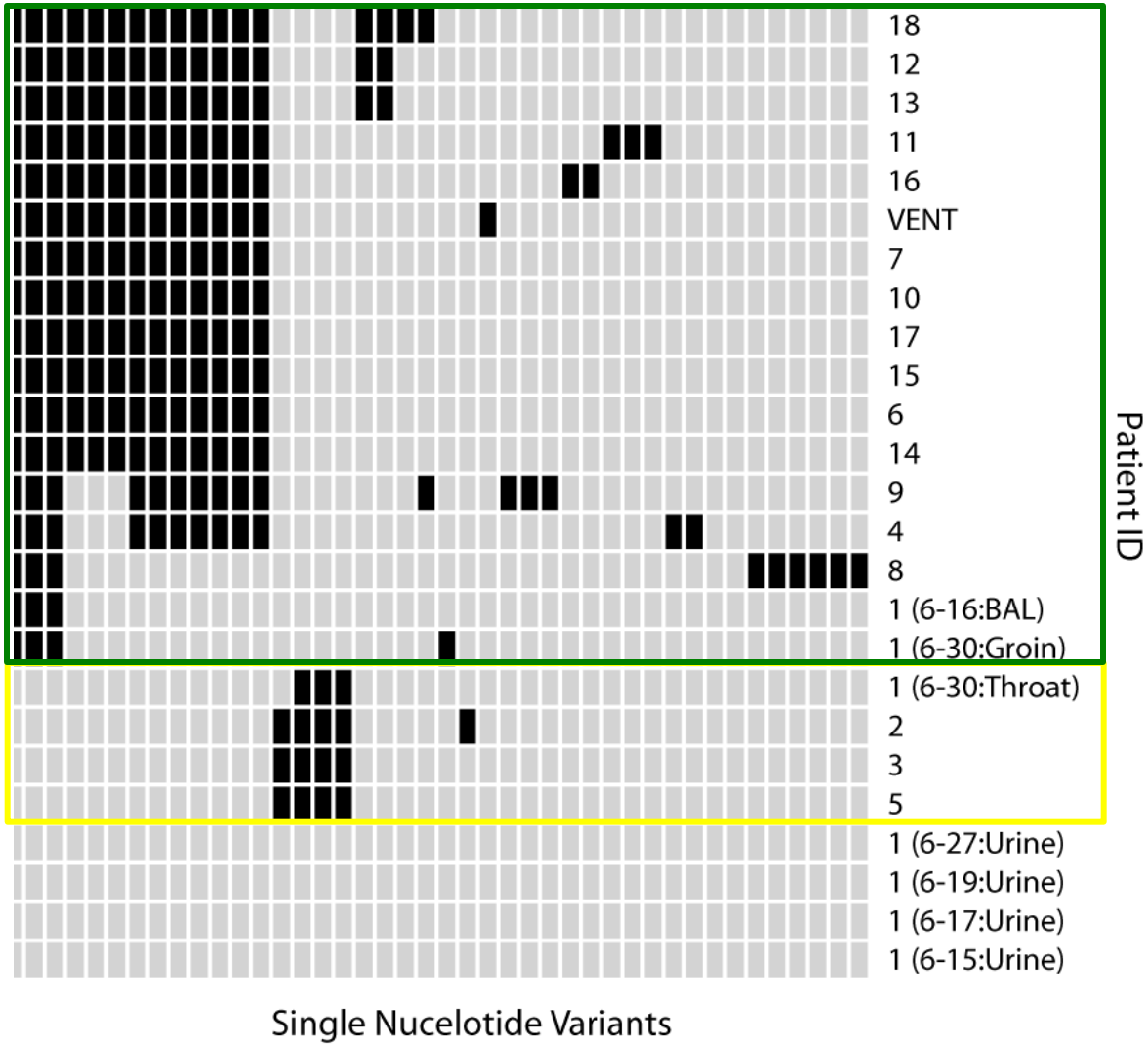
Reconstructing transmission



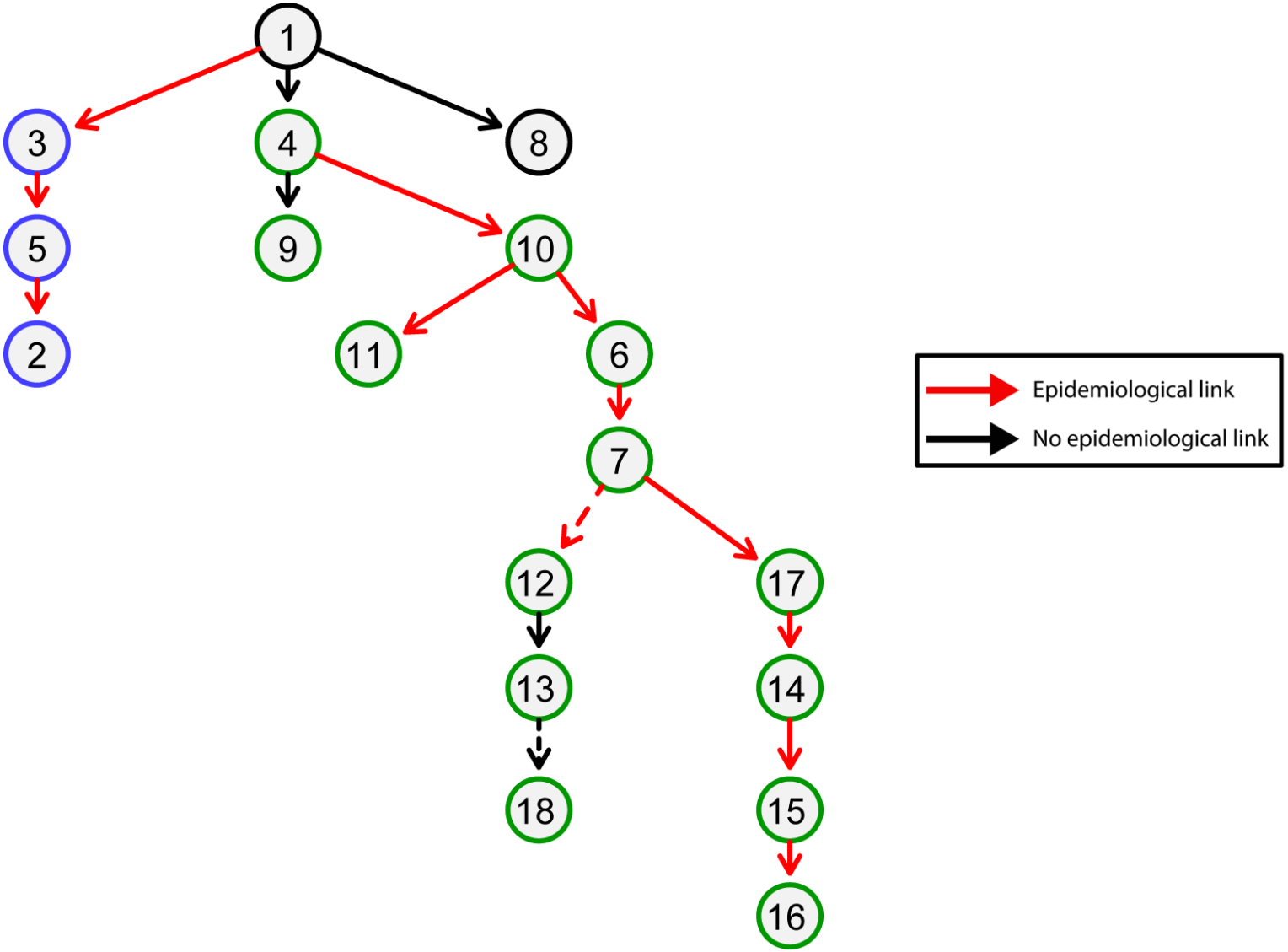
Evidence for transmissions originating from distinct sites on patient 1







Reconstructing transmission



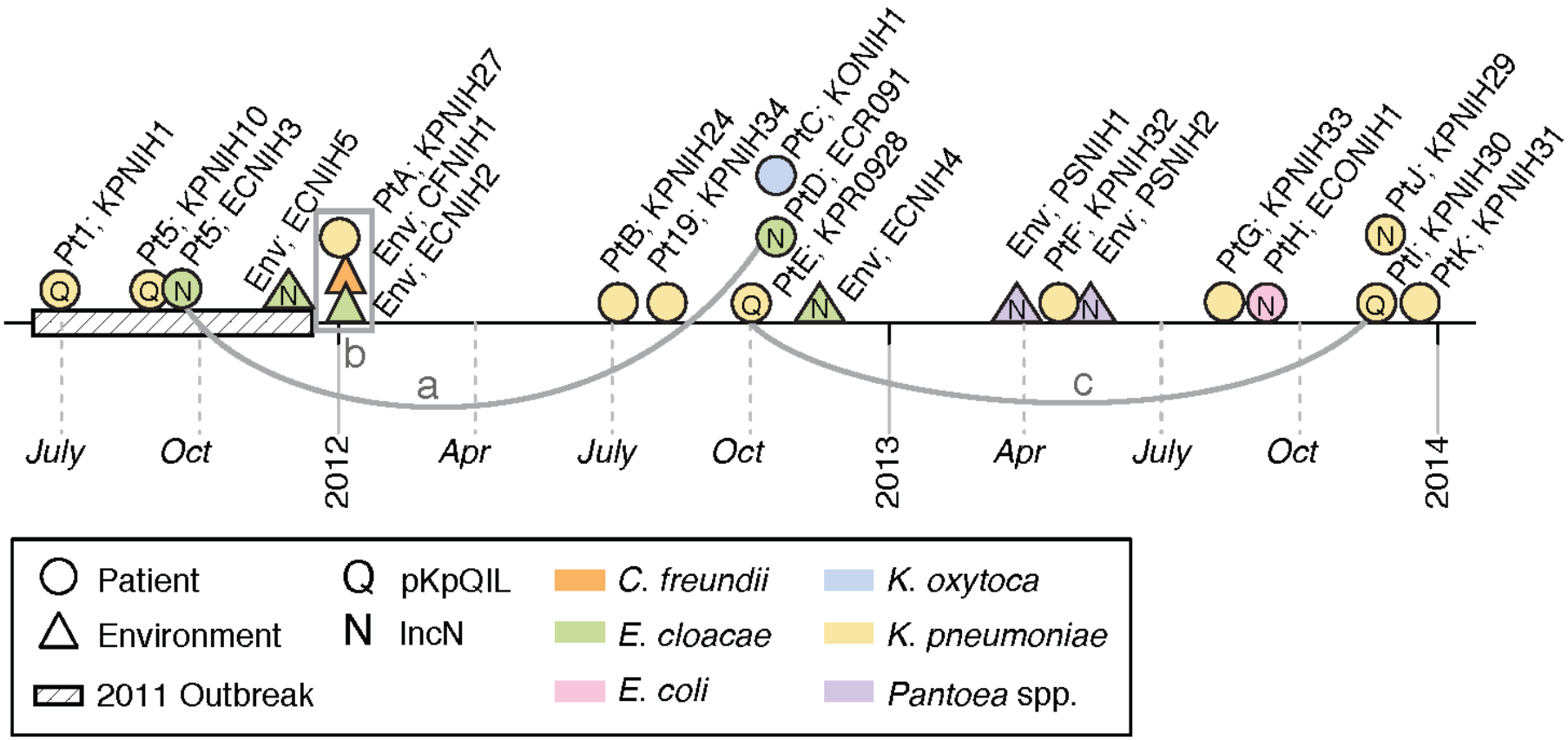
Enhanced hand hygiene practice

Enhanced contact isolation for KPC-colonized patients

(CRE) Carbapenem-resistant Enterobacteriaceae Open Questions?

- What is the rate of CRE+ patients entering our hospital? How many of them do we detect?
- Why are some patients negative on surveillance culture and then convert to positive?
- Are healthcare workers colonized with CRE?
- Are there CRE in the hospital environment? If yes, are these isolates colonizing patients.
- Is there plasmid transfer between Enterobacteriaceae species?

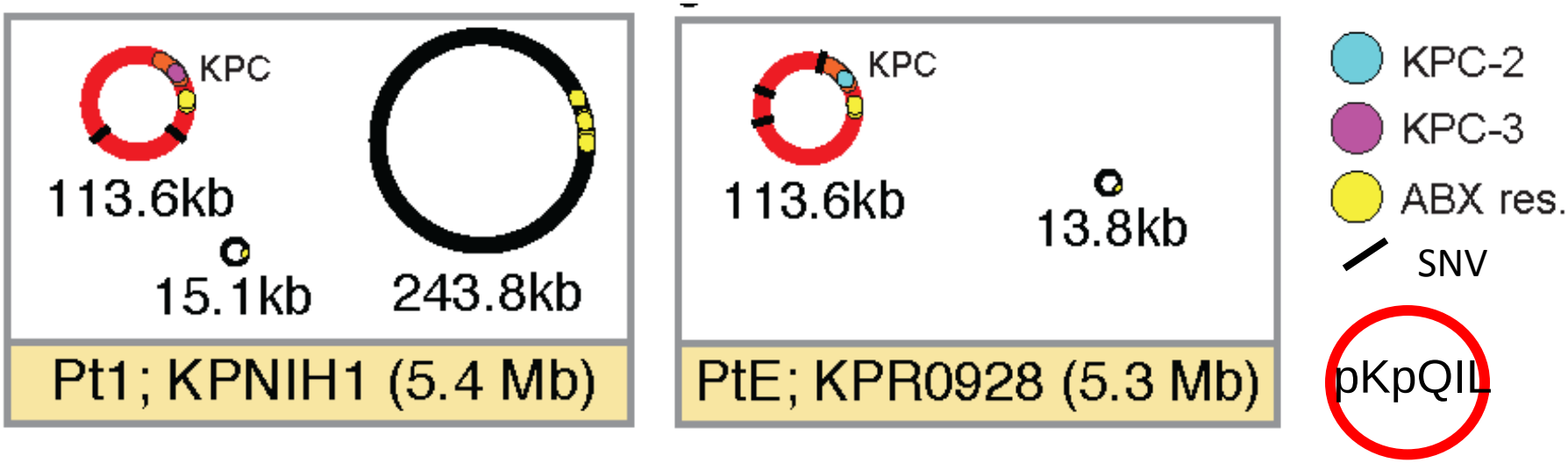
Timeline of KPC+ Enterobacteriaceae organisms from 2011-2013



Conlan,…Frank, Palmore, Segre
Science Translational Medicine, 2014

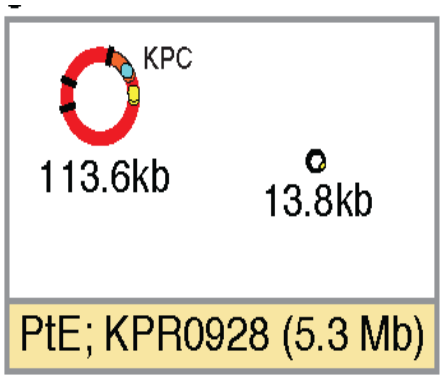
Undetected transmission or dominant clone of KPC+ *Klebsiella pneumoniae?*

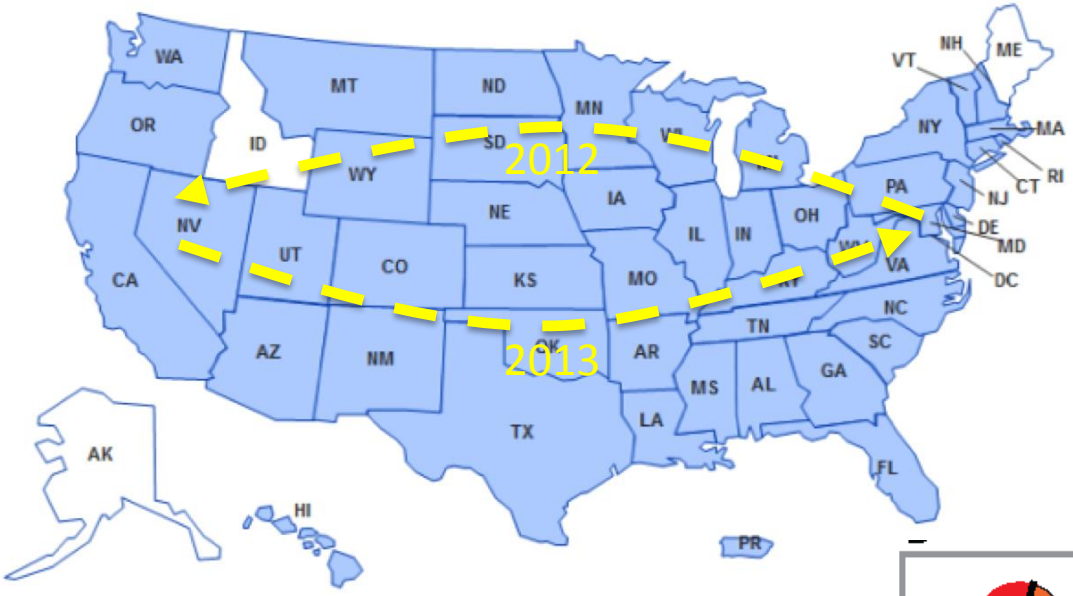
- Patient E treated at NIH CC during the 2011 outbreak
- Multiple negative surveillance cultures at NIH
- Sought medical care at West Coast hospital
- Tested positive for KPC+ *Klebsiella pneumoniae*

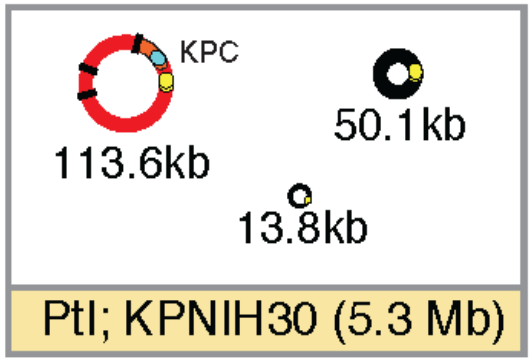


Genome	16797	40547	67342	67348	72669	73758	73831	73887	73893	73901	80525	91699	91723	91777	91798	91801	91807
patient #1	--	C->A	C->A	G->.	T->C	T->C	T->C	.->G	.->G	.->G	--	--	--	--	--	--	--
patient #61	T->C	--	--	--	--	--	--	--	--	--	A->T	C->T	T->C	G->C	T->C	G->C	G->C

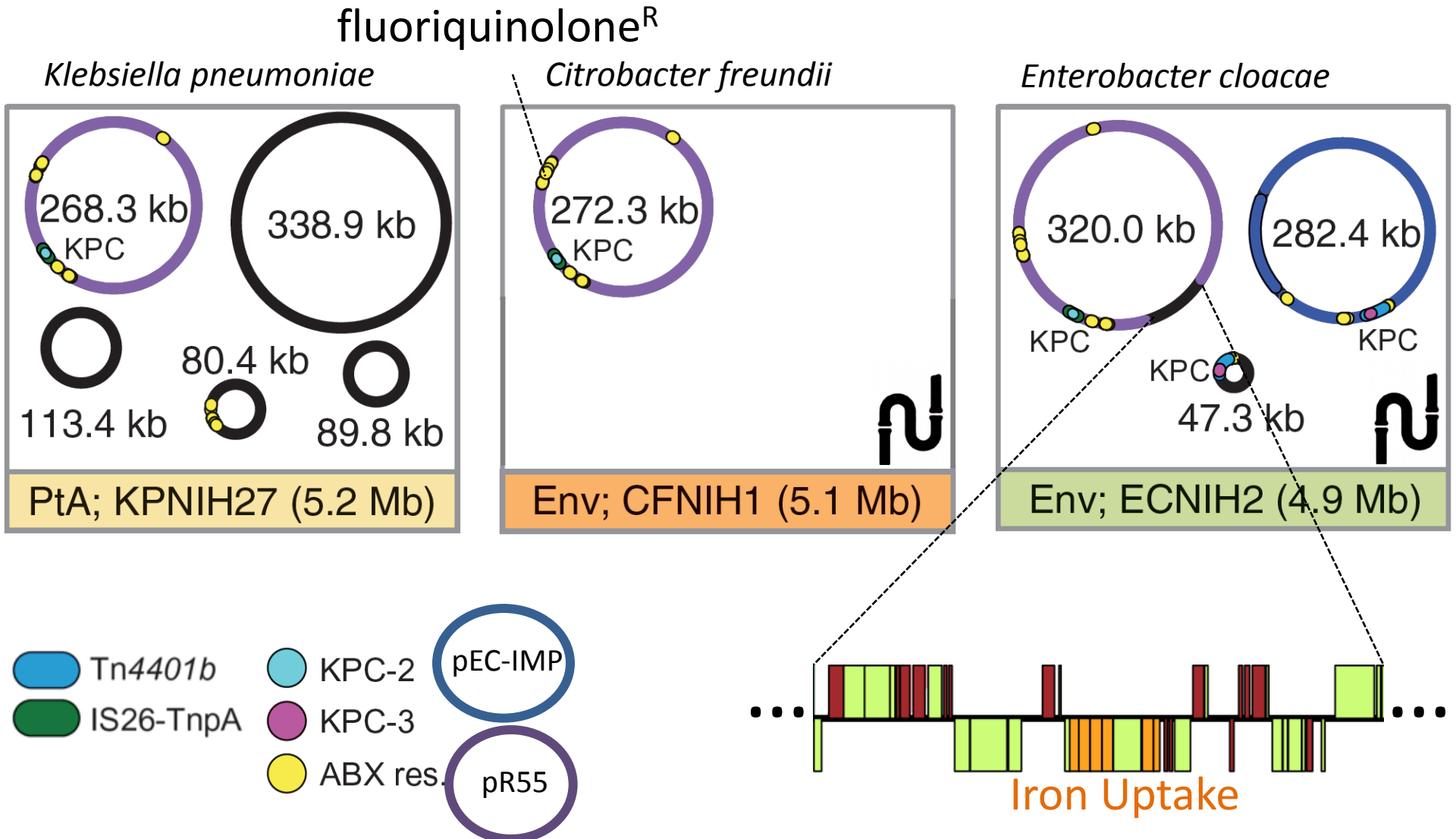
Circulating strains



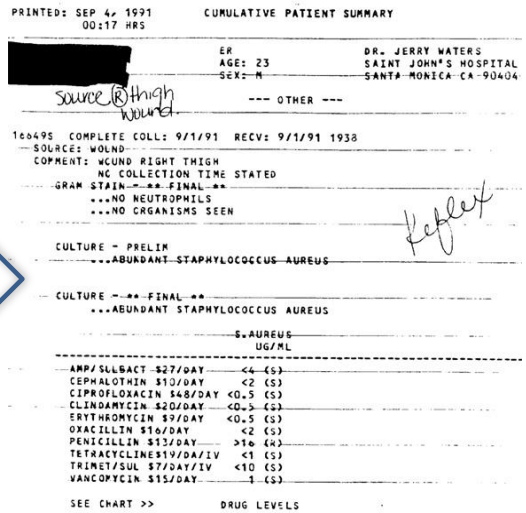




Horizontal gene transfer from patient to environment



In what space will sequencing be a disruptive technology that replaces existing methods?



PRINTED: SEP 4, 1991 00:17 HRS

CUMULATIVE PATIENT SUMMARY

[redacted]
AGE: 23
SEX: M

ER
DR. JERRY WATERS
SAINT JOHN'S HOSPITAL
SANTA MONICA CA 90404

Source (R) thigh wound

--- OTHER ---

16649S COMPLETE COLL: 9/1/91 RECV: 9/1/91 1938
SOURCE: WOUND
COMMENT: WOUND RIGHT THIGH
NO COLLECTION TIME STATED
GRAM STAIN - ** FINAL **
...NO NEUTROPHILS
...NO ORGANISMS SEEN

Reflex

CULTURE - PRELIM
...ABUNDANT STAPHYLOCOCCUS AUREUS

CULTURE - ** FINAL **
...ABUNDANT STAPHYLOCOCCUS AUREUS

	S.AUREUS UG/ML	
AMP/SULBACT $27/DAY	<4	(S)
CEPHALOTHIN $10/DAY	<2	(S)
CIPROFLOXACIN $48/DAY	<0.5	(S)
CLINDAMYCIN $20/DAY	<0.5	(S)
ERYTHROMYCIN $9/DAY	<0.5	(S)
OXACILLIN $16/DAY	<2	(S)
PENICILLIN $13/DAY	>16	(R)
TETRACYCLINE$19/DA/IV	<1	(S)
TRIMET/SUL $7/DAY/IV	<10	(S)
VANCOMYCIN $15/DAY	1	(S)

SEE CHART >> DRUG LEVELS

What is it going to take?

1. Reference database with complete genomes
2. Molecular diagnostics based on genomic sequence
3. Streamlined analysis methods.
4. Standardized clinical report
5. Robust DNA prep for primary specimens; Hospital access to sequencer and data analysis

Acknowledgments

Segre Lab

Julia Oh

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Sara Cassidy

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Clay Deming

Former members of Segre Lab

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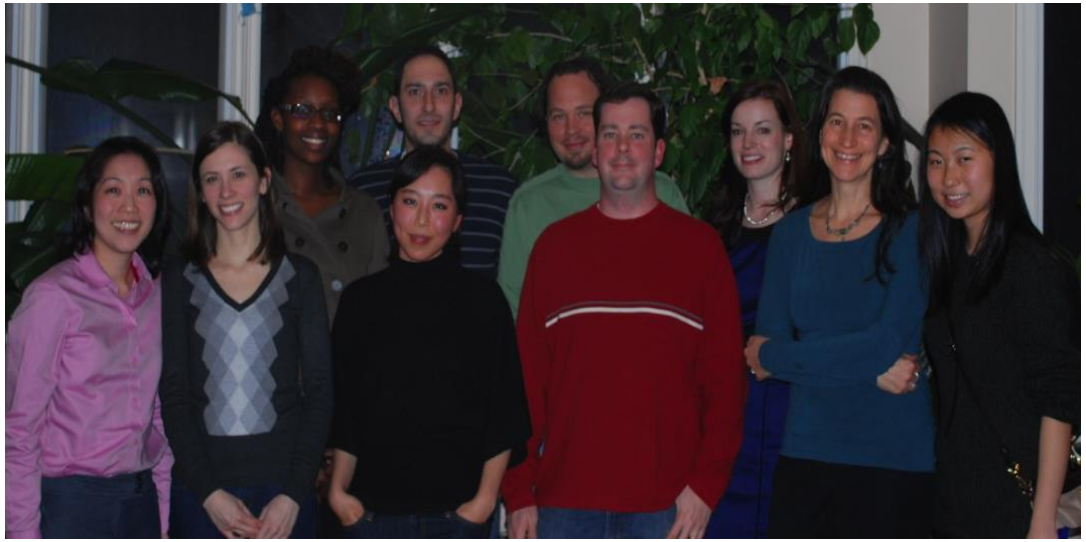
Evan Snitkin, Asst. Prof. Univ of Michigan

Keisha Findley, Health Policy, NHGRI

Joy Yang, graduate student at MIT

Fabulous collaborators who were absolutely essential to these studies

NCI: Heidi Kong

NIAID: Shruti Naik, Nicholas Bouladoux, Yasmine Belkaid