U.S. FOOD & DRUG ADMINISTRATION

Screening biological products for antimicrobial resistant genes using the HIVE AMR pipeline

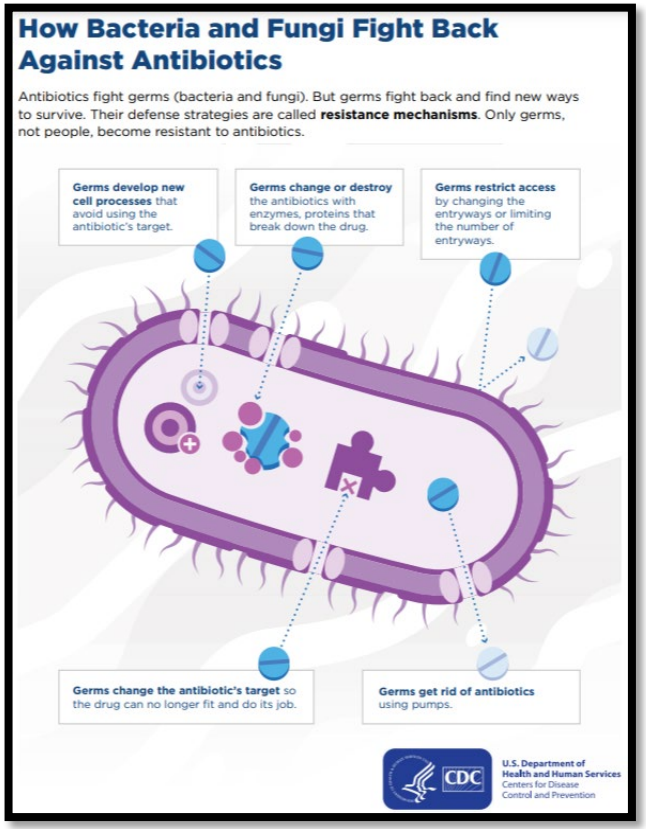
Sean Smith, Ph.D.
Office of Biostatistics and Pharmacovigilance
Center for Biologics Evaluation and Research

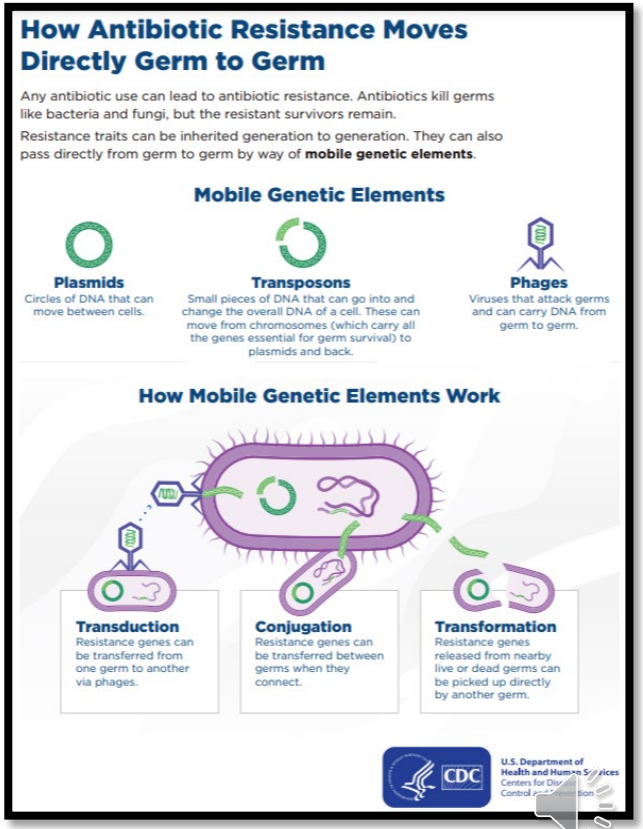
Disclaimer: *The information in this presentation represents the opinions of the speaker and does not necessarily represent FDA's position or policy.*

Introduction

Definition

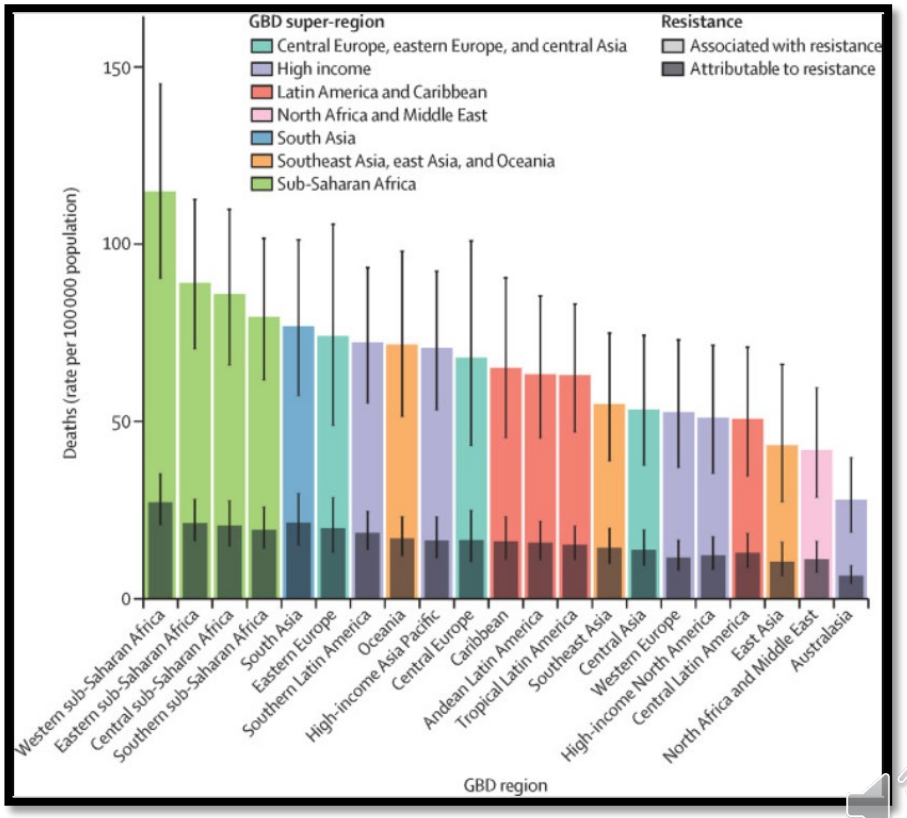
Bacterial antimicrobial resistance (AMR) occurs when changes in bacteria increase resistance of the bacteria to drug treatments





Introduction, cont.

- **World-wide impact**
  - AMR estimates for year 2019
    - 4.95 million AMR-associated deaths[1]
    - 1.27 million AMR-attributable deaths[1]
  - Predicted estimates for 2050
    - 10 million AMR-associated deaths annually (more than currently die from cancer)[2]



[1]Murray, 2021, Lancet. [2]O'Neill, Review on Antimicrobial Resistance.

Objectives

- **FDA Review**
  - Biologic therapeutics (bacteria, phages) reviewed at FDA
    - Screen for safety issues, antibiotic resistance genes

- **Application of technology / scientific computing**
  - Developed **antimicrobial resistance pipeline** through the **High-performance Integrated Virtual Environment (HIVE)**
  - Accepts high-throughput sequencing (HTS) reads as input and provides a **detailed report** on **potential antibiotic resistant** (ABR) genes present in the biologic product

AMR Pipeline

Designed, optimized for different high-throughput sequencing technologies:

Short reads (dark blue)

Illumina, Ion Torrent

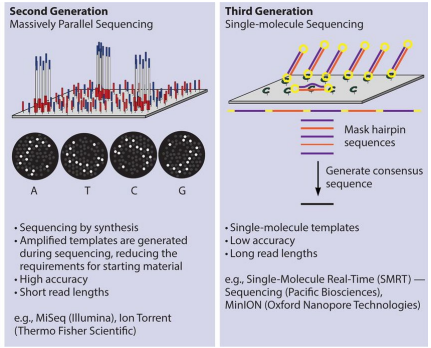
Implemented in HIVE

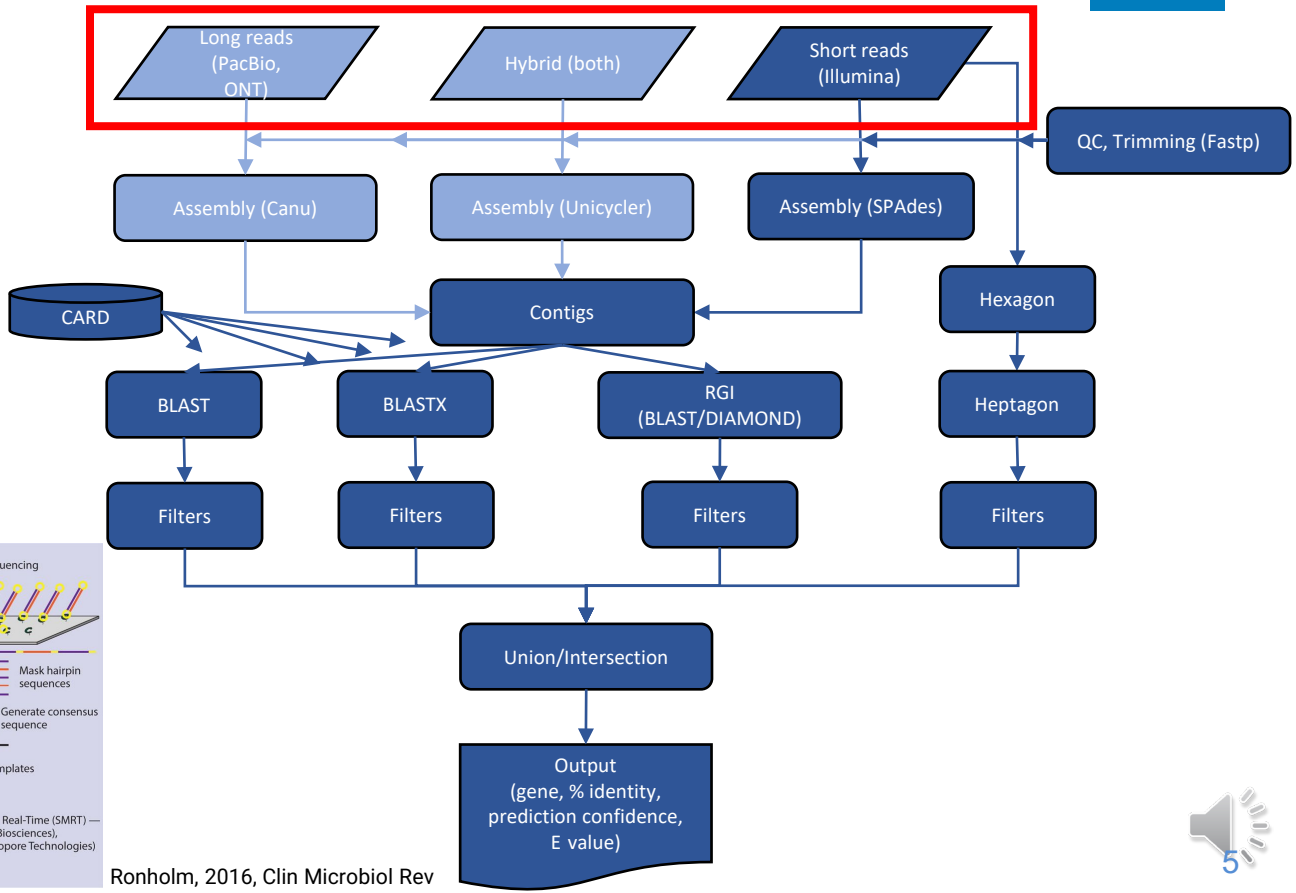
Long reads

PacBio, Oxford Nanopore

Hybrid

Both

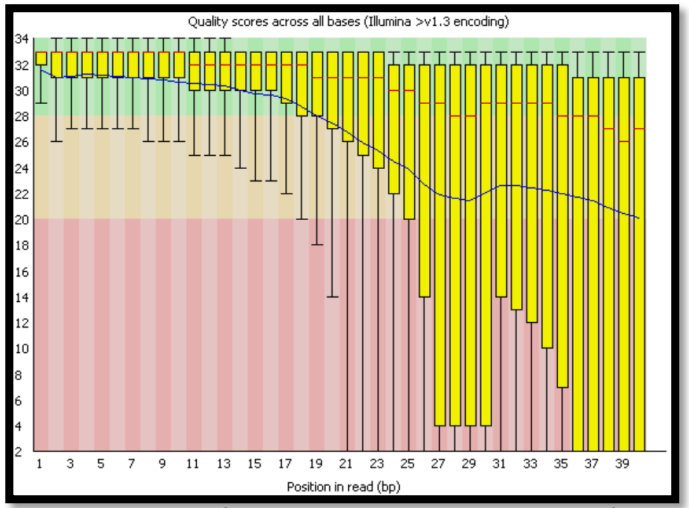




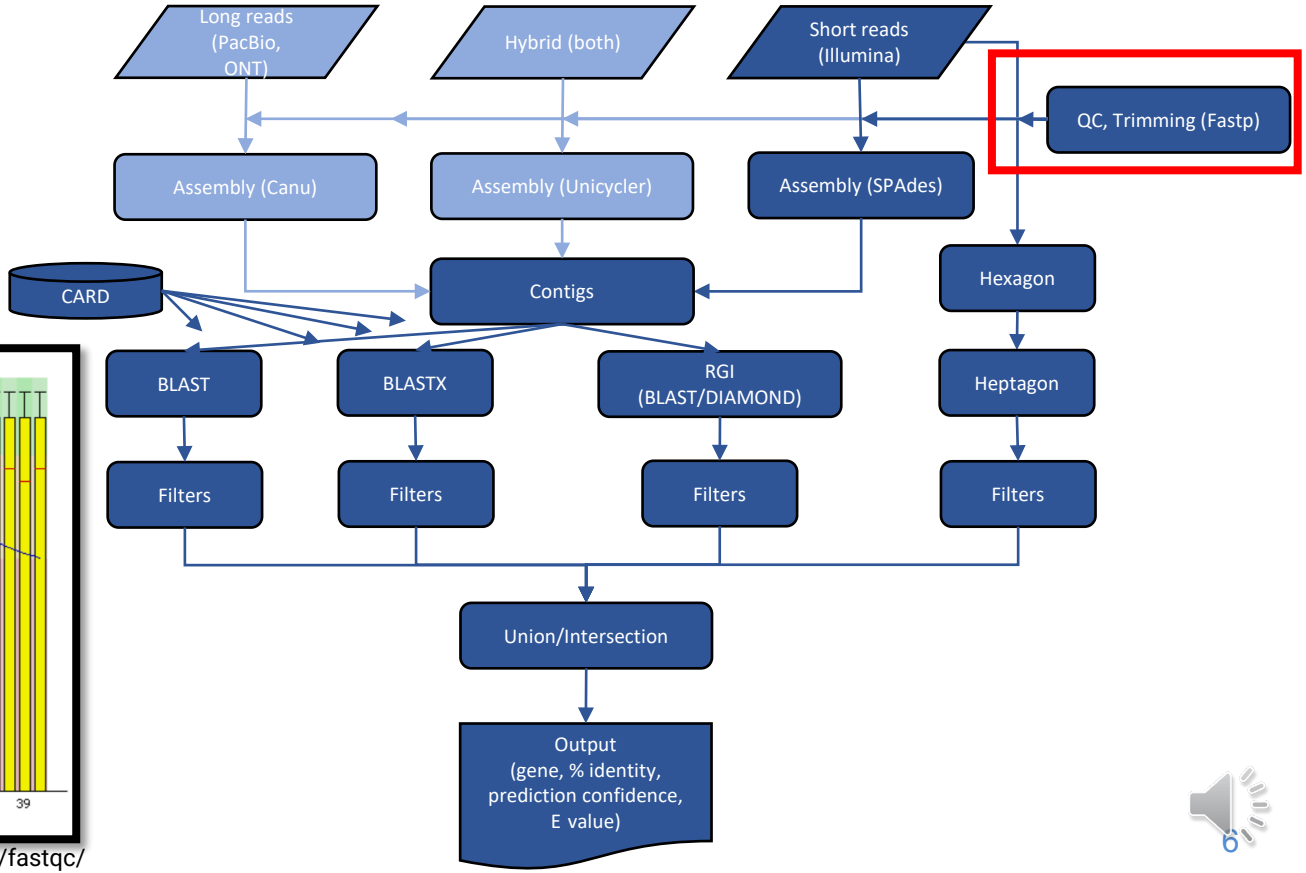
Ronholm, 2016, Clin Microbiol Rev

AMR Pipeline

Quality control and trimming of reads

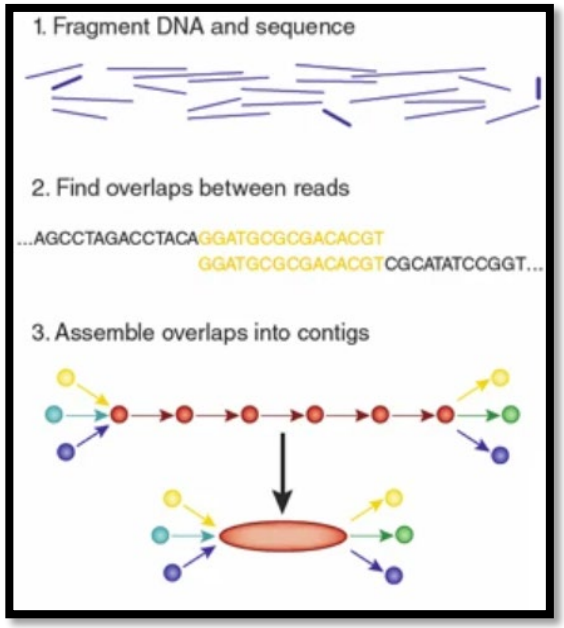


https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

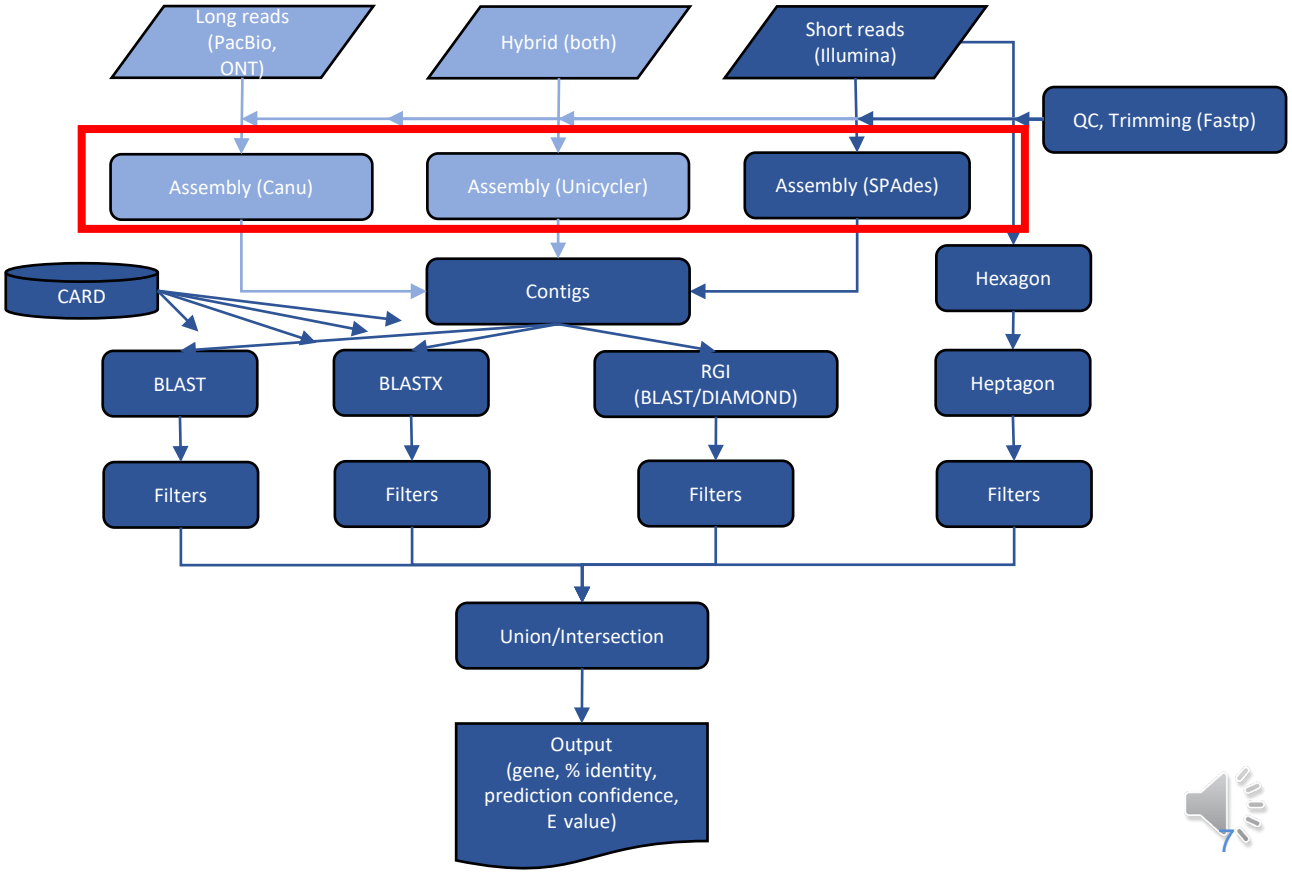


AMR Pipeline

Assemble reads into contigs

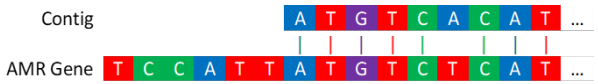


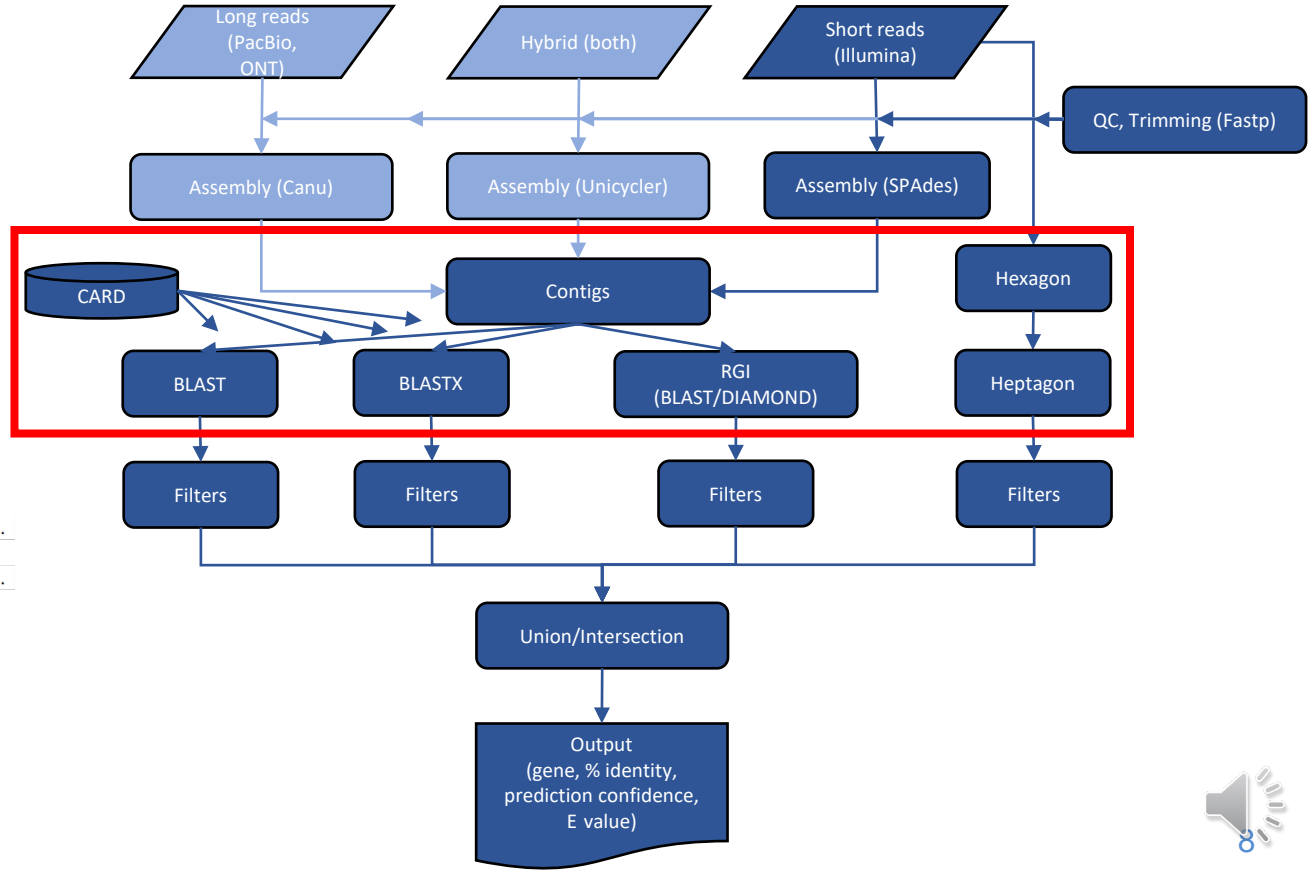
Baker, 2012, Nature Methods



AMR Pipeline

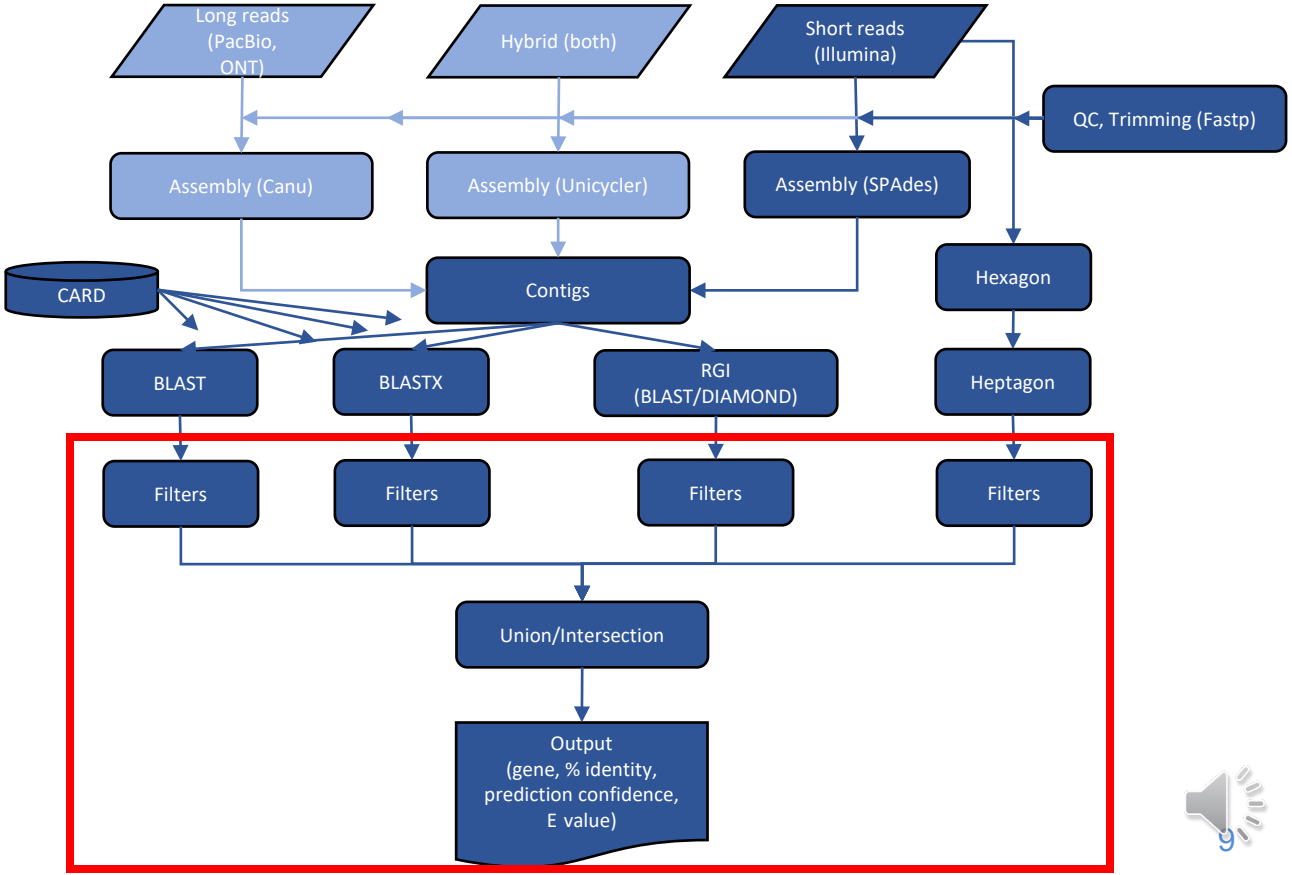
Align contigs to CARD database





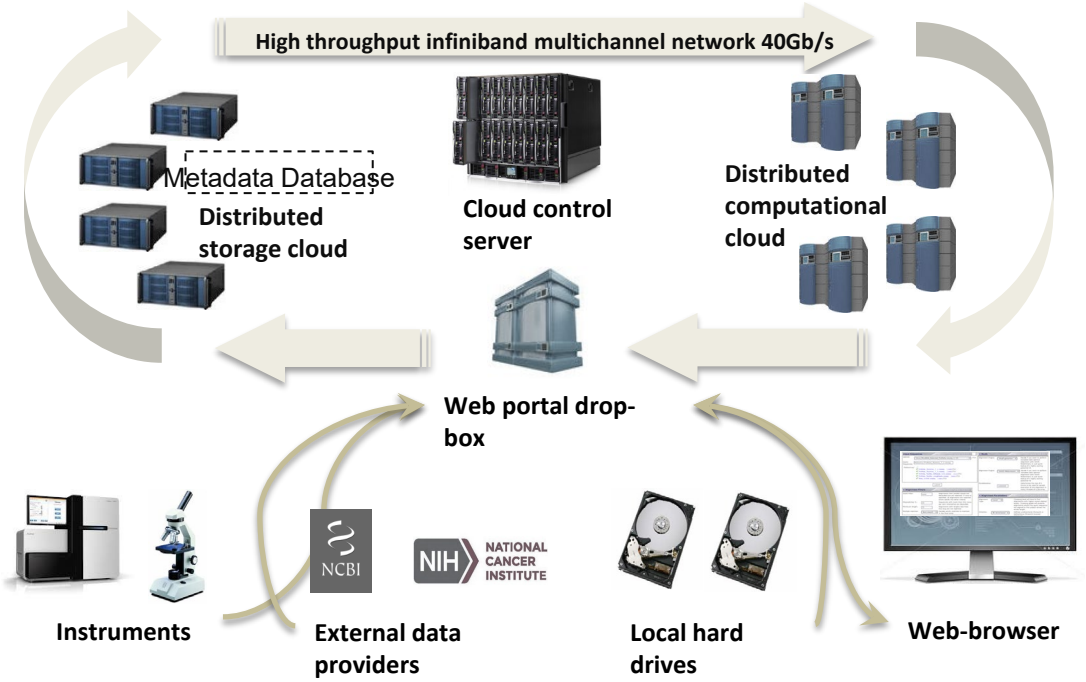
AMR Pipeline

Python script (developed internally) to filter/merge/format results for output to user

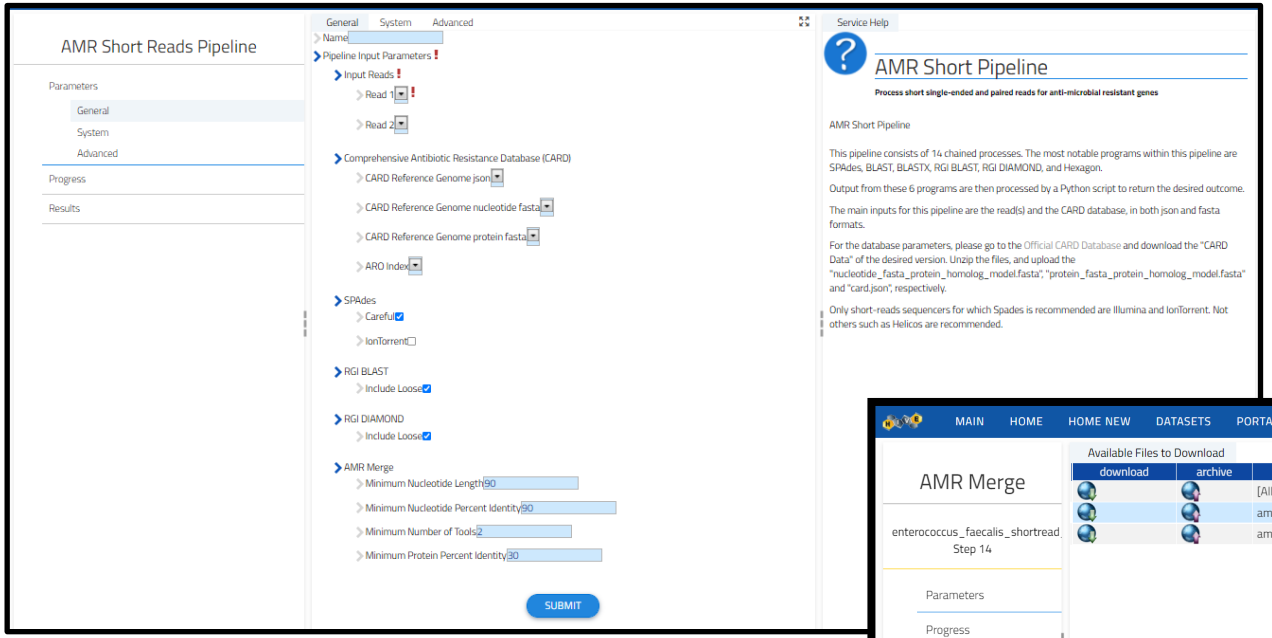


HIVE Platform

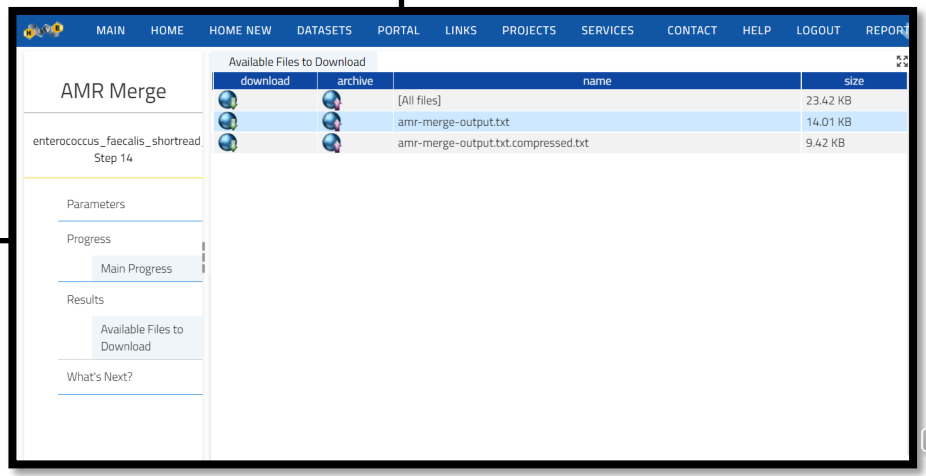
- A **cloud-based environment** that comprises both a storage library of data and a powerful computing capacity.
- Can **consume**, **digest**, **analyze**, **manage**, and **share all** this data.



HIVE Interface



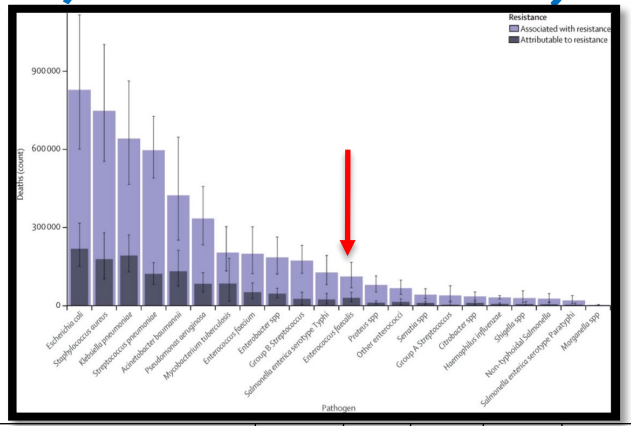
Output



Output (*Staphylococcus aureus*)

ARO	Gene	Protein Product	Protein Accession	Drug Class	Resistance Mechanism	Tool	Length	Pident	Bitscore	Number of Tools Identifying Potential AMR Gene
3000839	arlS	conserved hypothetical protein	ABD30512.1	acridine dye;disinfecting agents and intercalating dyes;fluoroquinolone antibiotic	antibiotic efflux	Blastx	451	100	812	5
3000026	mepA	MepA	AAU95768.1	glycylcycline;tetracycline antibiotic	antibiotic efflux	Blastx	451	100	885	5
3004661	fosB	fosfomycin resistance protein FosB	EHS19134.1	fosfomycin	antibiotic inactivation	Hexagon	420	97.38		5
3005097	blaZ	beta lactamase protein BlaZ	CBZ41939	penam	antibiotic inactivation	Blast	405	99.01		5
3000617	mecA	MecA	AGC51118.1	carbapenem;cephalosporin;cephamycin;monobactam;penam	antibiotic target replacement	Hexagon	2007	99.85		5
3000250	ErmC	macrolide-lincosamide-streptogramin B-resistance protein	AAA98296.1	lincosamide antibiotic;macrolide antibiotic;streptogramin antibiotic	antibiotic target alteration	Hexagon	735	99.86		5
3004572	LmrS	drug resistance transporter, EmrB/QacA subfamily	AAW38464.1	aminoglycoside antibiotic;diaminopyrimidine antibiotic;macrolide antibiotic;oxazolidinone antibiotic;phenicol antibiotic	antibiotic efflux	Blast	1443	99.86		5
3004667	norA	norA	BAA14147.1	fluoroquinolone antibiotic	antibiotic efflux	Hexagon	1167	99.91		5

Output (*Enterococcus faecalis, Blautia obeum*)



Murray, 2021, Lancet

Enterococcus faecalis

ARO	Gene	protein Product	Protein Accession	Drug Class	Resistance Mechanism	Tool	Length	Pident	Bitscore	Number of Tools Identifying Potential AMR Gene
3002875	dfrE		EOD99669.1	diaminopyrimidine antibiotic	antibiotic target replacement	Blastx	164	98.171	335	5
3003948	efrA		WP_104671188.1	fluoroquinolone antibiotic;macrolide antibiotic;rifamycin antibiotic	antibiotic efflux	RGI-Blast		99.83	1168.3	5
3003551	emeA	multidrug efflux pump	BAC11911.1	acridine dye;disinfecting agents and intercalating dyes	antibiotic efflux	Blast	736	99.46		5
3000300	lsaA	Lsa	AAO43110.1	lincosamide antibiotic;macrolide antibiotic;oxazolidinone antibiotic;phenicol antibiotic;pleuromutilin antibiotic;streptogramin antibiotic;tetracycline antibiotic	antibiotic target protection	Blastx	498	98.193	954	5
3000186	tetM	Tet(M)	BAB82500.1	tetracycline antibiotic	antibiotic target protection	Hexagon	1920	96.77		5

Blautia obeum

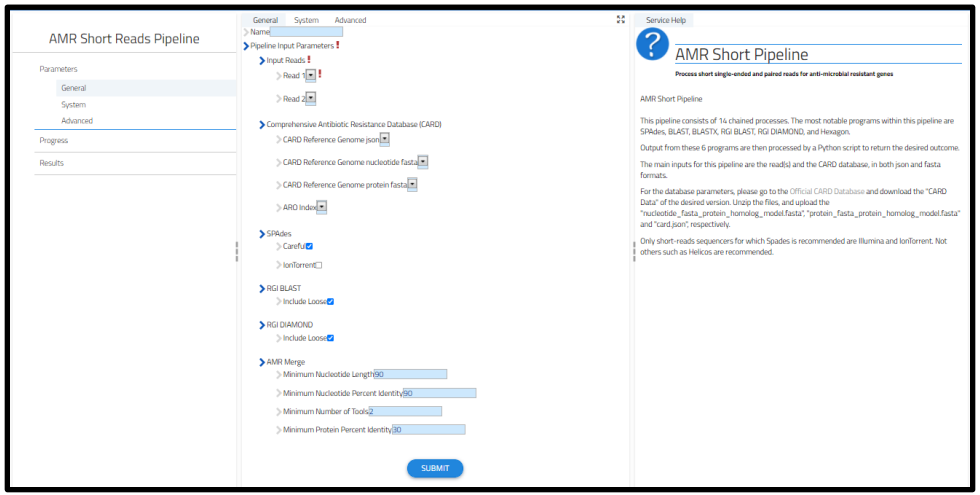
ARO	Gene	protein Product	Protein Accession	Drug Class	Resistance Mechanism	Tool	Length	Pident	Bitscore	Number of Tools Identifying Potential AMR Gene
	none									

Conclusion

- Antibiotic resistance is a serious global health concern
- **AMR pipeline** developed and optimized, hosted on the **HIVE** platform, and available to registered users
- Pipeline identified antibiotic resistant genes in agreement with current knowledge of the bacterial species analyzed
- Pipeline is ready to be utilized by FDA reviewers
  - Applies technology and scientific computing to provide **fast**, **efficient**, **consistent**, and **unbiased** results to validate that products are free from antibacterial resistant genes
  - User friendly, bioinformatics knowledge not needed

Availability

- sciHIVE
  - scihive.fda.gov
- regHIVE



Acknowledgements

Paul Carlson Group (FDA)
Deepika Prasad

HIVE (FDA)
Grace Alterovitz
Arya Eskandarian
Tigran Ghazanchyan
Anton Golikov
Hannah Howell
Konstantinos Karagiannis
Luis Santana-Quintero
Sean Smith

U.S. FOOD & DRUG ADMINISTRATION

Thank you!

Disclaimer: *The information in this presentation represents the opinions of the speaker and does not necessarily represent FDA's position or policy.*


FDA
U.S. FOOD & DRUG
ADMINISTRATION