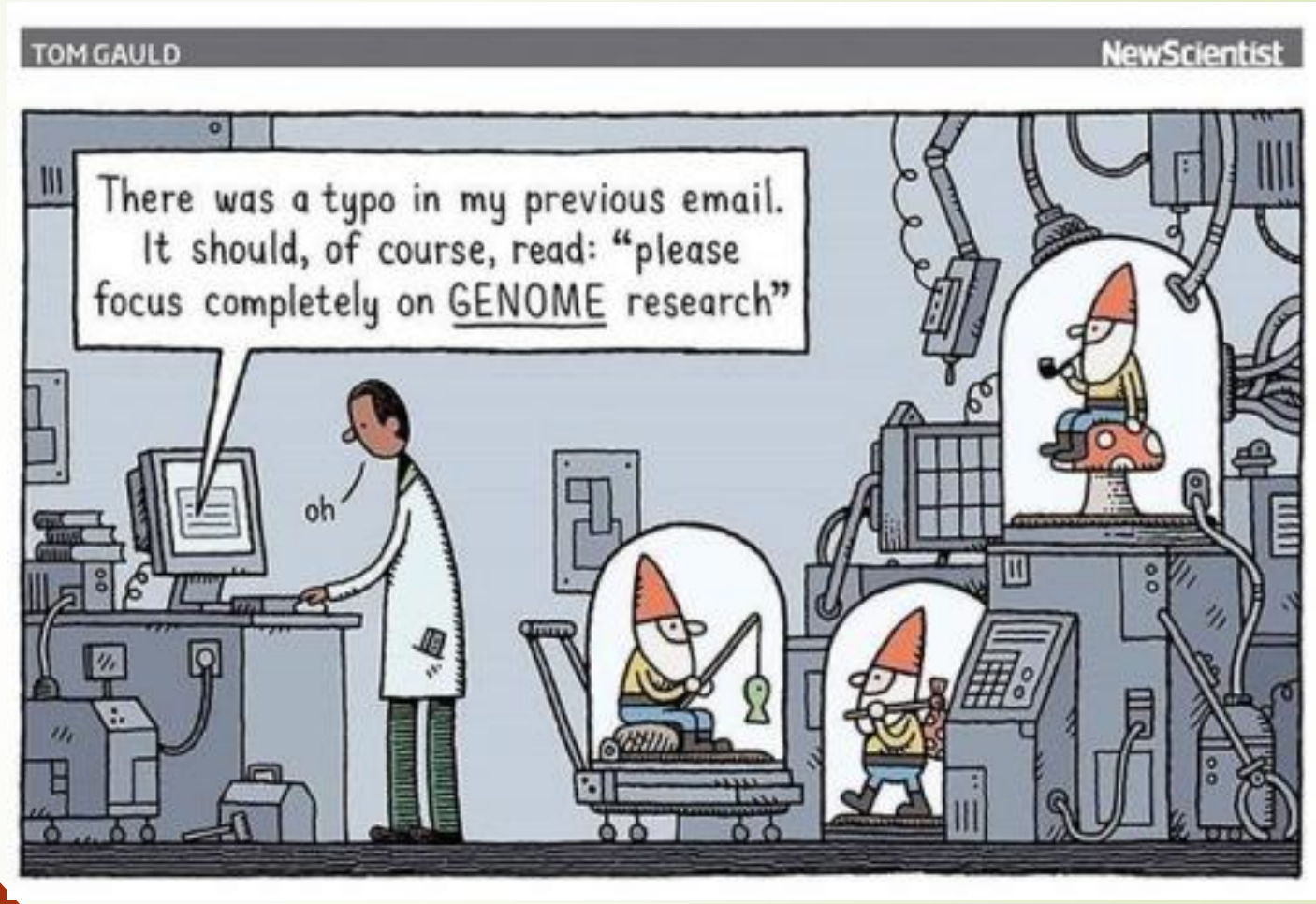
Framework for the Use of Genomic Data to Inform Human Safety Assessment of Microbial Products



Proposed by
Dr. Tim Gant – Public Health England
and Dr. Dan Goldstein – Monsanto.

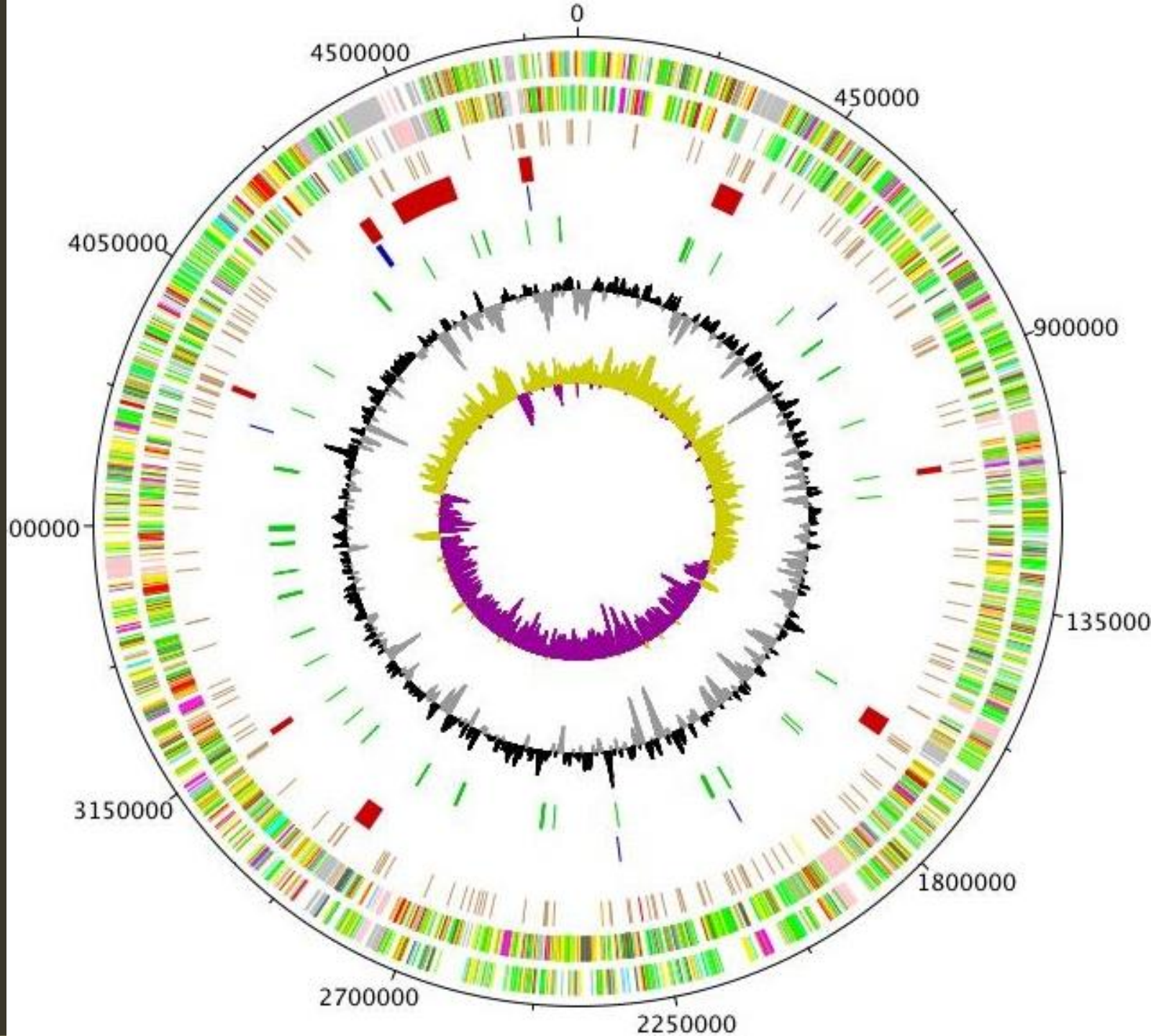
HESI Emerging Issues Proposal to HESI Membership.
June 2018

Imagining the Issue…

You are sitting at your desk one day, and someone brings you a microbial DNA sequence.

QUESTION:

Is it Safe?



Who may be asked to consider safety of microbial sequences?

- Agriculture
  - Crop production
  - Animal agriculture?
- Pharmaceutical
  - Risk of PRODUCTION organisms
  - Assessment of pro-biotics / microbiome modification agents
- Food
  - Food safety
  - Food production organisms (not present in final product)
  - Food-related organisms (present in final product)
- Academic
  - New approaches and methods needed

A problem in many forms…

FOODS

- An existing food organism in your yoghurt product turns out to carry the ampicillin resistance gene (amp, chromosomal)
- A gram positive spore forming rod in sausage turns out to be 99.3% homologous to Clostridium Botulinum. It is toxin negative.

CROPS

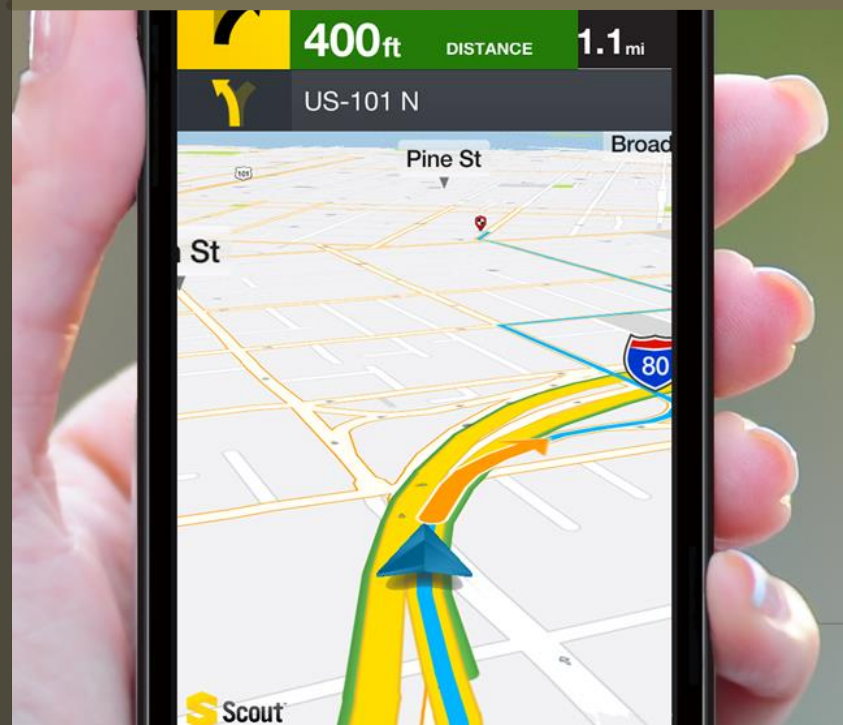
- A pseudomonad proposed for use as a viable seed treatment for crop production turns out to carry hyaluronidase, a known pathogenicity factor for other organisms.
- A gram positive spore forming organism for agricultural nutrient mobilization carries an expressed but non functional deletion mutant of the toxin from *B. Cereus,* with greater than 100 bp remaining homology on both ends of the deletion (allowing for homologous recombination)

DRUGS/THERAPEUTICS

- A probiotic organism carries the full coding sequence for vancomycin resistance, but does not express the gene due to a deletion in the promotor.
- A gram negative enteric for use as a probiotic carries a 200 bp non-expressed central fragment of the KPC gene. It is not likely to become a functional gene recipient, but could re-constitute another organism.

Evaluating Risk via Traditional Approaches-
A Well Defined Route

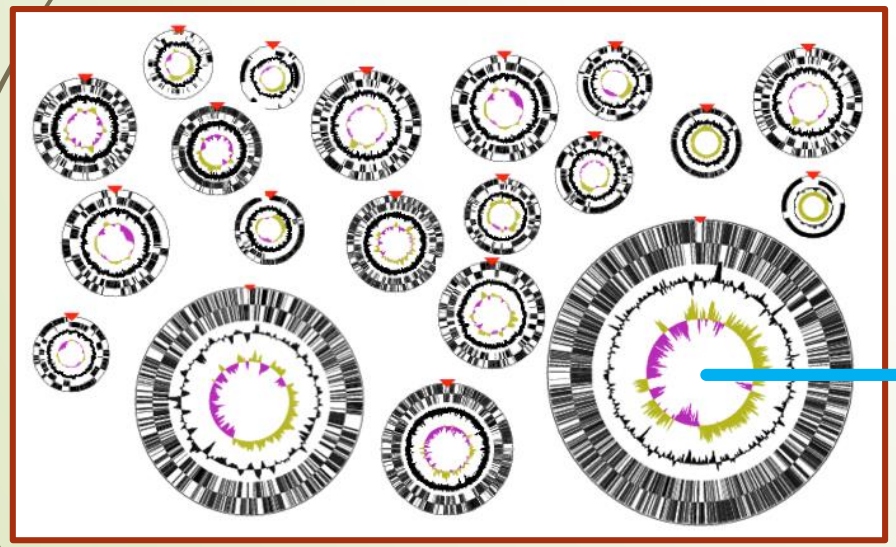
- Declared Genus/Species
  - Fermentation / phenotypic testing in culture.
  - Possibly 16s Ribosomal RNA sequence
    - Likely to match a pathogen because that is what we have sequenced.
- Temperature/growth requirements
- +/- Pathogenicity testing (animal/plant)
- “Exotic” vs not for APHIS/Plant Pathogens
- Probably misses the majority of microbial organisms



Genomic Approaches: *Hic Sunt Dracones*

(There be dragons!!)

- **Genomic Approach: Unknown territory….**
  - **Routinely collected for characterizing micro-organisms.**
  - **Undoubtedly will work in Parallel with traditional approaches for some time.**
  - **Genus/species rapidly re-arranging, disappearing, dividing, merging, and emerging.**
  - **Metagenomic identification of most or all organisms…. BUT**
  - **Genus/Species and "Exotic" status…. Down the tubes….**



La Carta Marina de Olaus Magnus, 1539

Does the Genus/Species binomial model fit in the context of an effectively continuous spectrum of microbial life??

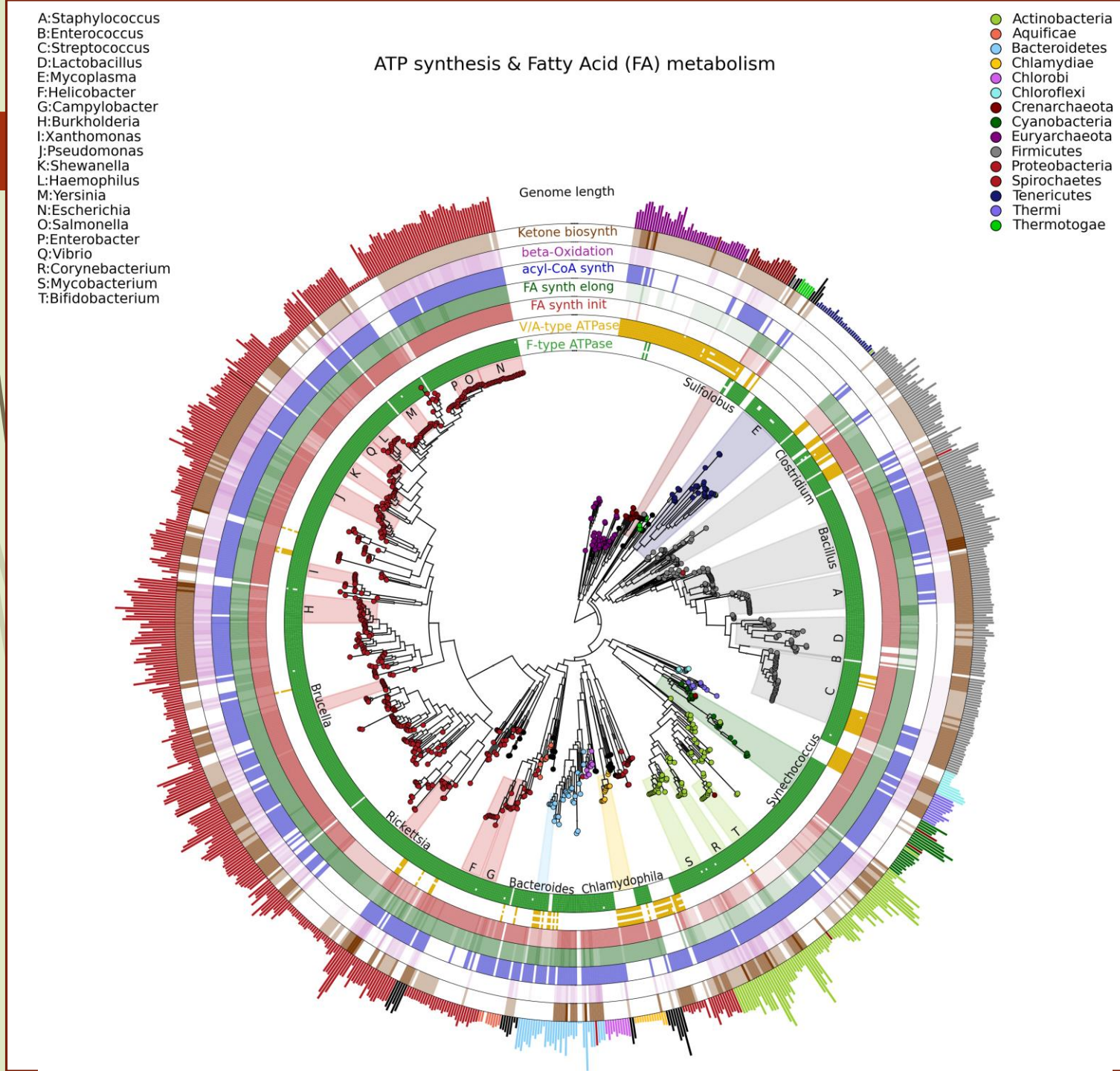
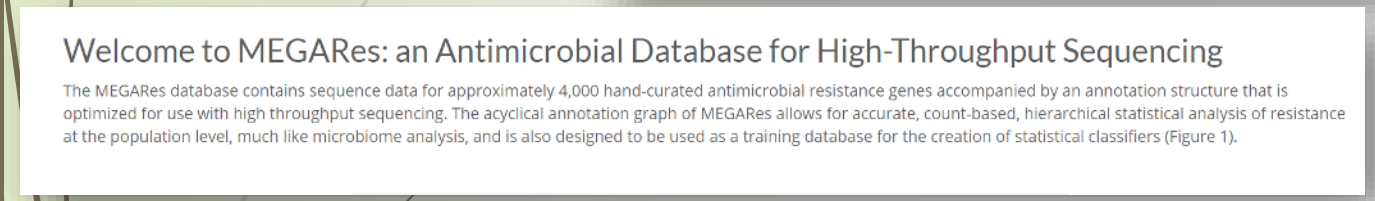


Figure 2: A large, 3,737 genome phylogeny annotated with functional genomic properties. We used the phylogenetic tree built using PhyloPhlAn (Segata et al., 2013) on all available microbial genomes as of 2013 and annotated the presence of ATP synthesis and Fatty Acid metabolism functional modules (as annotated in KEGG) and the genome length for all genomes. Colors and background annotation highlight bacterial phyla, and the functional information is reported in external rings. ATP synthesis rings visualize the presence (or absence) of each module, while Fatty Acid metabolism capability is represented with a gradient color.

https://doi.org/10.7717/peerj.1029/fig-2

Lots of data sources, but how useful for safety assessment?



"As these new predicted resistance genes can be incorporated into databases under the antibiotic resistance heading without further functional verification, databases are becoming increasingly comprehensive and contain a large amount of 'noise'." *Martínez JL, Coque TM, Baquero F. What is a resistance gene? Ranking risk in resistomes. Nat Rev Microbiol [Internet]. 2015;13(2):116–23.*

International Safety Assessment Community Recognizes the Need for Guiding Methods and Standards


efsa
European Food Safety Authority

SCIENTIFIC COLLOQUIUM SERIES

EFSA Scientific Colloquium N°24

'OMICS in risk assessment: state-of-the-art and next steps'

24-25 April 2018, Berlin, Germany

PROGRAMME

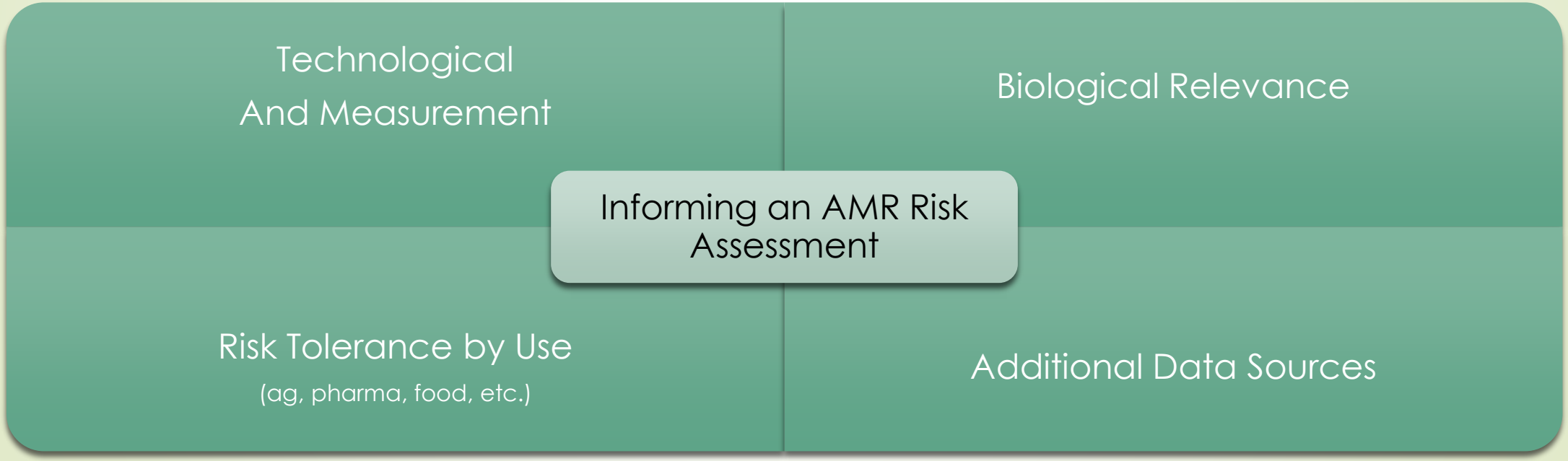
- …Identification and Characterization…impacts further steps of evaluation and in many cases determines the need for tests to address safety…
- …If the strain is capable of producing toxic compounds, the product should be free of those compounds…
- …If the strain carries antimicrobial resistance genes, those genes should not be present in the product.
- Genetically modified strains require a full characterization of the introduced modifications.

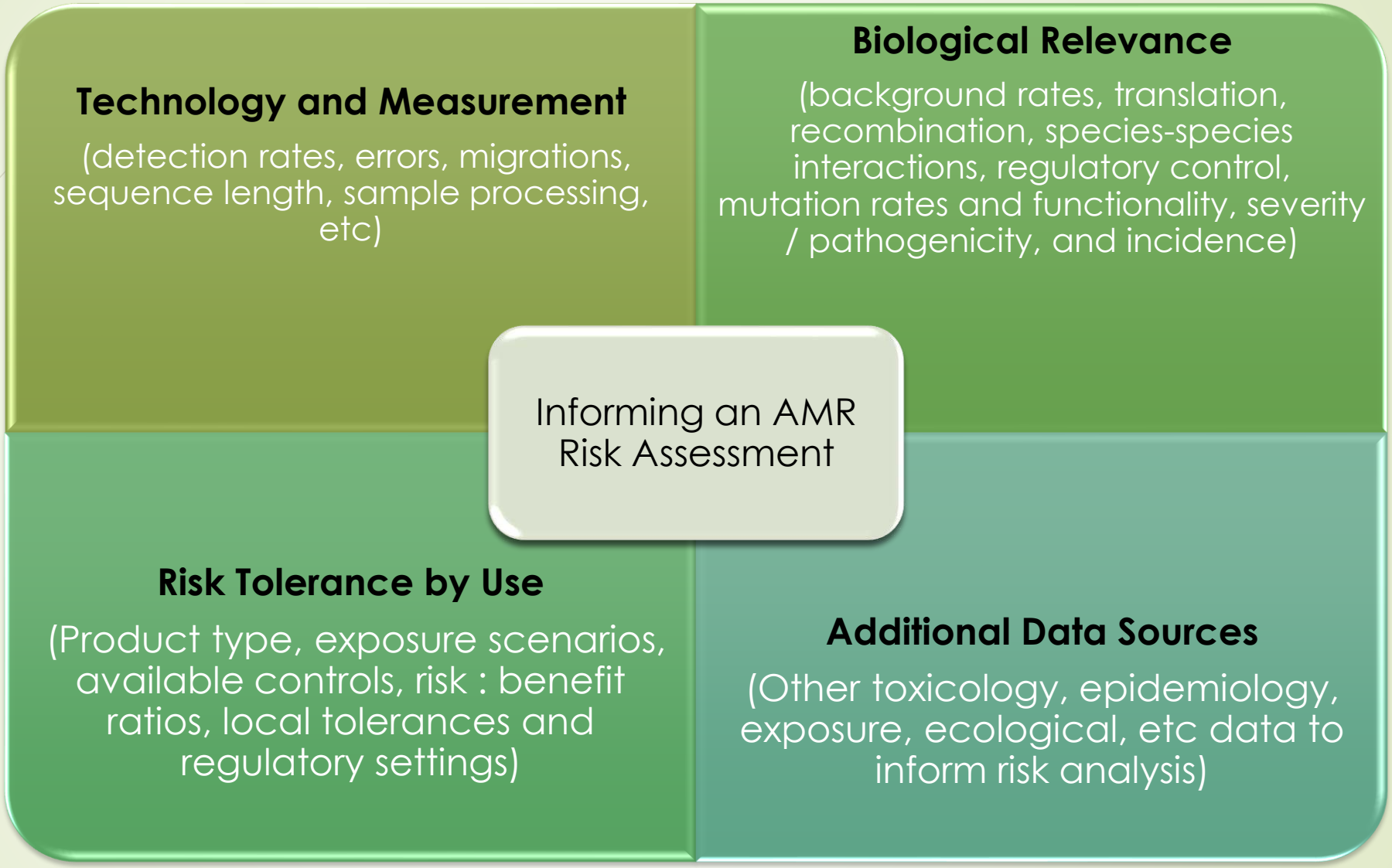
Problem Statement: Our ability to make informed safety assessment decisions on products containing microbes is limited because the technology to measure and characterize microbes exceeds our ability to understand their potential to confer a safety risk.

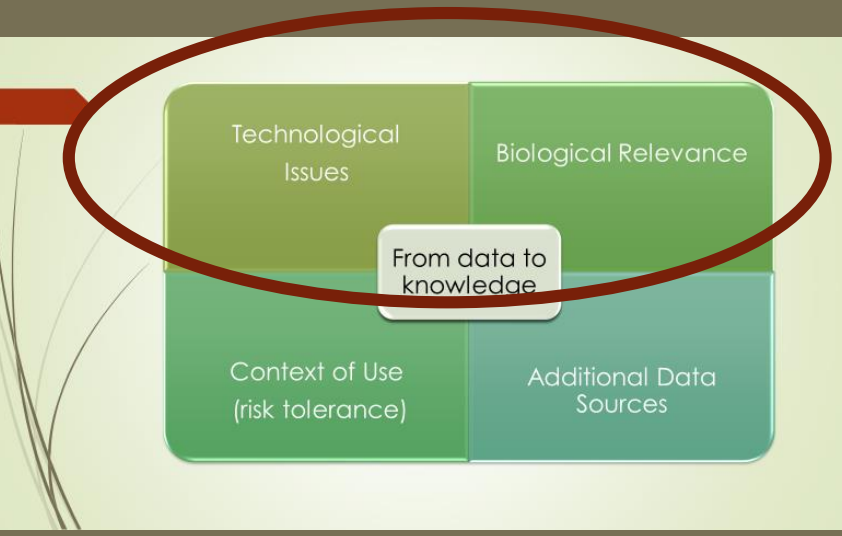
Proposed Stage One Focus

Interpretation of **AMR or putative-AMR** genomic sequences as potential safety risks **to humans**.

Conceptual model of information components to inform AMR risk assessment




Technology and Measurement
(detection rates, errors, migrations, sequence length, sample processing, etc)
Biological Relevance
(background rates, translation, recombination, species-species interactions, regulatory control, mutation rates and functionality, severity / pathogenicity, and incidence)
Informing an AMR Risk Assessment
Risk Tolerance by Use
(Product type, exposure scenarios, available controls, risk : benefit ratios, local tolerances and regulatory settings)
Additional Data Sources
(Other toxicology, epidemiology, exposure, ecological, etc data to inform risk analysis)



Proposal for HESI Program

Phase I Focus

Convene team of experts to build consensus on data needs and current best practices around 'technology and measurement' and 'biological relevance' that would be needed to inform human safety assessment.

Potential Outcomes of Phase 1

- Criteria for evaluating quality and relevance of AMR sequence data from a technological and biological standpoint.
- Focused direction to future research on biological relevance of AMR genes in settings of specific relevance for human safety assessment;
- Identification of tools and methods that work (or need improvement) for detection and evaluation of AMRs for safety assessment purposes.
- Consensus and data driven steps towards building a comprehensive data integration framework for safety assessment of AMR genes detected in products

Timeline


July–Sep. 2018
Voting by HESI Membership in July-Sep 2018
Launch of EI (if adopted) in November 2018
Nov. 2018
Jan.–June 2019
Form working groups (technology and biology), conduct literature evaluation and expert consultation
Publish Gap Analysis
Nov. 2019
Early 2020
Workshop on Findings and Develop Consensus on Acceptance/ Evaluation Criteria

At completion of Phase 1 – we might move to Phase 2….it could look like this

Genomic Microbial Safety Assessment

BACKGROUND vs FOREGROUND

Key Background Information needed for safety assessment:

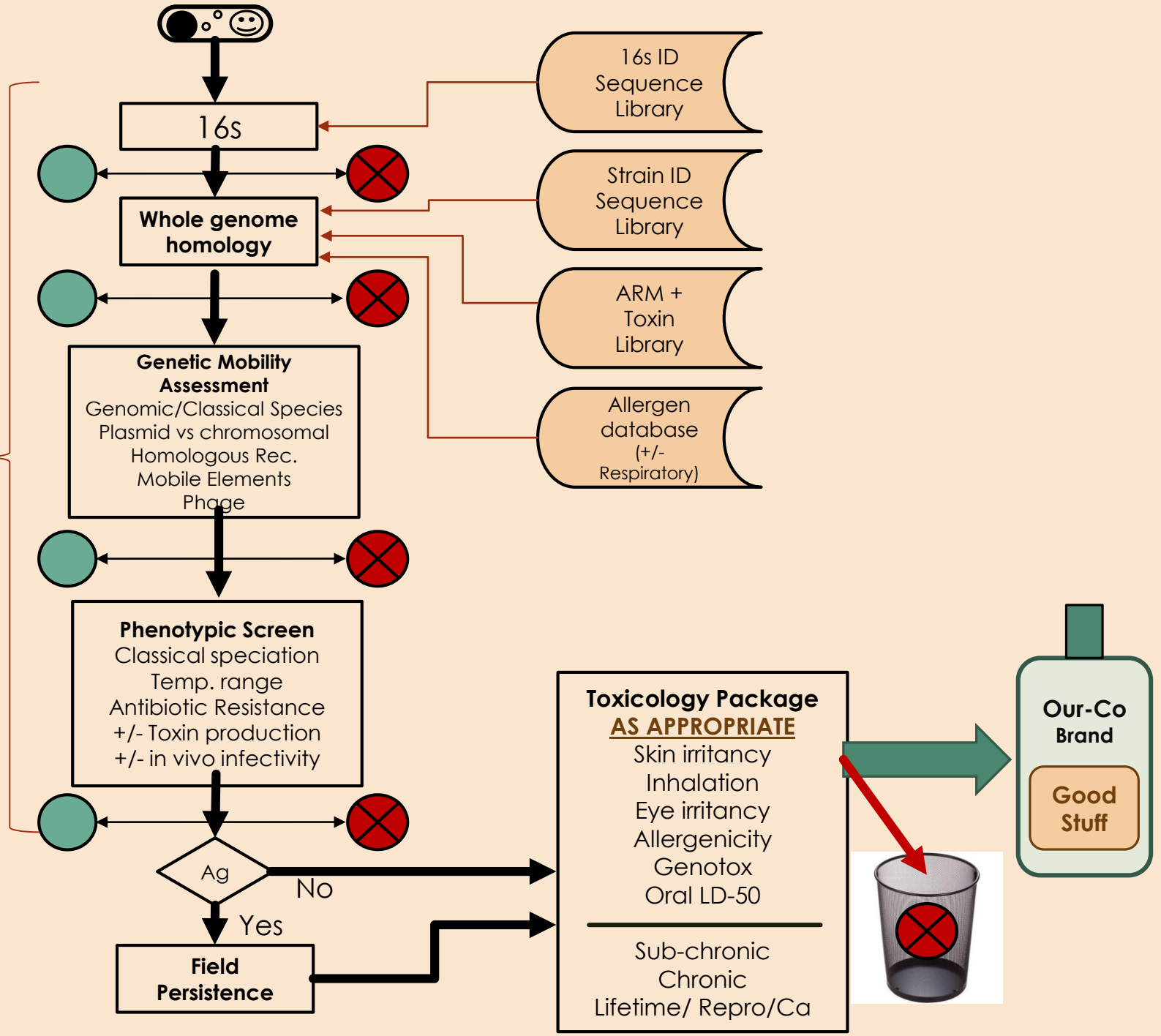
Environmental ARMs and other genes of concern

Gene mobility in the environment

Stability / Recovery of relevant microbiomes

Survival of the Organism

Pre-Regulatory Work Safety Assessment
Depending on source and exposure potential, adjust assay timing as needed.



Ye Olde Discussion

(Hic Sunt Disputantionibus)