



UNIVERSITÄT
HOHENHEIM



H O L M I R
Hohenheim Center for
Livestock Microbiome Research

STREAMLINING PIG MICROBIOME STUDIES:

Improving Efficiency Through Reproducibility And Comparability.

Samuel Onyilokwu Enokela, Timur Yergaliyev, Amélia Camarinha Silva

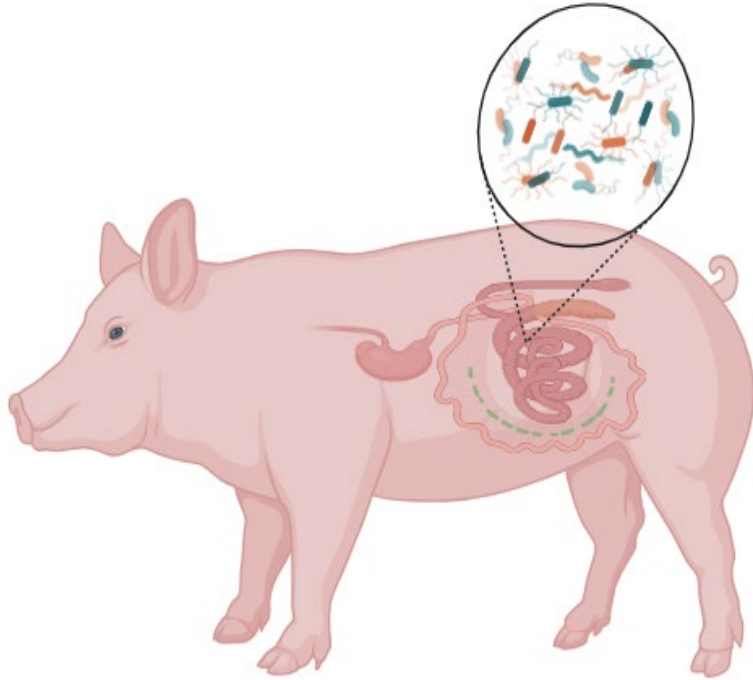
Department of Livestock Microbial Ecology

Institute of Animal Science

University of Hohenheim

INTRODUCTION

○ Porcine Gut Microbiome



Biorender.com

- Essential for health and performance.
- Genes: 17.2 million vs. pig genome: 25,000.
- Recent studies have focused on understanding how as a biomarker it reflects physiological state and identifies environmental factors that affect performance of the animal.

Proper and accurate characterization of the microbiome in pigs can only be achieved when standardized protocols are applied across the various facets of microbiome studies.

Without standards, inconsistencies can compromise research reliability!!!

- Published Standards/ Protocols on Studying the Microbiome



Human Microbiome Project



- In pigs.....?



Development of methodological standards as a prerequisite for meaningful, reproducible and application-oriented microbiome research on livestock

- Phase 1: Literature Research, Evaluation and Standardization
 - Develop a template for metadata collection and standardize it in a way that covers:
 - Biological Elements
 - Environmental Variables
 - Technical Variables

DATA COLLECTION AND ELIGIBILITY CRITERIA

➔ Pubmed Database Search <https://pubmed.ncbi.nlm.nih.gov/>

Search	Actions	Details	Query	Results
#9	...	>	Search: (pig microbiome) AND (pig microbiota) Filters: Full text, from 2003 - 2023	2,939
#8	...	>	Search: (pig microbiome) AND (pig microbiota) Filters: Full text, Associated data, from 2003 - 2023	893
#7	...	>	Search: (pig microbiome) AND (pig microbiota) Filters: Full text, Associated data, from 2001 - 2023	895
#6	...	>	Search: (pig microbiome) AND (pig microbiota) Filters: Full text, Associated data	895
#5	...	>	Search: pig microbiome Filters: Full text, Associated data, from 2003 - 2023	977
#4	...	>	Search: pig microbiome Filters: Full text, from 2003 - 2023	3,232

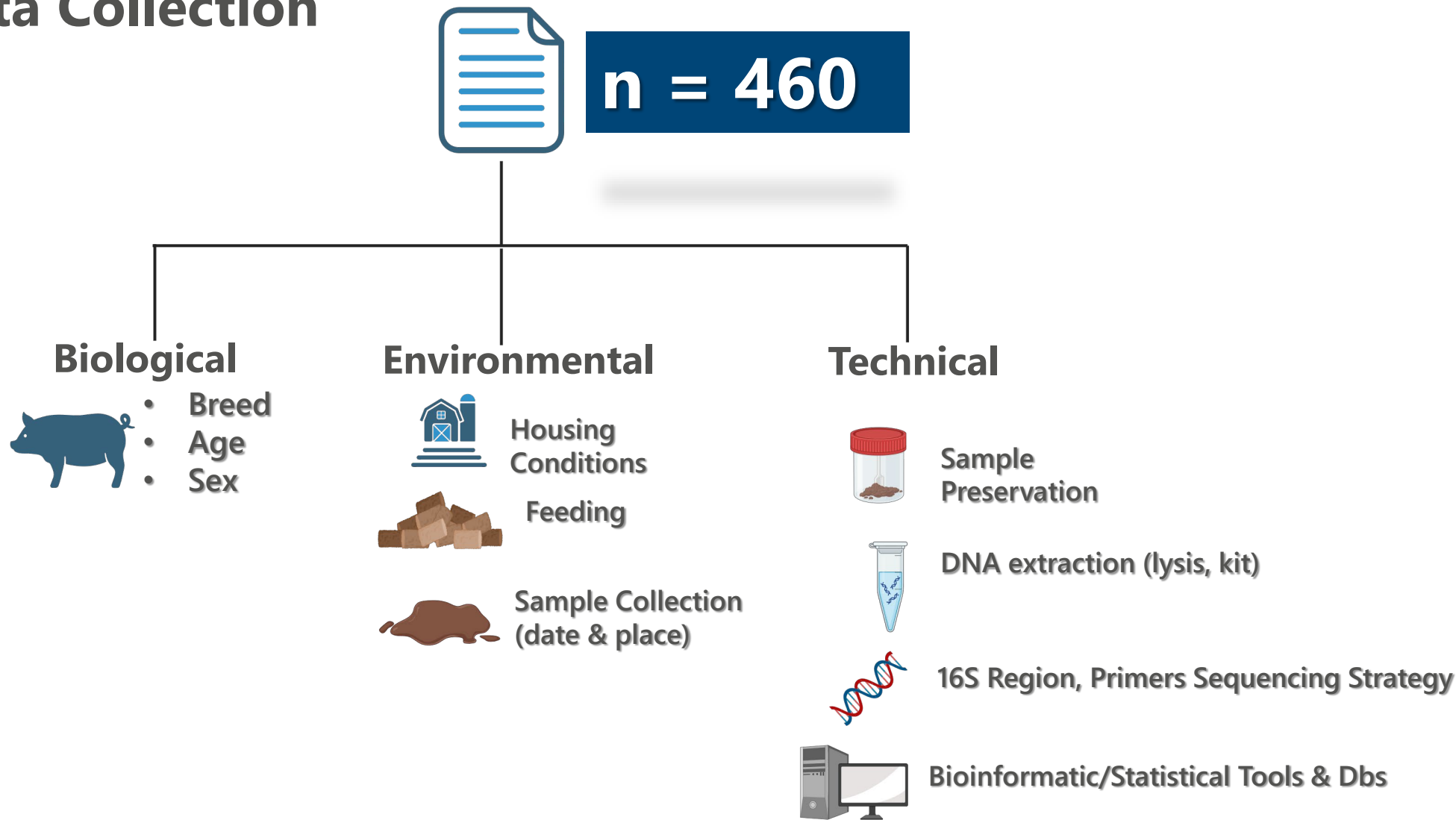
Timeframe: 2003- 31st April, 2023

2939

➔ In-vivo studies

➔ Sequencing Data available and accessible

➔ Metadata Collection



Standardization ➔

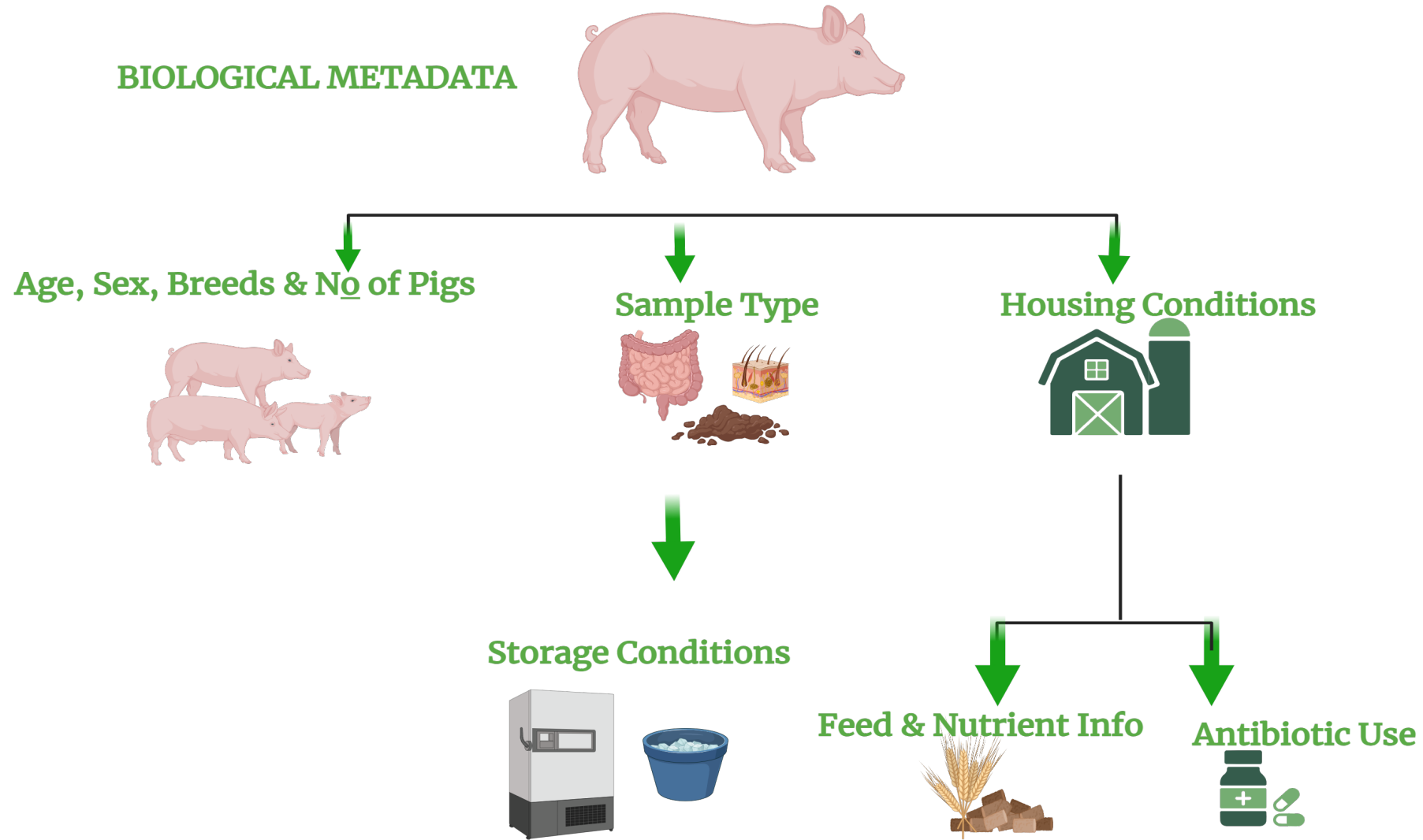
STANDARDIZING THE METADATA

Article Name	Feed Frequency daily	Sample Location	Storage Medium	Storage Temperature (°C)	Type of Analysis	16S rRNA Gene Region	DNA Extraction Kit	Forward Primers
Identification of the relationship between the gut microbiome and feed efficiency in a commercial pig cohort	Ad libitum	feces	Liquid Nitrogen	-80	16s rRNA	V3-V4	QIAamp Fast DNA Stool Mini Kit (Qiagen)	338F
Genetic relationships between feed efficiency and gut microbiome in pig lines selected for residual feed intake	Ad libitum	feces	Dry Ice	-80	16s rRNA	V3-V4	Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research)	343F
Effects of Clostridium butyricum on growth performance, metabonomics and intestinal microbial differences of weaned piglets	Unavailable	feces	Unavailable	Unavailable	16s rRNA	V4-V5	TIANamp Stool DNA Kit (Tiangen), QIAamp PowerFecal DNA Kit (Qiagen)	515F
Barcoded pyrosequencing-based metagenomic analysis of the faecal microbiome of three purebred pig lines after cohabitation	Unavailable	feces	Unavailable	Unavailable	16s rRNA	V1-V3	UltraClean Fecal DNA Isolation Kits (MO BIO Laboratories)	Unavailable

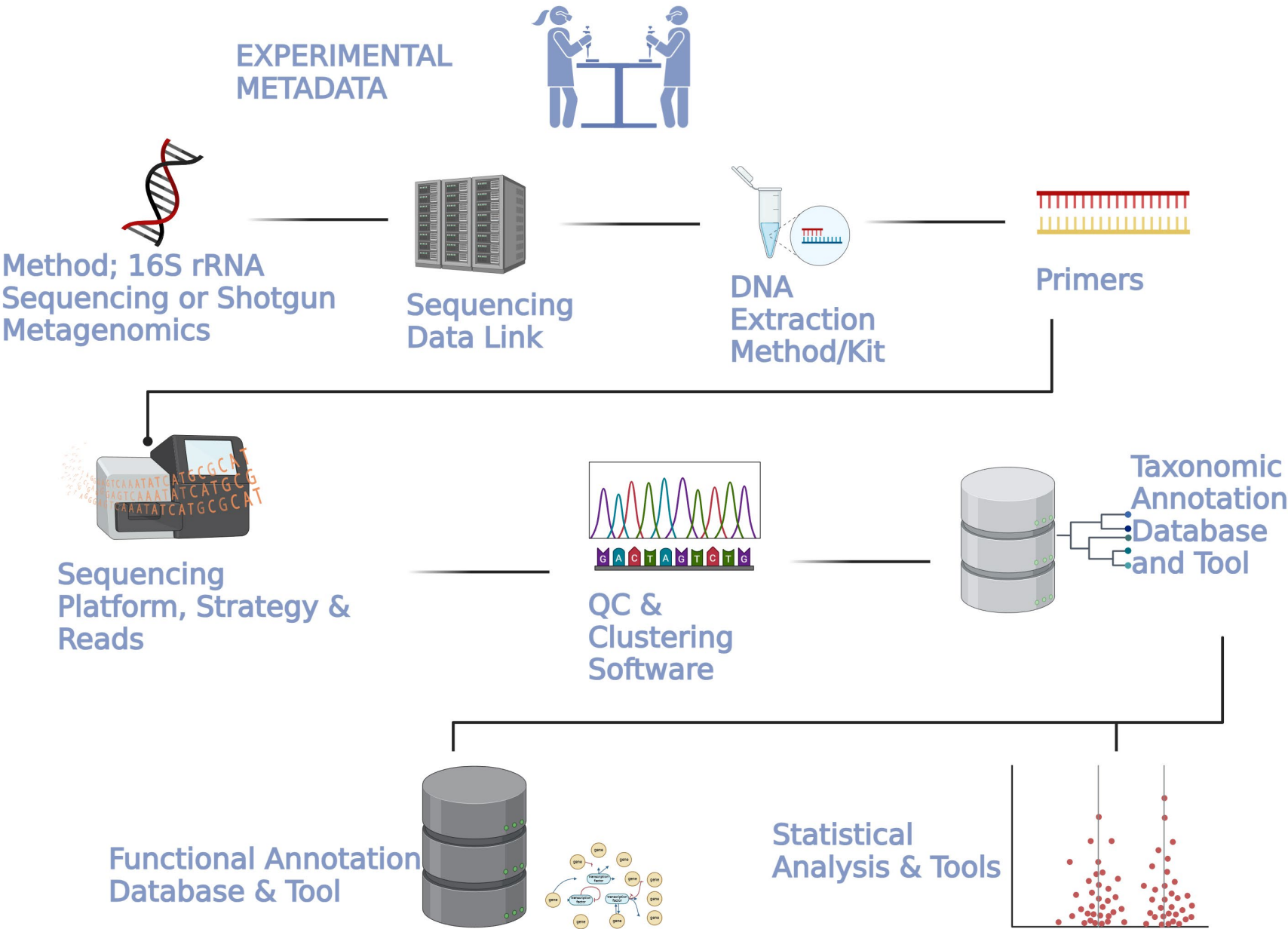
STANDARDIZING THE METADATA

Article Name	Quality Control	Classification Metrics	Classification Software	Taonomic Annotation Database	Taxonomic Annotation Tool
Characterization of Bacterial Microbiota Composition in Healthy and Diarrheal Early-Weaned Tibetan Piglets	Unavailable	Operational Taxonomic Units	Uclust	Unite	Unavailable
Use of Host Feeding Behavior and Gut Microbiome Data in Estimating Variance Components and Predicting Growth and Body Composition Traits in Swine	Unavailable	Amplicon Sequence Variants	Unavailable	Unavailable	Unavailable
Gut microbiota and host genetics contribute to the phenotypic variation of digestive and feed efficiency traits in growing pigs fed a conventional and a high fiber diet	DADA2(R)	Amplicon Sequence Variants	DADA2(R)	SILVA v132	DADA2(R)
Effects of dietary supplement with a Chinese herbal mixture on growth performance, antioxidant capacity, and gut microbiota in weaned pigs	DADA2	Amplicon Sequence Variants	DADA2	Unavailable	Unavailable
A comparison of dynamic distributions of intestinal microbiota between Large White and Chinese Shanxi Black pigs	Mothur	Operational Taxonomic Units	Mothur	GreenGenes	RDP Classifier

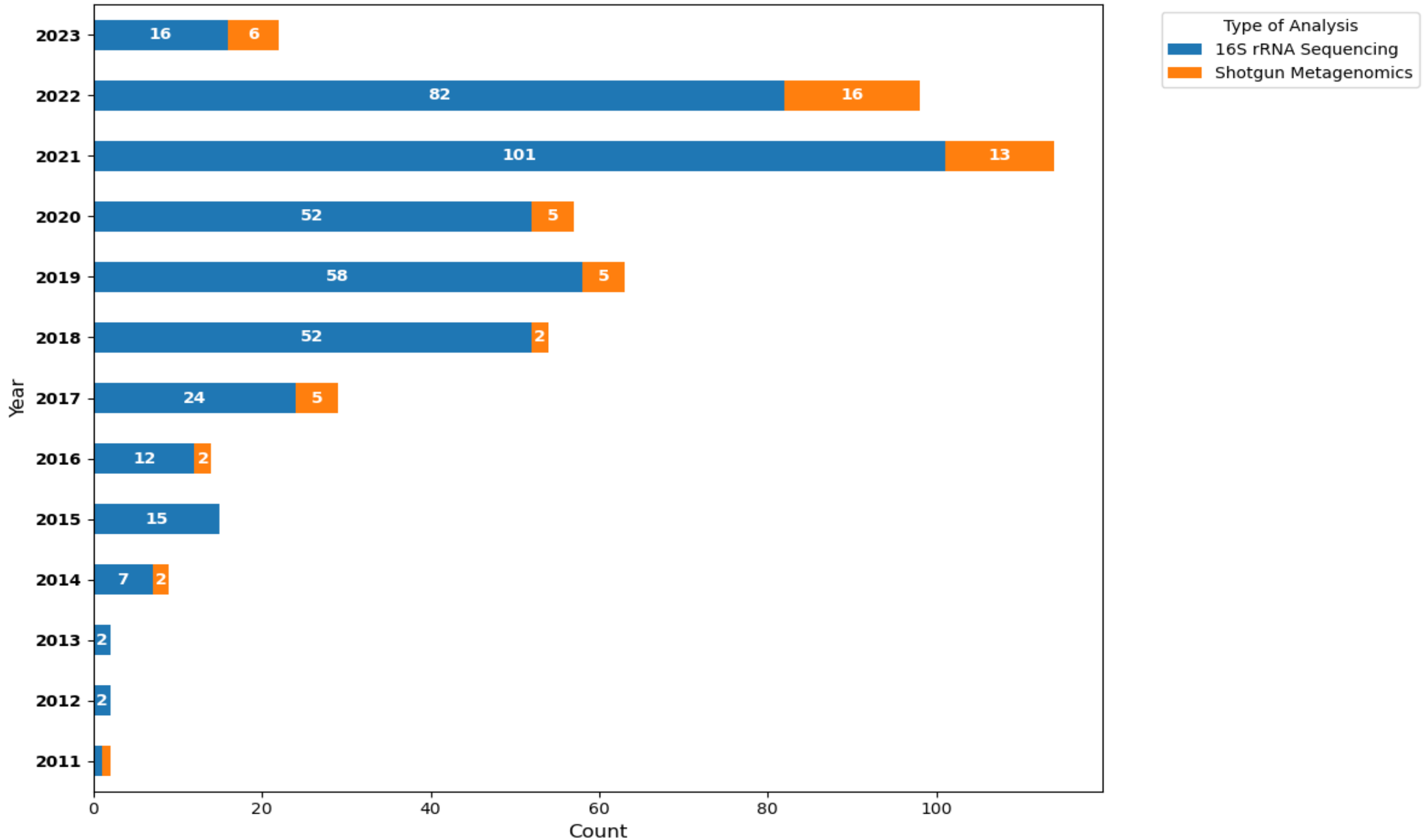
STANDARDIZED METADATA TEMPLATE



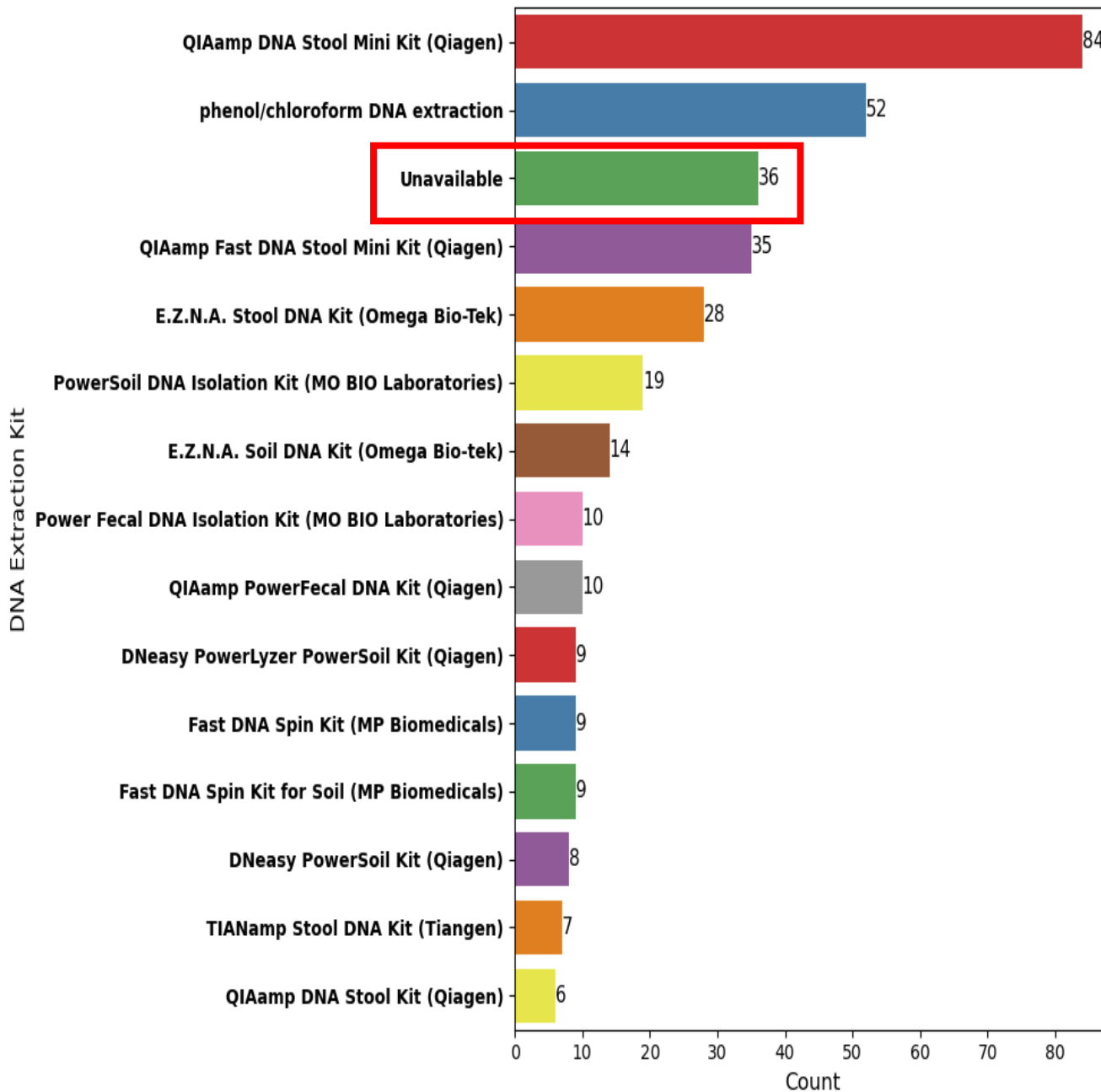
STANDARDIZED METADATA TEMPLATE



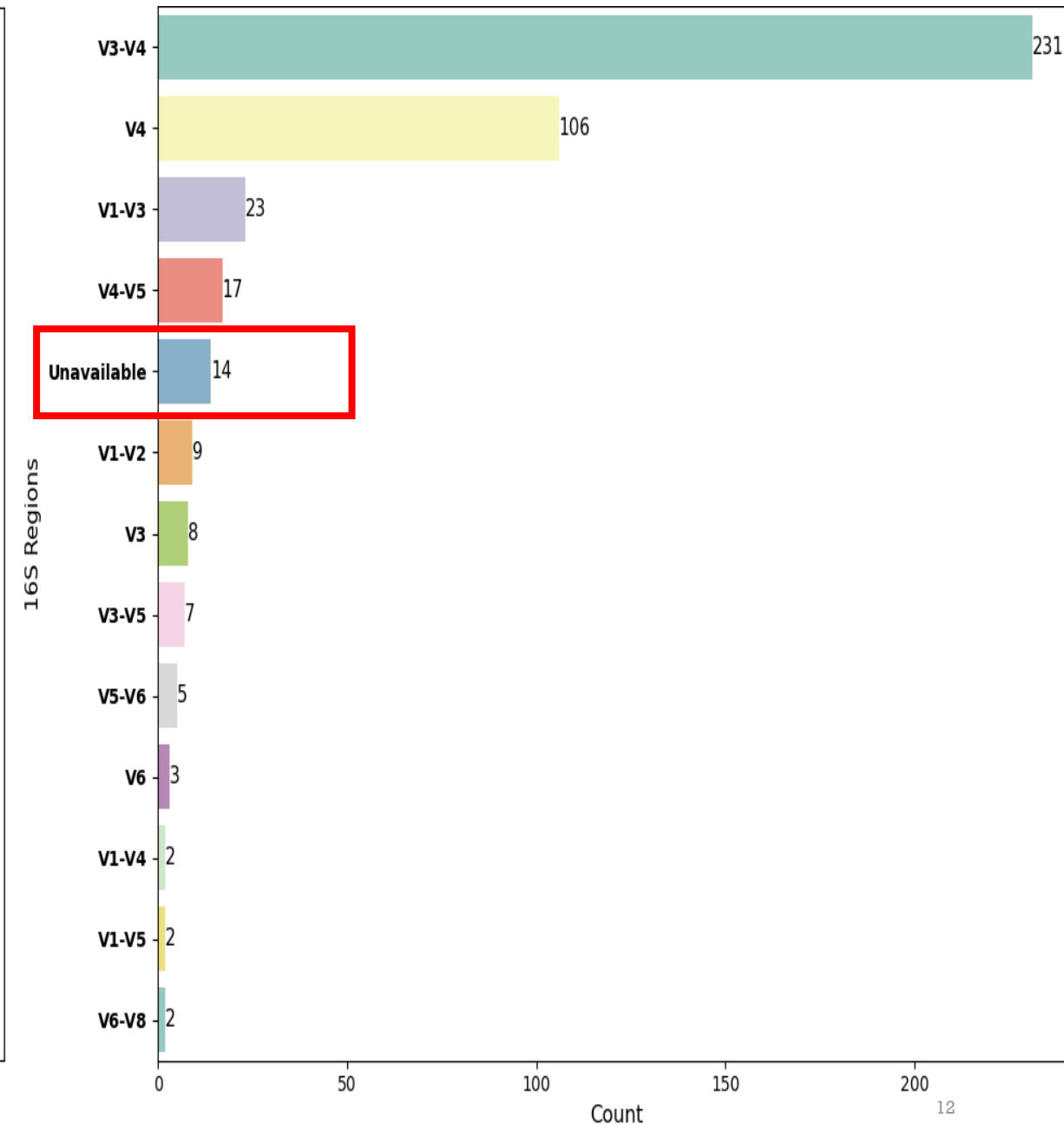
Publications Reviewed Per Year



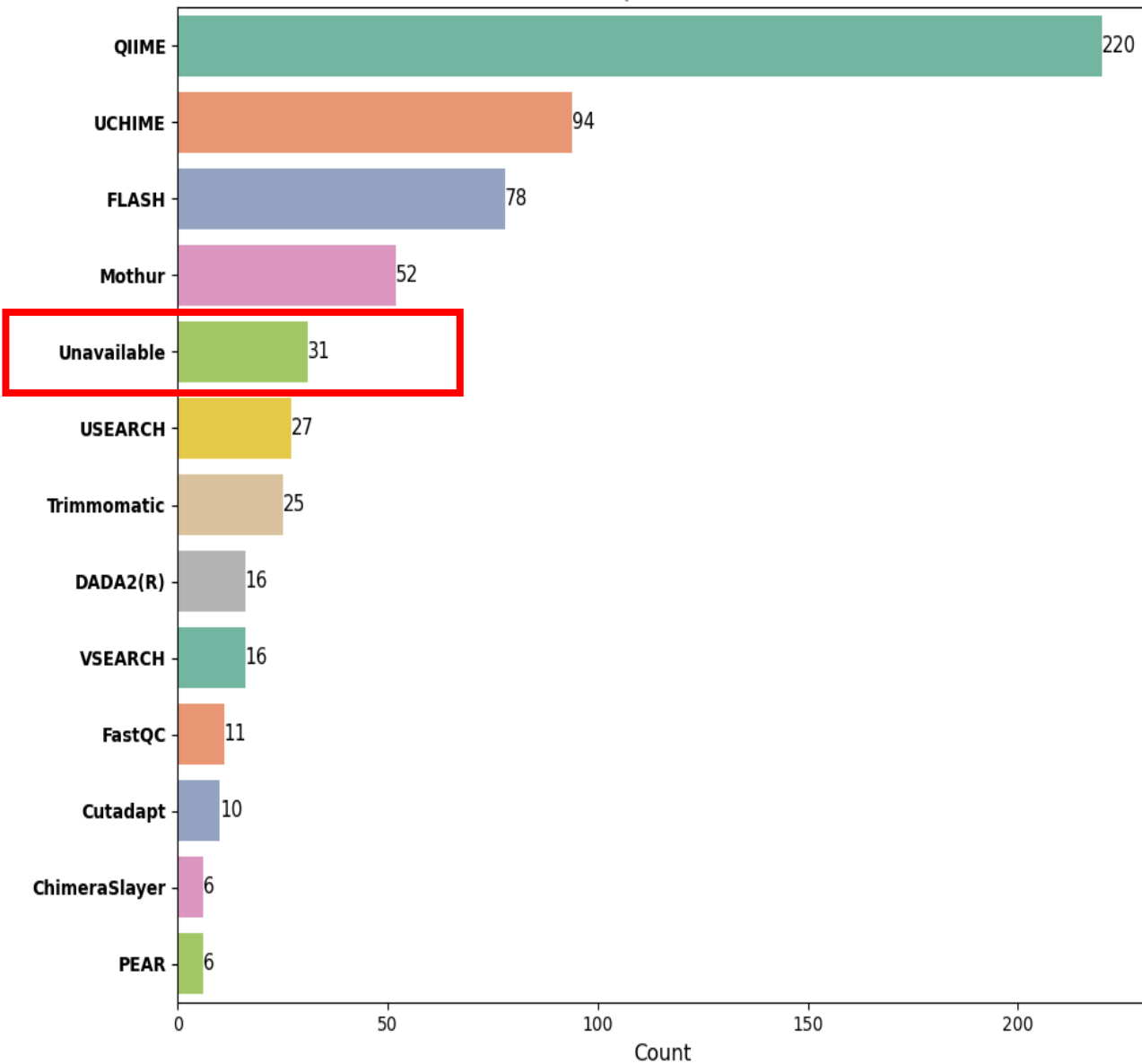
DNA Extraction Kits/Methods



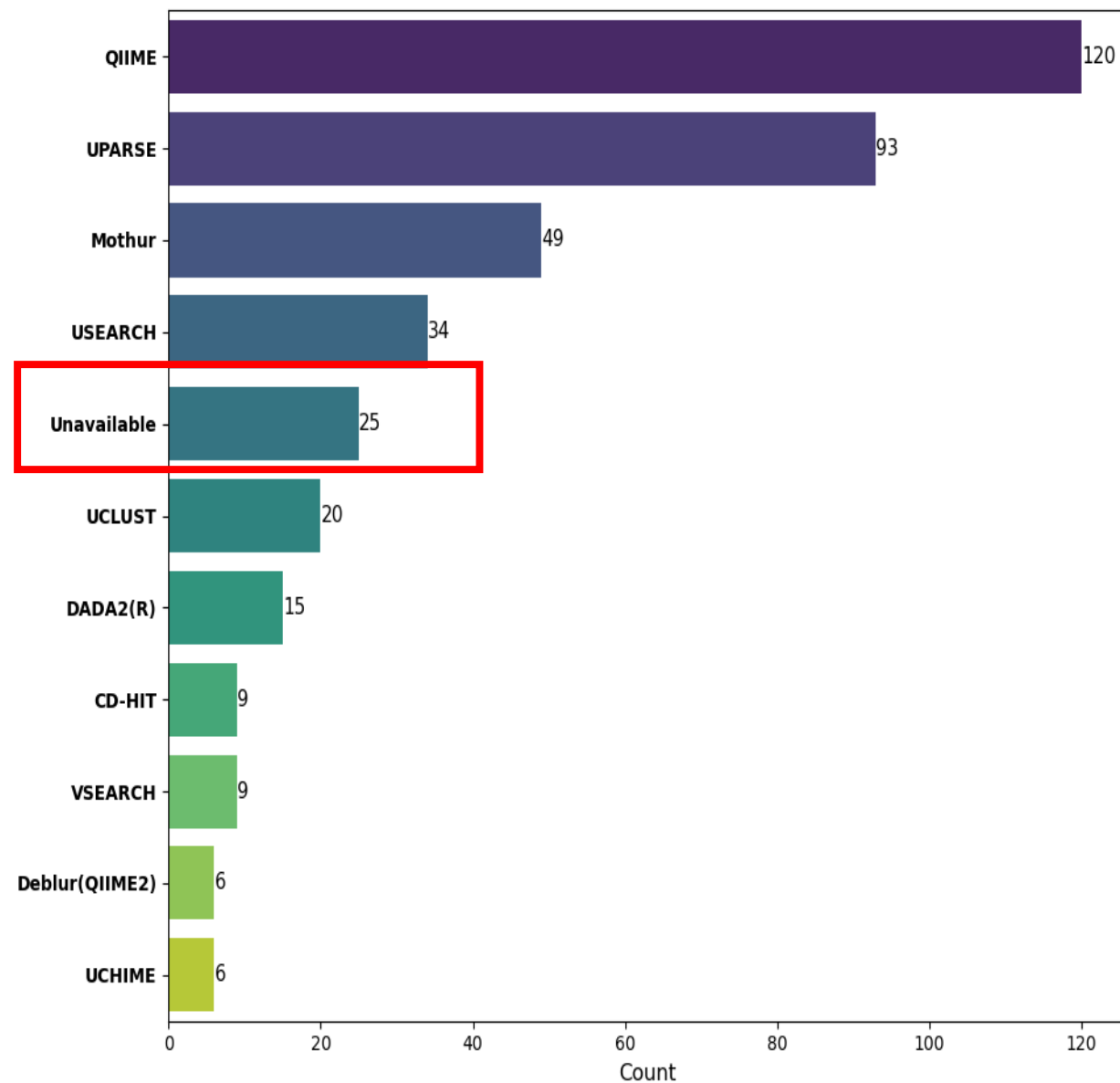
Targeted 16S rRNA Gene Regions



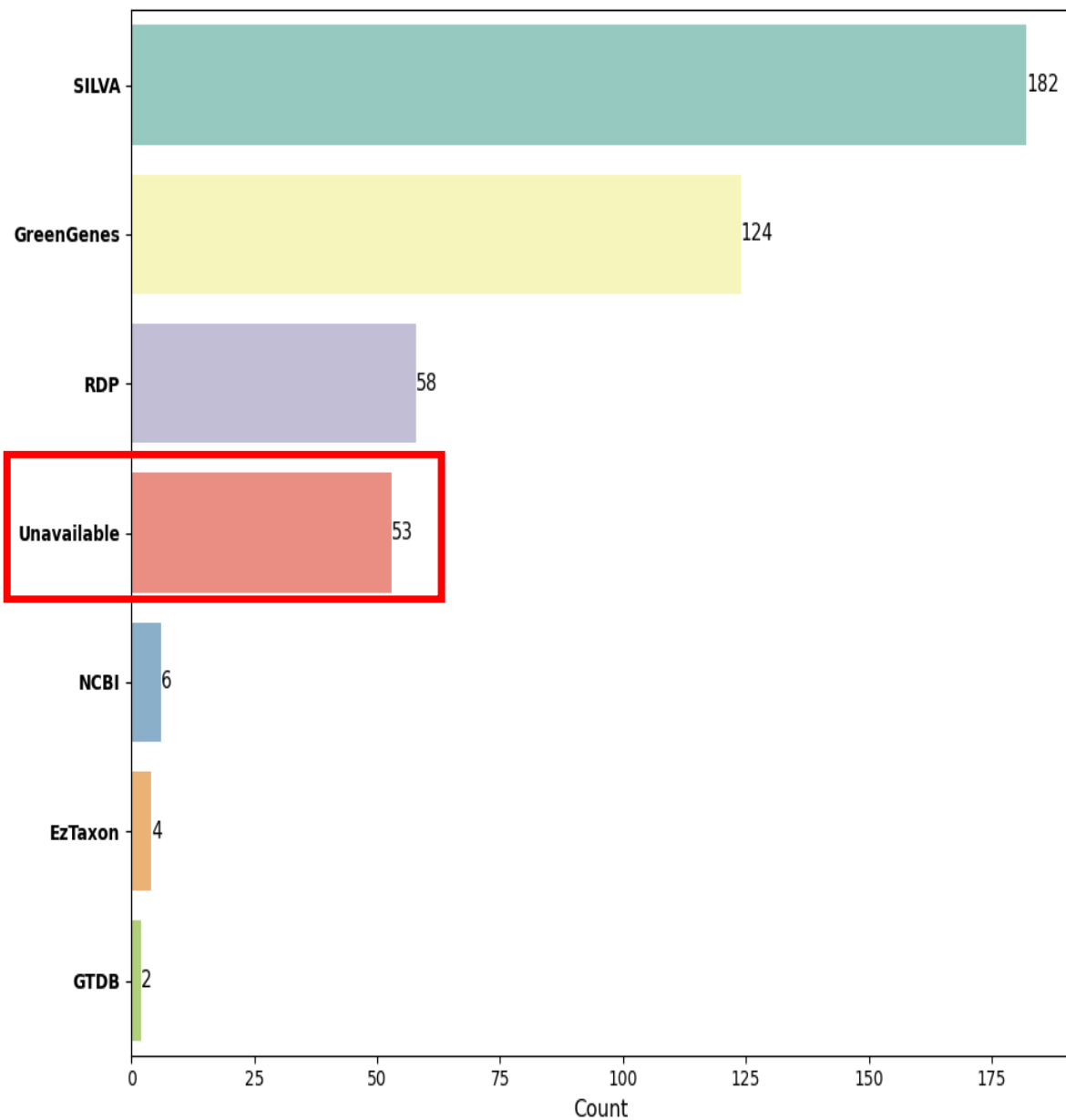
Quality Control Tools



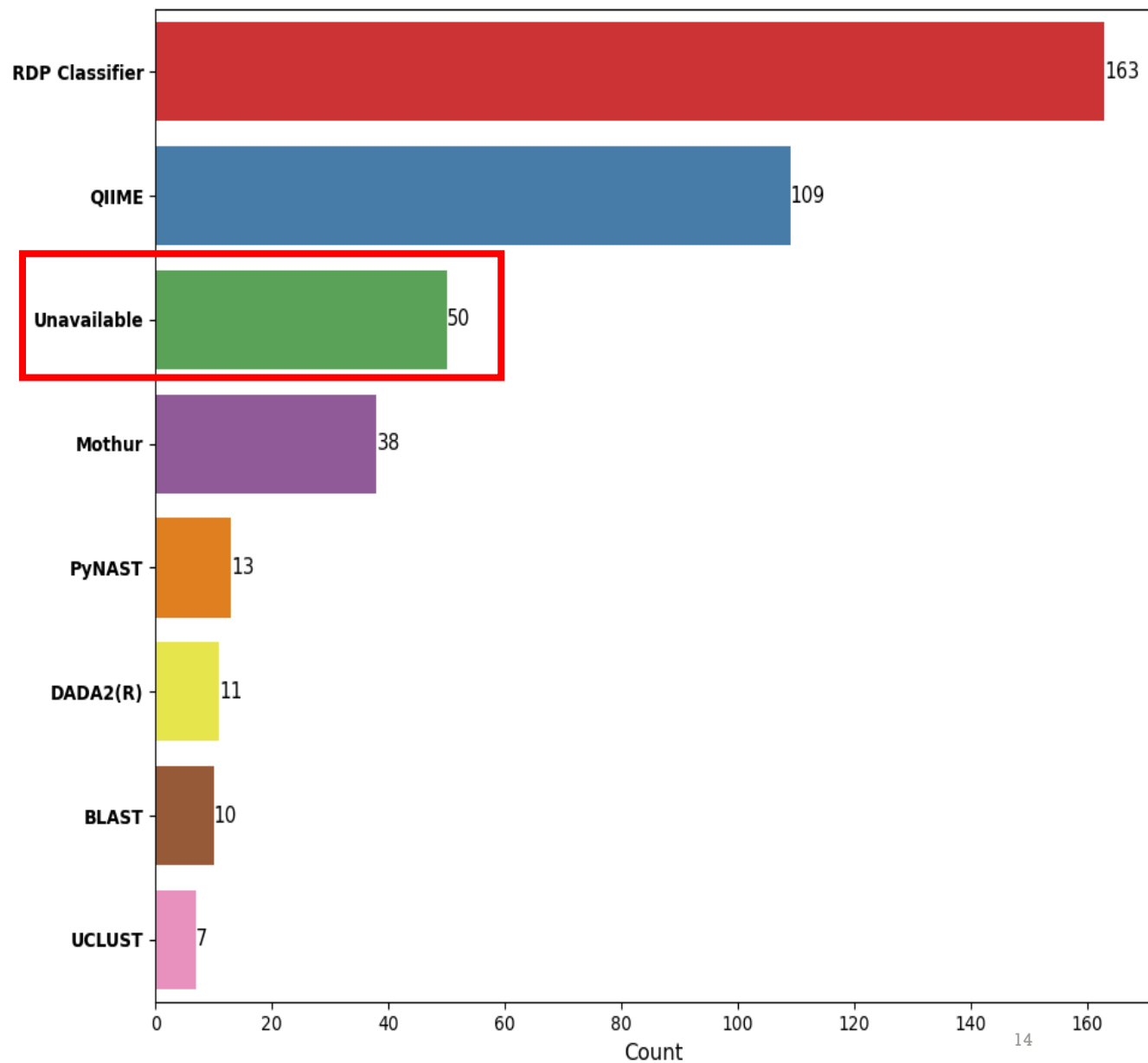
Classification Tools



Databases for Taxonomic Annotation



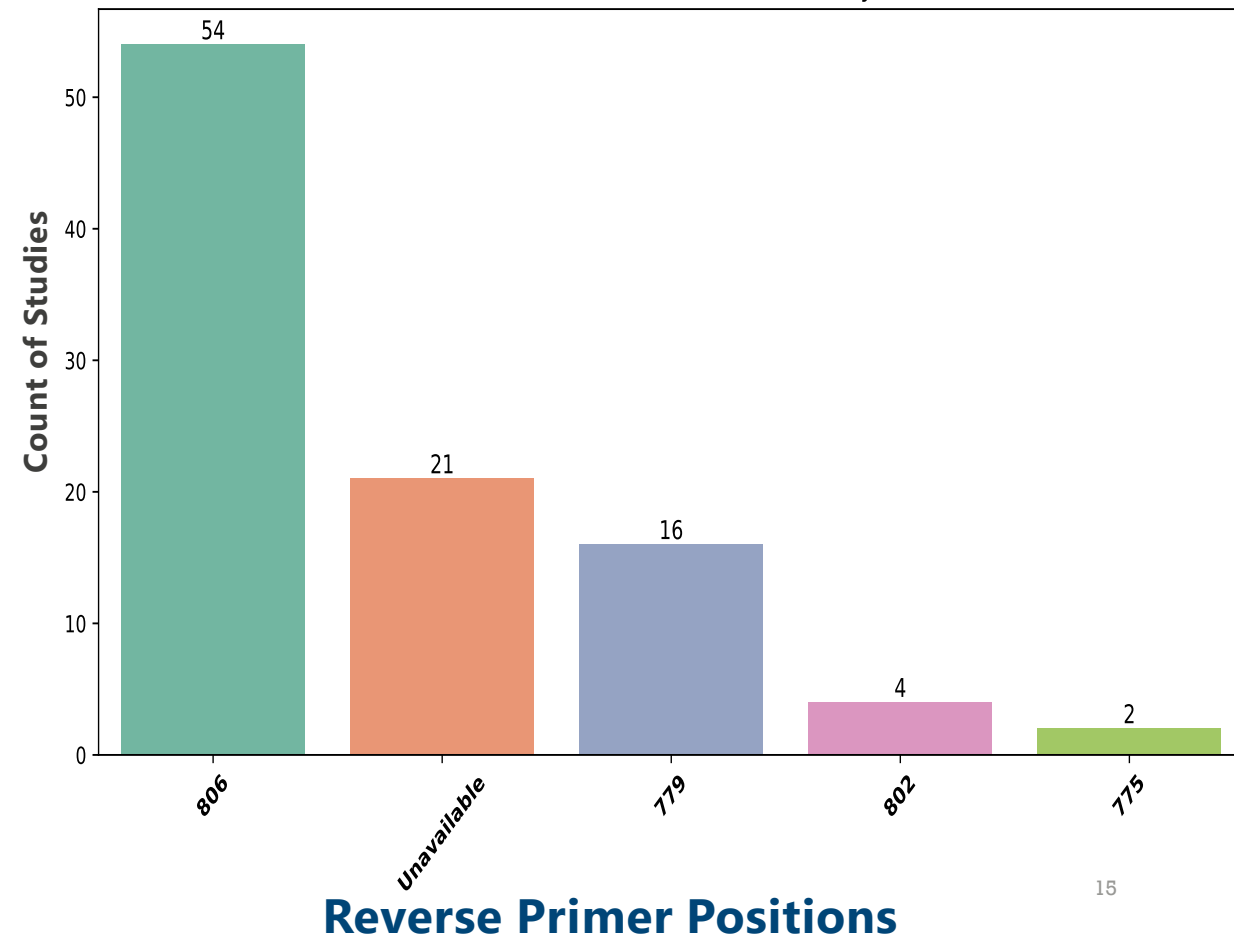
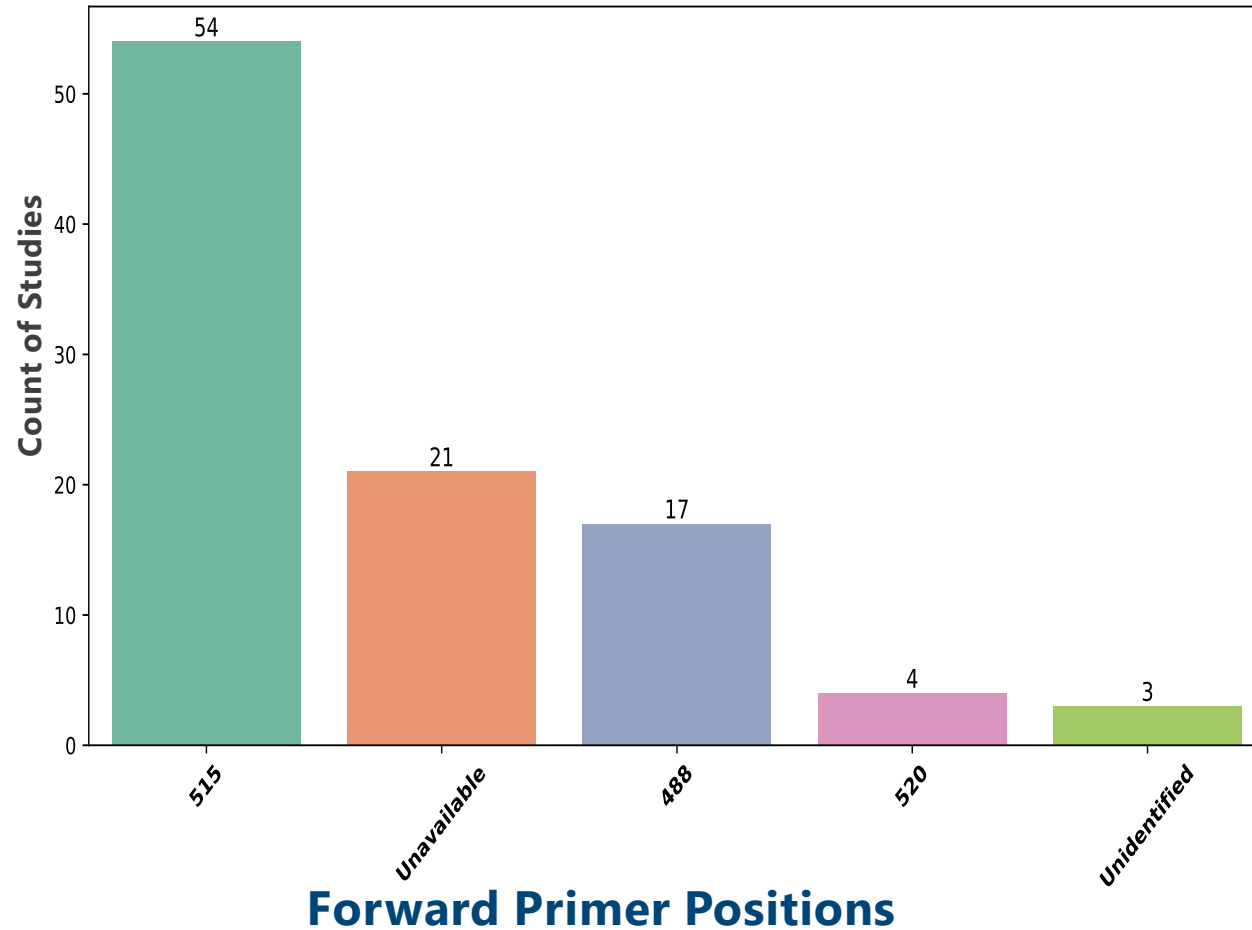
Tools for Taxonomic Annotation



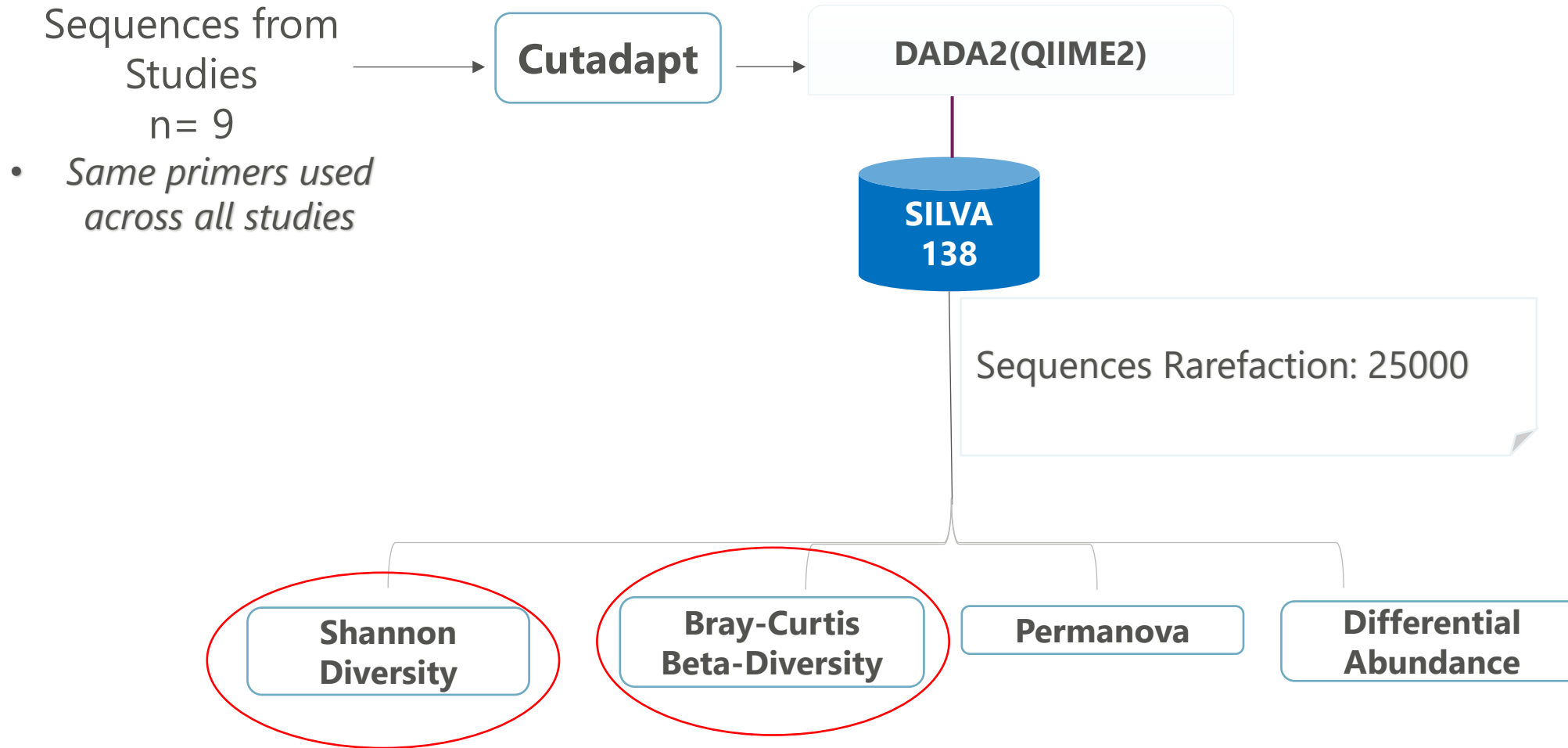
META-ANALYSIS

Will there be differences in microbial diversity based on DNA Extraction Kit used?

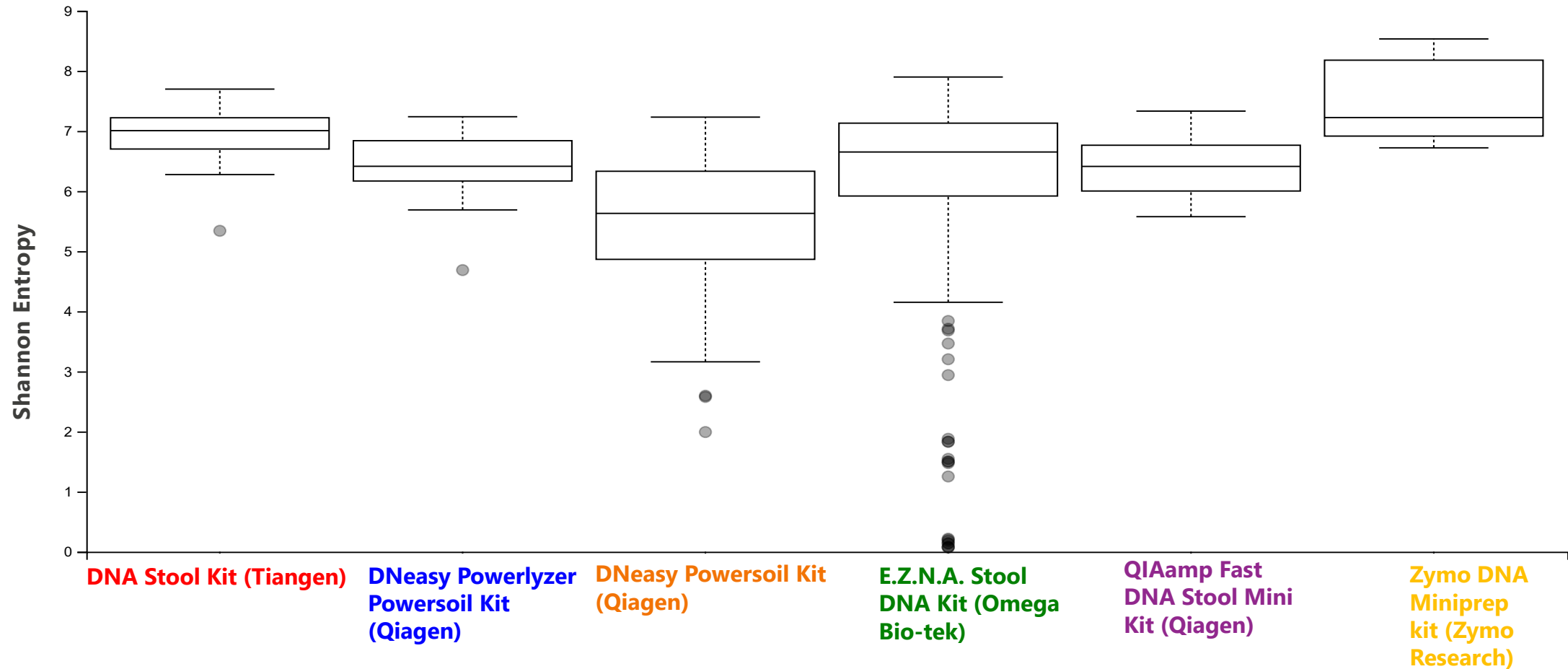
V4 Region (n = 106)



META-ANALYSIS - V4 REGION

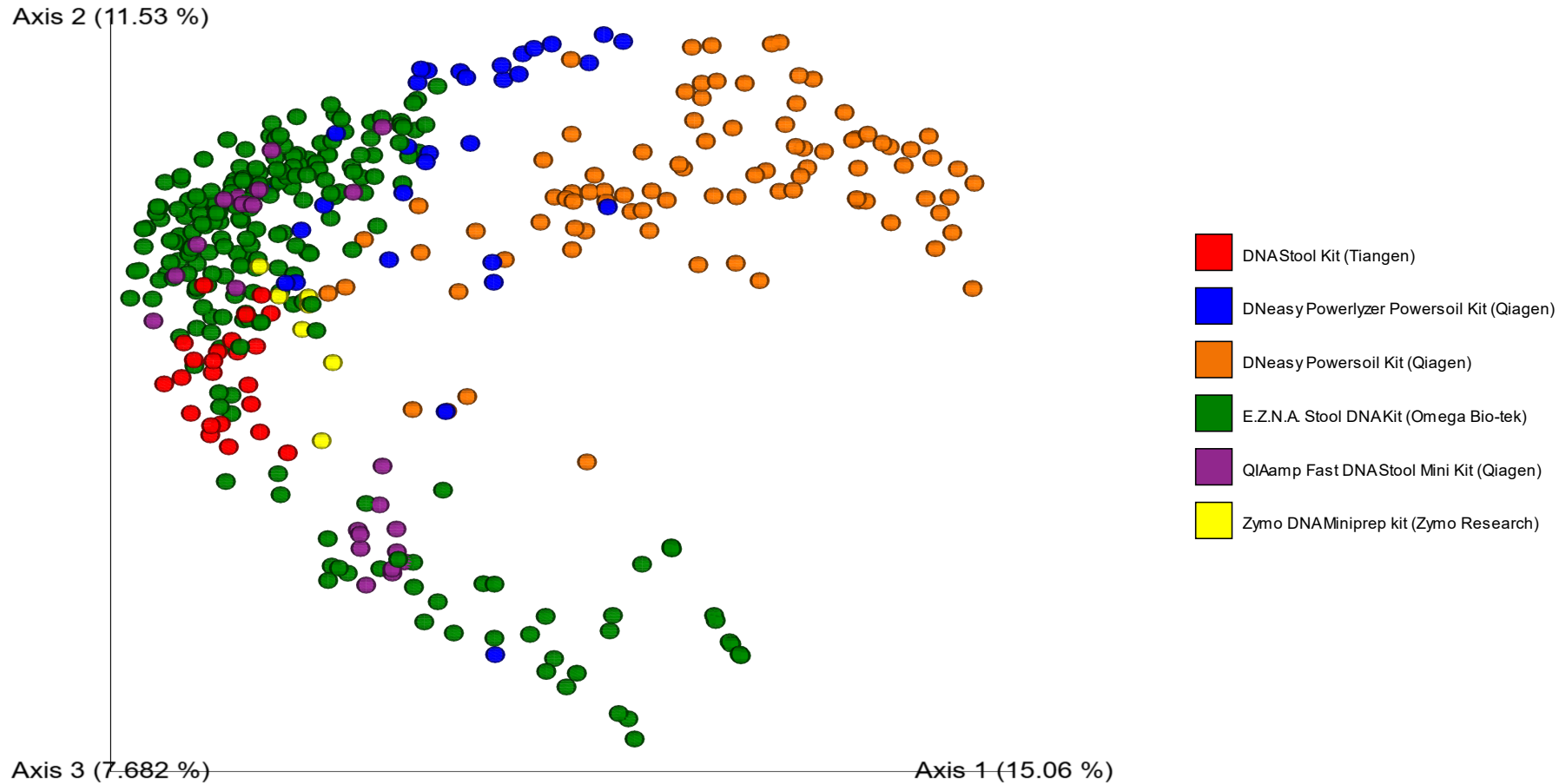


Shannon Alpha Diversity



Kruskal-Wallis test revealed significant differences in Shannon alpha diversity across different DNA extraction kits ($H = 72.91$, $p < 0.001$), with multiple significant pairwise comparisons after correction.

Beta-Diversity – Bray Curtis : Genus Level



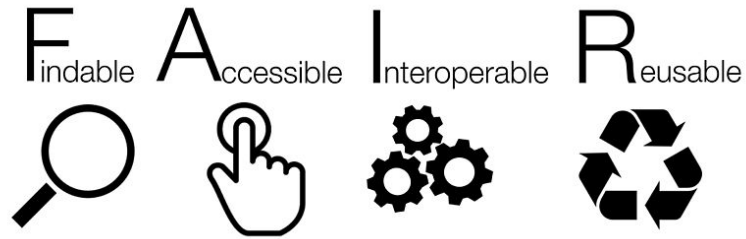
Different studies, same region, same primers



Sequences cluster based DNA kit used!!!

CHALLENGES AND FUTURE DIRECTION

- ❖ Significant constraints due to the inaccessibility of sequencing data from most of the publications that appeared in the search results.



- ❖ The template developed from this review provides a foundation for developing a standardized protocol in Pig Microbiome Studies
- ❖ Next Step:
 - ❖ Complete Meta-Analysis to note trends for other regions of the 16S rRNA
 - ❖ Pilot Study to compare variability and composition among most used DNA extraction kits & 16S Regions

ACKNOWLEDGEMENT



H o L M i R
Hohenheim Center for
Livestock Microbiome Research

With support from



by decision of the
German Bundestag

Project manager





UNIVERSITÄT
HOHENHEIM

THANK YOU FOR YOUR ATTENTION

samuel.enokela@uni-hohenheim.de