# AMRtime

Precise identification of antimicrobial resistance determinants from metagenomic data

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June 11, 2019

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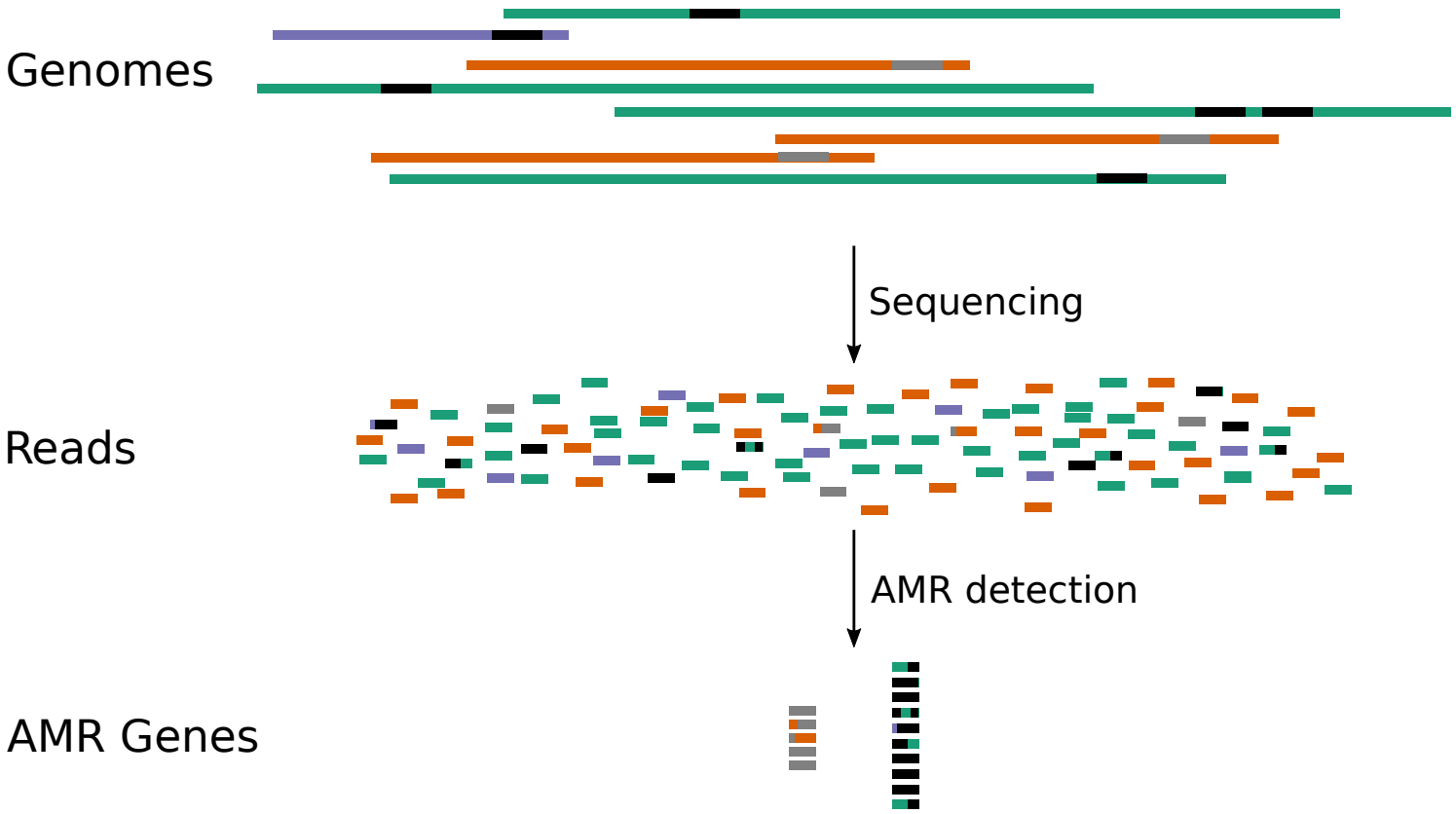
# Background

# AMR-metagenomics



# Comprehensive Antibiotic Resistance Database

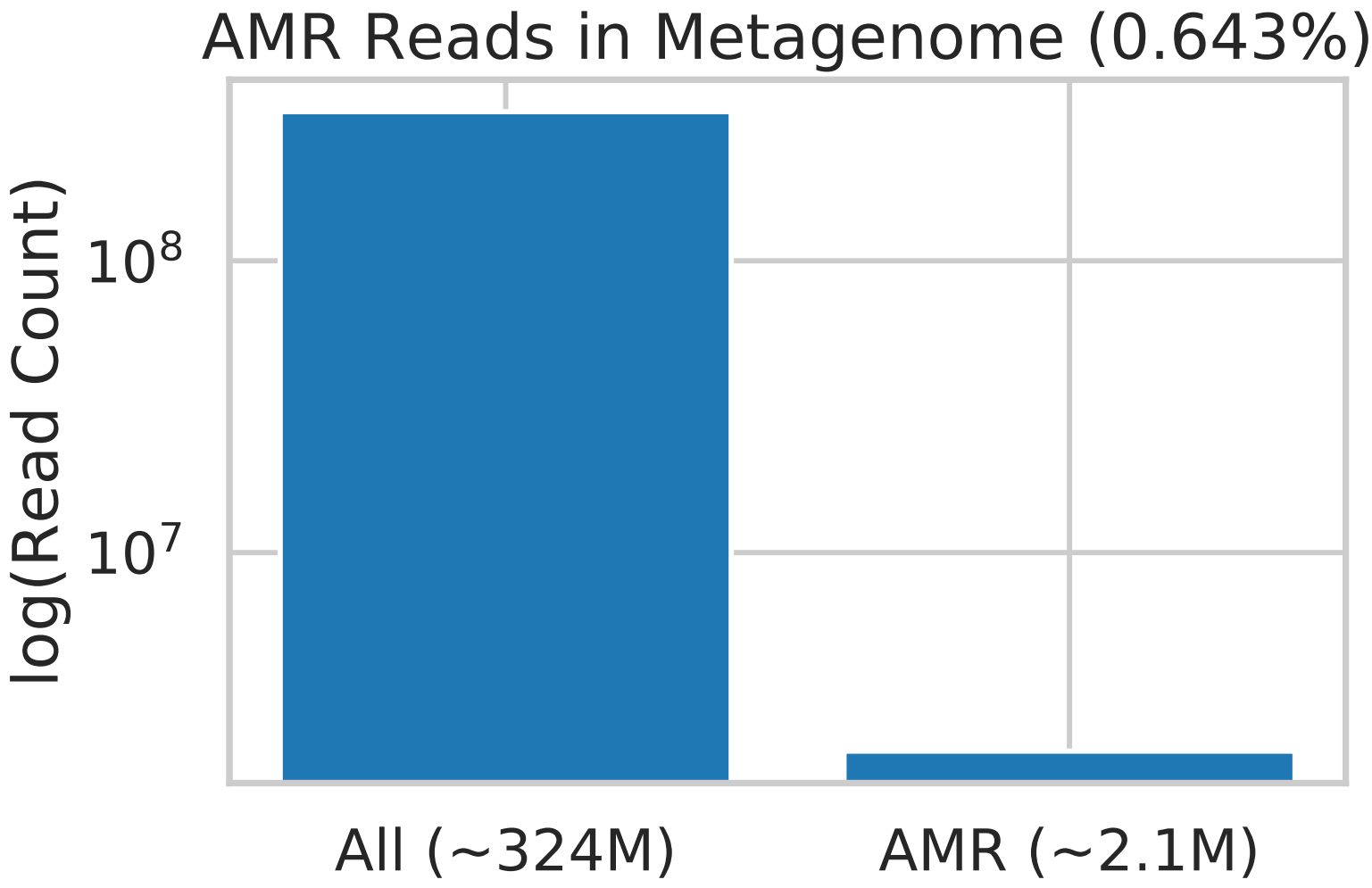
## cmlA1

Download Sequences

| | |
|---|---|
| Accession | ARO:3002693 |
| Definition | cmlA1 is a plasmid or transposon-encoded chloramphenicol exporter that is found in Pseudomonas aeruginosa and Klebsiella pneumoniae |
| AMR Gene Family | major facilitator superfamily (MFS) antibiotic efflux pump |
| Drug Class | phenicol antibiotic |
| Resistance Mechanism | antibiotic efflux |
| Efflux Component | efflux pump complex or subunit conferring antibiotic resistance |
| Classification | **7 ontology terms** \| Hide<br>+ process or component of antibiotic biology or chemistry<br>+ mechanism of antibiotic resistance<br>+ determinant of antibiotic resistance<br>+ antibiotic molecule<br>+ antibiotic efflux [Resistance Mechanism]<br>+ phenicol antibiotic [Drug Class]<br>+ efflux pump complex or subunit conferring antibiotic resistance [Efflux Component] |
| Parent Term(s) | **2 ontology terms** \| Hide<br>+ major facilitator superfamily (MFS) antibiotic efflux pump [AMR Gene Family]<br>+ *confers_resistance_to_drug* chloramphenicol [Antibiotic] |
| Publications | Bissonnette L, et al. 1991. J Bacteriol 173(14): 4493-4502. Characterization of |

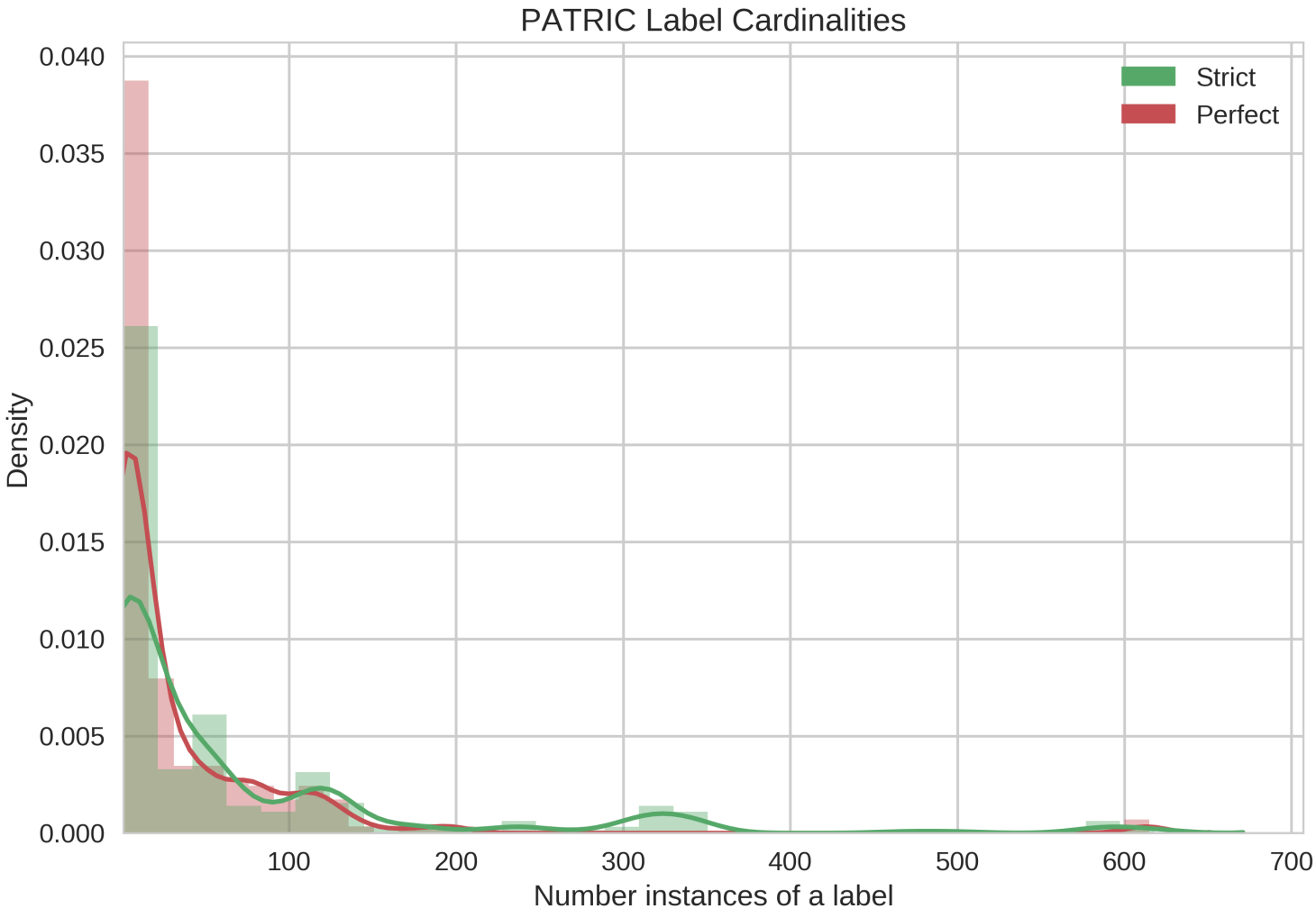
# Why is AMR metagenomics difficult?

# AMR genes are rare genomically



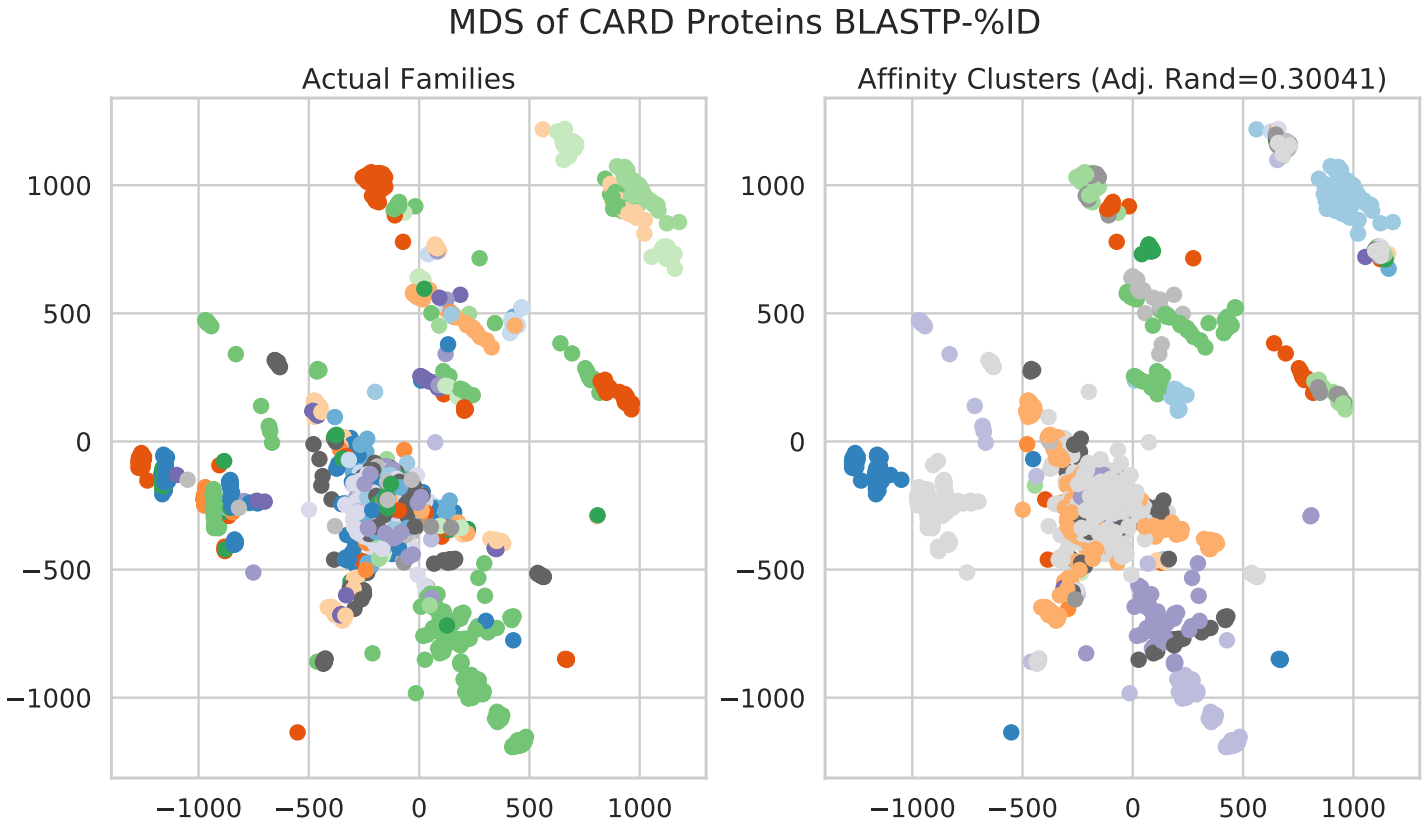

2184 CARD-Prevalence Genomes at 1-10X abundance

# AMR genes have wildly different abundances




1236 AMR PATRIC genomes

# AMR sequence space overlaps

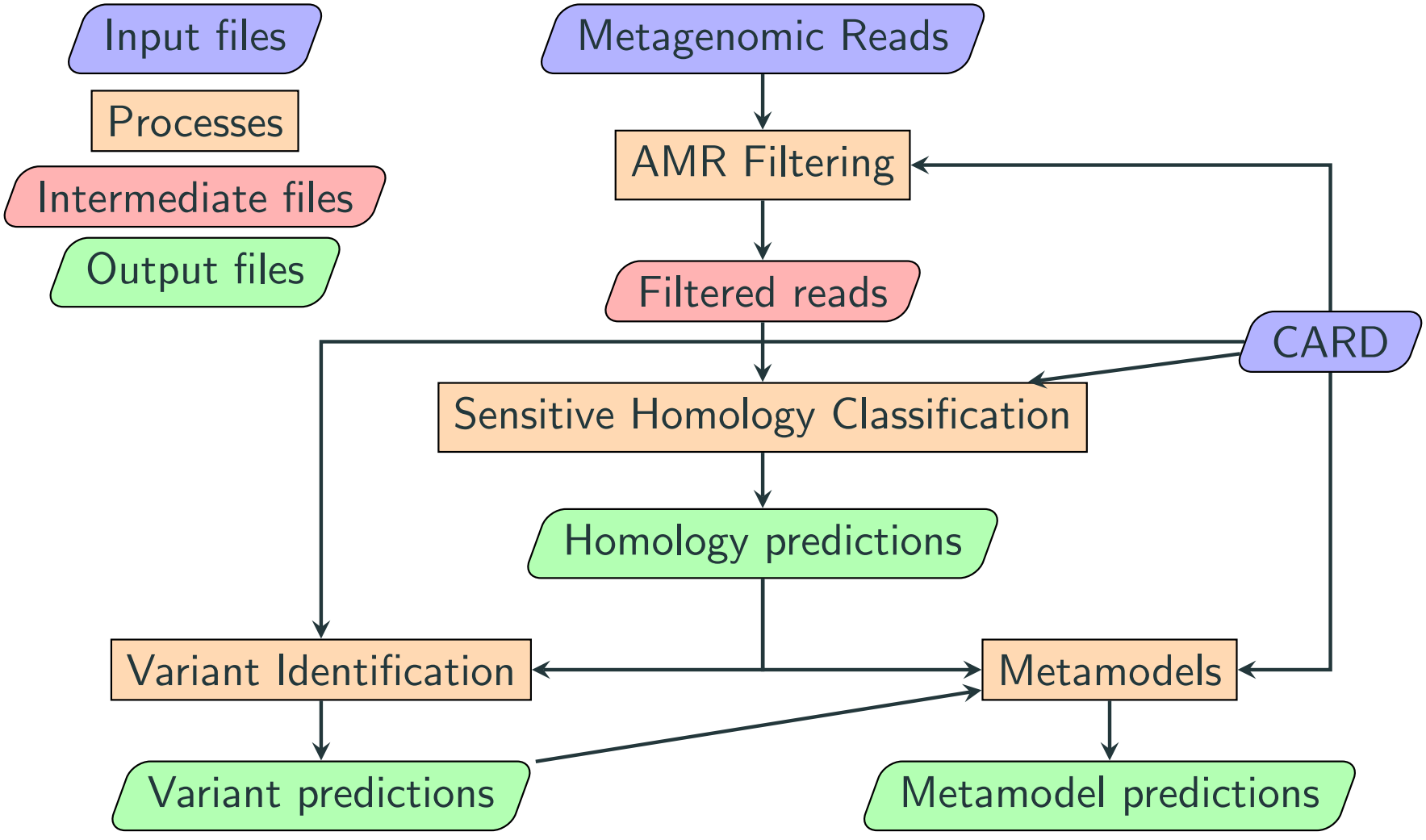


# Other constraints

- No point doing what we do if people can't use it.
- Limited hardware requirements (a standard workstation or instance $< 8 - 12$Gb, $1 - 8$ cores).
- Fast enough ($< 12$ hours).
- Easy to install/configure.
- Easy to use.
- Easy to update.

# AMRtime Overview

# AMRtime structure



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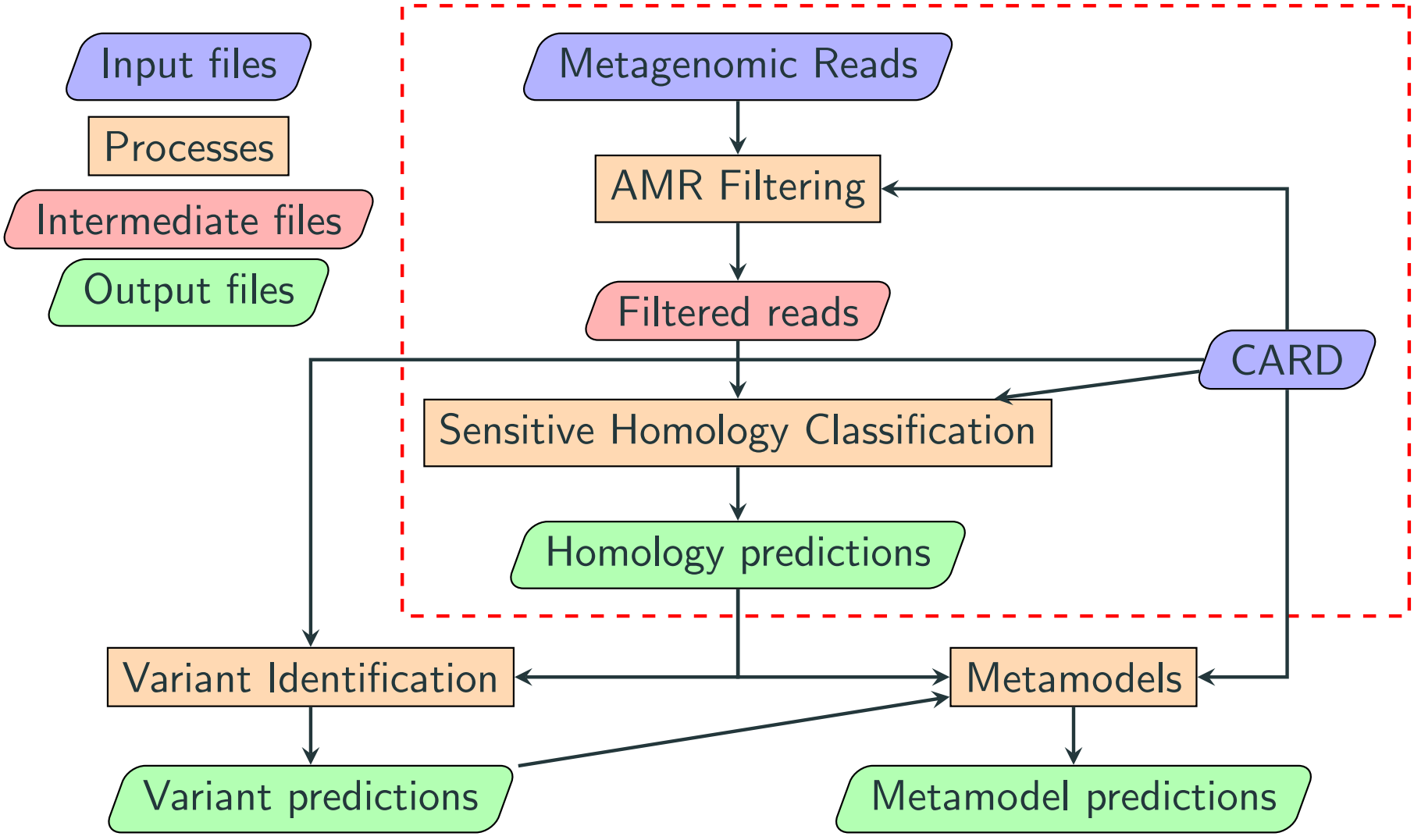

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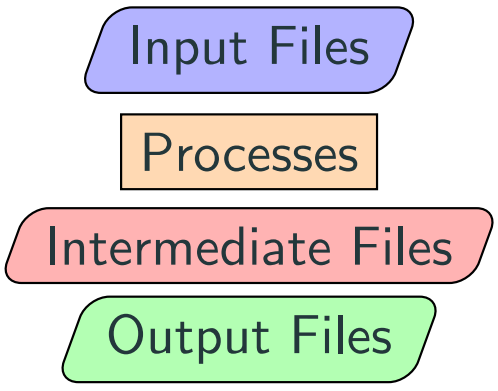
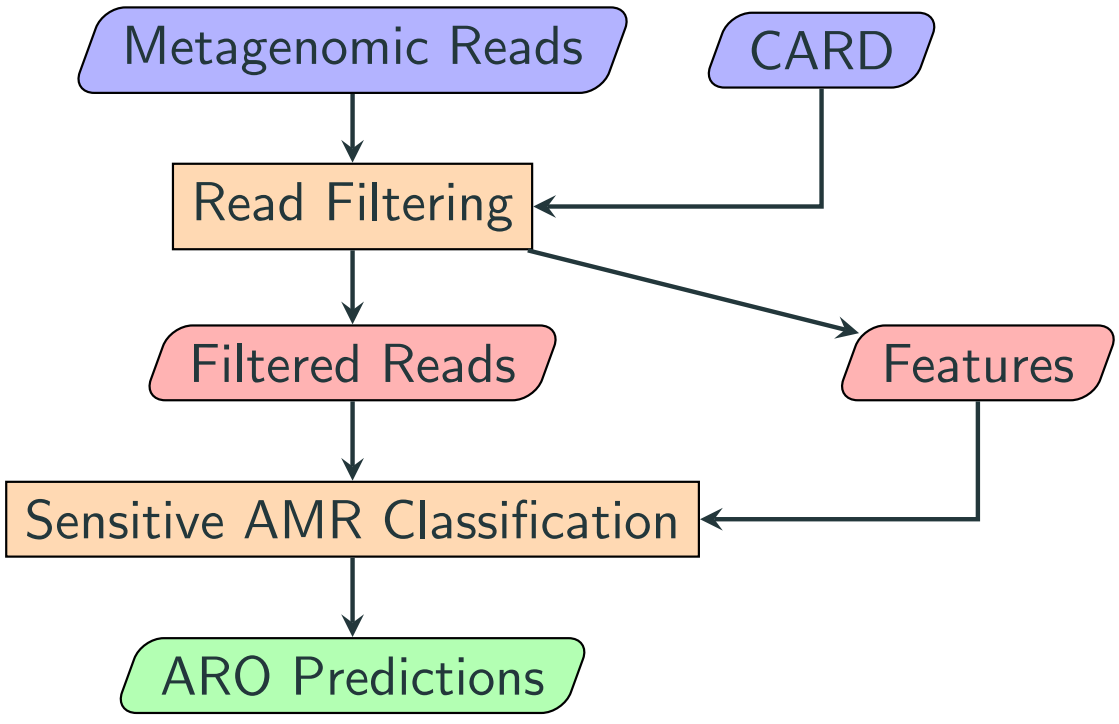
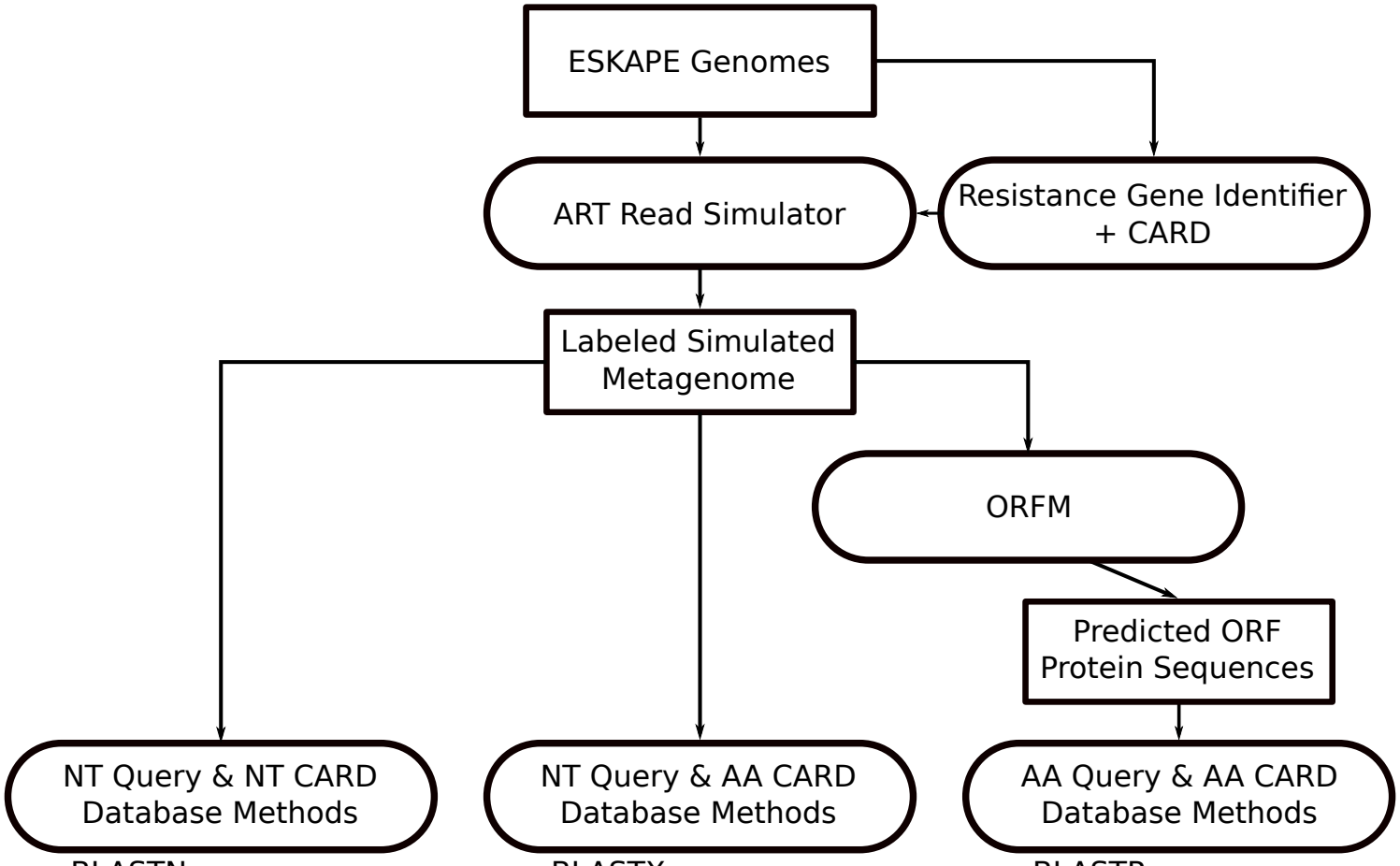


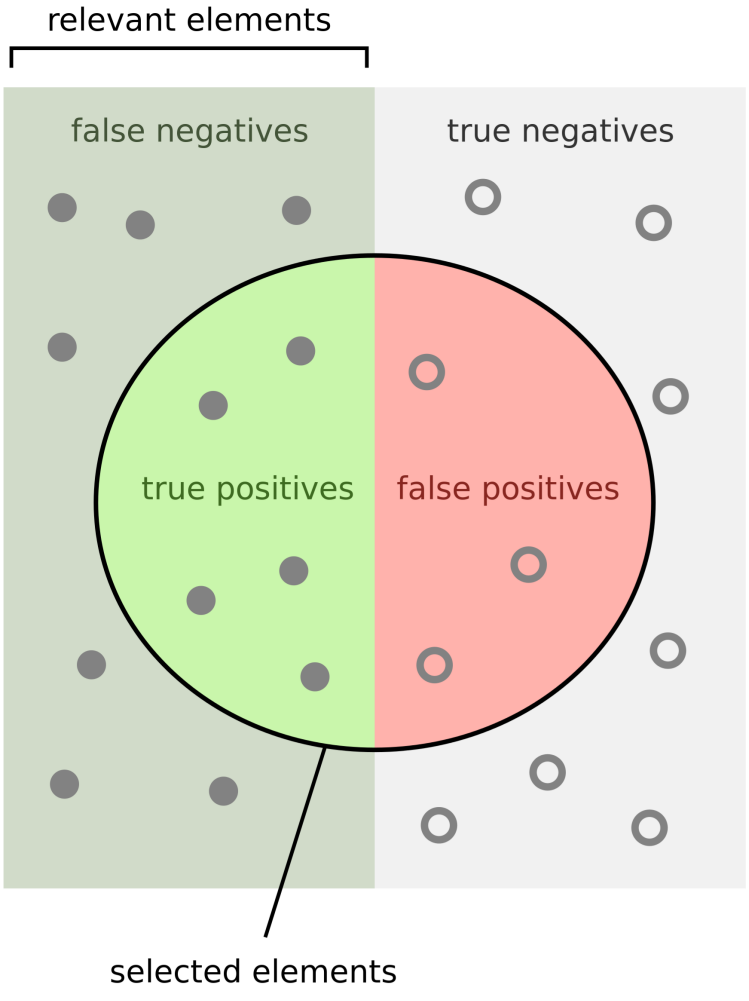



# Filtering out non-AMR reads

# Testing sequence similarity search tools



# Terminology refresher interlude




How many selected items are relevant?

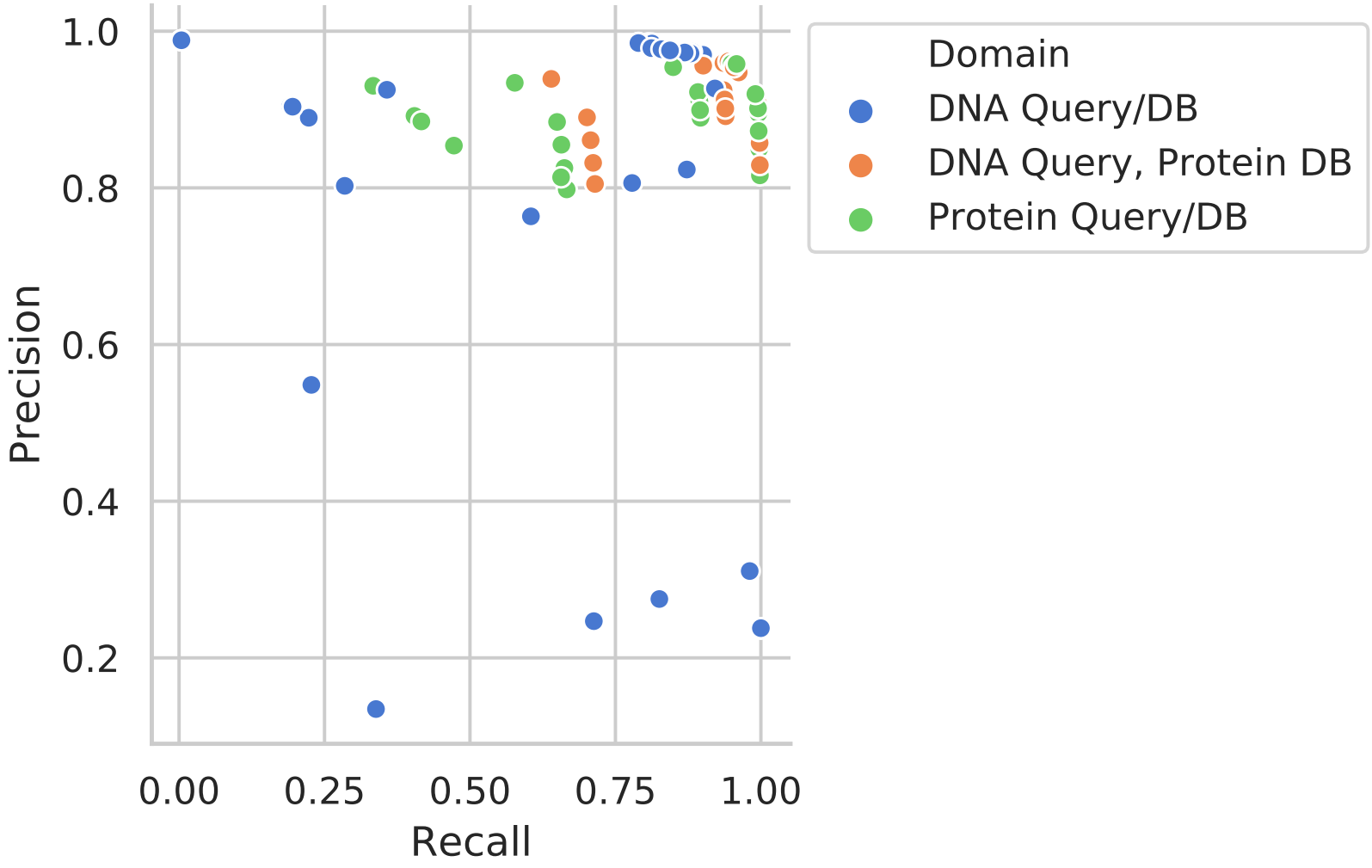
$$\text{Precision} = \frac{\text{true positives}}{\text{true positives} + \text{false positives}}$$

How many relevant items are selected?

$$\text{Recall} = \frac{\text{true positives}}{\text{true positives} + \text{false negatives}}$$

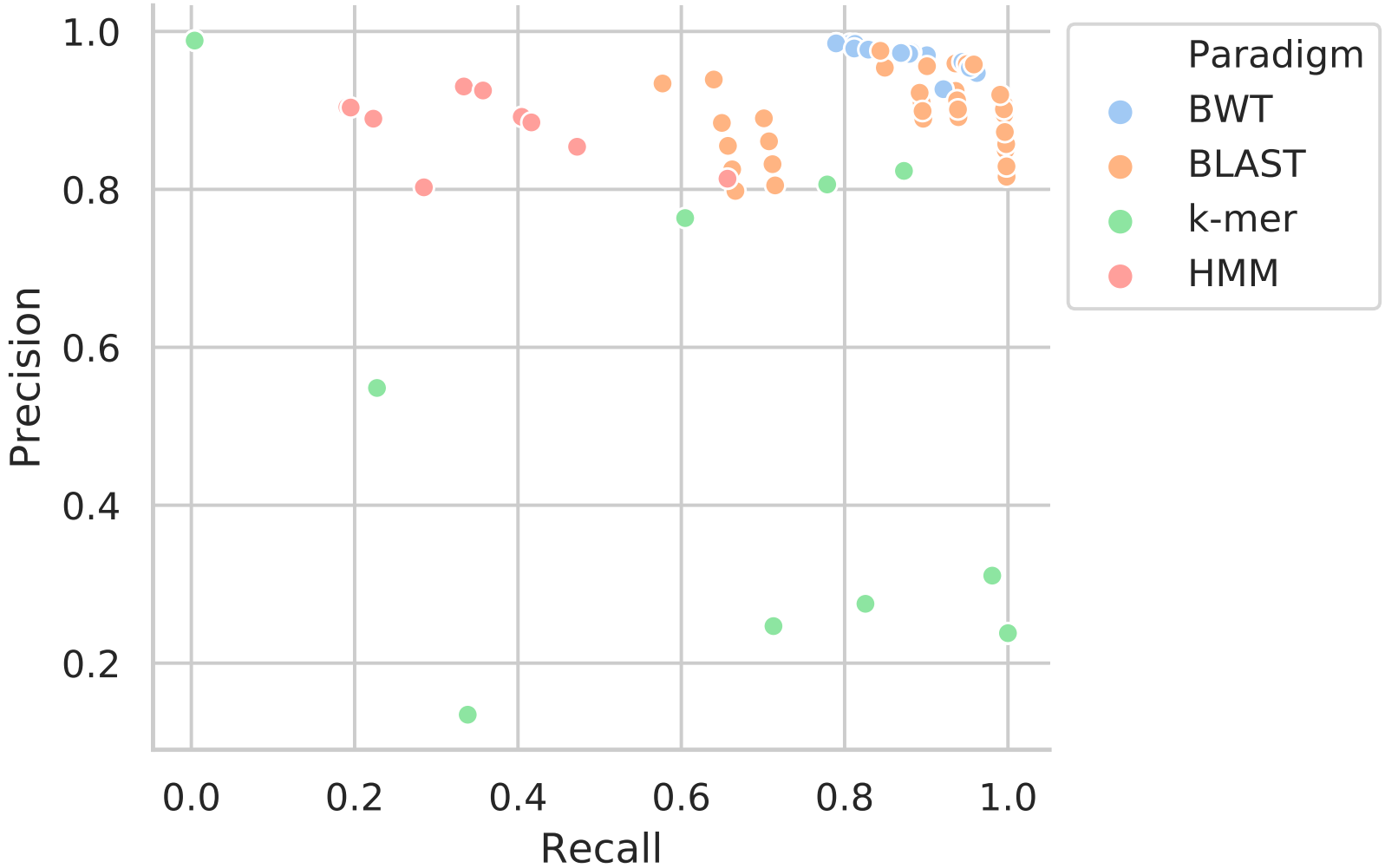
https://commons.wikimedia.org/wiki/File:Precisionrecall.svg

# DNA subject best for precision, Protein subject best for recall



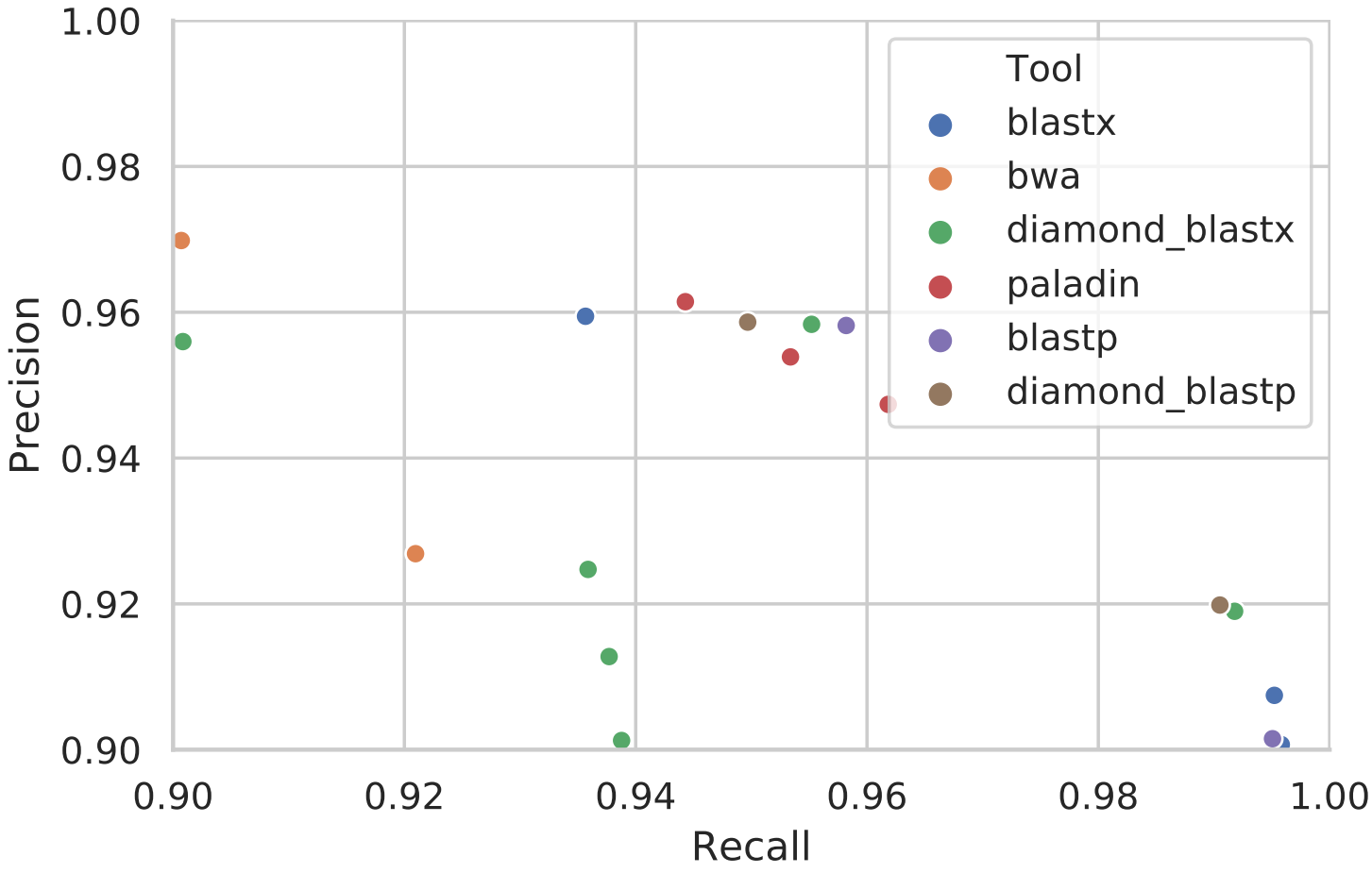

Simulated MiSeq v3 250bp reads, 30.31M reads (7.21M AMR derived)

# K-mer methods perform poorly




**BWT:** bowtie2, bwa-mem, paladin; **BLAST:** blast, diamond; **HMM:** hmmsearch; **K-MER:** biobloom, groot.

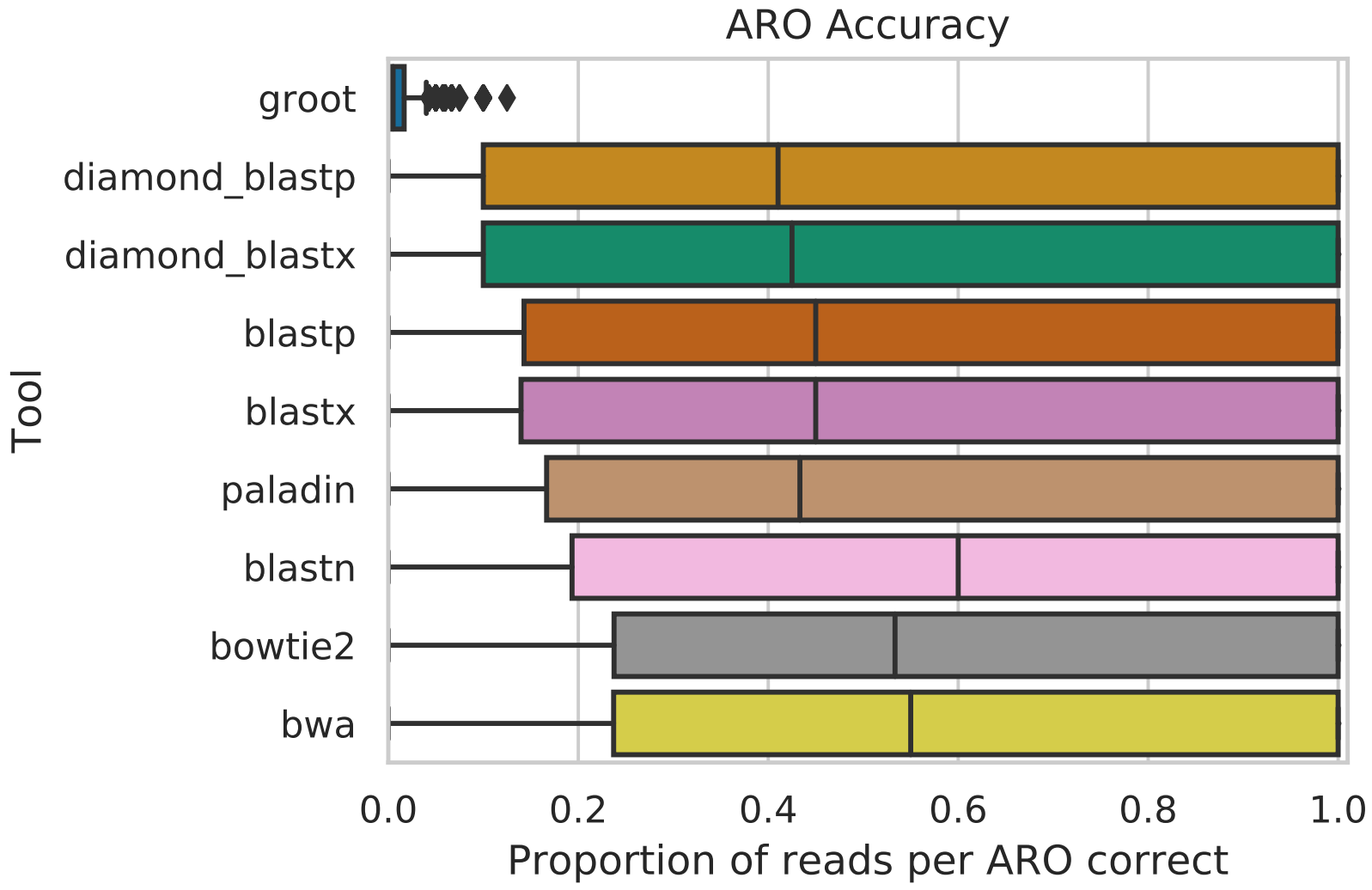
# DIAMOND-BLASTX best compromise




DIAMOND-BLASTX 'more sensitive' setting (min $< 1e^{-10}$): 4.926 hours with 2 cores and 8.3Gb of memory. AMR Reads: 7.15M detected, 59.26K missed, 1.87M false positives.

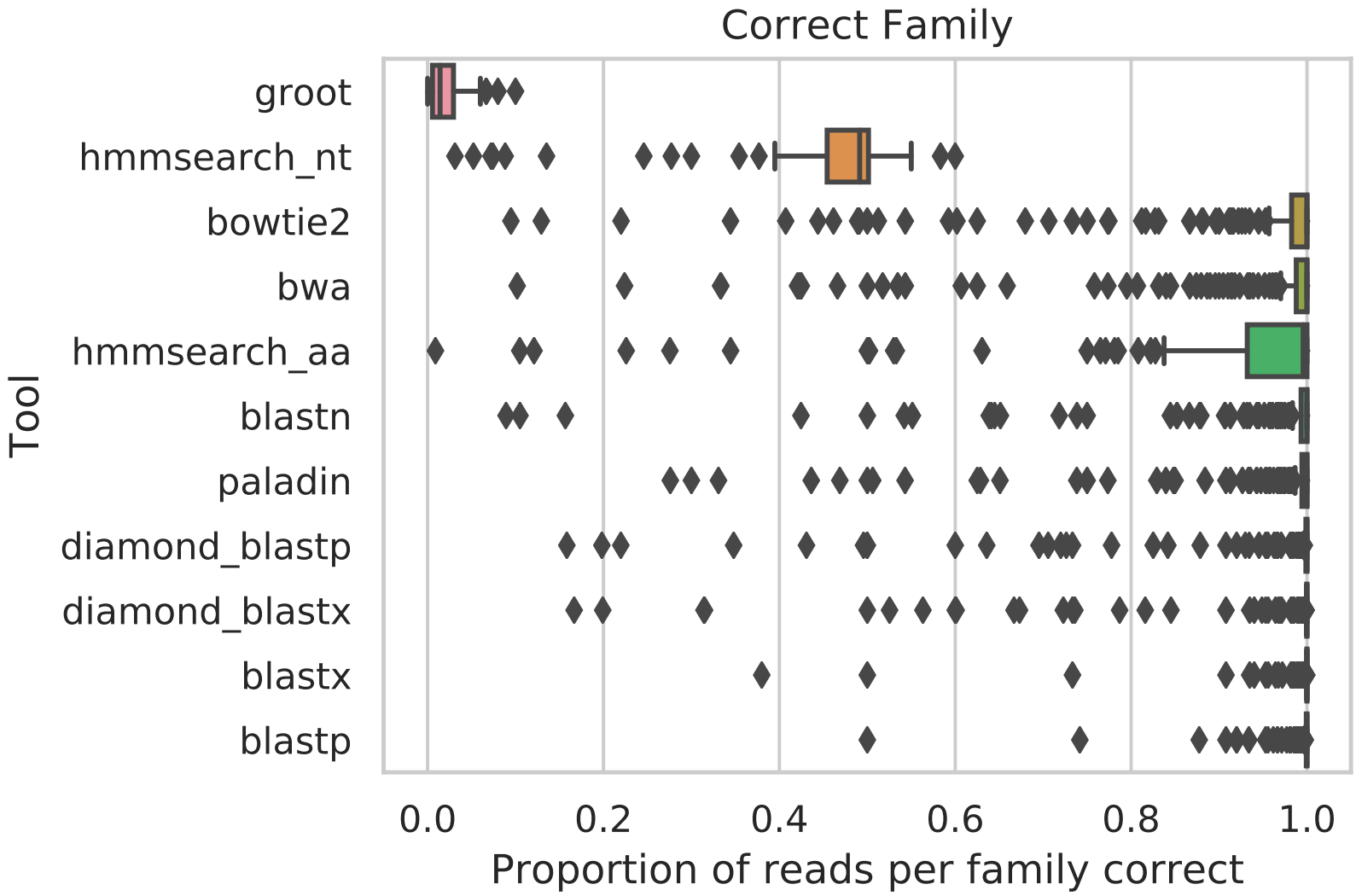
# Why not just use these sequence searches?

# Poor gene-level accuracy




Performance at optimal settings for ARO accuracy

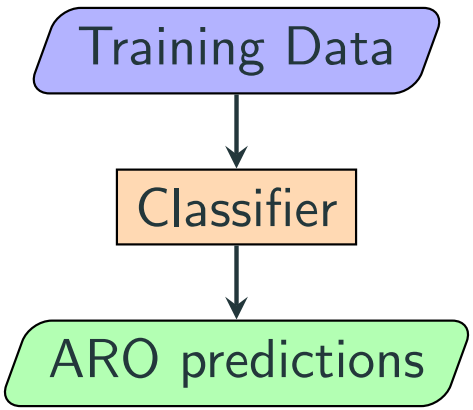
# Good family-level accuracy



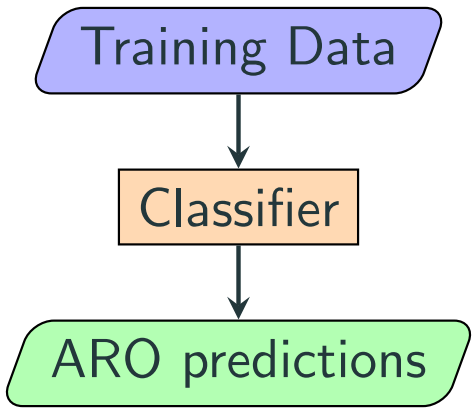

Performance at optimal settings for Family accuracy

# Sensitive Homology Classification

# Initial classifier

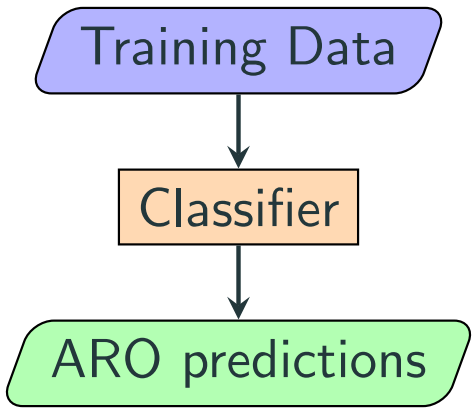


# Initial classifier




NB 7-mer Average Precision: 0.63

# Initial classifier

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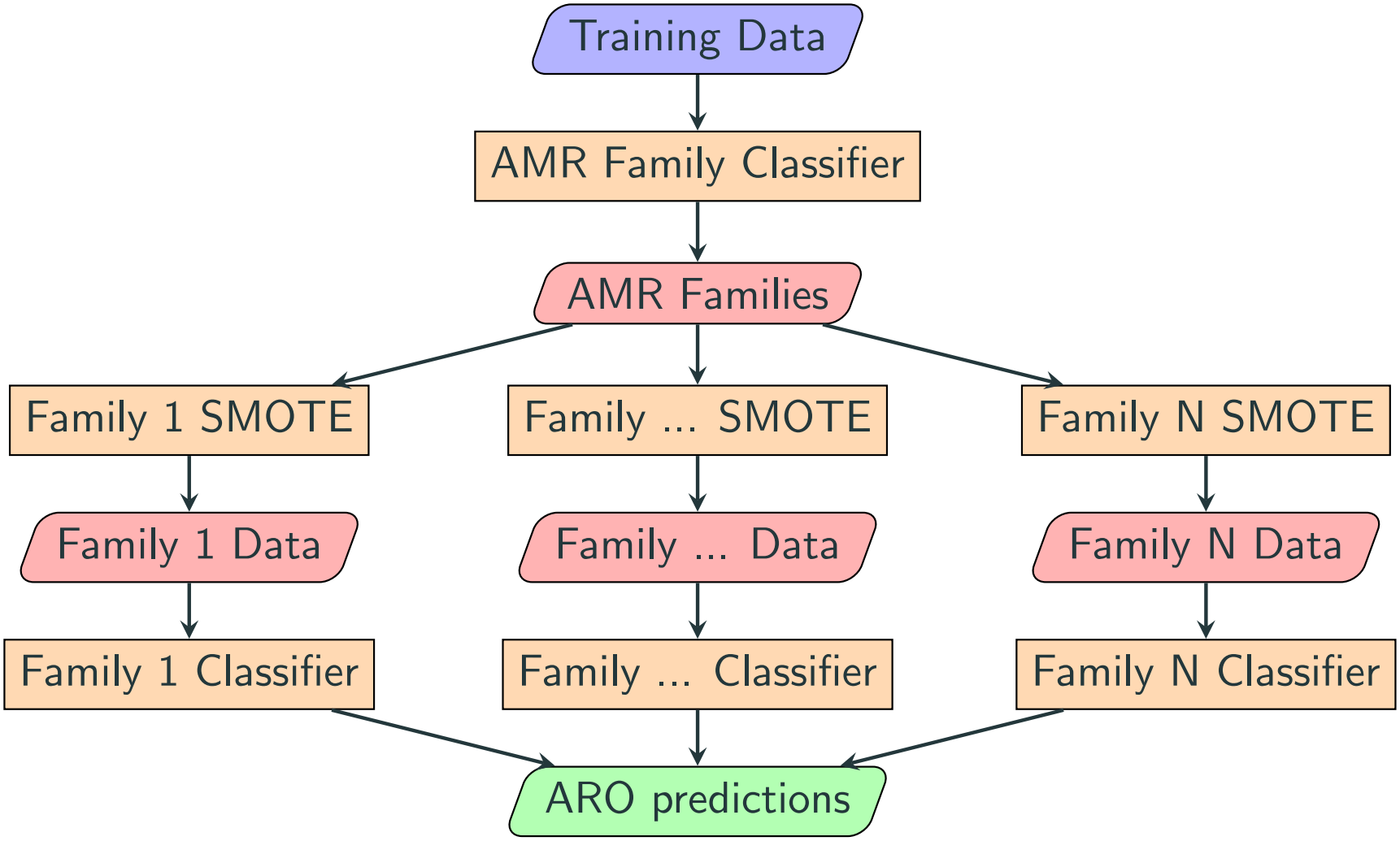
# Revised classifier structure: exploiting the ARO

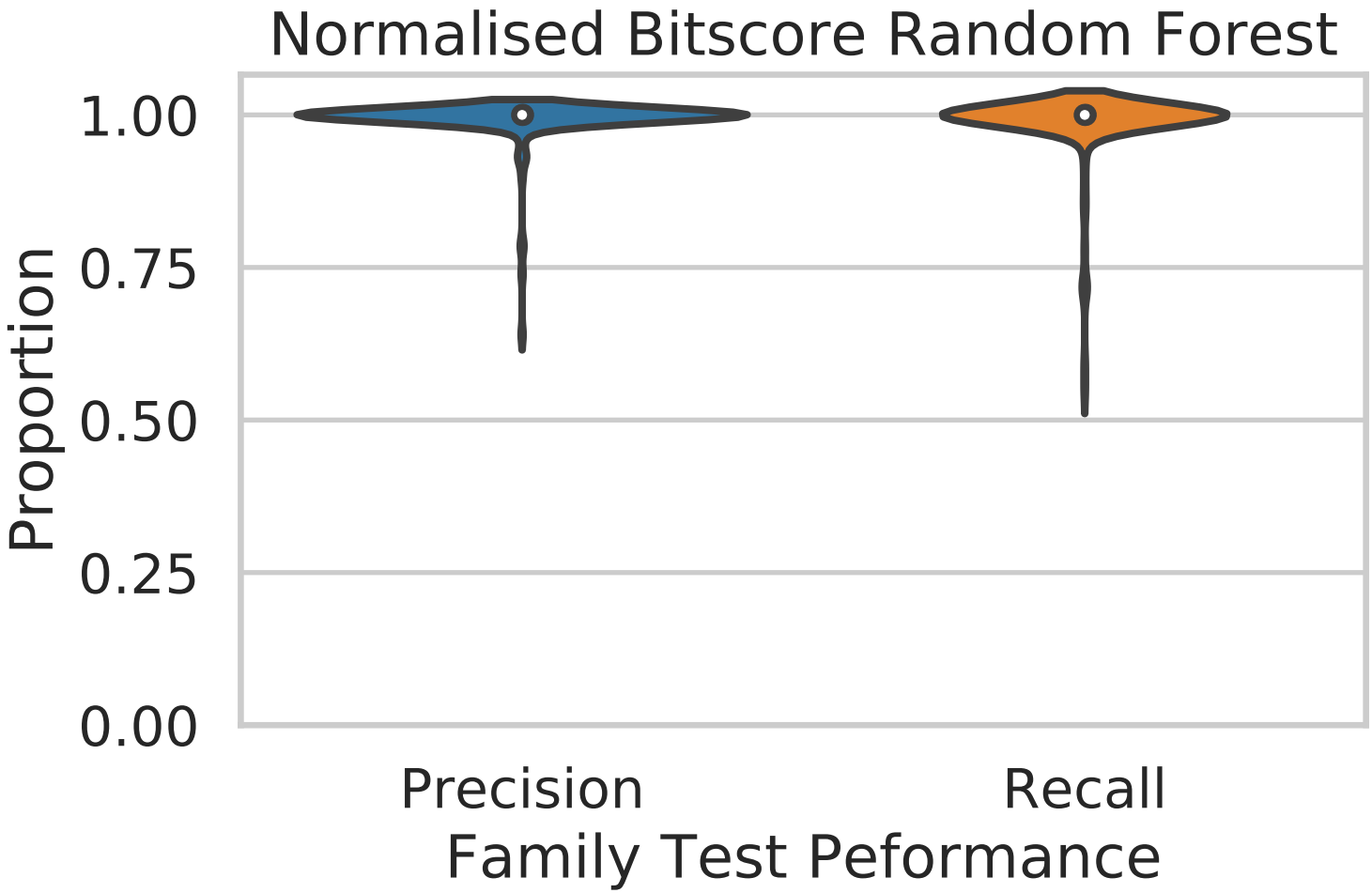


# Read encoding

$$\text{Sequence bitscore matrix} = \begin{array}{c} \\ read_1 \\ read_2 \\ \dots \\ read_{i-1} \\ read_i \end{array} \begin{array}{c} \begin{array}{ccccc} gene_1 & gene_2 & \dots & gene_{j-1} & gene_j \end{array} \\ \begin{pmatrix} 1256 & 0 & \dots & 0 & 63 \\ 0 & 0 & \dots & 0 & 0 \\ \dots & \dots & \dots & \dots & \dots \\ 0 & 512 & \dots & 0 & 0 \\ 0 & 0 & \dots & 785 & 129 \end{pmatrix} \end{array}$$

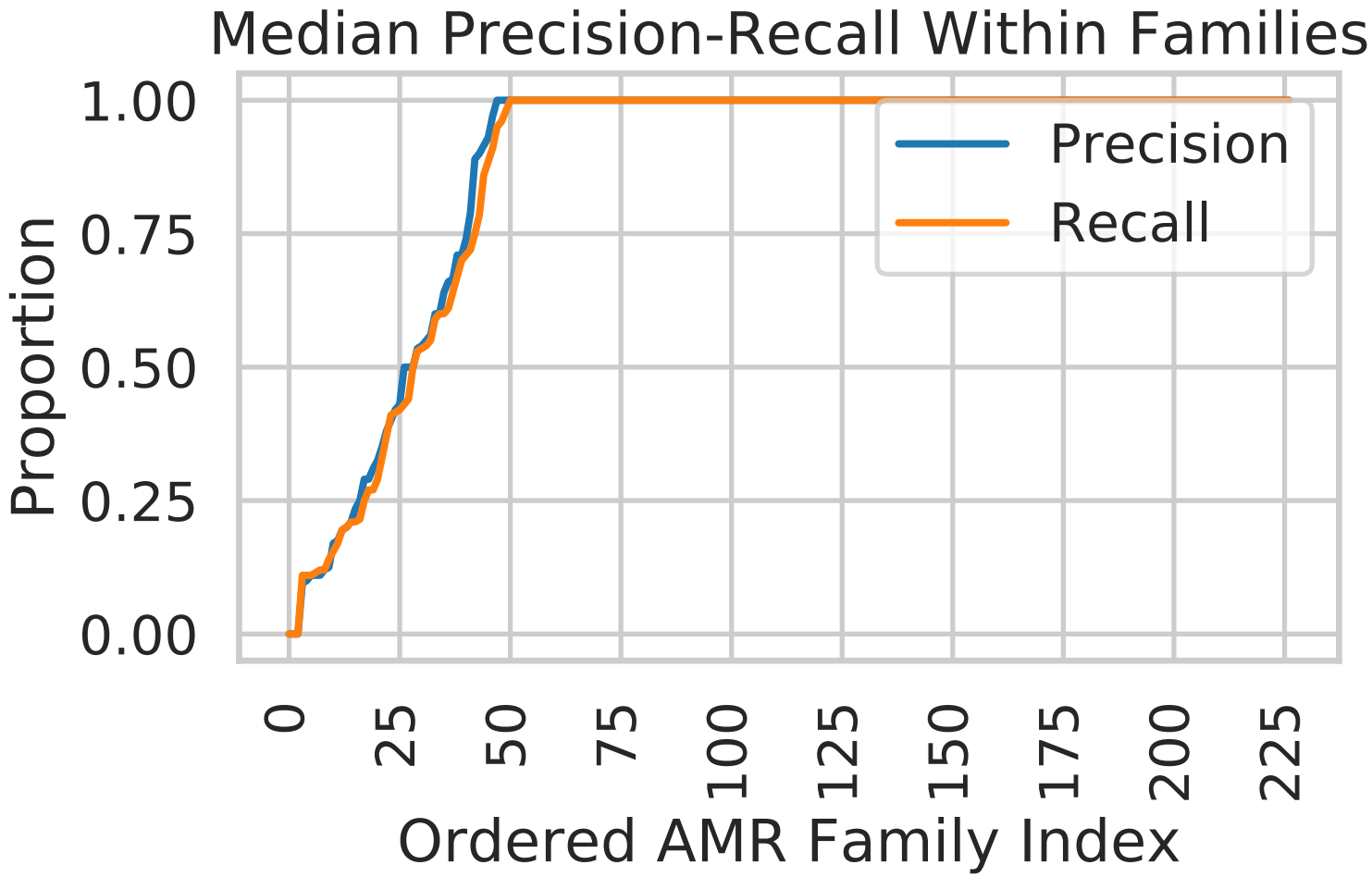
Advantages: read length invariant, low dimensionality, uses filtering data

# Held-out test results




Mean Precision: 0.995, Mean Recall: 0.985

# ARO level classification more variable



# On-going work

- Soft-threshold (i.e. propagating probabilities through layers)
- Multiset labels based on sequence redundancy within families.
- Threshold identification for variant model counts.
- Metamodel rule parsing.
- Galaxy bindings (CARD/IRIDA integration).

# Summary

# Conclusions

- Direct homology searches are suprisingly poor for AMR metagenomics.

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- The Antibiotic Resistance Ontology provides useful structure to improve predictions.

# Conclusions

- Direct homology searches are suprisingly poor for AMR metagenomics.
- K-mer based approaches fall flat with sequencing error, low coverage and sparse labels.
- Direct homology search results ARE useful when combined with machine learning.
- The Antibiotic Resistance Ontology provides useful structure to improve predictions.
- AMRtime: coming soon to CARD and your local government genomic epidemiology platform.

# Acknowledgements

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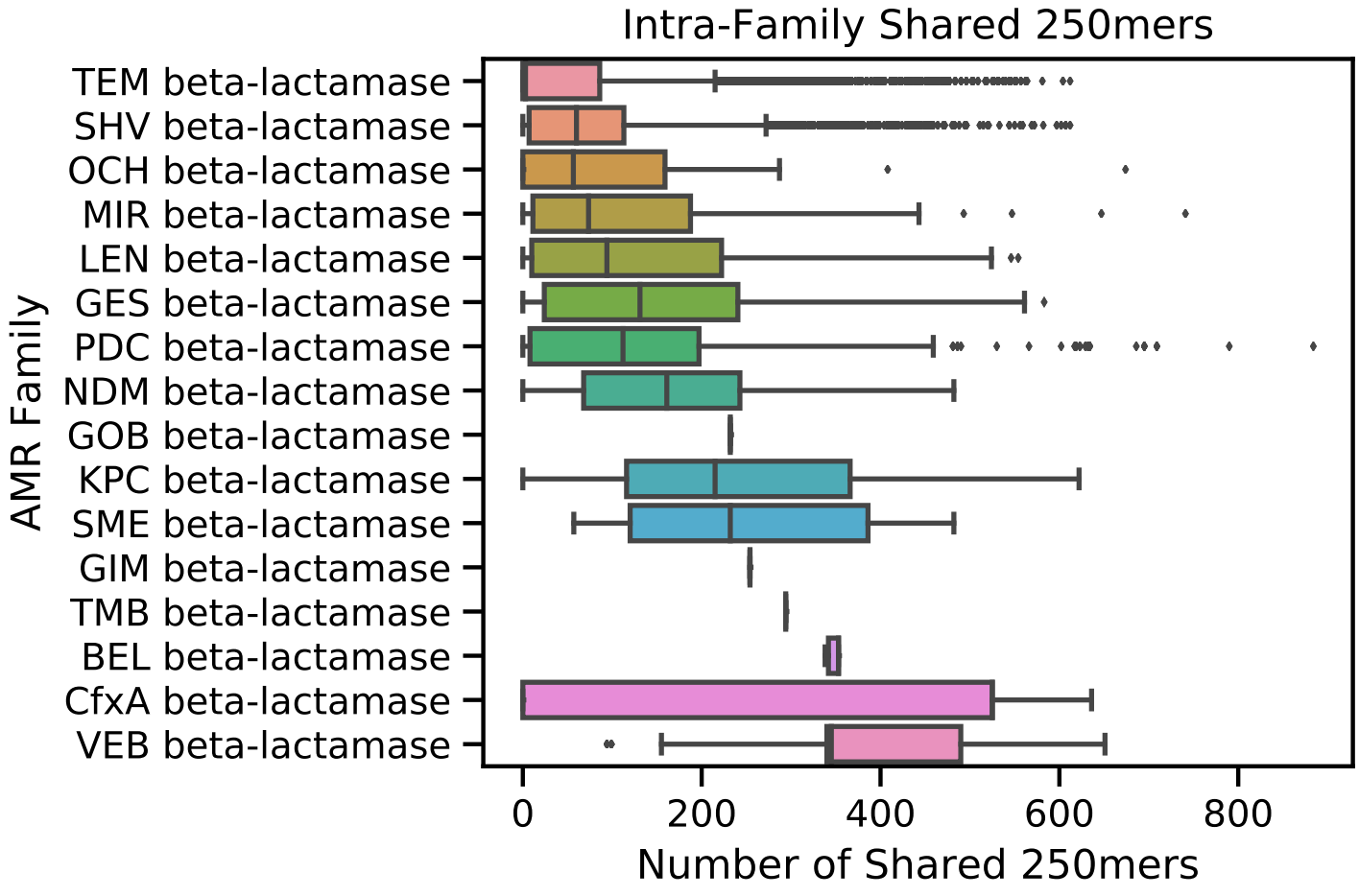
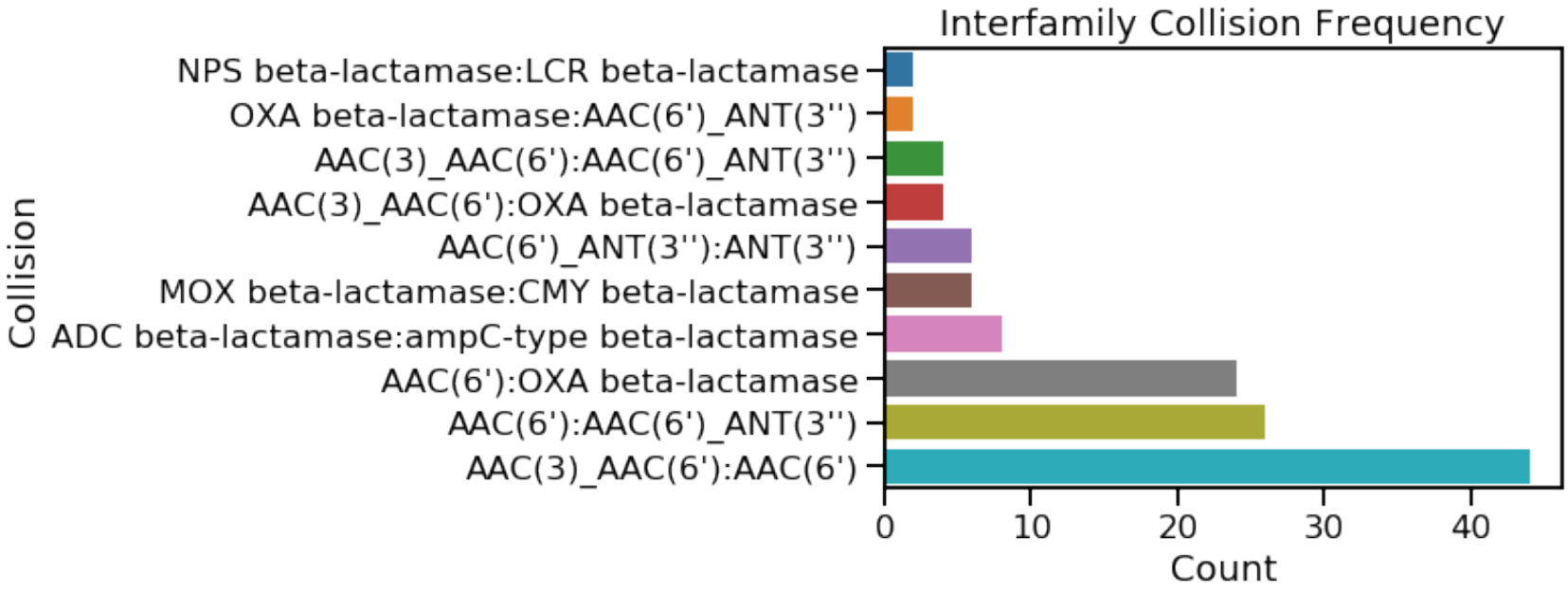
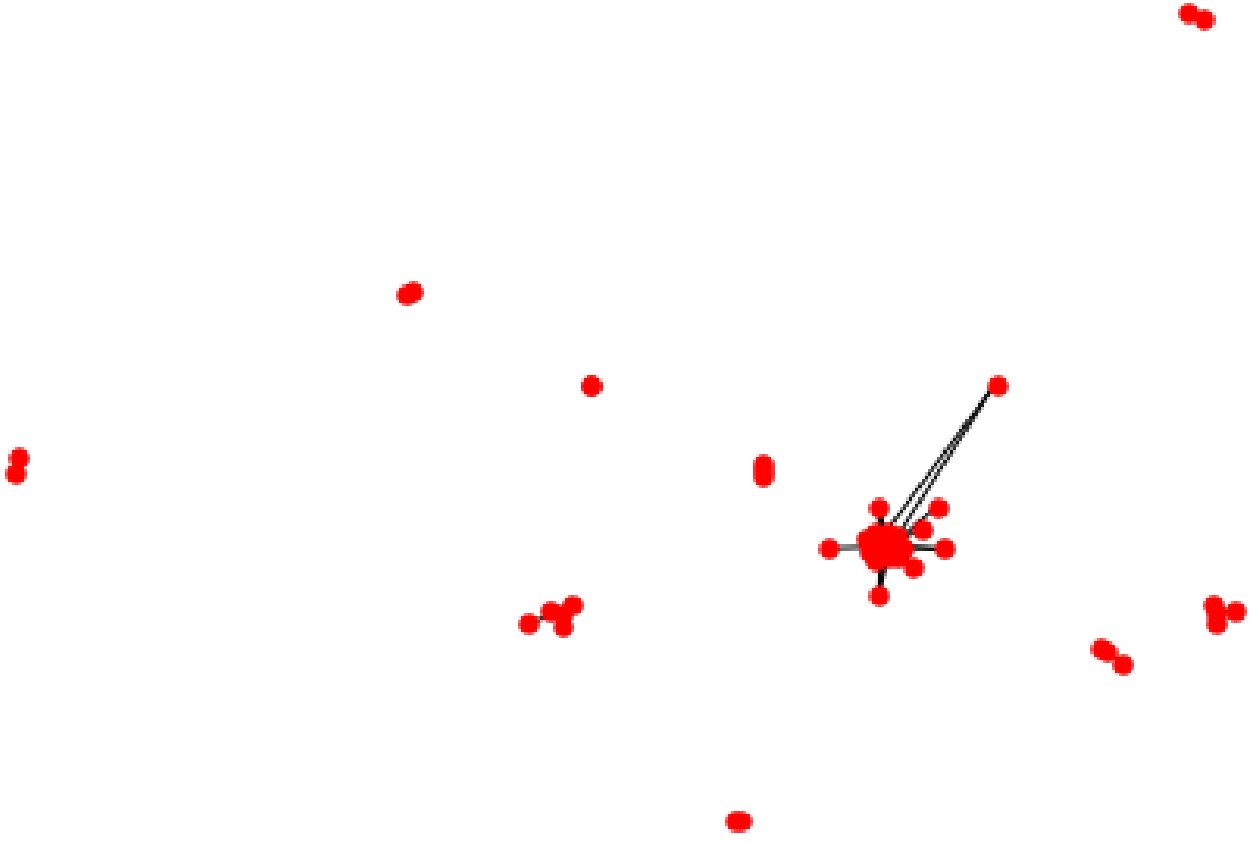
- McMaster University: Brian Alcock and Andrew McArthur
- Simon Fraser University: Fiona Brinkman
- Dalhousie University: Robert Beiko
- Funding: Donald Hill Family Fellowship, Genome Canada Grant.

# Questions?

# Insufficient Intrafamily Signal



# Interfamily Collisions



# Interfamily Collisions