L-EasyARG: A Long-read Metagenomics Tool for Rapid Antibiotic-Resistance-Gene Profiling

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Introduction

Emergence and Hazards of ARG

- Antibiotic resistance (AMR) is listed by WHO as a major global health threat.
- Widespread antibiotic use increases environmental concentrations, exerting selective pressure on bacteria.
- Bacteria can spread ARGs via vertical transmission and HGT

Advantages of Long Reads

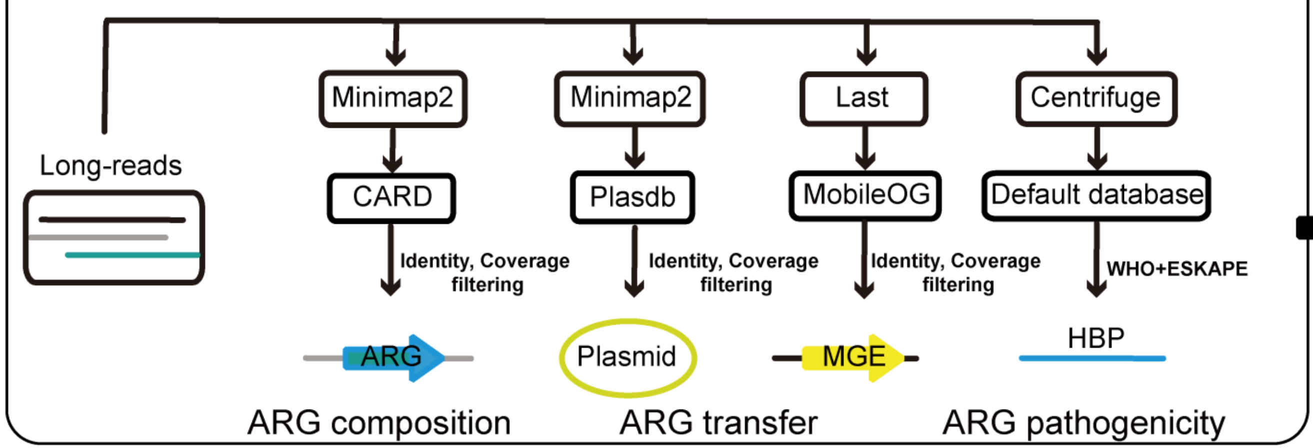
- Short-read assembly is computationally intensive.
- Fragmented assembly from short reads makes it difficult to link ARGs to their host organisms.
- Long reads generated by Nanopore and PacBio sequencing require no assembly, thus avoiding assembly-related issues.

Limitations of Common Tools

- DeepARG only identifies ARGs, with no further analysis; ARGpore2 lacks visualization output; NanoARG has outdated databases and no local version.
- There is a lack of systematic threshold optimization, tool performance benchmarking, and evaluation of error-rate impacts.

Highlights

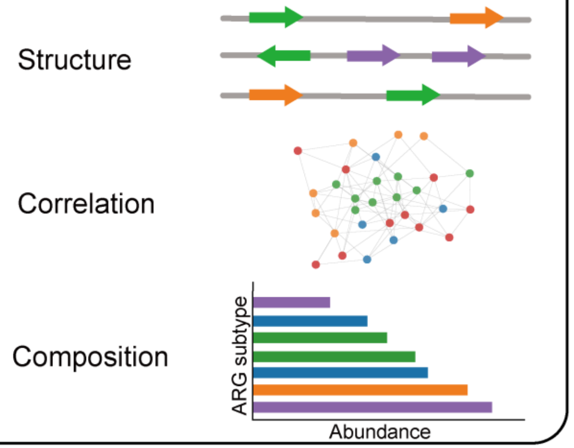
Workflow



Long-reads
Minimap2 → CARD → Identity, Coverage filtering → ARG
Minimap2 → Plasdb → Identity, Coverage filtering → Plasmid
Last → MobileOG → Identity, Coverage filtering → MGE
Centrifuge → Default database → WHO+ESKAPE → HBP

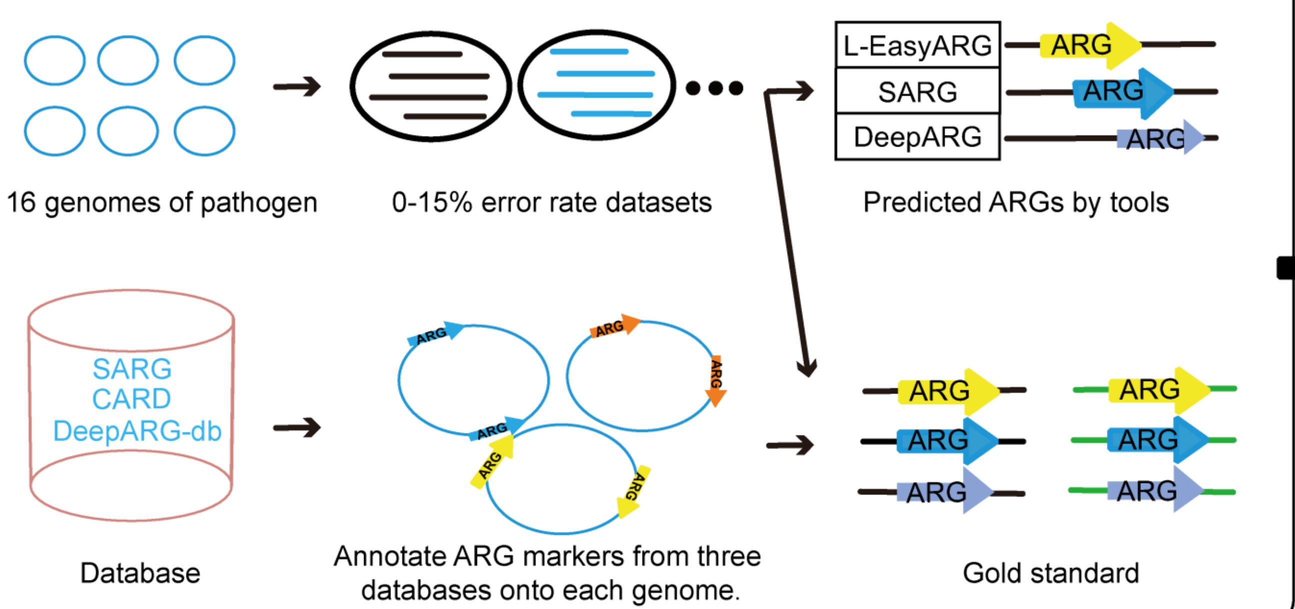
ARG composition
ARG transfer
ARG pathogenicity

Visualization



Structure
Correlation
Composition

Benchmarking



16 genomes of pathogen
0-15% error rate datasets
Predicted ARGs by tools

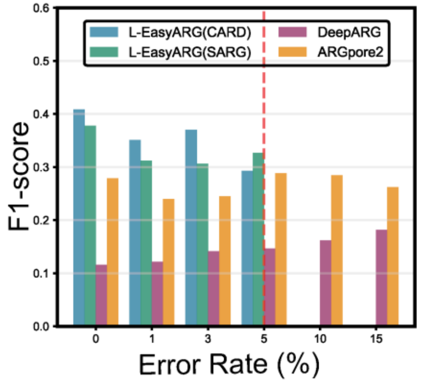
L-EasyARG
SARG
DeepARG

Database

Annotate ARG markers from three databases onto each genome.

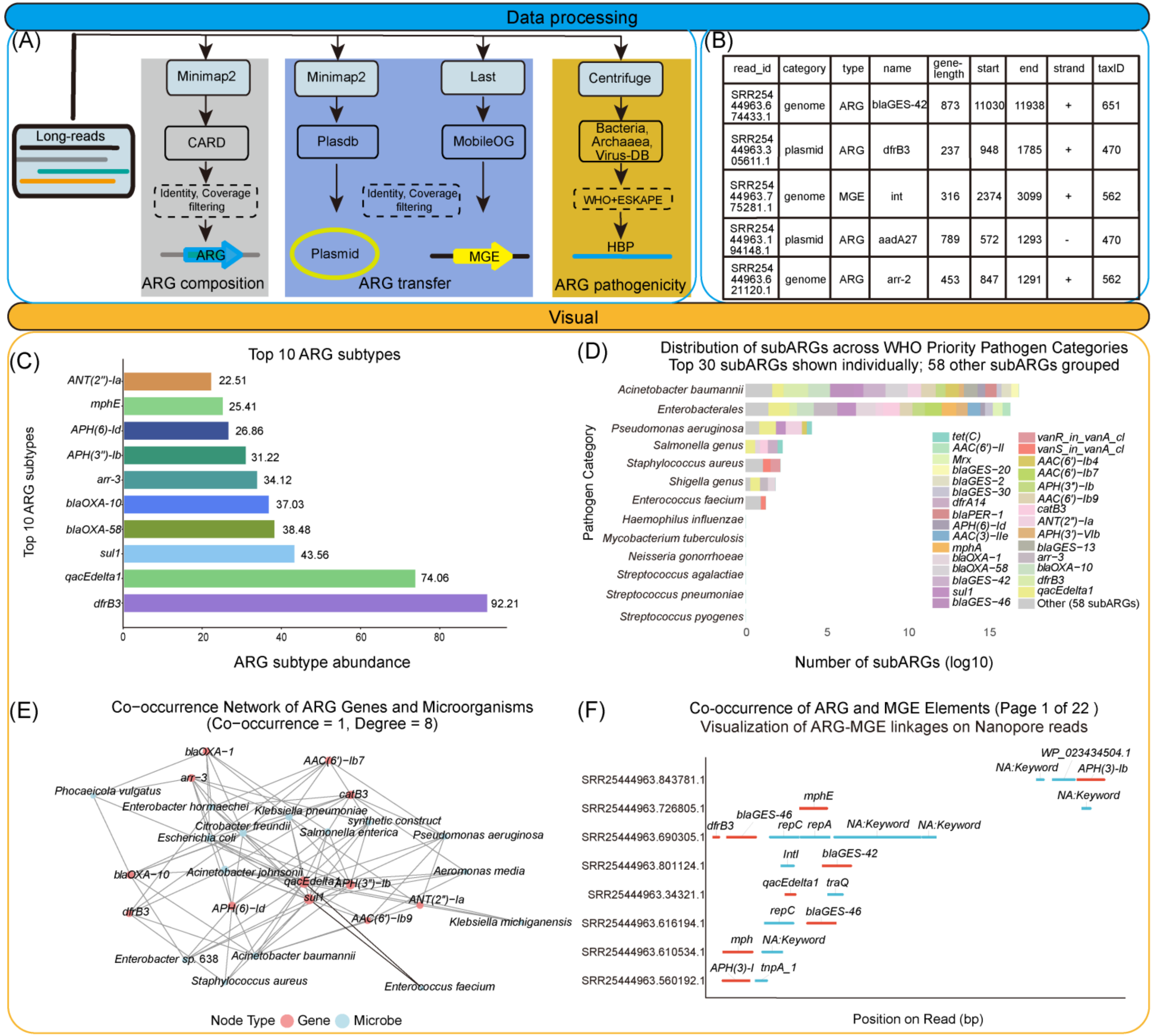
Gold standard

Benchmarking results



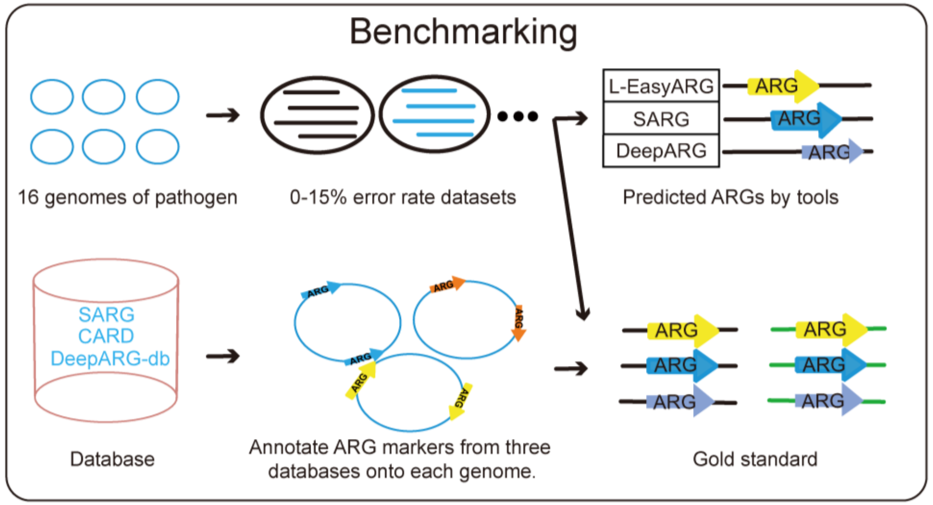
✓ Optimal for 0-5% error rate data
✓ Identifies ARG genetic context
✓ Local execution
✓ Validated on real dataset

Workflow of L-EasyARG



read_id	category	type	name	gene-length	start	end	strand	taxID
SRR25444963.674433.1	genome	ARG	blaGES-42	873	11030	11938	+	651
SRR25444963.305611.1	plasmid	ARG	dfrB3	237	948	1785	+	470
SRR25444963.775281.1	genome	MGE	int	316	2374	3099	+	562
SRR25444963.194148.1	plasmid	ARG	aadA27	789	572	1293	-	470
SRR25444963.621120.1	genome	ARG	arr-2	453	847	1291	+	562

Benchmarking analysis



Benchmarking

16 genomes of pathogen

0-15% error rate datasets

Predicted ARGs by tools

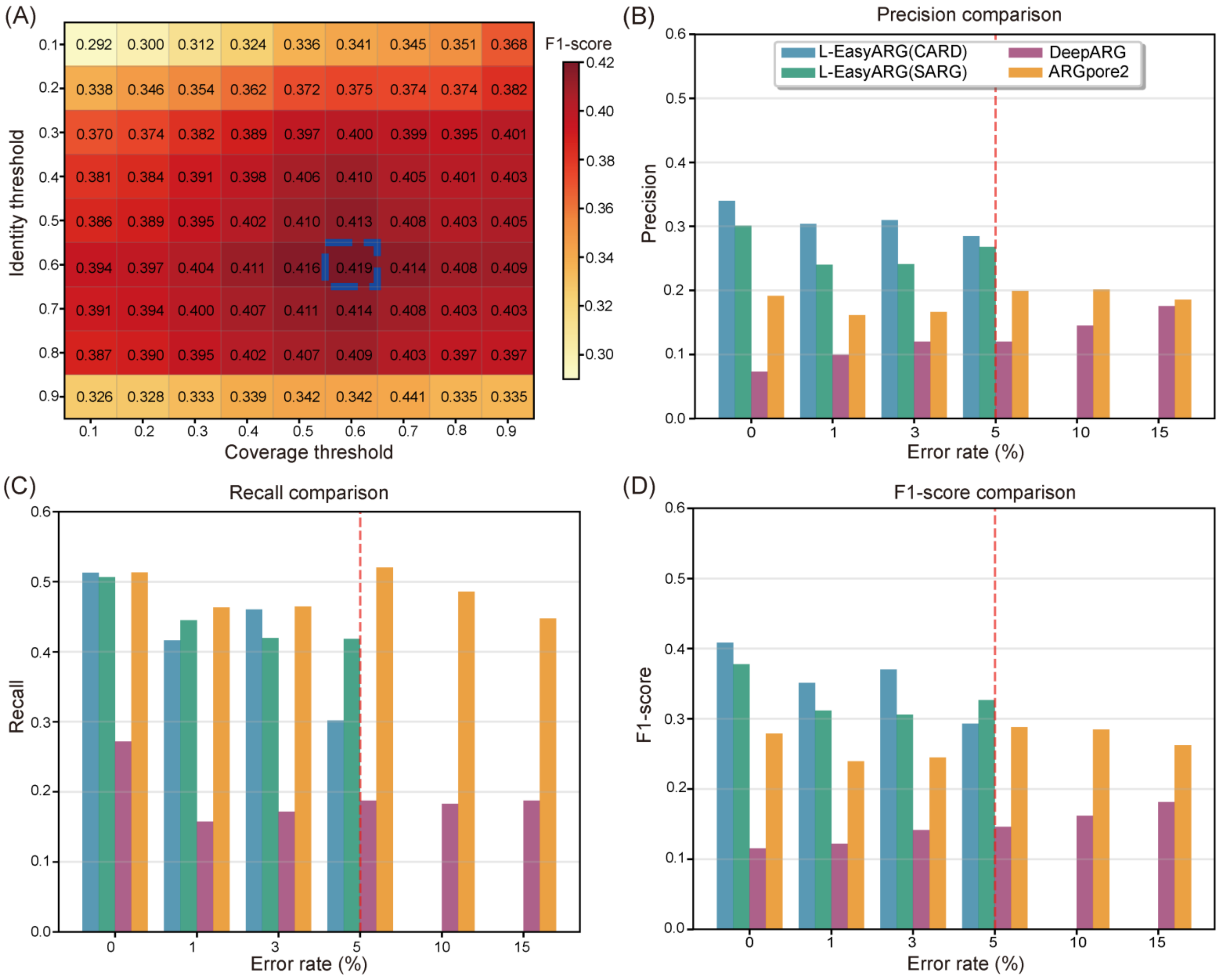
L-EasyARG
SARG
DeepARG

SARG
CARD
DeepARG-db

Database

Annotate ARG markers from three databases onto each genome.

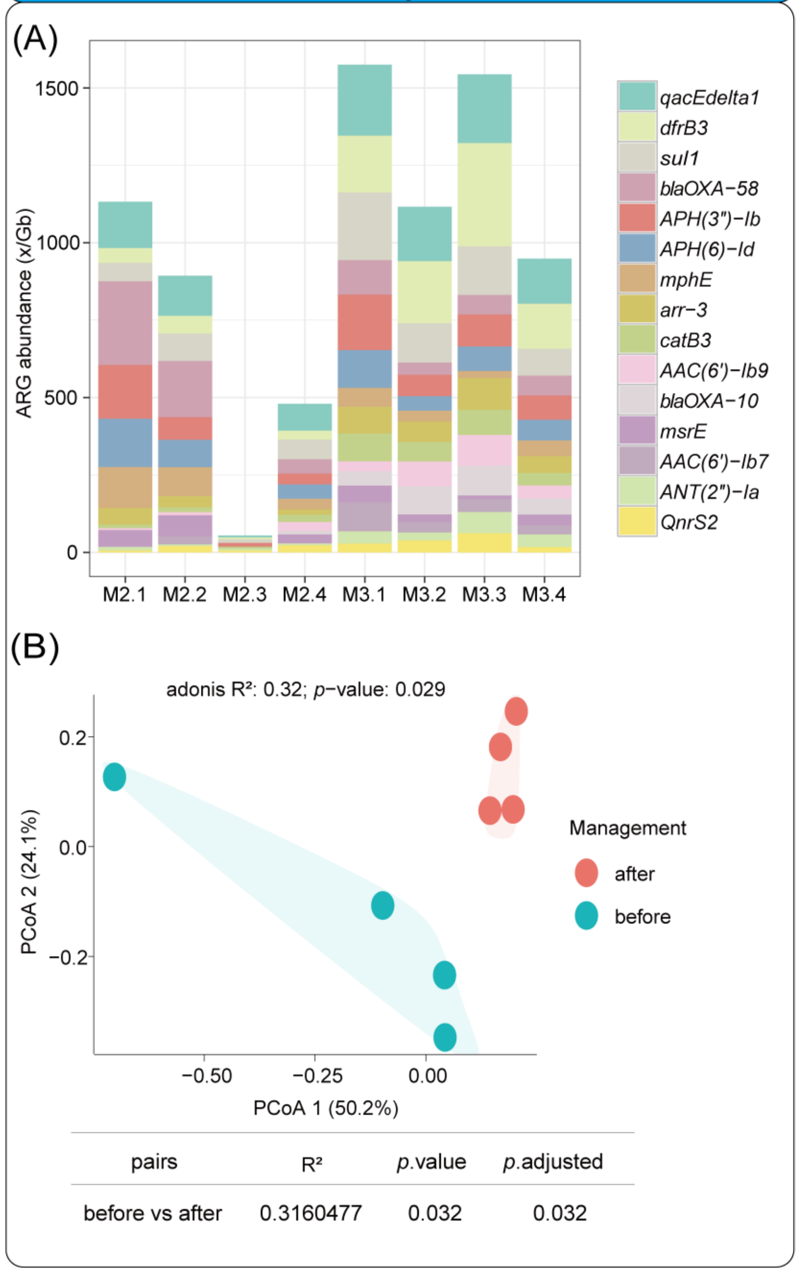
Gold standard



Identity threshold \ Coverage threshold	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0.1	0.292	0.300	0.312	0.324	0.336	0.341	0.345	0.351	0.368
0.2	0.338	0.346	0.354	0.362	0.372	0.375	0.374	0.374	0.382
0.3	0.370	0.374	0.382	0.389	0.397	0.400	0.399	0.395	0.401
0.4	0.381	0.384	0.391	0.398	0.406	0.410	0.405	0.401	0.403
0.5	0.386	0.389	0.395	0.402	0.410	0.413	0.408	0.403	0.405
0.6	0.394	0.397	0.404	0.411	0.416	0.419	0.414	0.408	0.409
0.7	0.391	0.394	0.400	0.407	0.411	0.414	0.408	0.403	0.403
0.8	0.387	0.390	0.395	0.402	0.407	0.409	0.403	0.397	0.397
0.9	0.326	0.328	0.333	0.339	0.342	0.342	0.441	0.335	0.335

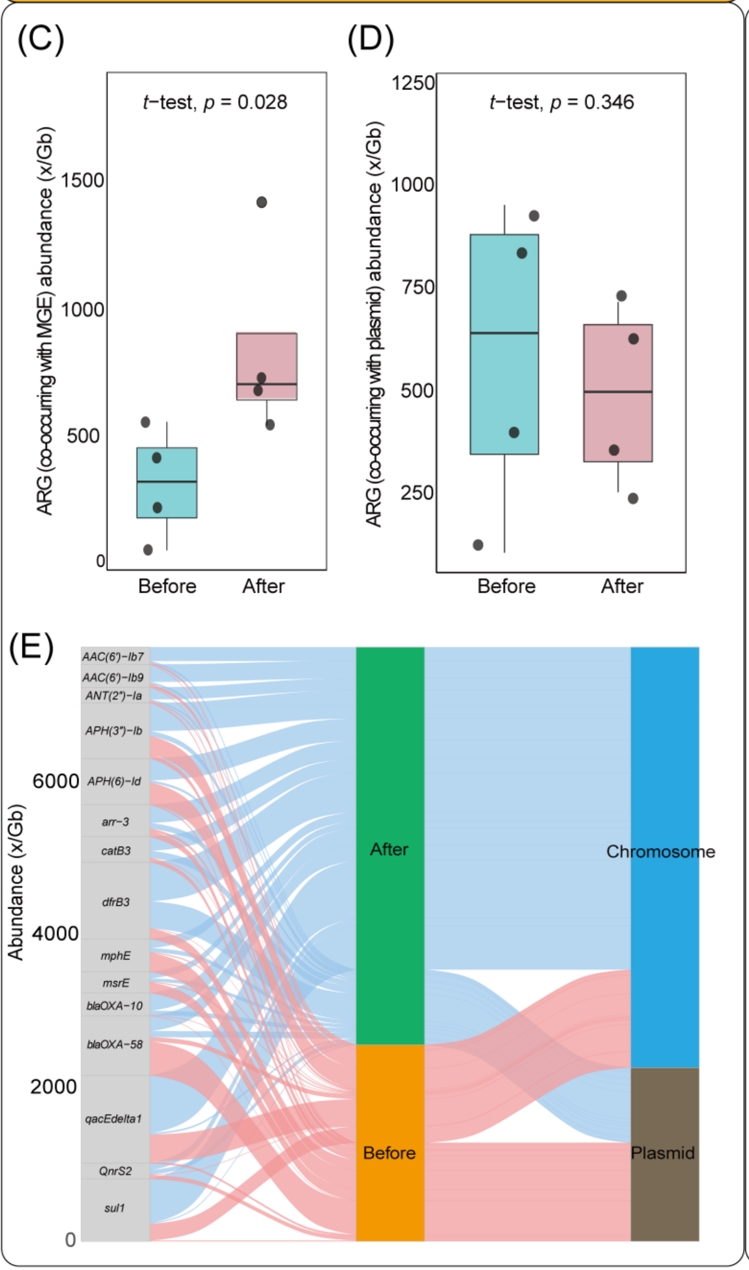
Application to environmental samples

ARG composition

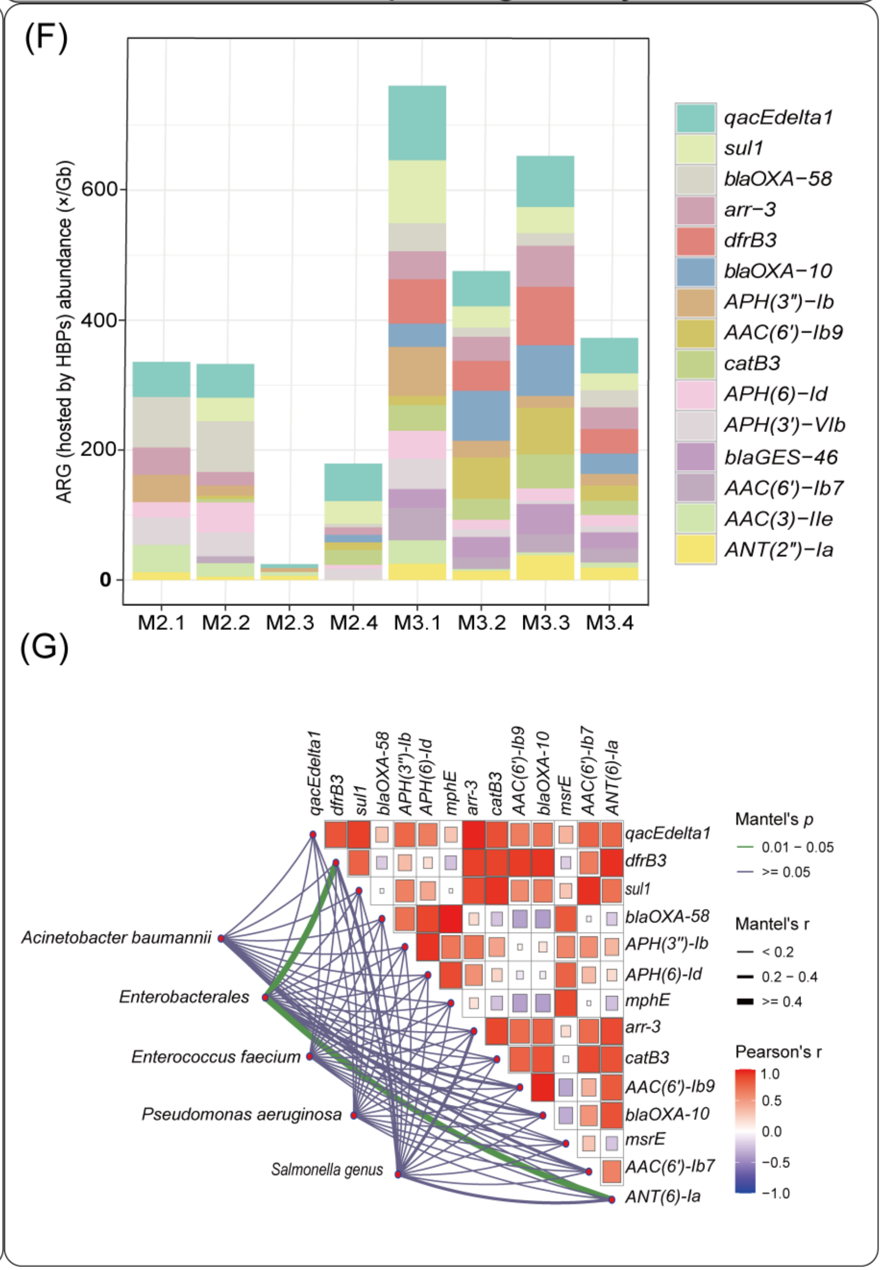


pairs	R²	p.value	p.adjusted
before vs after	0.3160477	0.032	0.032

ARG transfer



ARG pathogenicity



Summary

- First systematic cross-error-rate benchmarking of long-read ARG identification tools: built tool-specific gold standards across 0%-15% error rates, evaluating L-EasyARG, ARGpore2, and DeepARG to fill a critical gap.

- L-EasyARG achieves optimal F1-scores at low error rates (≤5%): it outperforms existing tools in precision and F1 on both simulated and real Nanopore data (error rates 2.57%-4.51%).

- Integrated pipeline covering ARG, MGE, plasmids and host identification, assesses migration potential and pathogenic risk through visual output.

- Website: https://github.com/liyonxin/L-EasyARG

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