healthy all life long

NGS-based workflow to improve the detection of antimicrobial resistance: from wet-lab to data analysis

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Context

AMRseq PhD project:
- AMR on chromosome and/or on plasmids
- Plasmid-mediated transfer AMR genes to bacteria of different origins (One-health): public health risk factor
- Currently most AMR testing is done with cultures (MIC) and PCR tests
- These lack flexibility to look for new mutations and identify carrier plasmids
- Alternative needed: NGS approach → problem: difficult plasmid reconstruction

Objective 1:
Build a generic NGS workflow for characterization of circulating plasmids (from sample preparation to bioinformatics analysis), based on 3 case studies: GMM, pathogens from food and from human, all important in the public health context

Objective 2:
Develop a database with full circular plasmid sequences including antibiotic resistance profiles and metadata (such as location, host bacteria and type of sample)

NGS strategy for plasmid reconstruction: combining short and long reads

MiSeq:
- Short reads (25-250 bp)
+ High accuracy (99.9%)
+ Affordable
+ Standardized protocols

MiSeq

MinION:
+ Long reads (>8000 bp)
- Low accuracy (75-90%)
- Expensive (~1000 euro for 1 flowcell)
- Protocols constantly evolving and improving
- Stringent requirements for input DNA

MinION

GMM study case

- GM *Bacillus subtilis* overproducing riboflavin (vitamin B2) imported in feed additives (RASFF 2014–1249)
Approach used: whole genome DNA extraction
- Paired MiSeq reads = not able to span repetitive regions of GM plasmids
- MinION reads = limited AMR gene detection due to error rate
- Hybrid assembly with Unicycler:
  able to identify antimicrobial resistance and reconstruct 3 plasmids
  To be confirmed with PacBio for integration of plasmid in chromosome

Miseq only	MinION only	Hybrid
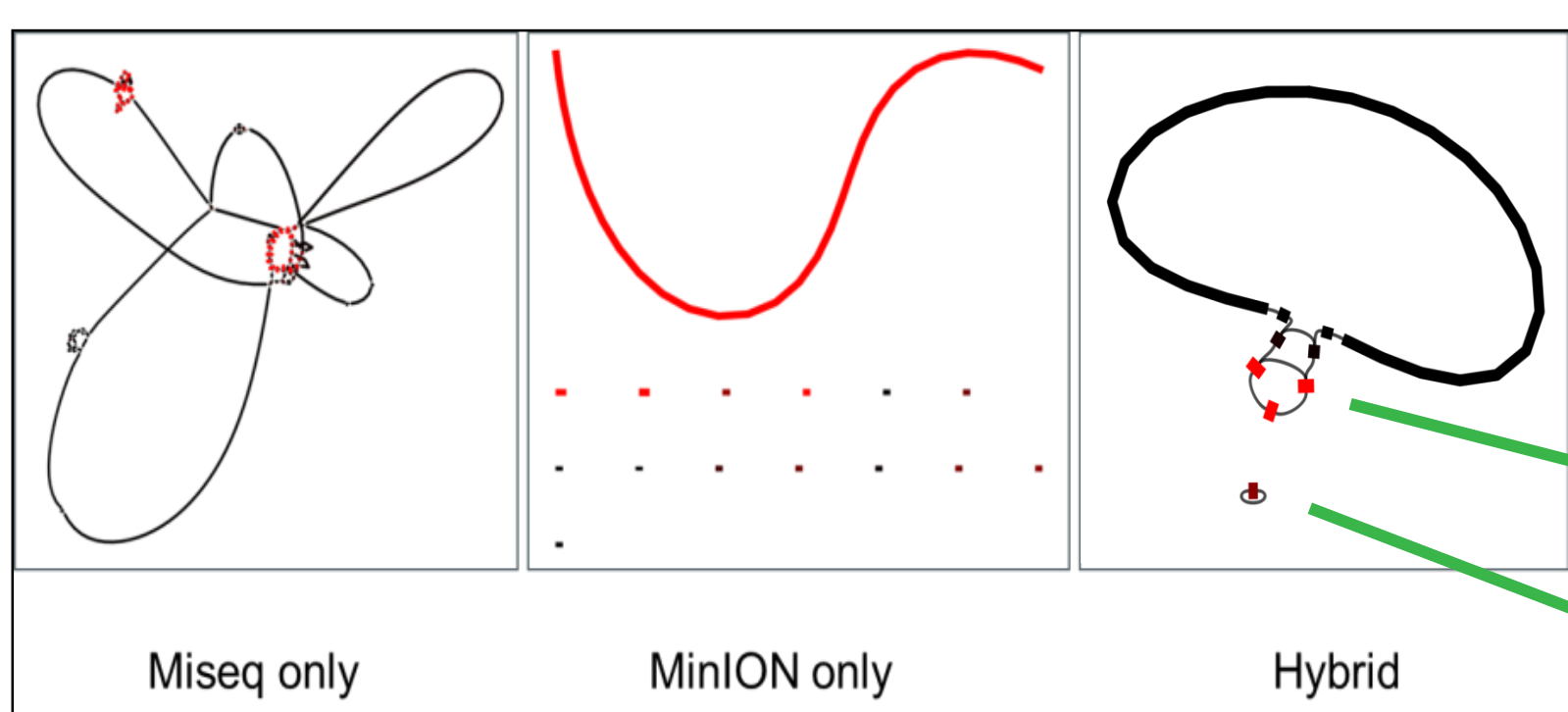
Assemblies made with Unicycler and visualized with Bandage. Each line represents a contig. Red contigs have a higher coverage than black contigs.

2x more coverage
5x more coverage
3x more coverage

AMR gene	AMR class	Hybrid assembly (genotype)				Culture-based (phenotype)
		chromosome	plasmid 1	plasmid 2	plasmid 3	
aadD	Aminoglycoside		x	x		x
aadK	Aminoglycoside	x				x
blaTEM-116	Beta-lactam		x	x	x	x
cat(pC194)	Phenicol	x				x
ErmB	Macrolide			x		x
tet(L)	Tetracycline	x		x		x

Antimicrobial resistance genes and classes found by the genotypic hybrid assembly and by a phenotypic culture-based test.
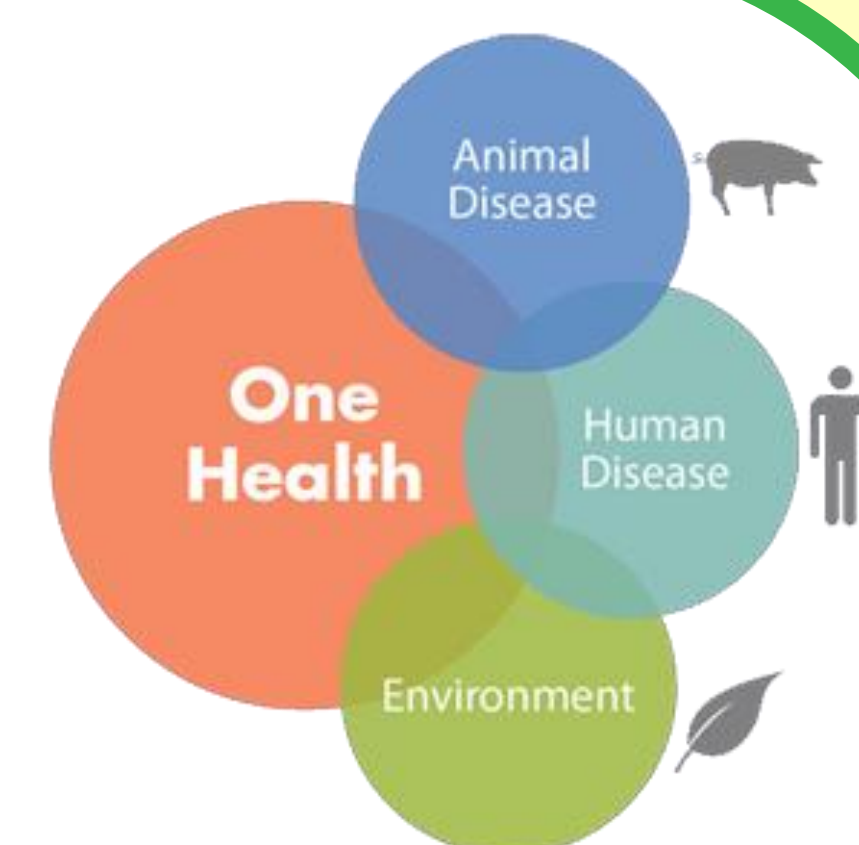
Workflow

Factors to be taken into account:
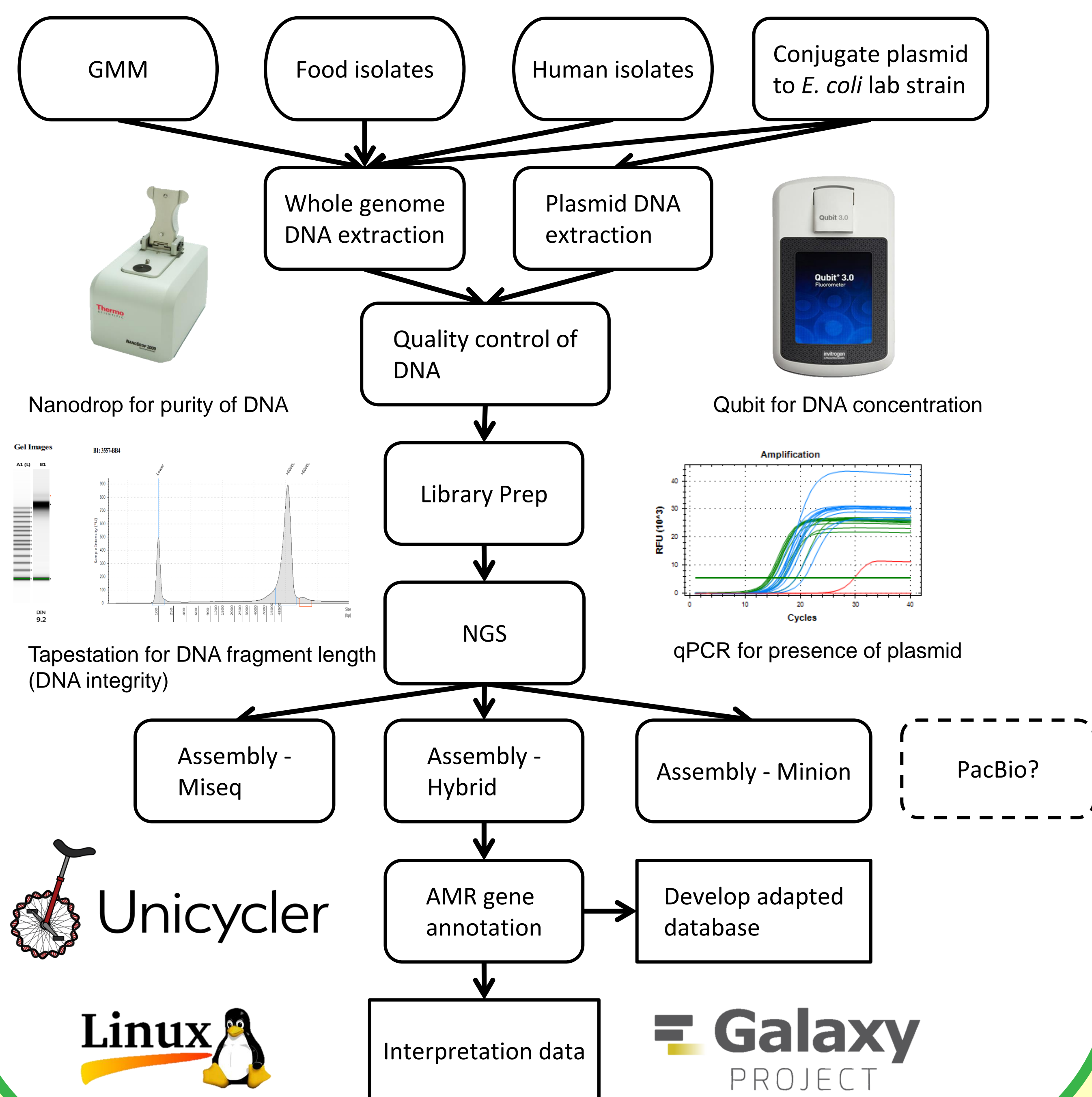- Accuracy
- Speed
- Complexity
- Cost

Case studies to be used:
- Genetically modified *Bacillus subtilis* from feed additive
- Colistin resistant pathogens from human samples
- VIM producing *Enterobacteriaceae* from food

	Whole genome DNA extraction	Plasmid DNA extraction	Conjugation plasmid → whole genome DNA extraction	Conjugation plasmid → plasmid DNA extraction
Wet-lab complexity	*	****	***	*****
Dry-lab complexity	*****	**	***	*

Complexity of the DNA extraction approaches. * = not complex, while ***** = very complex. Based on initial experiments and literature.

Nanodrop for purity of DNA

Qubit for DNA concentration

Tapestation for DNA fragment length (DNA integrity)

qPCR for presence of plasmid

Unicycler

Linux

Galaxy PROJECT

Conclusions & perspectives

- Not possible to reconstruct repetitive regions of plasmid without long reads, and short reads are necessary to improve the accuracy
- AMR with culture-based test (phenotype) = AMR with NGS (genotype)
- However NGS gives valuable information to facilitate an One Health approach
- First workflow tested on GM *Bacillus subtilis* and ongoing for other case studies
- Workflow will be applied to bigger collection for construction of plasmid database