



NGS for characterization of clinical class 1 integrons from hospital effluents

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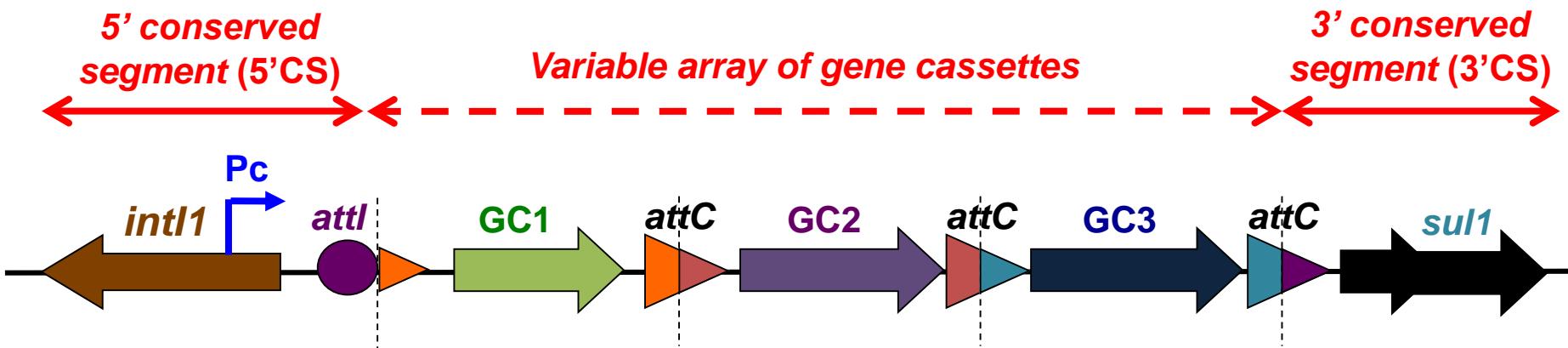
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Class 1 integrons, vehicles of antibiotic resistance

Bacterial genetic elements able to capture and express ATB-resistance genes embedded within gene cassettes (GC)

Stokes et al, MolMic, 1989



- a dynamic system
 ⇒ GCs and GC arrays are different between integrons
- > 350 GCs with 145 involved in antibiotic resistance
 ⇒ link between integrons and antibiotic resistance

Partridge et al, FEMS, 2009

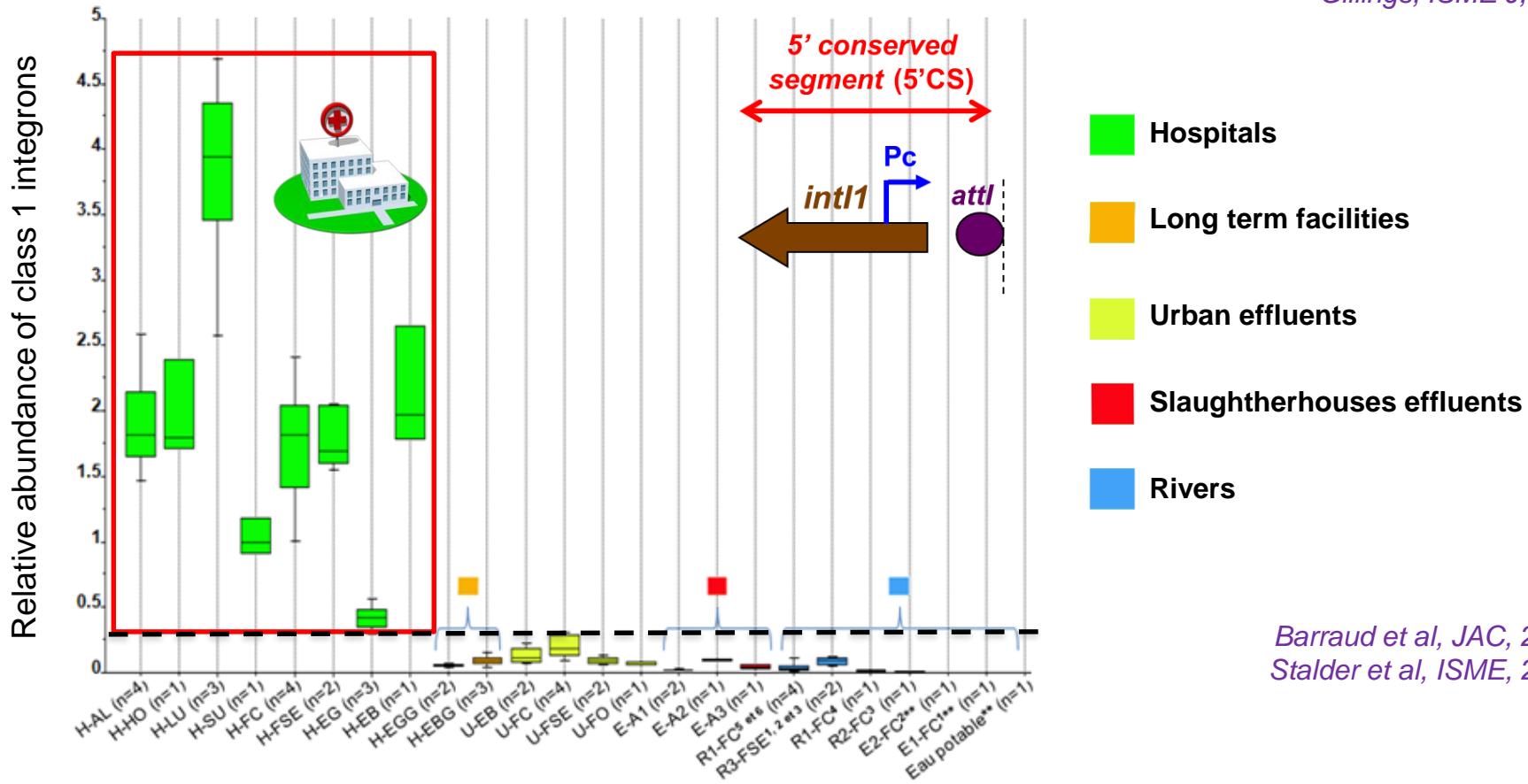
Leverstein-van Hall et al, JID, 2003, Barraud et al, IJAA, 2014



Class 1 integrons, markers of anthropic pollution

Relative abundance of *intI1* gene is a good proxy for anthropic pollution

Gaze et al, ISME, 2011
Gillings, ISME J, 2015



⇒ High quantities of class 1 integrons in hospital effluents

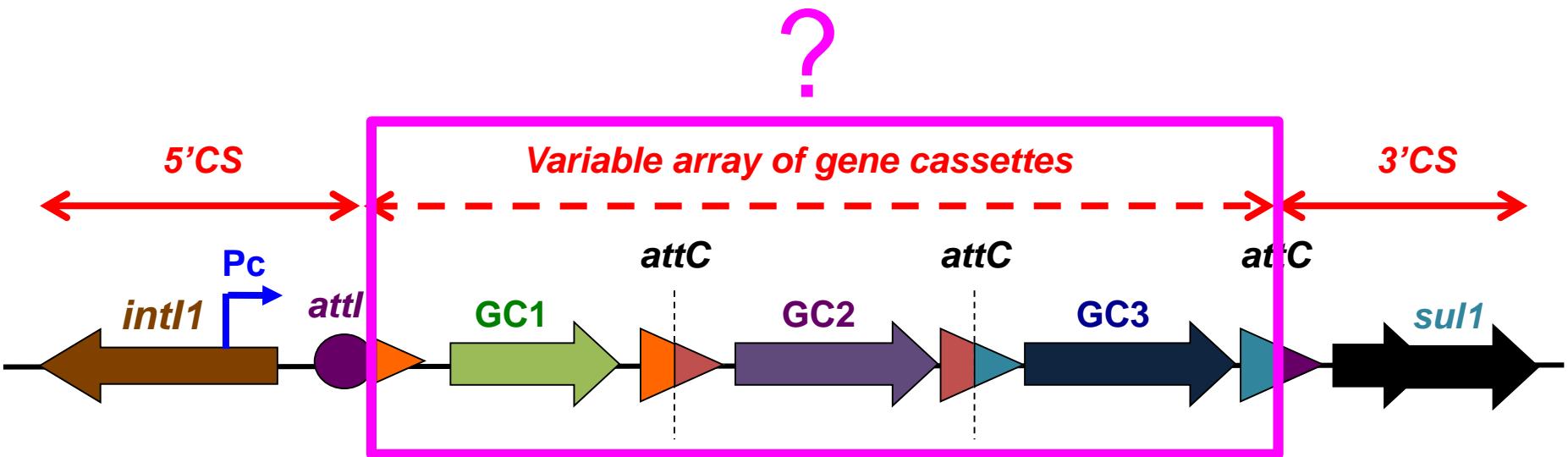


Barraud et al, JAC, 2010
Stalder et al, ISME, 2014

Objective of the study



To extensively characterize the pool of class 1 integrons GCs from hospital effluents using NGS

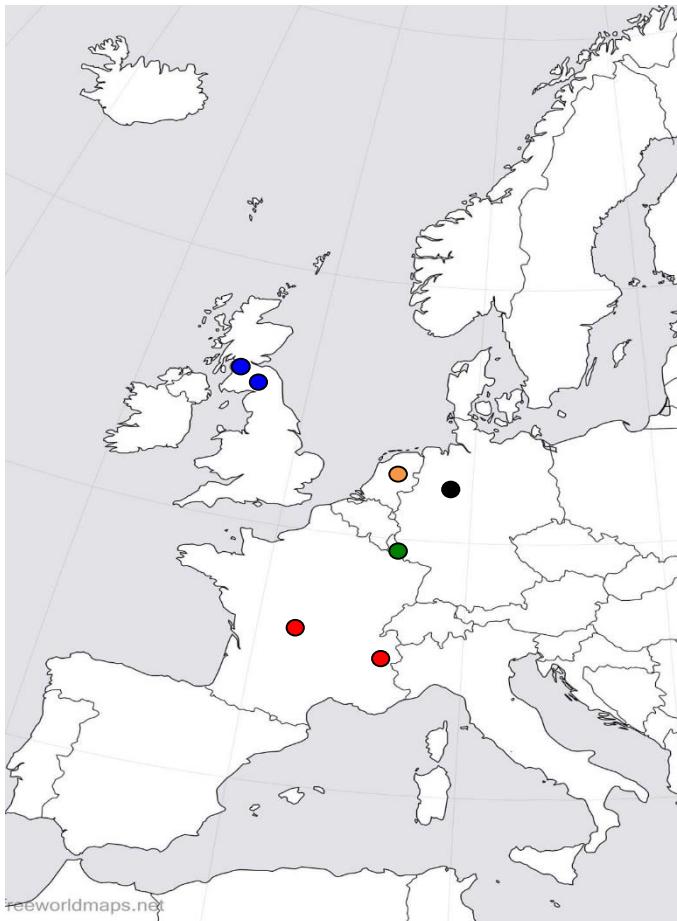


- Gene cassette content of each hospital effluent?
- Novel gene cassettes?



Methods: samples

10 European hospital effluents



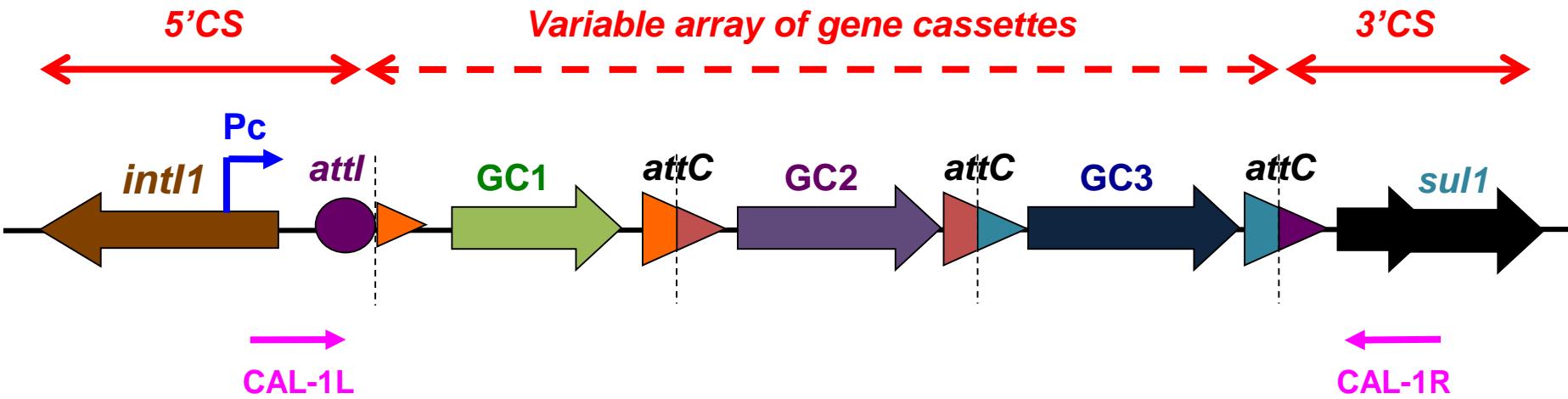
- France: **F1a, F1b, F2**
- Scotland: **S1, S2, S3a, S3b**
- The Netherlands: **N1**
- Luxembourg: **L1**
- Germany: **G1**

1) **Total DNA extraction**
Power-Water DNA isolation kit, MoBio



Methods: NGS on PCR products

2) Amplification of gene cassette arrays by end-point PCR



3) NGS from PCR products Ion Proton™ system



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Methods: Bioinformatic analysis

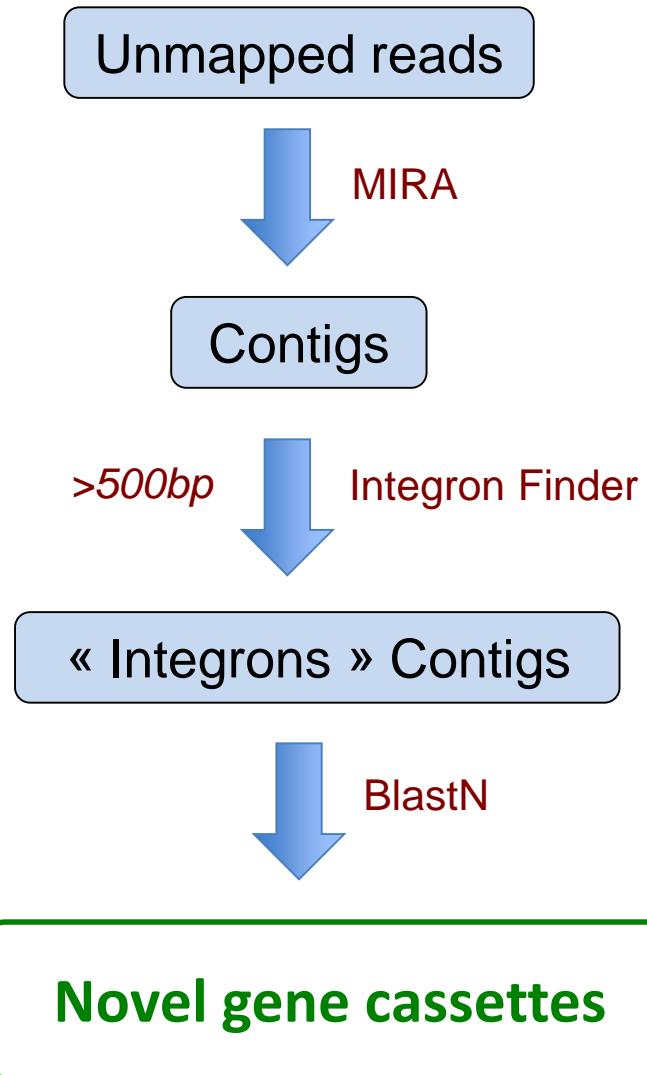
4) Raw read mapping

- ✓ against all known gene cassettes
 - 145 ATB resistance GC
 - 216 *gcu* (GC of unknown function)
- GCs with 100% identity beyond 50 bp were excluded



Quantitative GC content
of each effluent

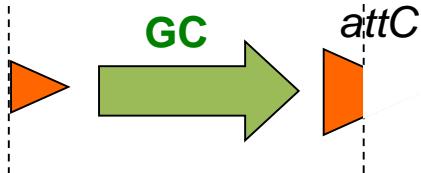
5) Assembly of unmapped reads



Results: raw read mapping

Quantitative
GC content

- 10^6 - 10^7 reads / sample
- 60 to 80% of all reads were mapped



	Min	Average	Max
Mapped GCs	72	101	139
Main GCs* (% total)	4 (52%)	7 (68%)	12 (78%)
Rarer GCs** (% total)	66 (4%)	94 (6%)	131 (9%)

* Mapped by >1% mapped reads ** <1% mapped reads



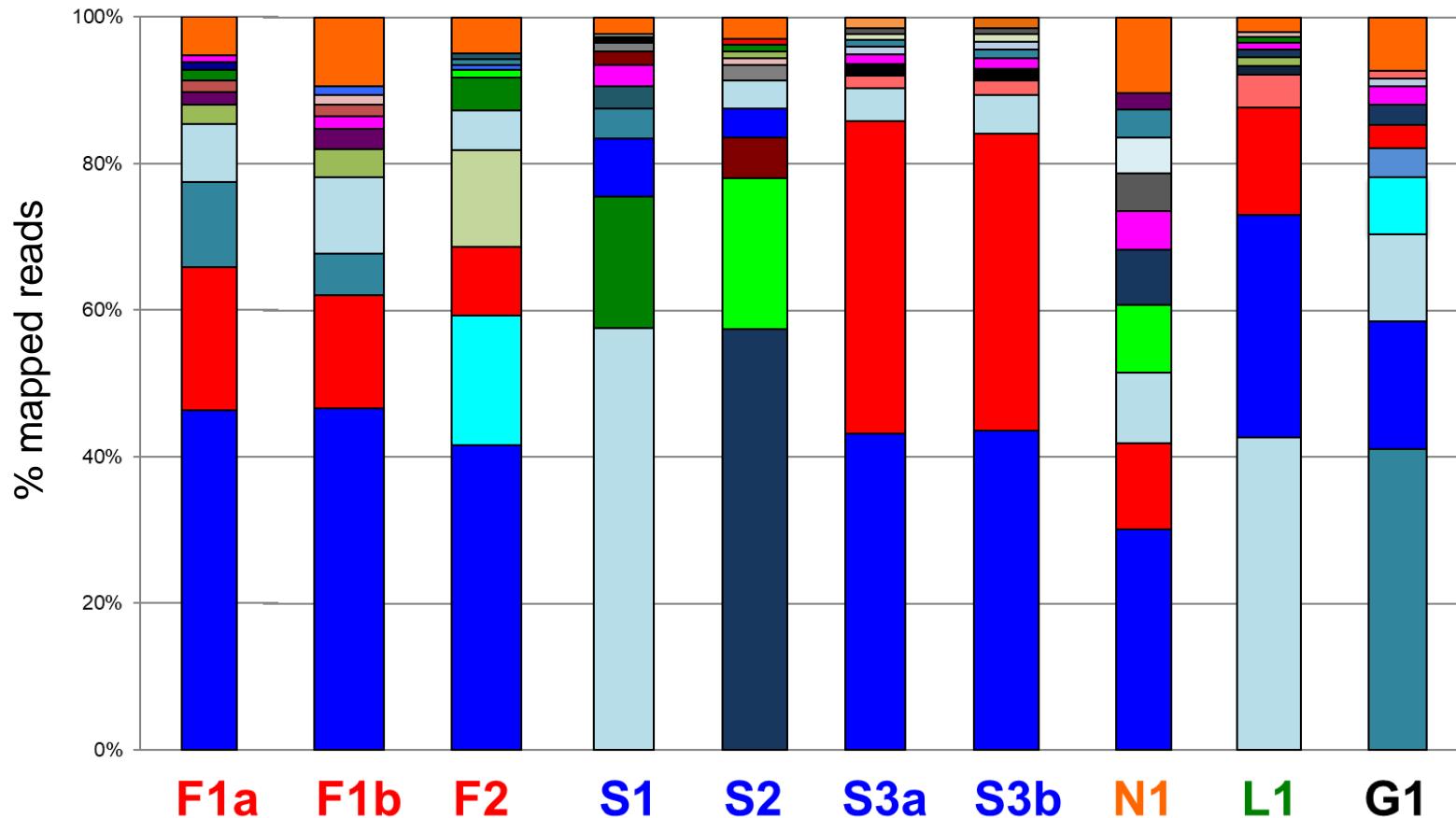
⇒ Main mapped GCs are antibiotic resistant GCs

- *aadA, aadB, aacA4...* (aminoglycosides)
- *bla_{OXA}, bla_{GES}* (β -lactams)
- *dfrA, dfrB* (trimethoprim)
- *catA, cmlA* (chloramphenicol)



Results: raw reads mapping

Quantitative
GC content

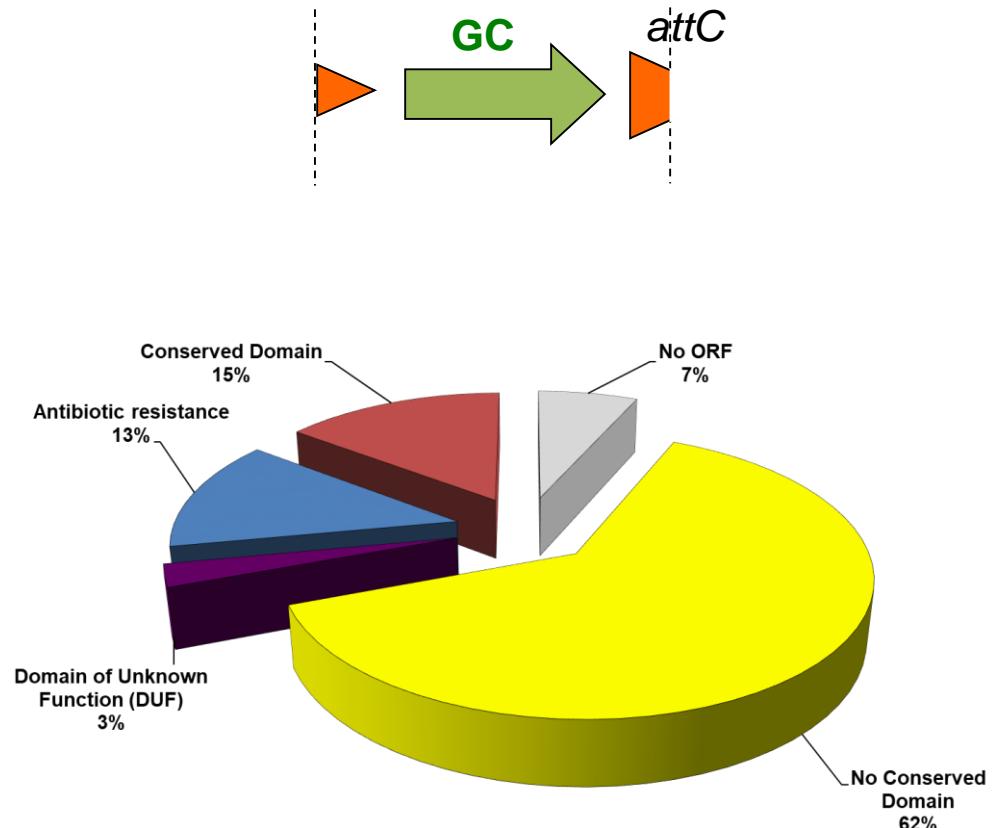
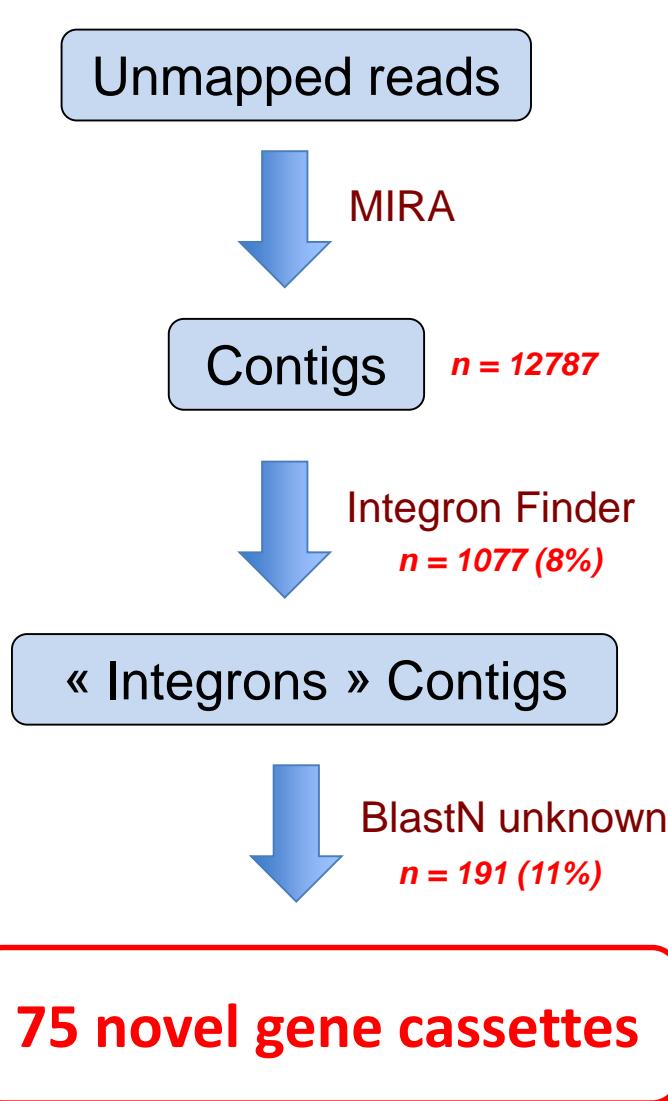


⇒ Each effluent has its own « GC signature »



Results: assembly of unmapped reads

Novel GCs



⇒ 10 novel ATB resistance GC



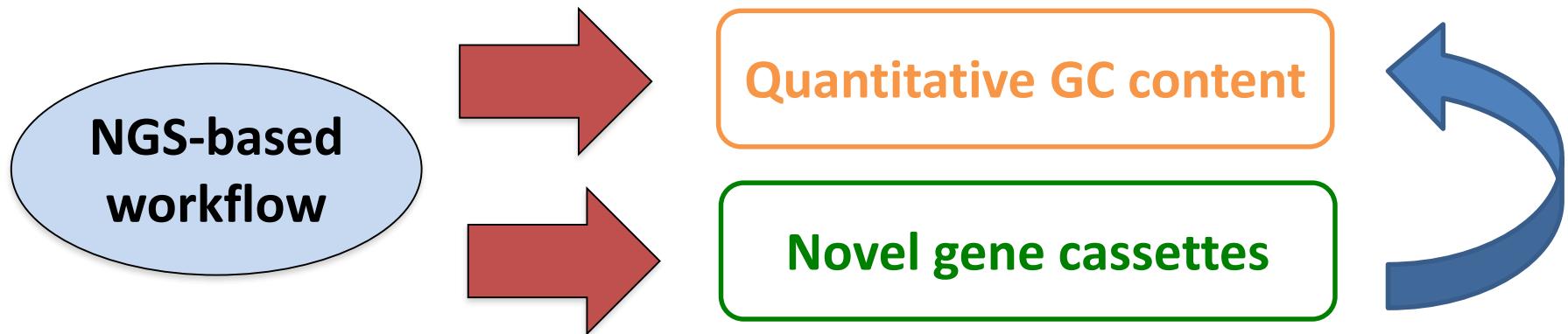
Results: ATB resistance gene cassettes

Novel GCs

Gene cassette	Antibiotic resistance	Max % mapped reads
A	Chloramphenicol	1%
B	β-lactams	0,44%
C	Quaternary ammonium/Small efflux	0,04%
D	Rifampicin	0,06%
E	Rifampicin	0,02%
F	Aminoglycosides	2%
G	Bleomycin	0,001%
H	Quartenary ammonium/Small efflux	0,2%
I	Aminoglycosides	0,002%
J	Quartenary ammonium/Small efflux	0,11%



Discussion - Conclusion



- 100 GCs per effluent but 10 of them represent 90% of mapped reads
- Most GCs are ATB-resistance GCs (aminoglycosides, β -lactams)
- Each effluent has its own GC signature « cassetome »
- Novel GCs were discovered, some of them involved in ATB resistance

⇒ **A powerful tool to extensively describe GCs of effluents**

Acknowledgments

UMR Inserm 1092

Anti-infectieux: Supports moléculaires des résistances et innovations thérapeutiques



Thomas Jové

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Emilie Guérin Valentin Tilloy



Thank you for your attention





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