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# What's going on in hospital effluent biofilm?

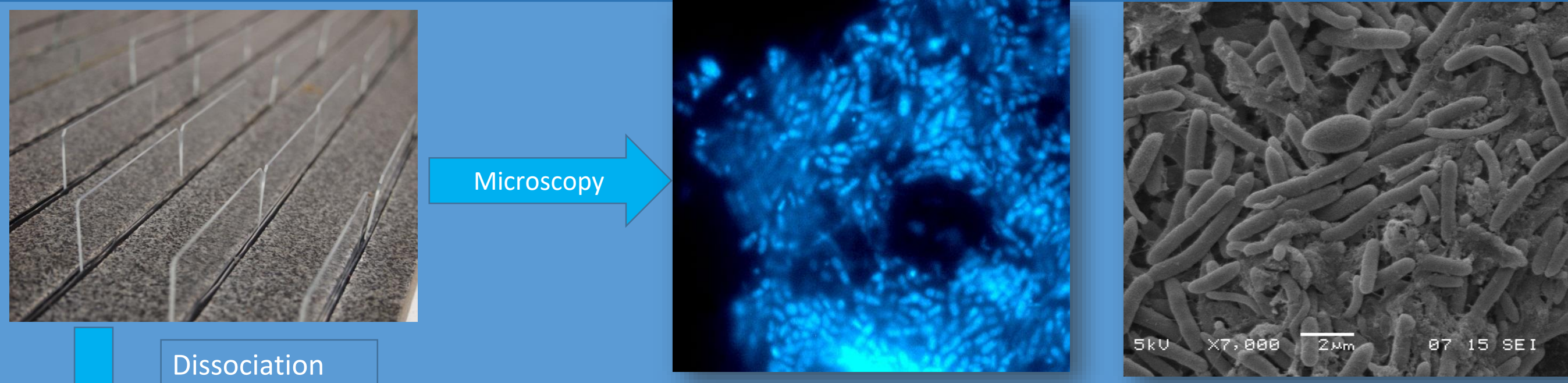
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**KNOW YOUR ENEMY** : Antibiotic resistance is a growing global public threat with serious health, political, and economic implications. Hospitals are a source of antibiotic molecules and resistance genes as their effluents are discharged into the sewer systems, rivers, lakes, and seas without prior treatment. However, bacteria in hospital effluent biofilms are exposed to stressful conditions leading to the survey of a limited number of species, i.e. the survivors, which could play a role of reservoir, "the place to be" to exchange resistance genes.

## In situ biofilm formation, microbial content analysis

Biofilms formed on glass slides in hospital effluent



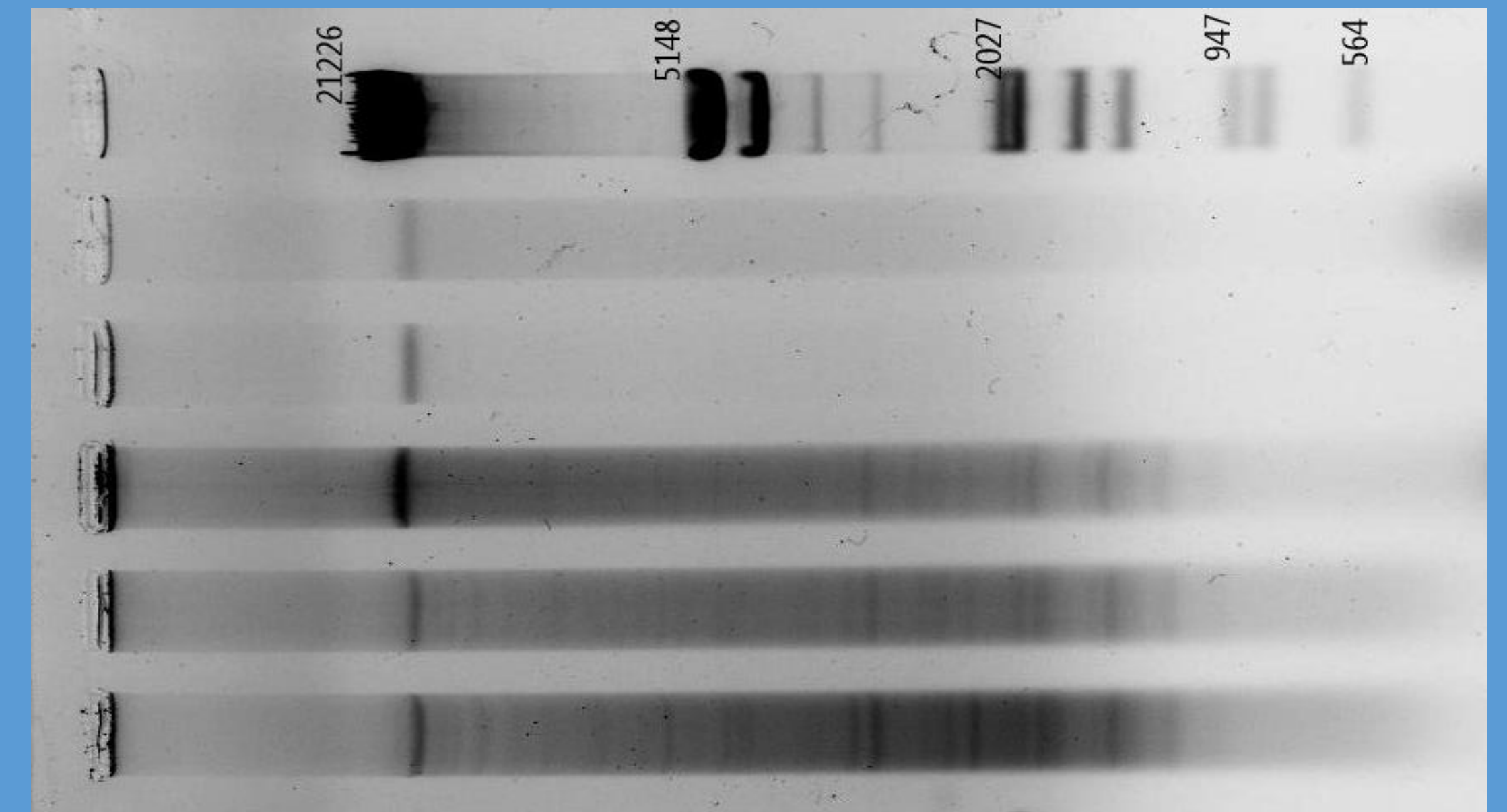
Microscopy

Dissociation  
Cytometry

Three-week old biofilms are composed of bacteria embedded in a rather thin matrix.

SYBR®Green and propidium iodide (PI) staining:  
2 populations: - Living cells 49% (white circle)  
- Damaged Cells 41% (Red circle)

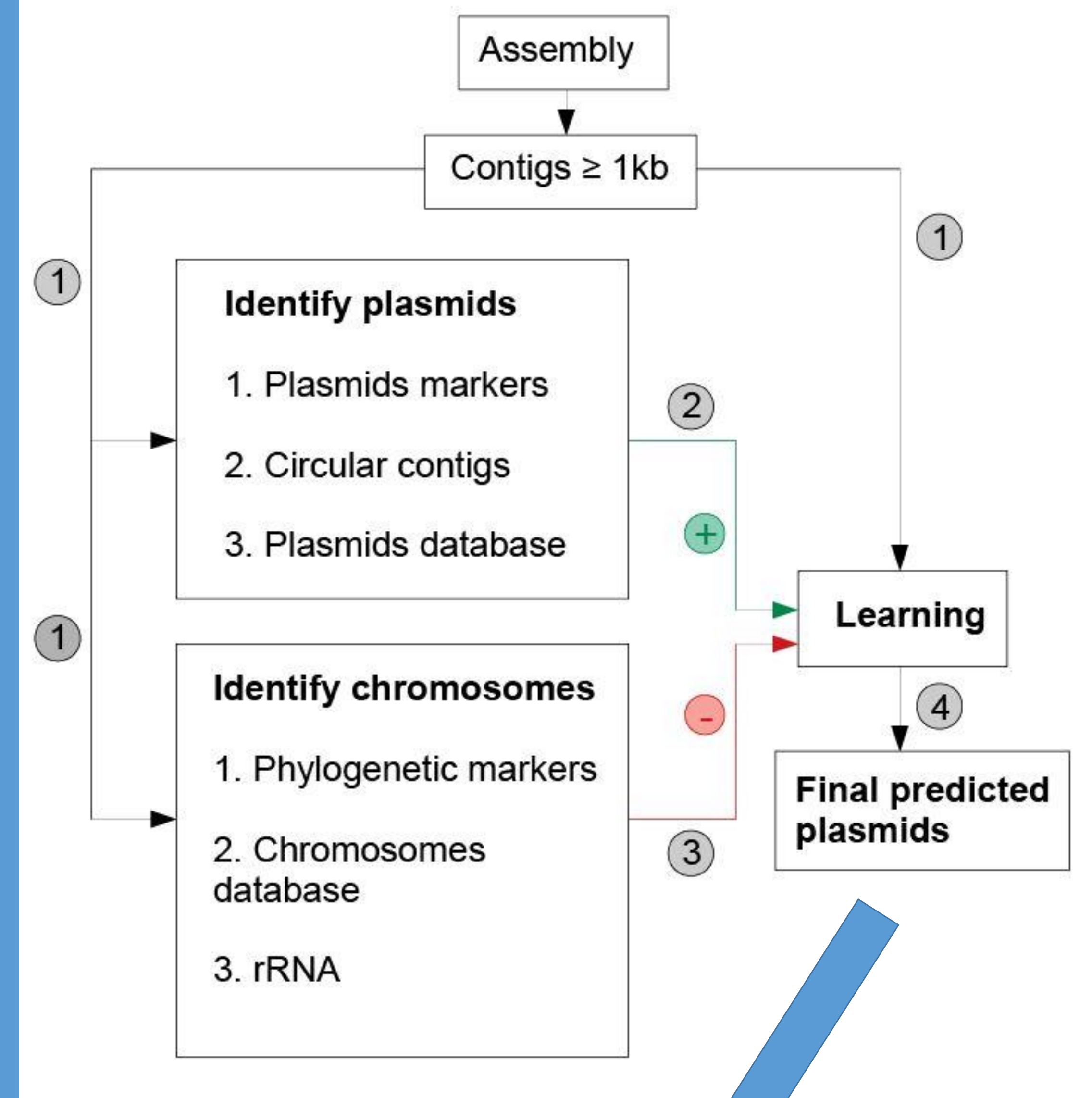
## Extraction using a manual alkaline mini-prep



## Illumina sequencing of plasmid extract:

Reads number : 107 734 274

Sequences treatment with **PlasPredict**



## Contigs plasmids:

Number : 8,267  
Total length: 20,546,830 bp  
Med size: 2,485 bp

381 circular plasmids (1,4 Kb to 236Kb)  
9490 linear contigs (74bp to 236Kb)  
Two contigs already in database:  
*E. coli* plasmid pE35BA\_1, complete sequence (5Kb)  
*E. coli* plasmid pEco70745\_4, complete sequence (2Kb)

Resfams Ab classification	%	Antibiotic resistance genes
Others Efflux	56.0	<i>EmrE, AdeS, AdeR</i>
ABC Transporter	12.3	<i>ABC efflux, macA-B, msbA, tolC</i>
RND Antibiotic Efflux	9.5	<i>RND, AdeA-I-B, MexA-C-E-H, adeC-K-oprM</i>
Glycopeptide Resistance	8.3	<i>VanR-S-H-D</i>
Beta-Lactamase	6.38	<i>Class A-B, subclass B1-B3</i>
Aminoglycoside Modifying Enz	2.88	<i>AAC3, ANT, APH3</i>
Gene Modulating Resistance	2.11	<i>baeR-S, bla, marA, mecR1, RamA, robA, RomA, SoxR</i>
Quinolone Resistance	1.00	<i>Qnr</i>
MFS Transporter	0.52	<i>emrB, MFS</i>
Chloramphenicol Resistance	0.36	
rRNA Methyltransferase	0.24	
Tetracycline MFS Efflux	0.17	
Tet Ribosomal Protection		
Tet Inactivation		

**Efflux pump encoding genes** are the most frequently detected. They encode proteins involved in the secretion of toxic substances and antibiotics; a process considered to be a vital part of xenobiotic metabolism.

## Mobile repertoire

Contigs	Number
MOB	162 (103 MOB <sub>p</sub> , 30 MOB <sub>Q</sub> , 12 MOB <sub>F</sub> , 6 MOB <sub>H</sub> , 5 MOB <sub>V</sub> , 2 MOB <sub>C</sub> )
MPF	333 (106 MPF <sub>F</sub> , 40 MPF <sub>G</sub> , 7 MPF <sub>I</sub> , 176 MPF <sub>T</sub> )
OriT	296

The mobile repertoire is composed of conjugative and mobilizable plasmids, integrative and conjugative elements and integrative and mobilizable elements.

## Taxonomy

Taxon	Number of contigs	%
Actinobacteria	3	0,092
Bacteroidetes	4	0,12
Firmicutes	4	0,12
<b>Proteobacteria</b>	<b>2556</b>	<b>77,80</b>
unclassified	718	21,85
	3285	

The diversity of species is very low with mainly gammaproteobacteria