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# TN-PHYTO: ESSENTIAL GENOMES OF NINE BACTERIAL PHYTOPATHOGENS

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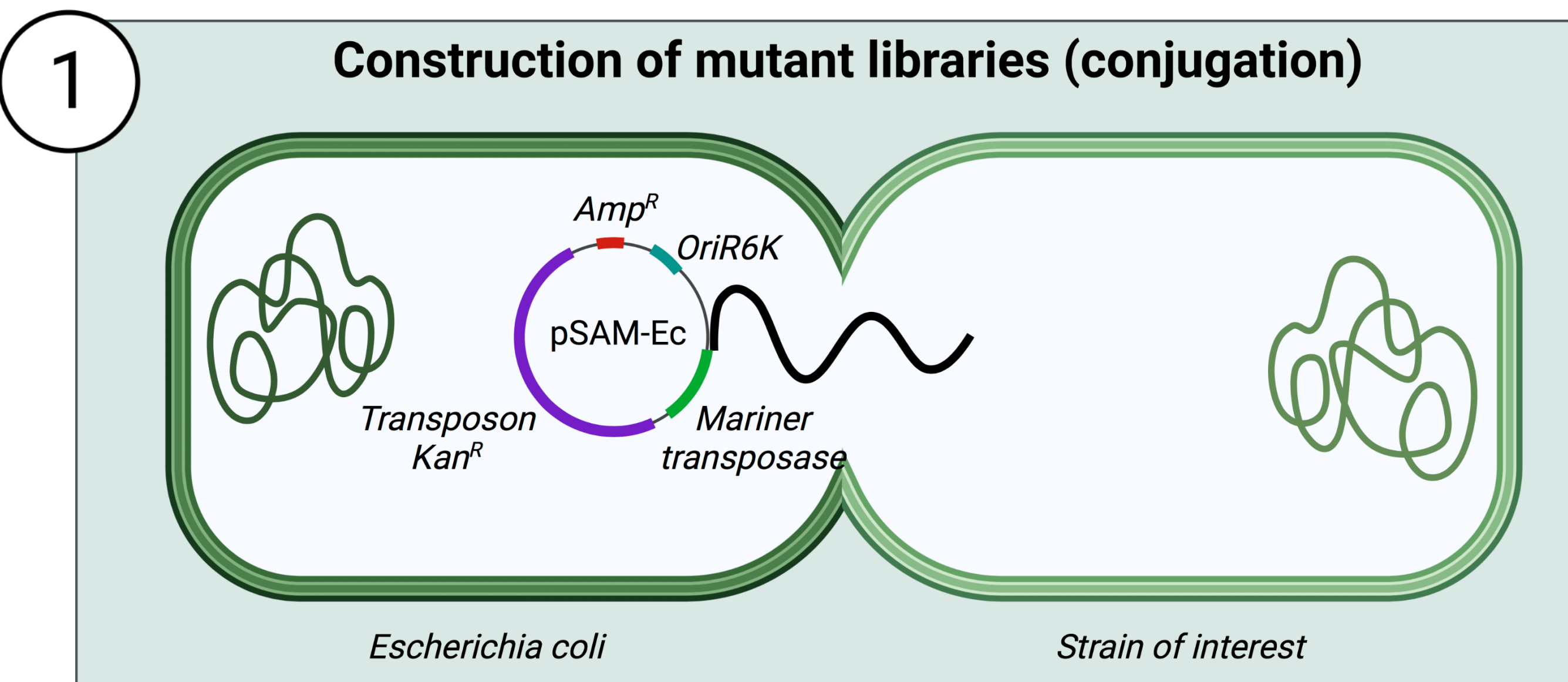
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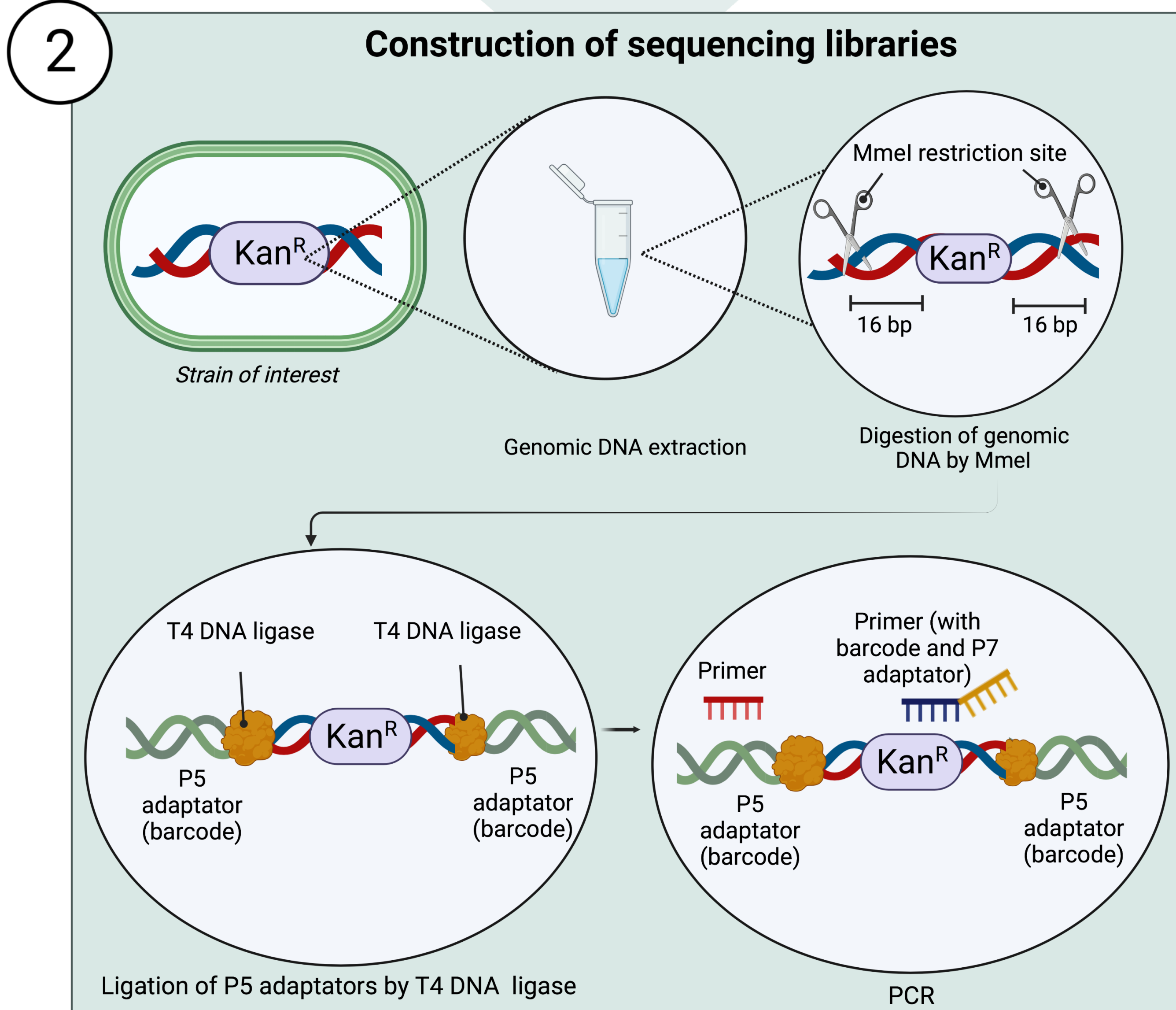
The aim of our project is to identify virulence factors of phytopathogenic bacteria isolated from the environment as well as those allowing their survival in water. For this purpose, we use Tn-seq (2), an innovative mutagenesis technique coupled with high throughput sequencing chosen to rapidly identify, on a large panel of strains, genes necessary for growth in a given condition, the plant or water. This study is carried out on strains of *Dickeya*, *Pectobacterium* and on the *Pseudomonas syringae* complex, all of which are capable of growing on a wide range of hosts as well as in water, thus allowing the most complete identification of genes of interest. Another axis of this ANR is the development of a complete bioinformatics pipeline, allowing to process data obtained under multiple conditions and for strains of different species. The results obtained will help us to predict the virulence of strains and/or to develop antibacterial strategies. Overall, this project will pave the way for studies of other environmental pathogens by proposing an original conceptual and technological framework that is extremely promising.

## Materials and methods

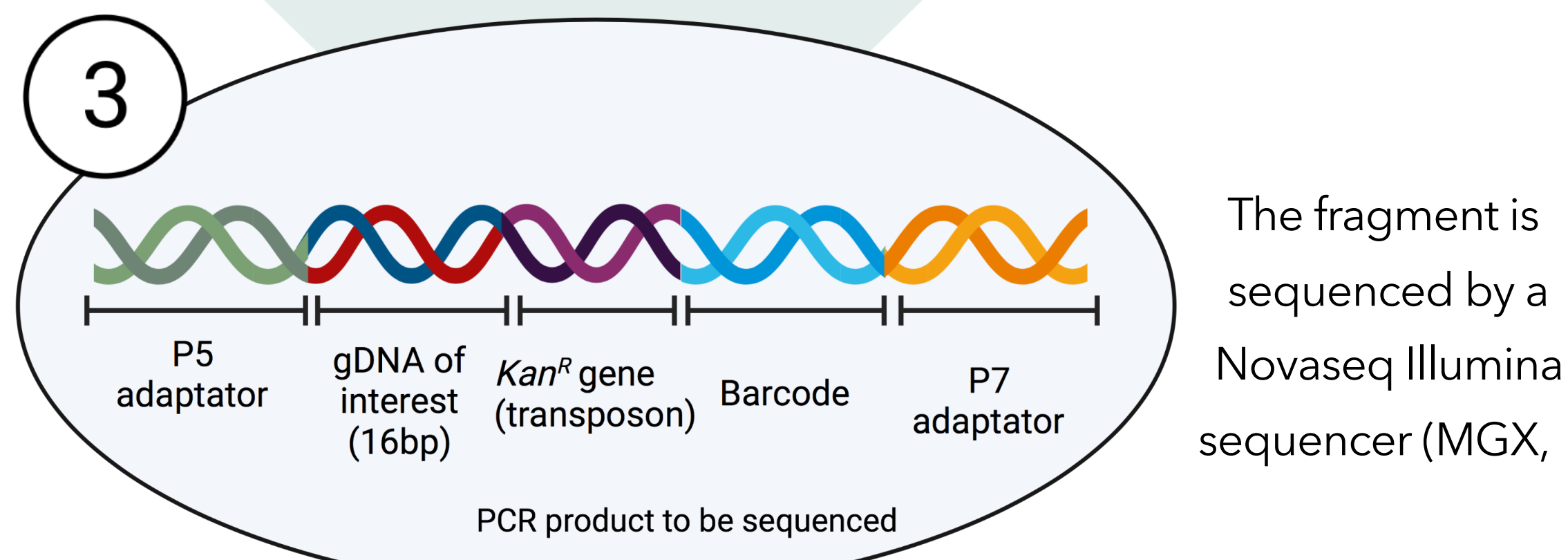
## Results



Mutant libraries are constructed by conjugation between an *Escherichia coli* MDFpir strain and each of the strains of interest. During this conjugation, the plasmid pSAM-Ec, which contains the mariner Himar9 transposon that confers kanamycin resistance (3), a transposase and an ampicillin resistance gene, is transferred to the strain of interest. It is a suicide plasmid that cannot replicate in the recipient strain. Thus, the transposon will integrate uniquely and randomly into the genome of the strain of interest. The mutants obtained can then be selected on medium containing kanamycin.



Genomic DNA from the mutant libraries is extracted and digested with the enzyme MmeI. Indeed, the transposon has MmeI restriction sites in each of its extremities. The particularity is that the enzyme will cut at 16 bp of its site. This will generate fragments containing the transposon and the adjacent 16 or 17 bp which corresponds to gDNA flanking the transposon. The P5 adaptors containing a barcode are ligated to the digested DNA fragments using a T4 DNA ligase. Then, PCRs are used to amplify the fragment which will be sequenced to determine the insertion gene of the transposon.

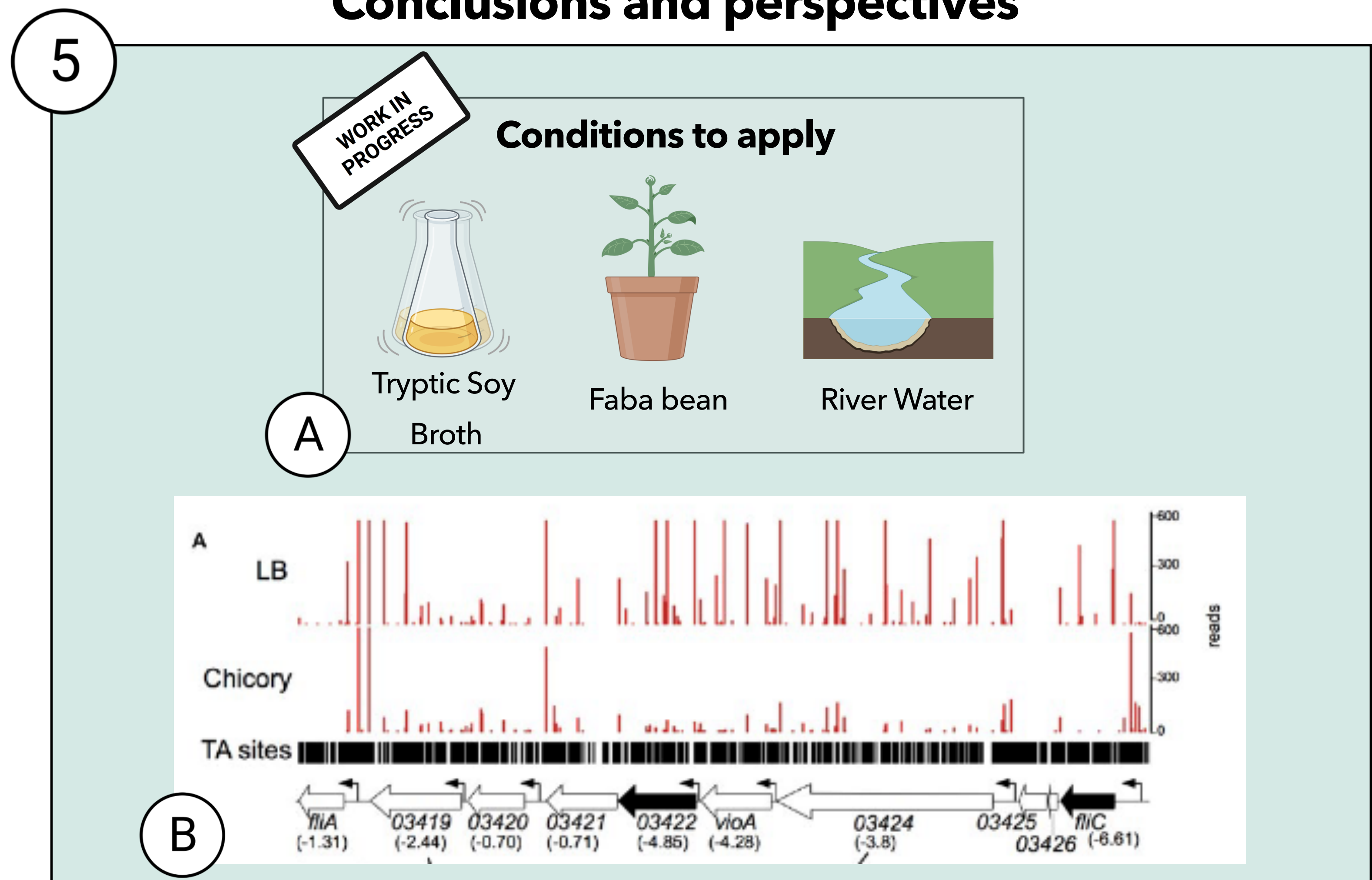


**4 Results of Novaseq sequencing : qualities of mutant libraries**

SPECIES	REPLICATE	INITIAL NUMBER OF READS	FINAL NUMBER OF READS	TAS	DENSITY	MEAN READ	PEARSON	# ESSENTIAL GENES / TOTAL GENES
<i>Dickeya dadantii</i> 3937	1	11 021 397	10 135 956 (91.97%)	171 791	50.1	124.6	0,98	416/4571
	2	11 657 925	10 686 008 (91.66%)	171 791	50.3	123.6		
<i>Dickeya zeae</i> A385	1	11 420 214	10 972 529 (96.08%)	194 008	52.6	129.4	0,96	428/4822
	2	11 814 804	11 251 444 (95.23%)	194 008	53.0	127.4		
<i>Dickeya fangzhongdai</i> B16	1	14 652 216	13 778 417 (94.04%)	166 816	38.1	122.2	0,99	447/4530
	2	11 834 554	11 129 805 (94.04%)	166 816	37.9	122.2		
<i>Pectobacterium atrosepticum</i> SCR1043	1	9 100 581	8 627 865 (94.81%)	232 639	45.9	124.5	0,97	406/4092
	2	10 454 137	9 831 239 (94.04%)	232 639	46.5	122.7		
<i>Pectobacterium versatile</i> A73	1	11 354 590	10 561 865 (93.02%)	219 901	55.5	124.3	0,99	398/4331
	2	10 215 789	9 496 298 (92.96%)	219 901	55.3	124.1		
<i>Pectobacterium aquaticum</i> A212	1	12 091 457	10 263 405 (84.88%)	199 301	62.7	124.0	0,99	428/4822
	2	11 184 408	9 441 354 (84.42%)	199 301	62.6	123.8		
<i>Pseudomonas syringae</i> CC440	1	15 818 299	6 954 441 (93.02%)	132 365	64.1	120.1	0,99	429/5062
	2	12 946 819	12 208 185 (94.29%)	132 365	63.8	120.1		
<i>Pseudomonas syringae</i> CC1557 chromosome	1	10 445 631	9 712 439 (92.98%)	131 221	65.5	120.8	0,99	443/5000
	2	10 820 446	10 140 094 (93.71%)	131 221	65.6	120.8		
<i>Pseudomonas syringae</i> pc1557	1	10 445 631	58 599 (0.56%)	1787	34.9	122.1	0,99	35/68
	2	10 820 446	57383 (0.53%)	1787	34.9	122.1		
<i>Pseudomonas viridiflava</i> TA043	1	9 425 329	8 872 242 (94.13%)	121 303	70.2	120.1	0,99	396/5384
	2	7 475 968	6 954 441 (93.02%)	121 303	69.7	120.0		

The quality of the libraries created for each strain was analyzed by Novaseq sequencing (MGX, Montpellier). It allowed to determine the percentage of transposon insertion in the genome of each strain. A good quality library is considered to have an insertion density of more than 30%. The correlation coefficient shows a high reproducibility of the transposition. These two elements attest to the good quality of the mutant libraries constructed.

## Conclusions and perspectives



(A) Now that the quality of the library is checked, the objective is to apply different conditions to the mutant libraries in order to determine the essential genes for bean infection or bacterial survival in a specific medium (TSB 50%, sterilized river water).

(B) Figure from Royet et al. (2019) (1). The results that will be obtained will provide a read number of the sequenced fragment. The presence of 16 bp adjacent to the transposon will allow to align these reads to the bacterial genome. The number of reads will reflect the importance of the mutated gene in under a certain condition. If the read count is low for a given gene, the mutant is not represented and the gene has an important role in that condition.

### References:

- (1) Royet et al. 2019. *Molecular Plant Pathology* : 287-306.
- (2) van Opijnen. 2009. *Nat Methods* : 767-772.
- (3) Wiles TJ. 2013. *PLoS Genet* 9(8): e1003716.