



**SEMA**

Sociedad Española de Mutagénesis Ambiental

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**Barcelona**

**The non-canonical SOS-system of DNA repair and  
mutagenesis in *Acinetobacter baumannii***

**Jesús Aranda Rodríguez**



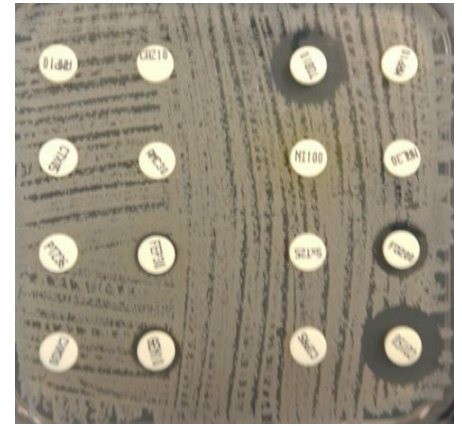
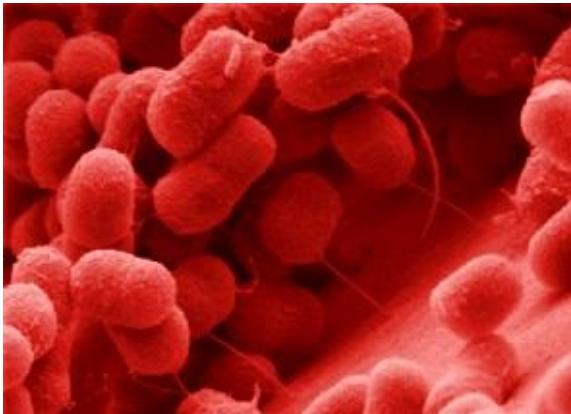
**Universitat Autònoma de Barcelona**

**Departament de Genètica i Microbiologia**

**Grup Microbiologia Molecular**

# *Acinetobacter baumannii*

- Gram-negative bacterium
- Nosocomial pathogen
- Resistant to most antibiotics



**Atypical SOS response**

# The bacterial SOS system in *E. coli*

Global DNA damage response



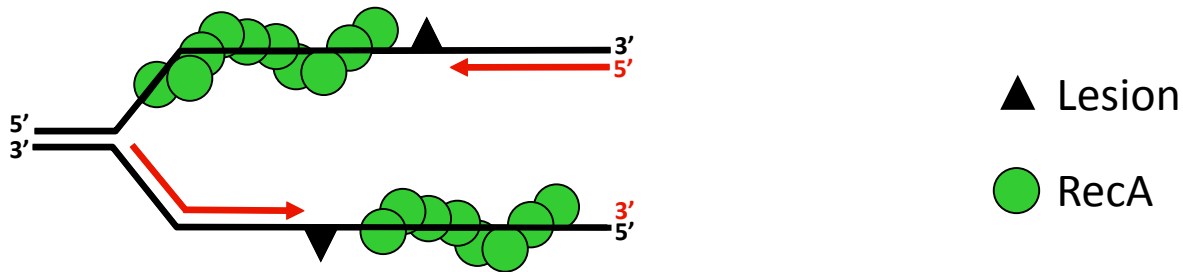
Repair of DNA lesions



Cell survival

# The bacterial SOS system in *E. coli*

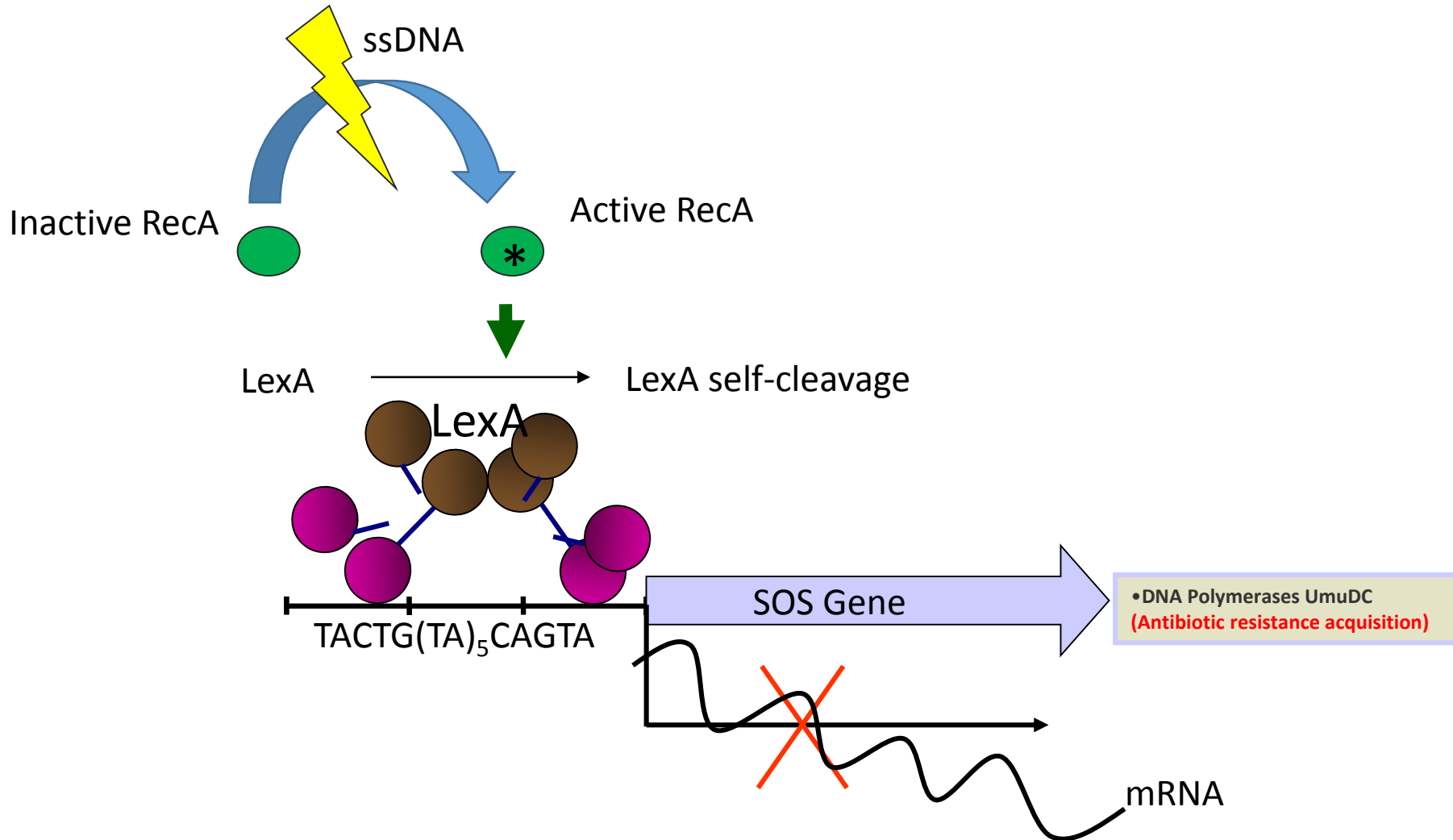
- DNA damage blocks DNA replication



- RecA forms a filament around these ssDNA regions becoming activated

Based on Little *et al.* Proc Natl Acad Sci U S A. 1980;77(6):3225-9.

# The bacterial SOS system in *E. coli*



Based on Little *et al.* Proc Natl Acad Sci U S A. 1980;77(6):3225-9.

# **A** *cinetobacter* spp.

- In *Acinetobacter* spp. no LexA homolog has been identified
- None of the promoters of genes of *Acinetobacter* spp. contain a known SOS sequence
- There is no damage-induced mutagenesis, with the remarkable exception of the opportunistic pathogens *Acinetobacter baumannii*, *Acinetobacter ursingii*, and *Acinetobacter beijerinckii*

# Objectives

- I. Construction and characterization of a *recA* knock-out mutant of *A. baumannii*
- II. Analysis of the transcriptional response of *A. baumannii* to DNA-damaging agents
- III. Analysis of the DNA damage-induced mutagenesis in *A. baumannii*

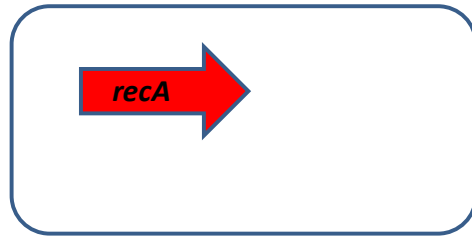
# Objective 1

Characterization of a *recA* knockout mutant of *A. baumannii*

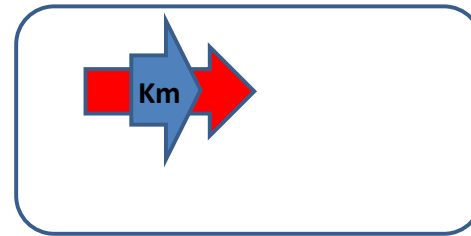


# The role of RecA in *A. baumannii*

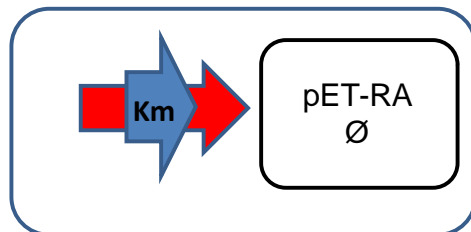
*A. baumannii* strains used in this work:



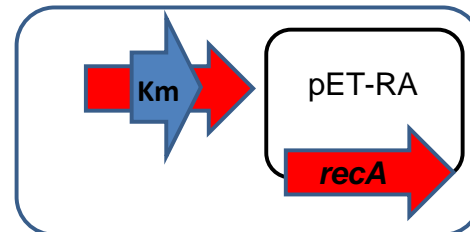
ATCC17978 (WT)



*recA* mutant

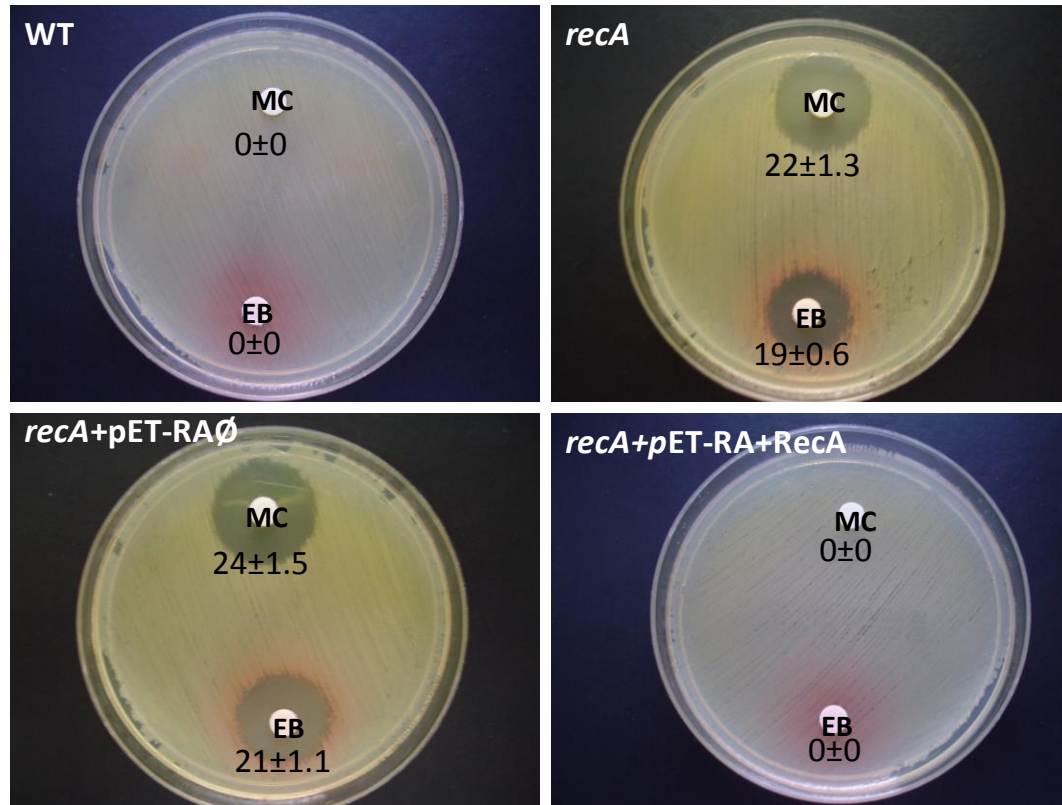


*recA* mutant + empty vector



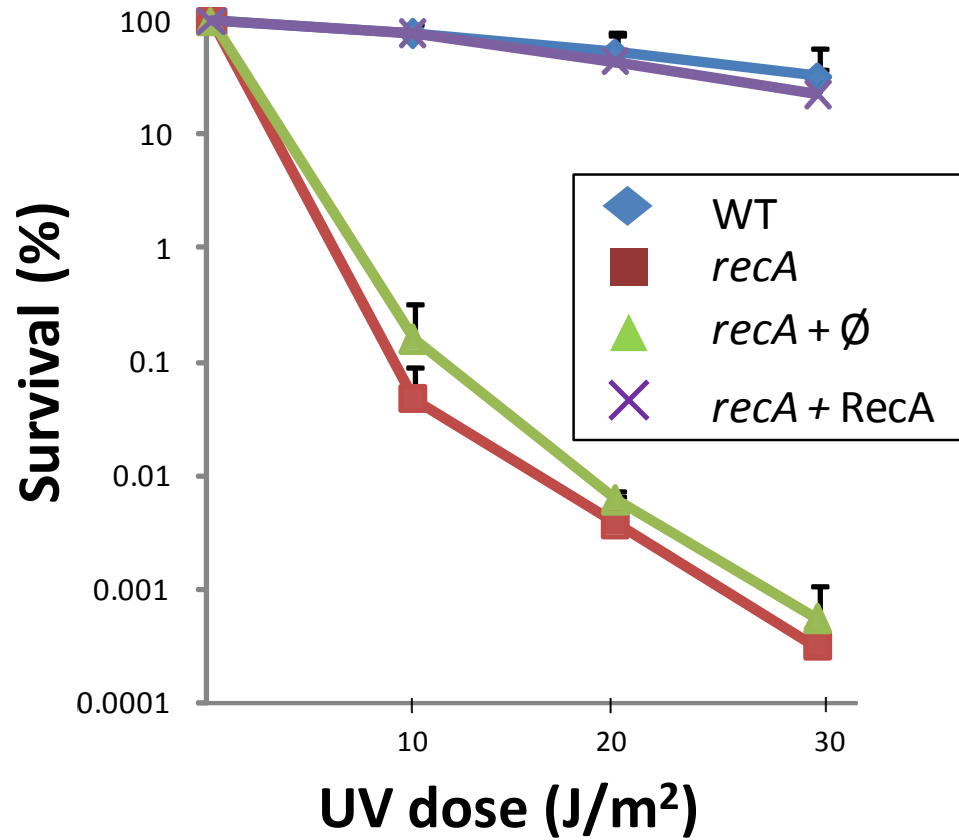
Complemented *recA* mutant

# DNA damaging agents

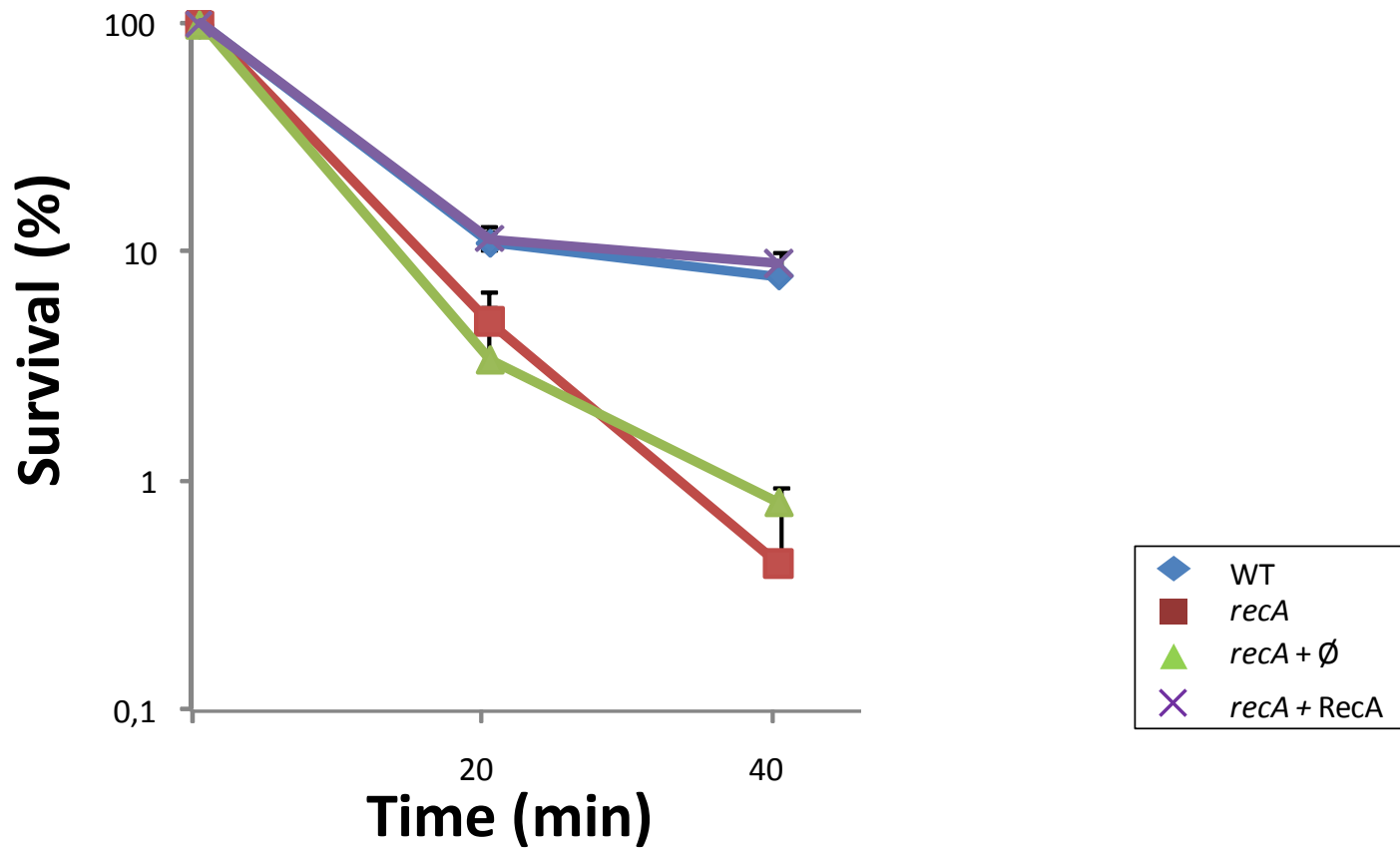


**MC:** MitomycinC; **EB:** Ethidium bromide  
(Inhibition halos are indicated in mm)

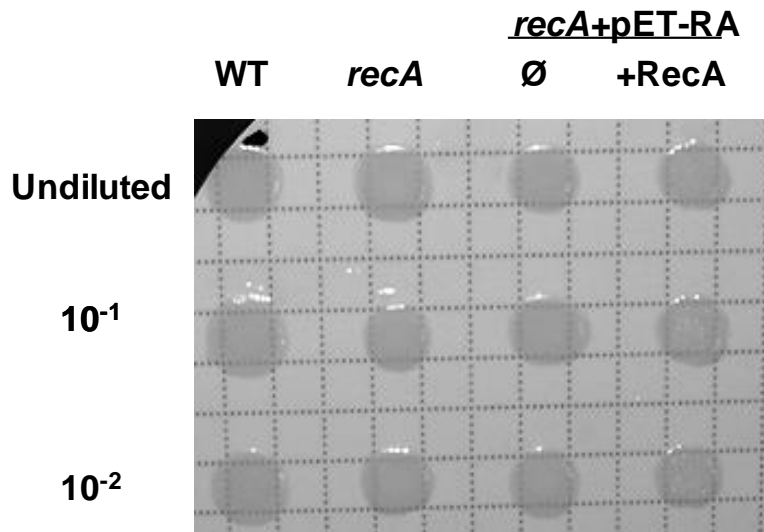
# DNA damaging agents: UV



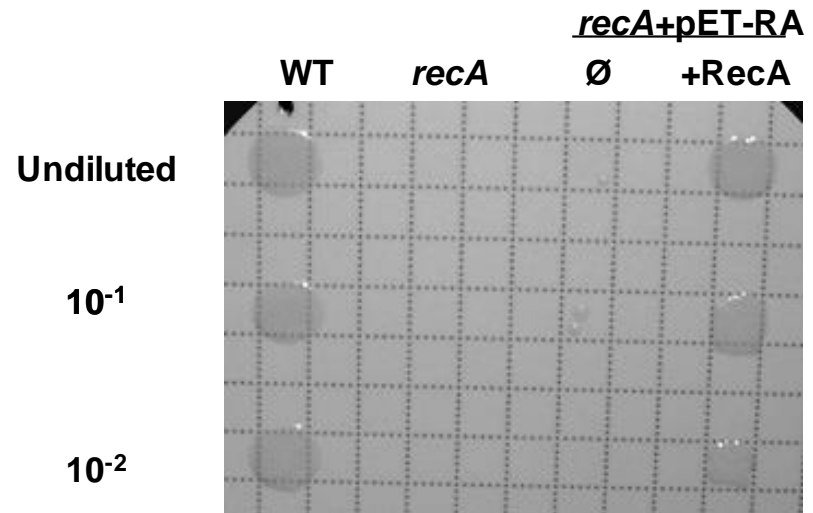
# Thermal stress (55°C)



# Desiccation



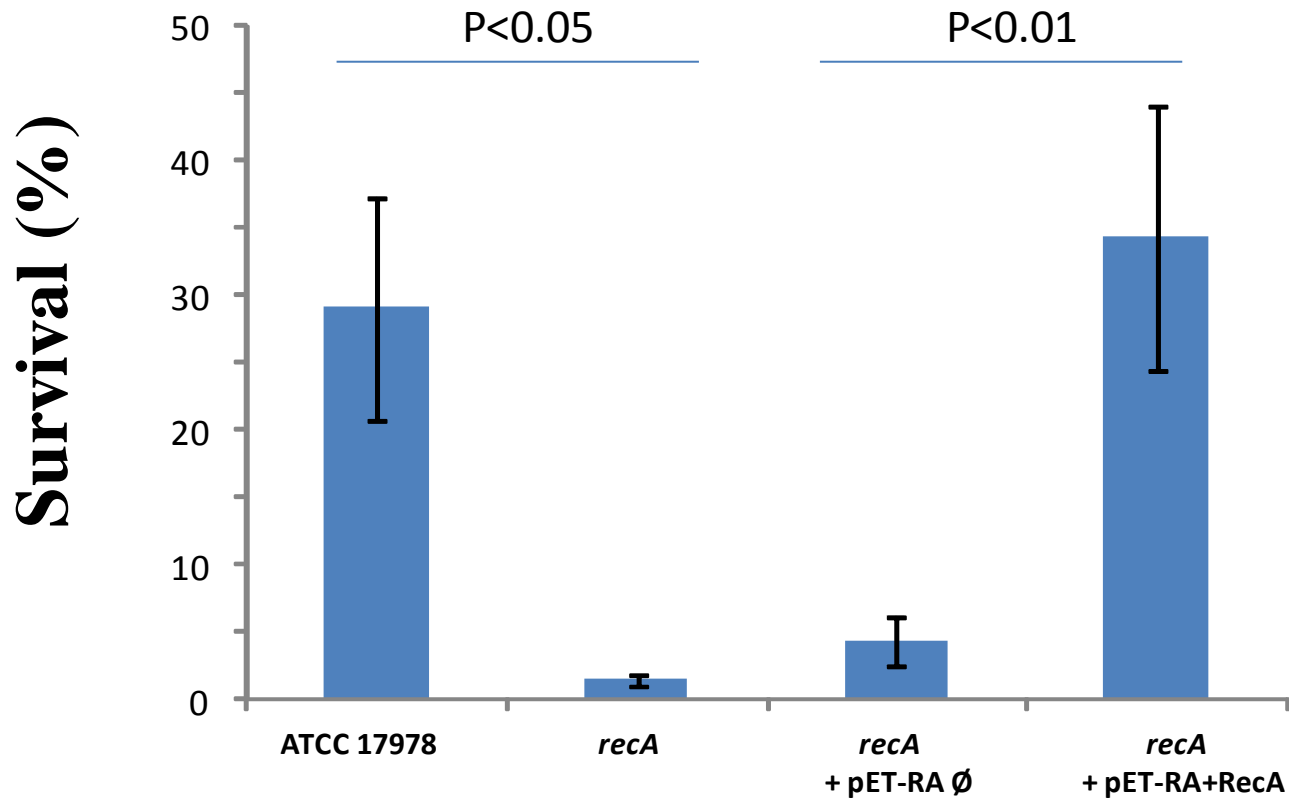
CONTROL



DROUGHT STRESS (24h)

**Desiccation resistance assays.** Dilution series of exponentially growing cells ( $OD_{600}$  of 0.5) were spotted on sterile cellulose filters (0.45- $\mu\text{m}$  pore size; Millipore). The filters were either not dried (control) or dried for 24 h inside a sterile petri plate at 37°C before they were placed on tryptic soy agar (TSA) plates and incubated for 24 h at 37°C.

# Virulence *in vitro* (macrophages)



Survival of the indicated strains of *A. baumannii* after 1-h incubation with macrophages (Cell line Raw 264.7).

# Virulence *in vivo* (mice)

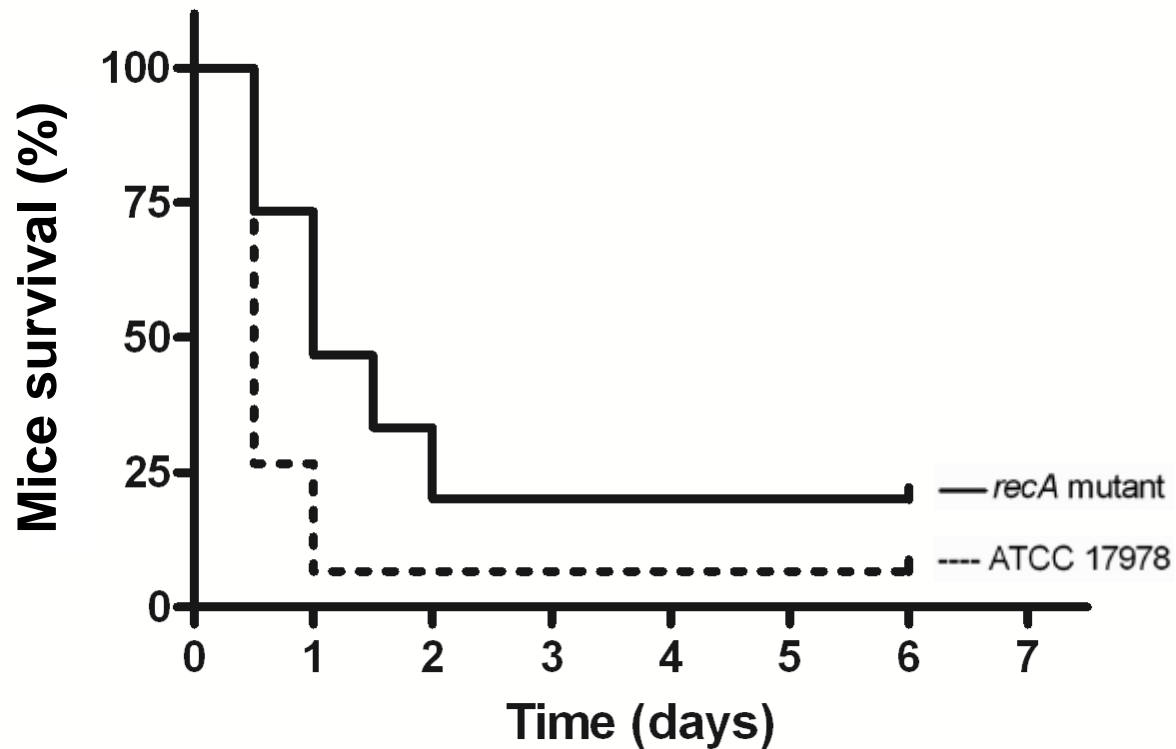


FIG. 7. Survival of mice ( $n = 15$  per group) inoculated with the *A. baumannii* WT or *recA* mutant strain. Significant differences in survival were noted (log rank test,  $P < 0.05$ ).

$2 \times 10^8$  CFU/mouse

ICR (CD1) female mice (Harlan Interfauna Ibérica, Barcelona, Spain)

# Antimicrobial susceptibilities

MICs of the antimicrobials used in this study for the indicated *A. baumannii* strains

Antibiotic	MIC of the indicated ATCC 17978 strain( $\mu\text{g/ml}$ ) <sup>a</sup>			
	Parent strain	<i>recA</i> mutant	<i>recA</i> pET-RA $\emptyset$ strain <sup>b</sup>	<i>recA</i> pET-RA-RecA strain
Piperacillin	32	12	12	24
Piperacillin/tazobactam	16	6	6	12
Amoxicillin/clavulanic acid	64	24	24	48
Imipenem	0.38	0.19	0.19	0.38
Ceftazidime	6	3	3	6
Cefotaxime	96	48	48	128
Colistin	0.5	0.25	0.25	0.38
Trimethoprim/sulfamethoxazole	4	0.75	0.75	6
Ciprofloxacin	0.25	0.016	0.016	0.25
Moxifloxacin	0.094	0.003	0.003	0.094
Erythromycin	4	4	4	6
Chloramphenicol	>256	>256	>256	>256
Tobramycin	0.38	0.38	0.38	0.38
Amikacin	1.5	1.5	1.5	2
Rifampin	4	4	NA	NA

<sup>a</sup> NA, not applicable (the pET-RA plasmid carries rifampin resistance).

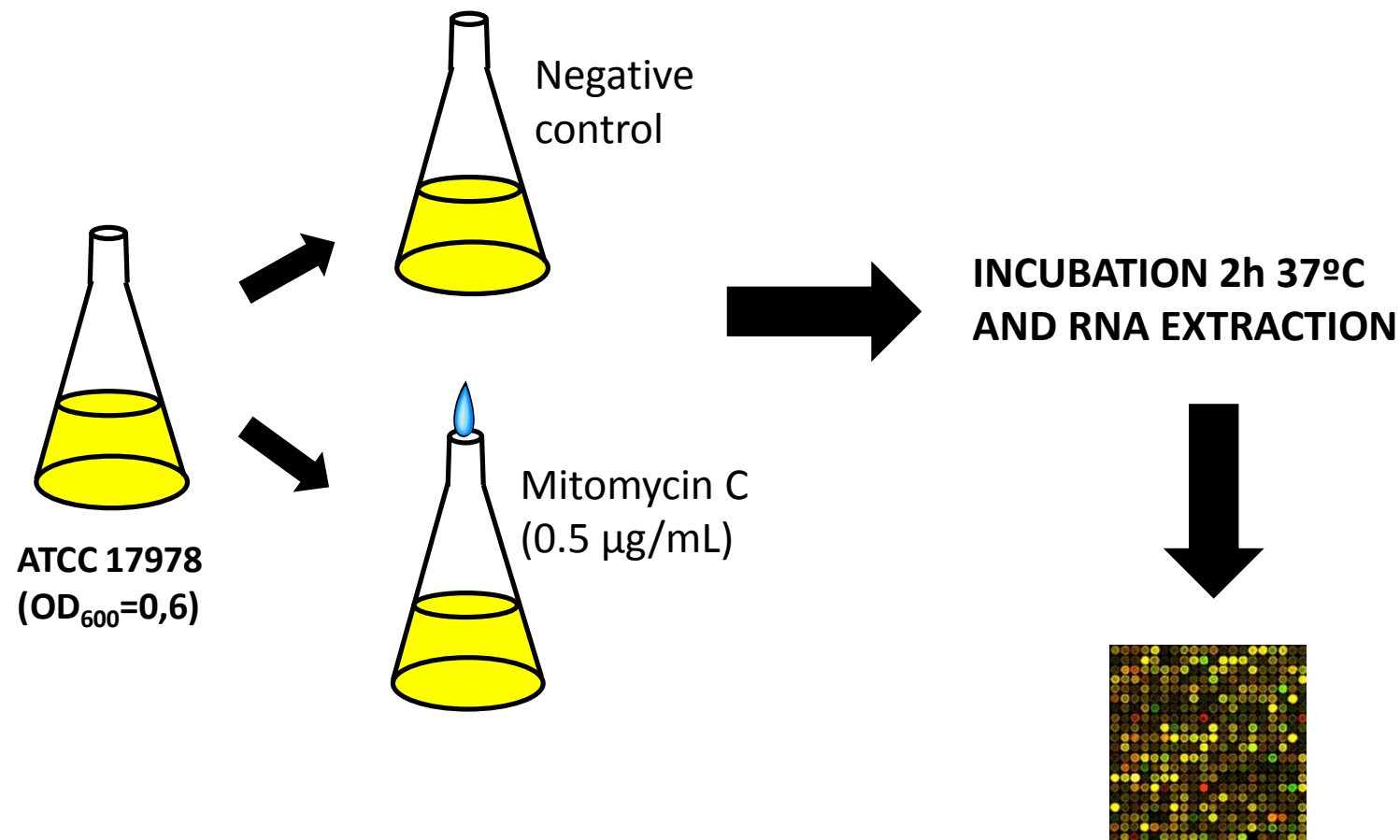
<sup>b</sup> pET-RA $\emptyset$ , empty pET-RA vector.



# Objective II

Analysis of the transcriptional response of *A. baumannii* to DNA-damaging agents

# Transcriptional response to MMC



# Transcriptional response to MMC

Genes deregulated in MMC-treated wild-type *A. baumannii*

Predicted functional group and gene <sup>a</sup>	Product description <sup>b</sup>	Expression level (fold change) <sup>c</sup>	Predicted functional group and gene <sup>a</sup>	Product description <sup>b</sup>	Expression level (fold change) <sup>c</sup>
Prophage			DNA repair		
<i>AIS_1145</i>	Putative Cro protein	2.4	<i>AIS_0636</i>	DNA polymerase V component UmuD	3.7
<i>AIS_1149</i>	Putative phage-related protein	1.6	<i>AIS_1174</i>	DNA polymerase V component UmuD	2.8
<i>AIS_1155</i>	Putative phage-related protein	1.5	<i>AIS_1389</i>	DNA polymerase V component UmuDAb	2.7
<i>AIS_1156</i>	Putative phage-related protein	1.6	<i>AIS_1962</i>	Recombinase A	2.1
<i>AIS_1581</i>	Putative methyltransferase	1.9	<i>AIS_2008</i>	DNA polymerase V component UmuC	3.1
<i>AIS_1583</i>	Hypothetical protein (putative family peptidase S24)	1.5	<i>AIS_2015</i>	DNA polymerase V component	1.5
<i>AIS_1587</i>	Phage terminase EsvK2	2	<i>AIS_2035</i>	Hypothetical protein (putative endonuclease)	2
<i>AIS_1588</i>	Phage terminase-like protein large subunit	1.9	<i>AIS_2036</i>	DNA cytosine methyltransferase	1.7
<i>AIS_1590</i>	Peptidase U35 phage prohead HK97	1.9	<i>AIS_2039</i>	Hypothetical protein (DNA polymerase III subunit-like)	3.2
<i>AIS_1591</i>	Phage major capsid protein HK97	2.8	<i>AIS_2586</i>	dGTP triphosphohydrolase-like protein	2.3
<i>AIS_1592</i>	Phage head-tail adaptor	2.1	<i>AIS_3115</i>	Hypothetical protein (putative DNA metabolism protein)	2.5
<i>AIS_1593</i>	Hypothetical protein (putative phage protein HK97 gp10 family)	2.3	<i>AIS_3116</i>	Hypothetical protein (putative DNA repair SAM protein)	3.7
<i>AIS_1594</i>	Hypothetical protein (putative phage protein)	2.2	<i>AIS_3295</i>	Nucleotide excision repair component UvrA	4.3
<i>AIS_1595</i>	Hypothetical protein (phage tail protein)	1.8	Unknown		
<i>AIS_1596</i>	Hypothetical protein (phage tail protein)	1.8	<i>AIS_1143</i>	Hypothetical protein	3.4
<i>AIS_1597</i>	Lambda family phage tail tape measure protein	1.7	<i>AIS_1226</i>	Hypothetical protein	1.9
<i>AIS_1598</i>	Hypothetical protein (putative phage protein)	1.9	<i>AIS_1388</i>	Hypothetical protein DdrR	4.2
<i>AIS_1599</i>	Hypothetical protein (putative phage protein)	2	<i>AIS_2033</i>	Hypothetical protein	1.5
<i>AIS_1600</i>	Lysozyme	2.1	<i>AIS_2038</i>	Hypothetical protein (putative lipoprotein)	4.2
			<i>AIS_3385</i>	Putative membrane protein	1.9

# **O**bjective III

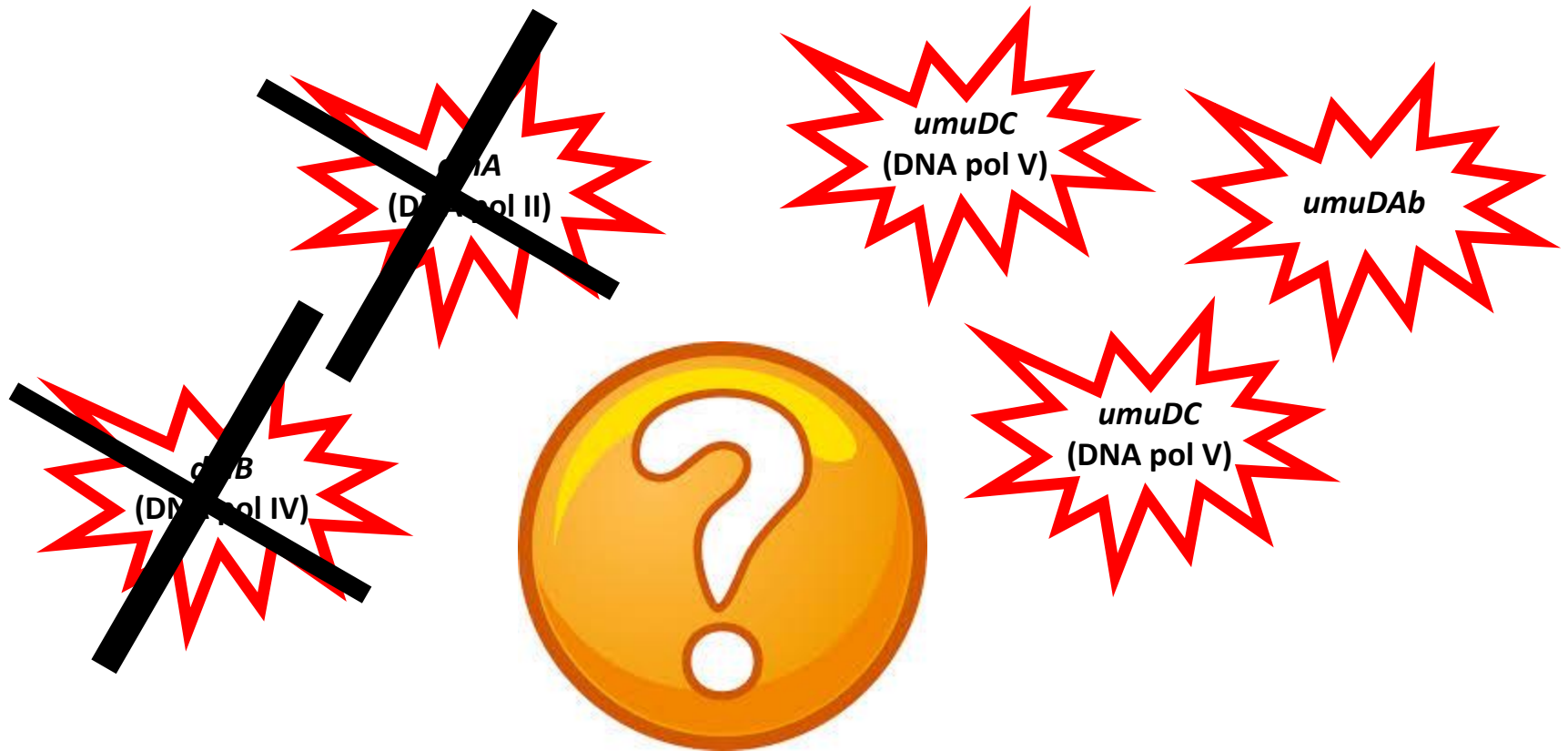
**Analysis of the DNA damage-  
induced mutagenesis in *A.*  
*baumannii***

# DNA damage-induced mutagenesis in *E. coli*

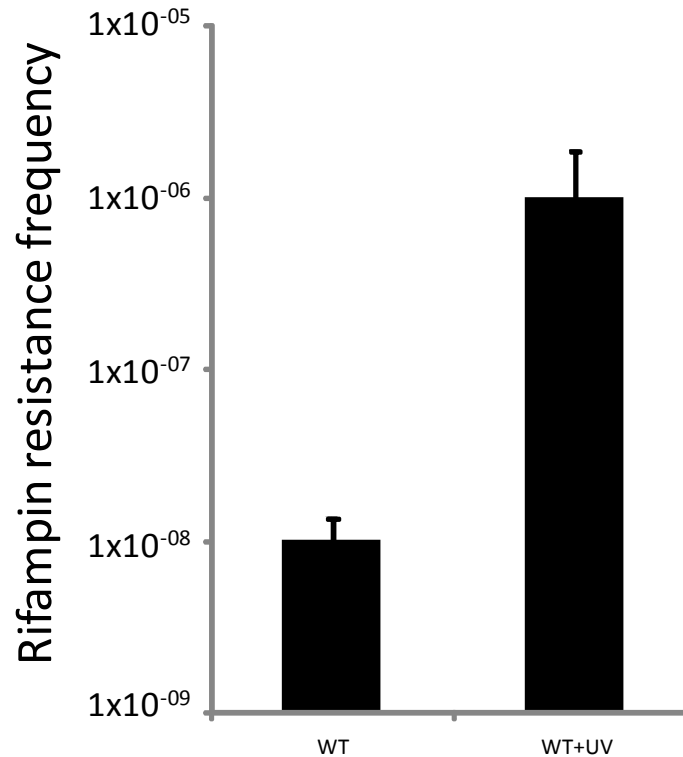


The induction of genes encoding error prone DNA polymerases causes an increase of the mutation rate

# DNA damage-induced mutagenesis in *A. baumannii*?



# DNA damage-induced mutagenesis in *A. baumannii*

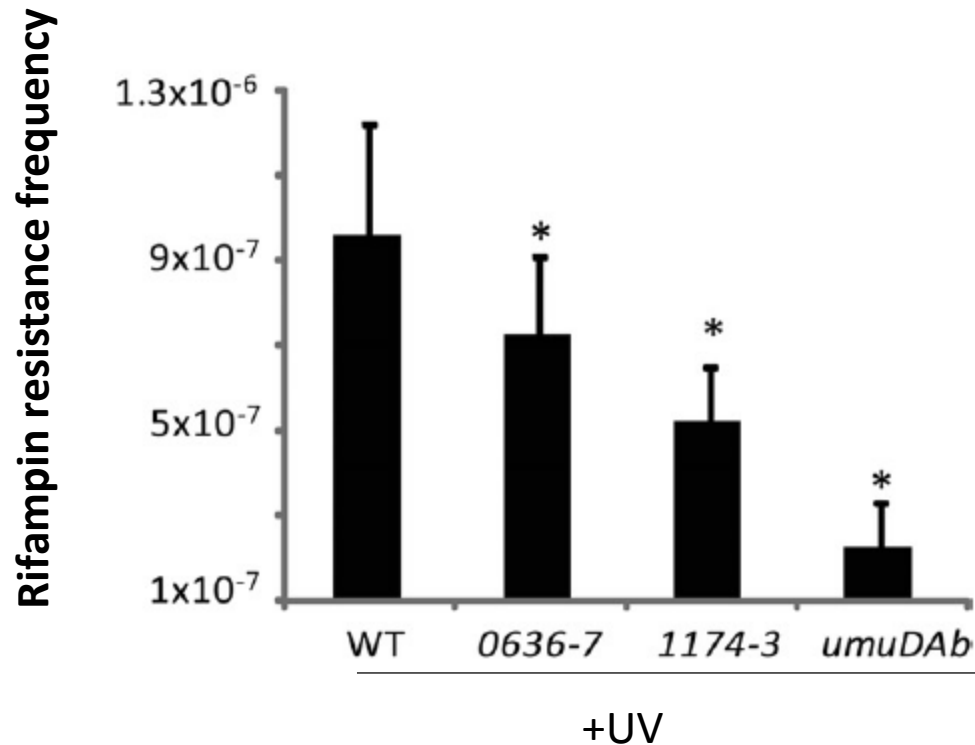


***A. baumannii* ATCC 17978**

UV: Treatment ( $100 \text{ J/m}^2$ )

\* $P < 0,01$

# DNA damage-induced mutagenesis in *A. baumannii*



UV: Treatment (100 J/m<sup>2</sup>)

\*P<0,05



# Transcriptional response to MMC

Genes deregulated in MMC-treated wild-type *A. baumannii*

Predicted functional group and gene <sup>a</sup>	Product description <sup>b</sup>	Expression level (fold change) <sup>c</sup>	Predicted functional group and gene <sup>a</sup>	Product description <sup>b</sup>	Expression level (fold change) <sup>c</sup>
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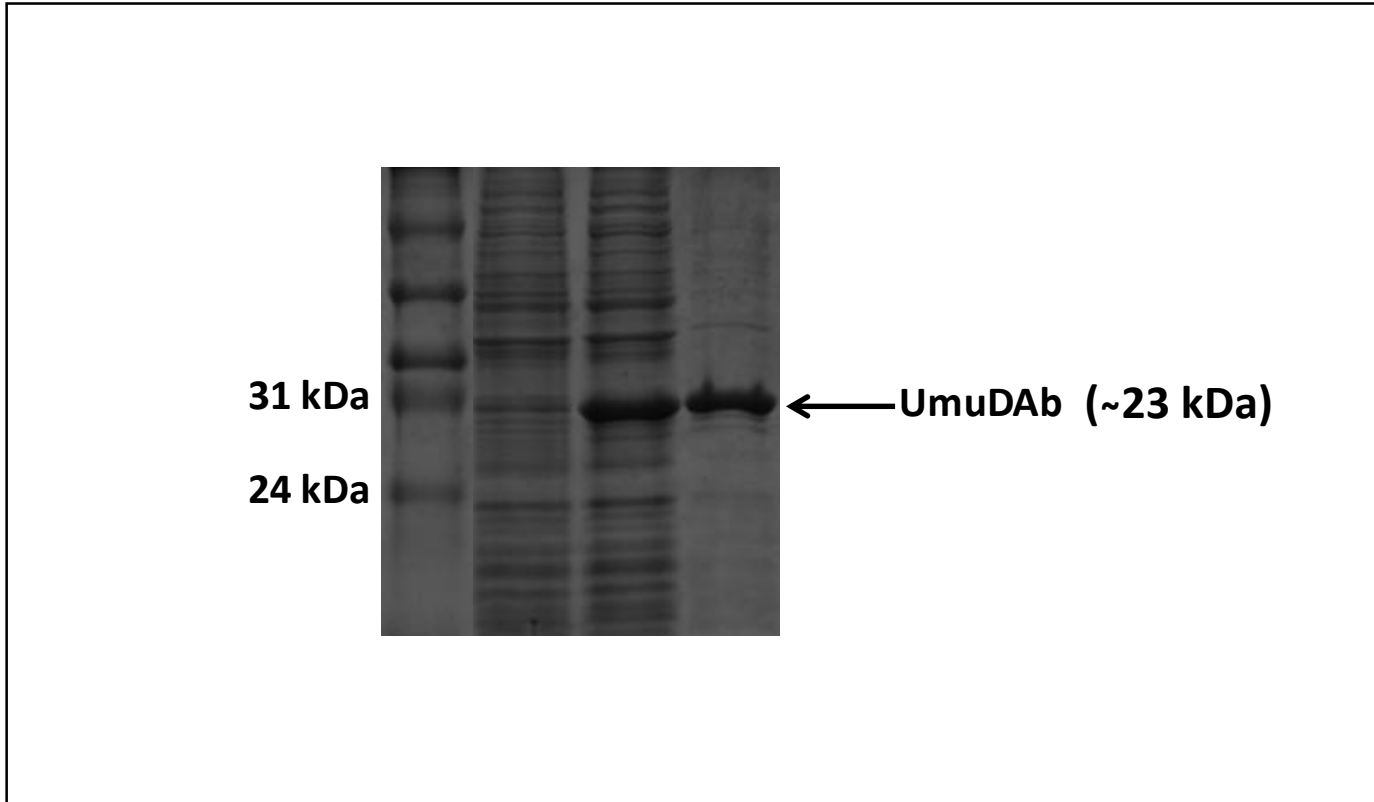
# UmuDAb

-Is present in practically all *Acinetobacter* spp.

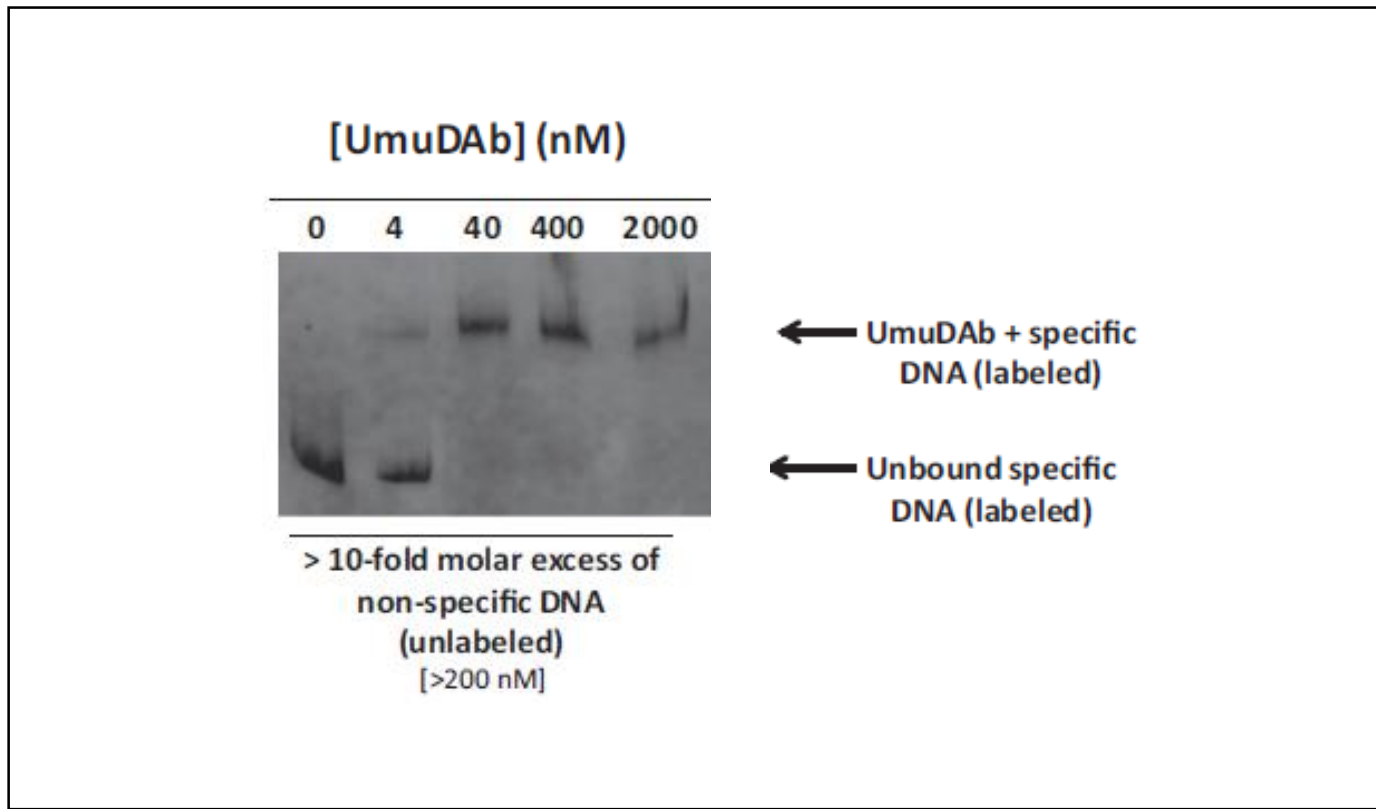
-Encodes an extra N-terminus region

```
UmuDAb      MPKKKEFEHGGARENAGRKAQFNEPTKVIRVPESQVNFIGNWLLNNVKTNNQTDFTSKLK 60
UmuDE.coli  -----MLFIKPAD 8
```

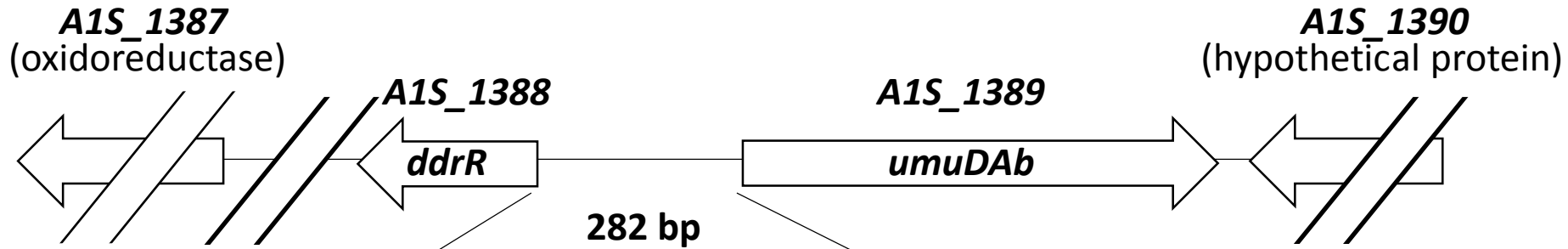
# Purification of UmuDAb



# UmuDAb specifically binds to its promoter region

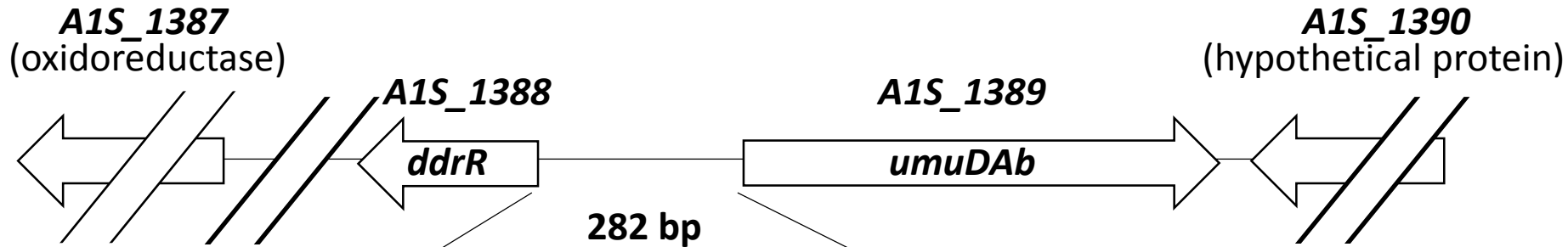


# The promoter region of *umuDAb*



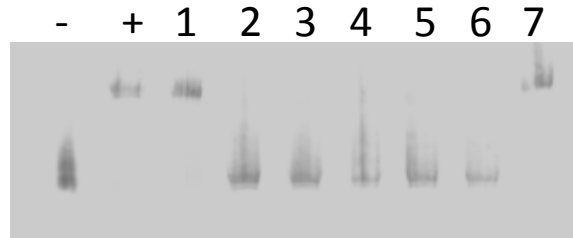
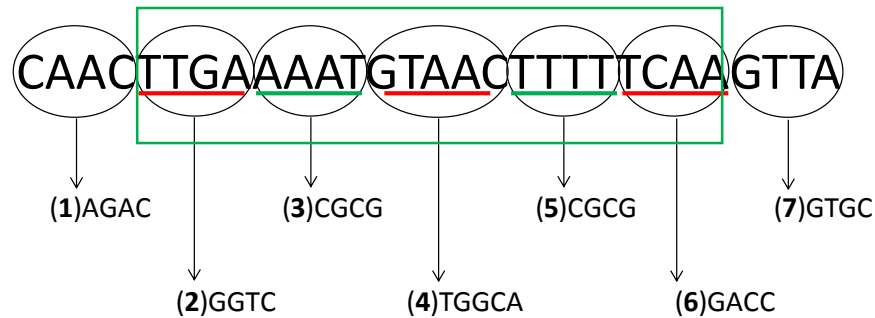
```
TTCGCATGTCTCCATACCAAAAAGTGAATCTAACTCATATTTA
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TAAGAACTATTTACAATAGGTTTTGATTCTCTTCAAATTACC
ATTTCCGTTTTAACATCCCTGTTAAAAGCAAAATGGATTAAA
ATCTGATCATGAAATTTATTGTTATGCAAAAAAATATTTTCGT
CAACTTGAAAATGTAACTTTTTCAAGTTACATTGTTTGTGTA
GGATTTGAACGGTGAAATGGAGGCGAT
```

# The promoter region of *umuDAb*



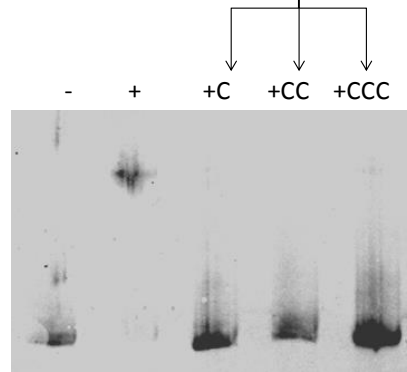
TTCGCATGTCTCCATACCAAAAAGTGAATCTAACTCATATTTA  
AGTGTTTCAGAATTTTCATTGATAAAAATCAAACAGTCAGTA  
ATAAGAACTATTTCACAATAGGTTTTGATTCTCTTCAAATTAC  
CATTTCCGTTTTAACATCCCTGTTAAAAGCAAATGGATTAA  
AATCTGATCATGAAATTTATTGTTATGCAAAAAAATATTTTCG  
TCAAC **TTGAAATGTAACTTTCAA** GTTA  
CATTGTTTGTGTAGGATTTGAACGGTGAAATGGAGGCGAT

# UmuDAb specifically binds to its promoter region



# UmuDAb specifically binds to its promoter region

TTGA   AAAT   GT AAC   TTTT   TCAA





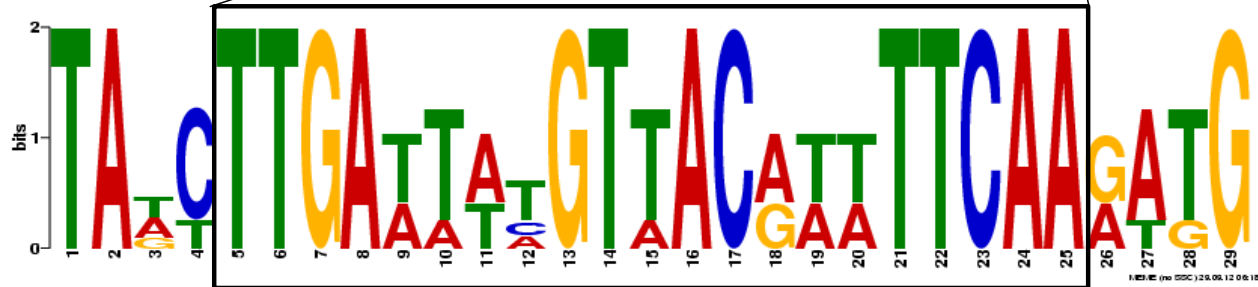
# The UmuDAb regulon

The MEME Suite

Motif-based sequence analysis tools

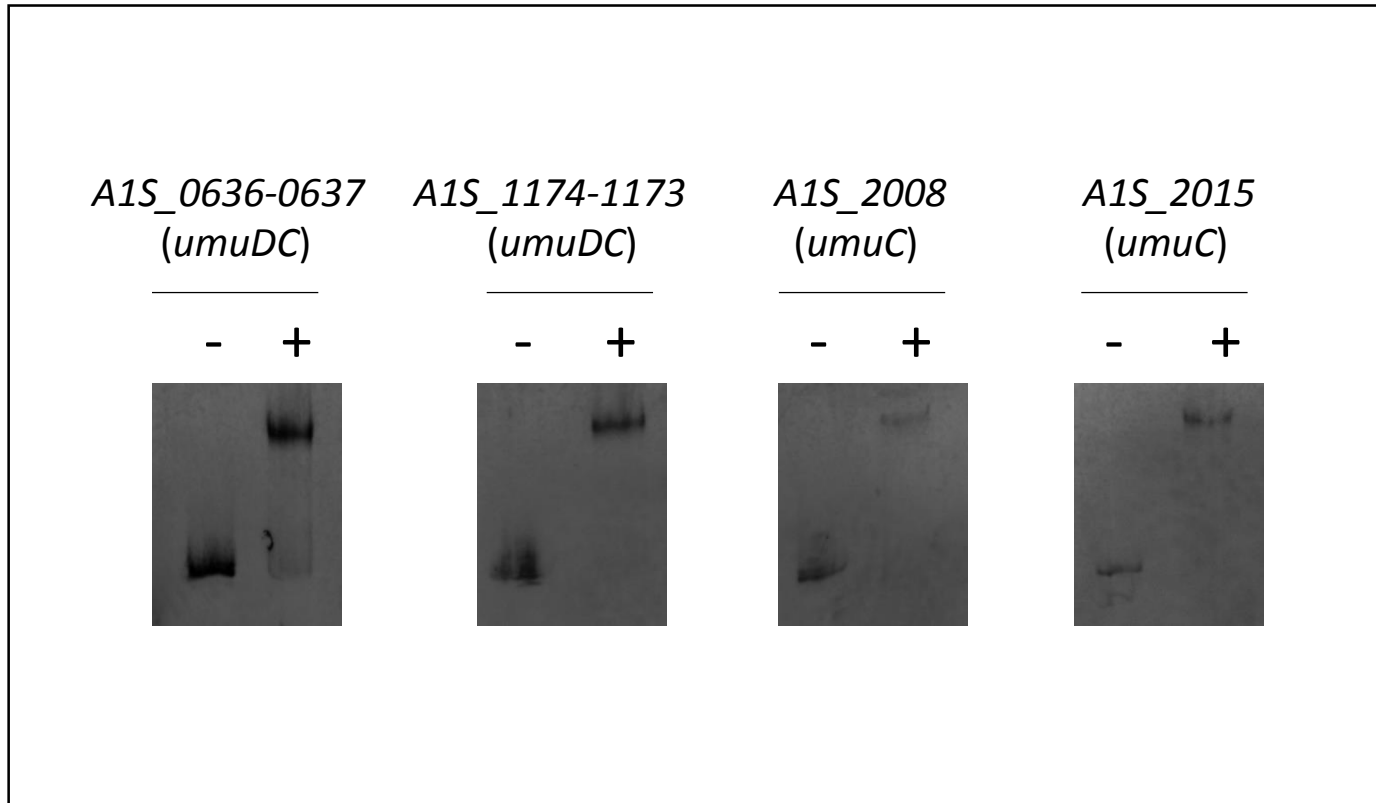
EMBL  
Australia Bioinformatics Resource

Gene	p-value	Sequence
A1S_2008 ( <i>umuC</i> )	1.43X10 <sup>-14</sup>	TAACTTGATTATGTTACAAATTCAAAATG
A1S_2015 ( <i>umuC</i> )	2.75X10 <sup>-14</sup>	TATCTTGAATTTGTTACGATTTCAAATG
A1S_1174 ( <i>umuD</i> )	3.22X10 <sup>-14</sup>	TAGCTTGAATTTTGTAAACATATTCAAGATG
A1S_0636 ( <i>umuD</i> )	8.20X10 <sup>-14</sup>	TATTTTGAATTACGTTACGTTTTCAAGAGG
A1S_1389 ( <i>umuDAb</i> )	1.92X10 <sup>-15</sup>	TAACTTGAAAAAGTTACATTTTCAAATTG



CONSENSUS: **TTGA NNNN GTNAC NNNN TCAA**

# The UmuDAb regulon



# The UmuDAb regulon

Inactivation of the *umuDAb* gene resulted in the deregulation of all DNA-damage-induced genes containing the described palindromic DNA motif

Gene	Designation	Product	Sequence	Position
<i>AIS_0636</i>	<i>umuD</i>	DNA polymerase V component	TTGATTACGTTACGTTTCAA	12
<i>AIS_0637</i>	<i>umuC</i>	DNA polymerase V component		
<i>AIS_1174</i>	<i>umuD</i>	DNA polymerase V component	TTGAATATGTTACAAAATCAA	15
<i>AIS_1173</i>	<i>umuC</i>	DNA polymerase V component		
<i>AIS_1389</i>	<i>umuDAb</i>	DNA polymerase V component	TTGAAAATGTAACTTTTCAA	45
<i>AIS_2008</i>	<i>umuC</i>	DNA polymerase V component	TTGATTATGTTACAAATCAA	212
<i>AIS_2015</i>	<i>umuC</i>	DNA polymerase V component	TTGAATTTGTTACGATTCAA	169

# Conclusions

I. The *A. baumannii* RecA protein is involved in repair of DNA damage, antimicrobial resistance, general stress response and virulence.

II. *A. baumannii* lacks the LexA repressor and the error-prone DNA polymerase UmuDAb carries out its functional role.

III. The *A. baumannii* UmuD homologs are involved in antibiotic resistance acquired through DNA damage-induced mutagenesis.