



**Vilnius  
University**

**Skoltech**

Skolkovo Institute of Science and Technology

*MSc Program*

# The Study of BREX System Proteins Functions

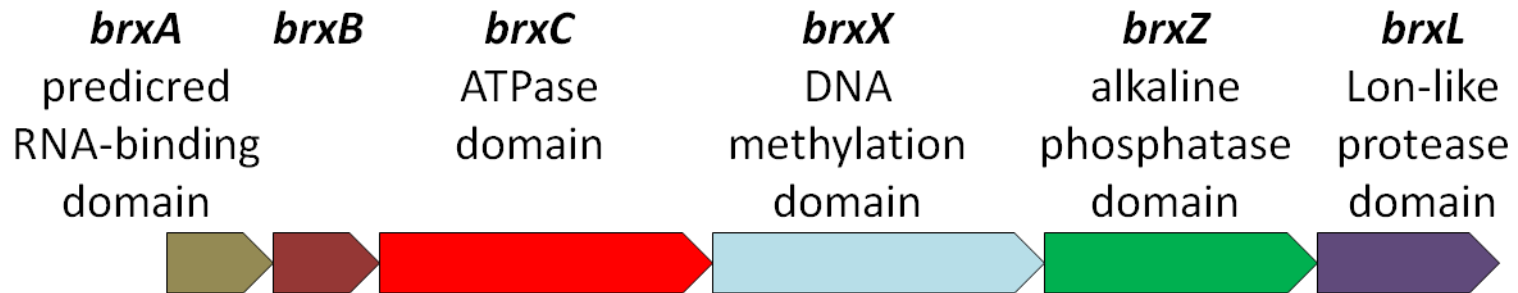
Student: *Alena Drobiazko*

Research Advisor: *Konstantin Severinov*

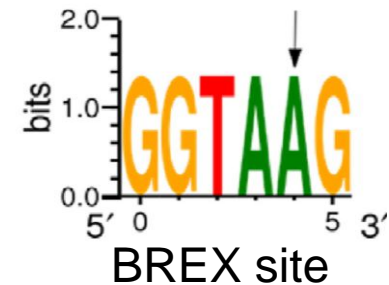
*Co-Advisor : Virginijus Šikšnys*

06, 2019

# BREX system type I



BREX system methylates the GGTAAG sites at the fifth adenosine residue



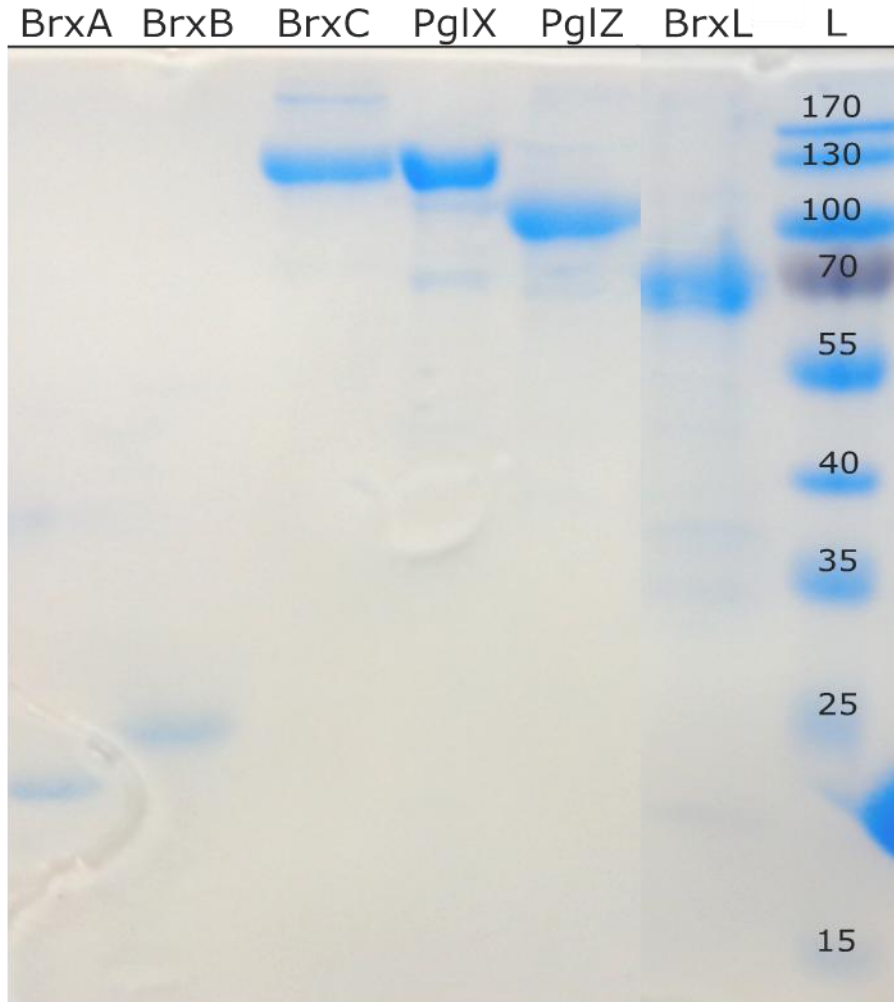
Based on (Goldfarb et al., *EMBO J.*, 34, 2015) and (Gordeeva et al., *NAR*, 47, 2019)

The goal of the project was to confirm the predicted function of some of BREX proteins individually and in complexes with other components of the system *in vivo* and *in vitro*.

## Objectives

- ❑ Expression and purification of recombinant BREX proteins;
- ❑ *In vitro* detection of methylation activity of methyltransferase or methylation complex;
- ❑ Identification of BrxX binding to DNA;
- ❑ Identification of the minimal BREX system methylation complex *in vivo*;
- ❑ Obtaining of the T7 phage mutants that can overcome the BREX system action and identification of the mutations responsible for the circumvent;
- ❑ Detection of potential toxin-antitoxin interaction.

# Purification of BREX proteins



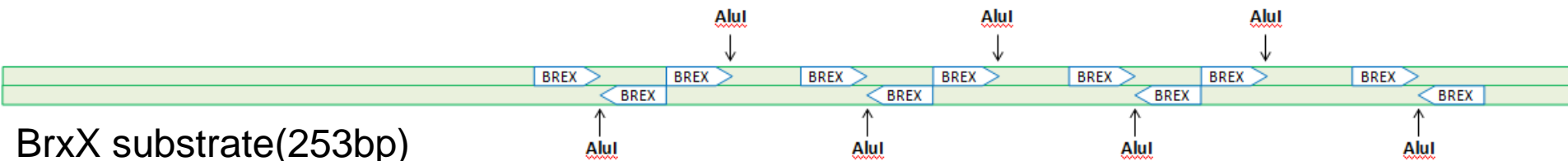
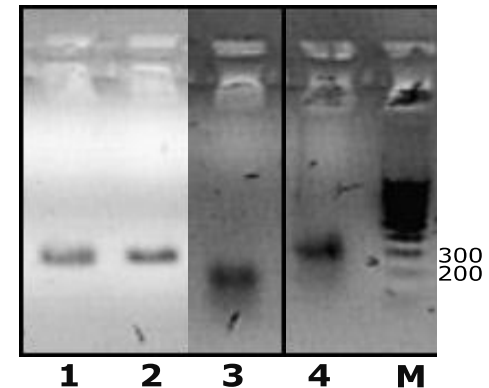
Protein	Mass (kDa)	Amount (mg)
BrxA	22.7	20
BrxB	22.8	5
BrxC	139.3	5
BrxX	138	1
BrxZ	100.2	18
BrxL	77.4	1

# BrxX *in vitro* methylation assay

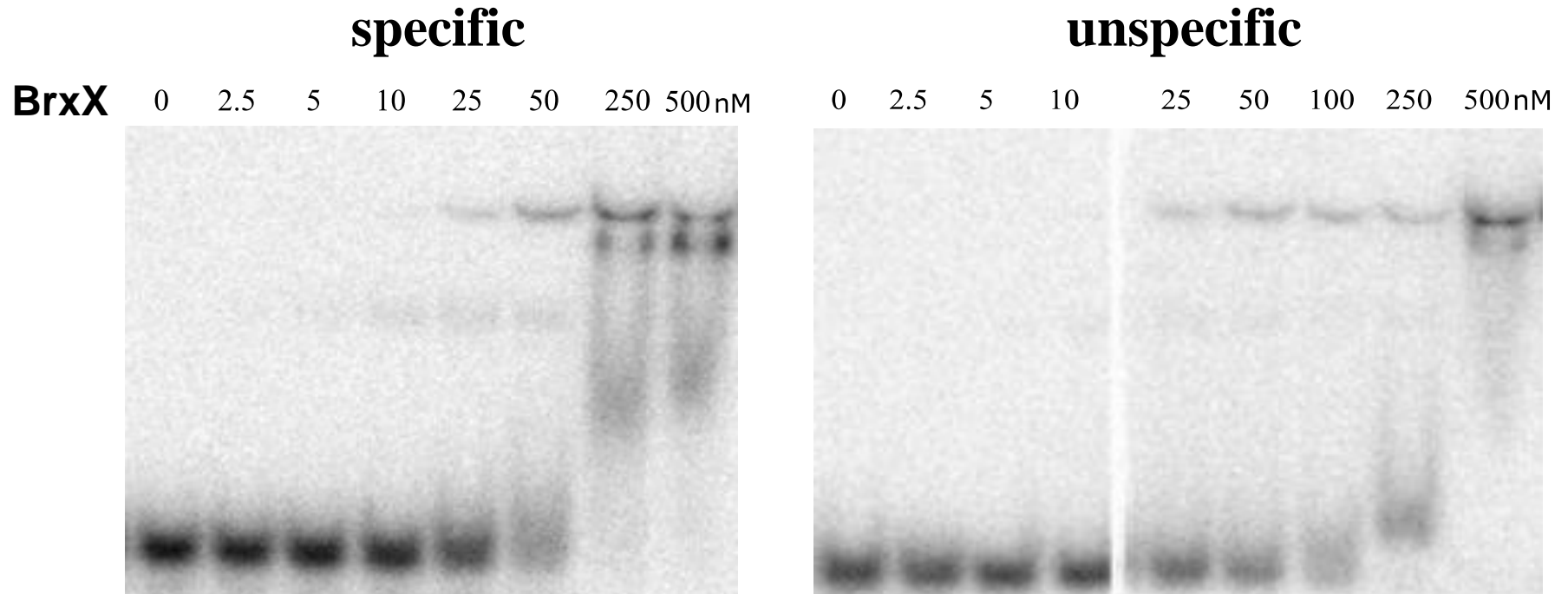
As AluI restriction endonuclease is sensitive to methylation, we expect that DNA substrate modified with BrxX would not be cleaved by AluI



BrxX	+	+	+	+
DNA substrate	+	+	+	+
SAM	-	+	-	+
AluI digestion	-	-	+	+



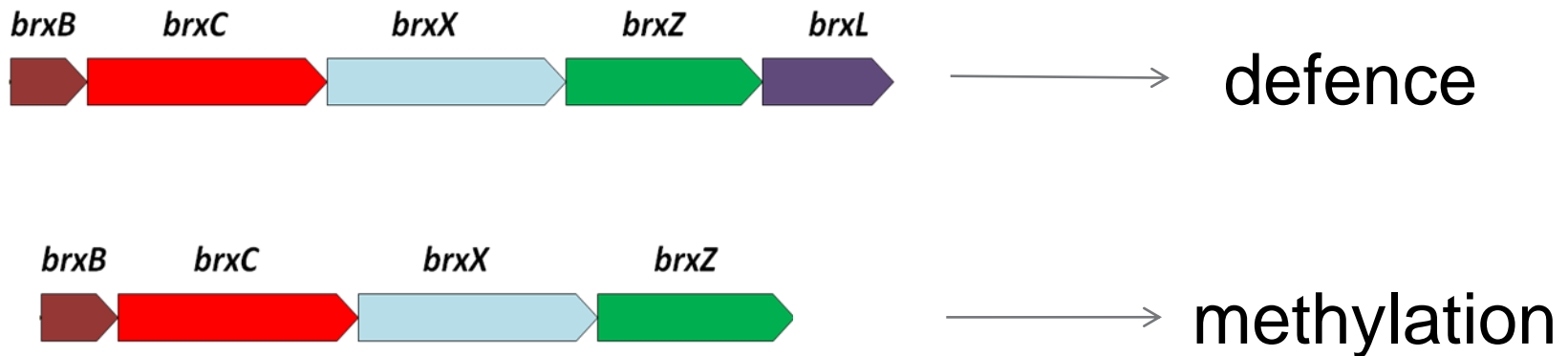
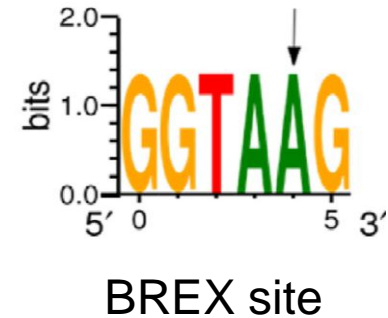
# Binding of the BrxX protein to DNA



BrxX is able to binds DNA, irrespective to BREX site

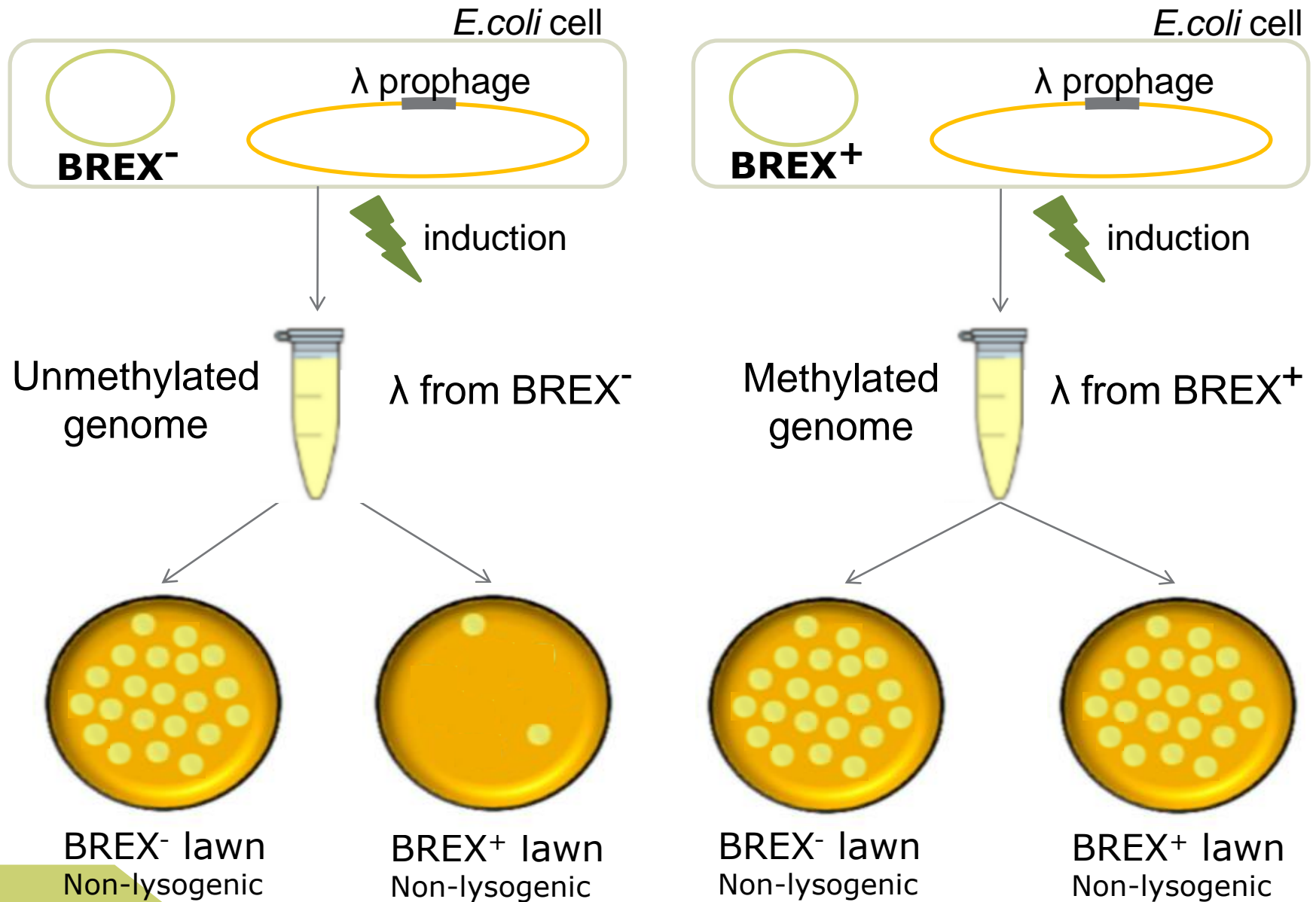
# Detection of the minimal BREX methylation complex *in vivo*

BREX system methylates the GGTAAG sites at the fifth adenosine residue



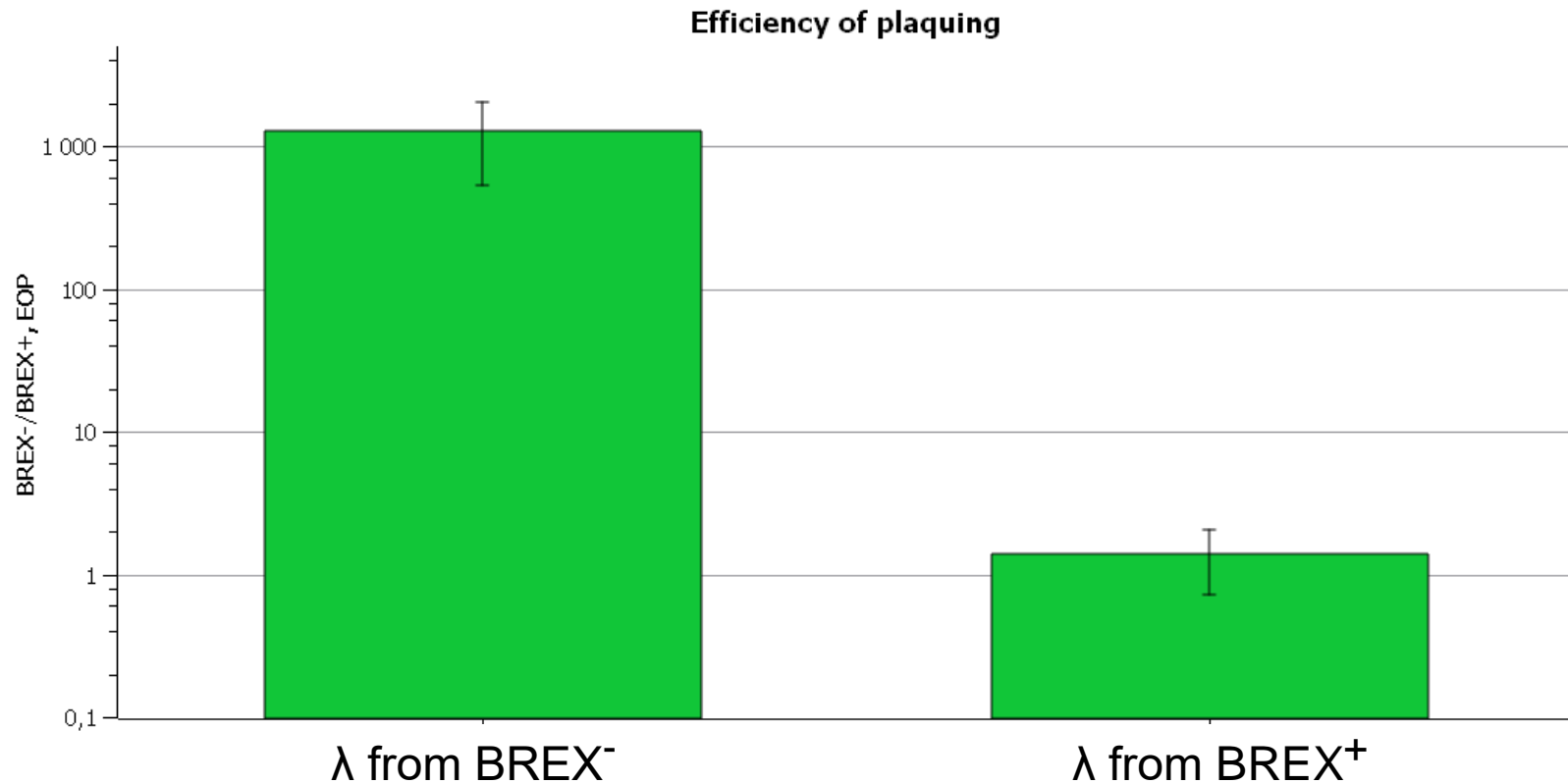
Based on (Gordeeva et al., *NAR*, 47, 2019)

# Detection of BREX modification in phage genome





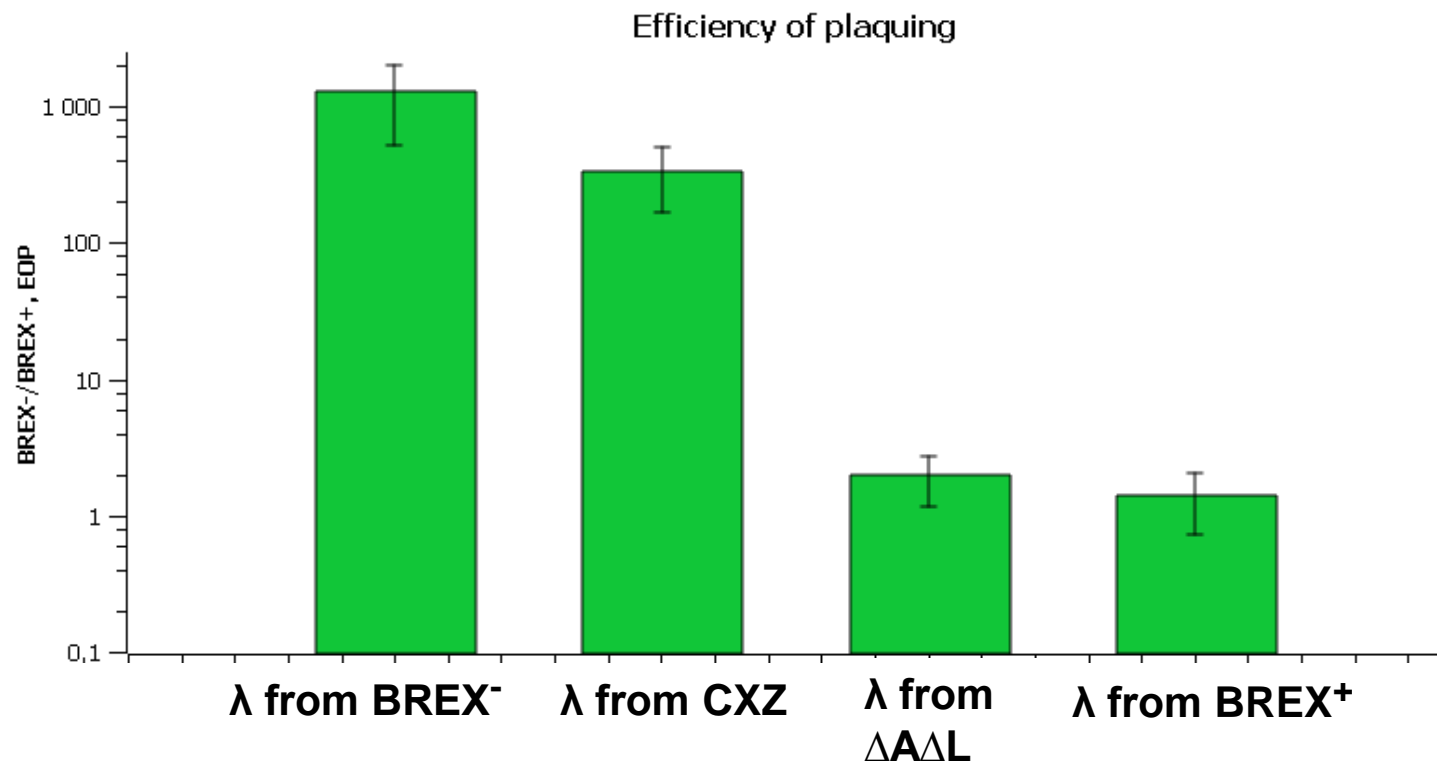
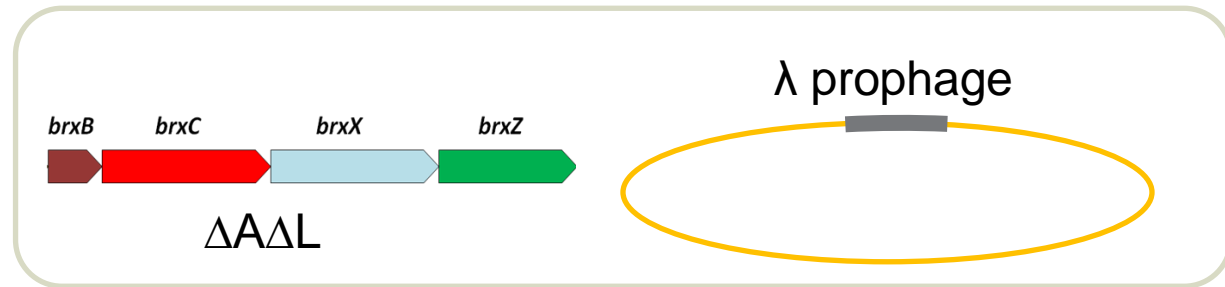
# Phage induced from BREX<sup>+</sup> cells is able to overcome BREX protection



$\lambda$  induced from BREX<sup>+</sup> lysogens infects both BREX<sup>+</sup> and BREX<sup>-</sup> non-lysogenic cultures with the same effectiveness.

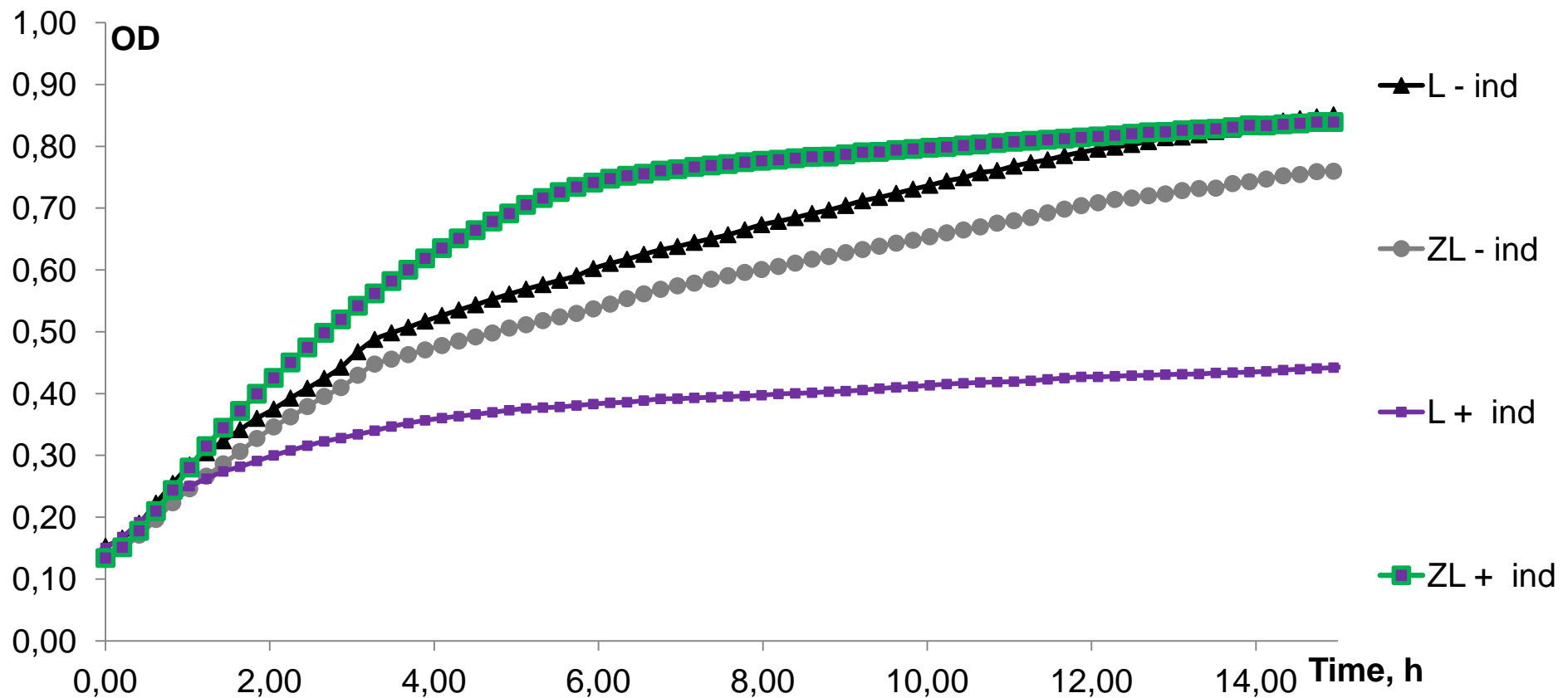
Expression of four BREX proteins is sufficient for methylation

*E.coli* cell



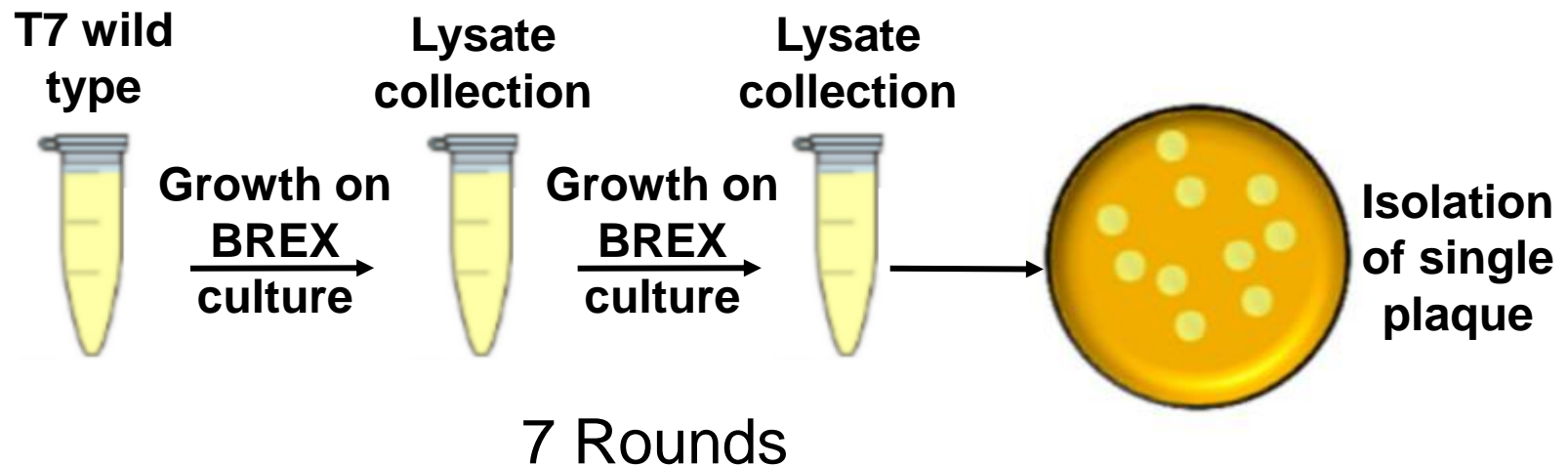
Expression of four BREX proteins is enough for modification, but is not enough to protect the cell from phage infection

# Growth curves of strains, expressing BrxZ and BrxL proteins

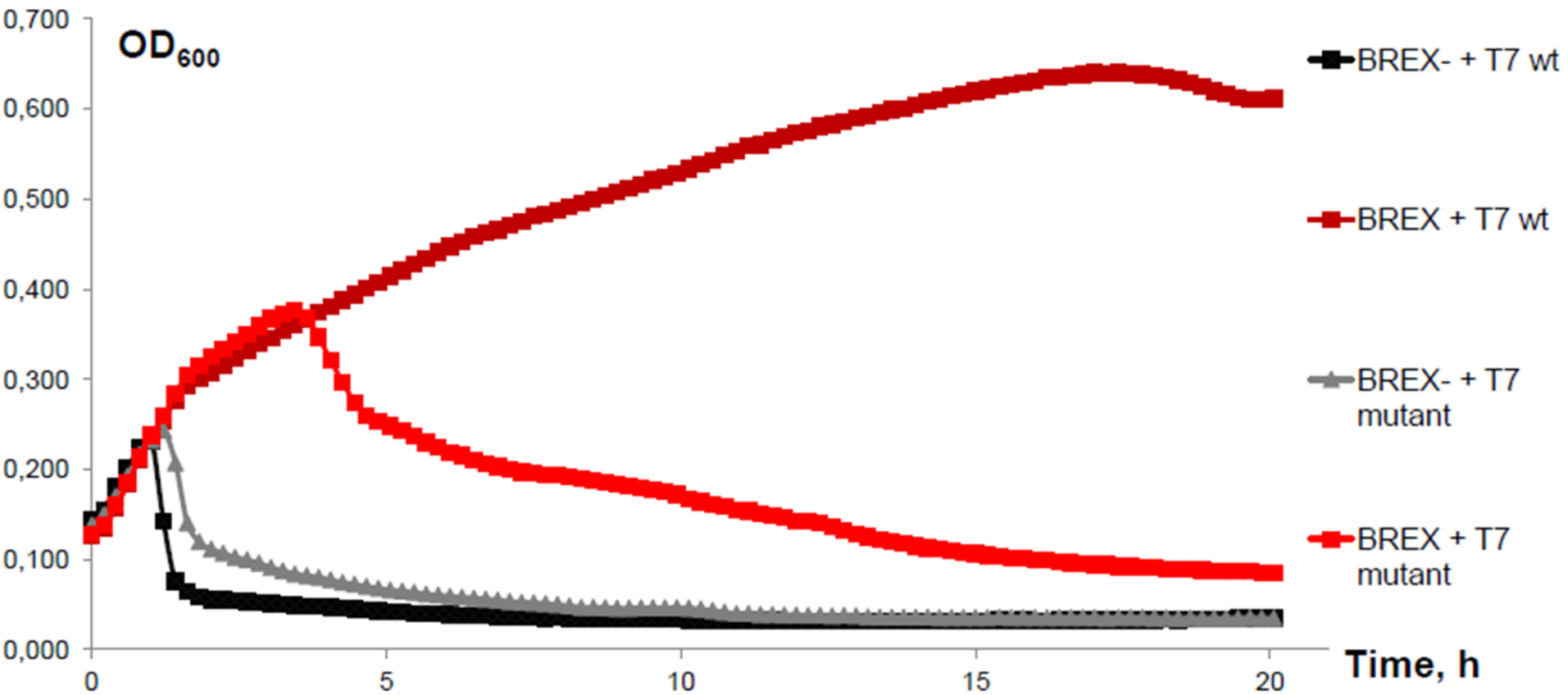


Expression of BrxZ alleviates toxic effect of BrxL

# Selection of BREX-resistant T7 phages



# Growth of BREX<sup>-</sup> and BREX<sup>+</sup> cultures infected with T7 wildtype and T7 mutant



Several mutants carry a mutation at the first BREX site



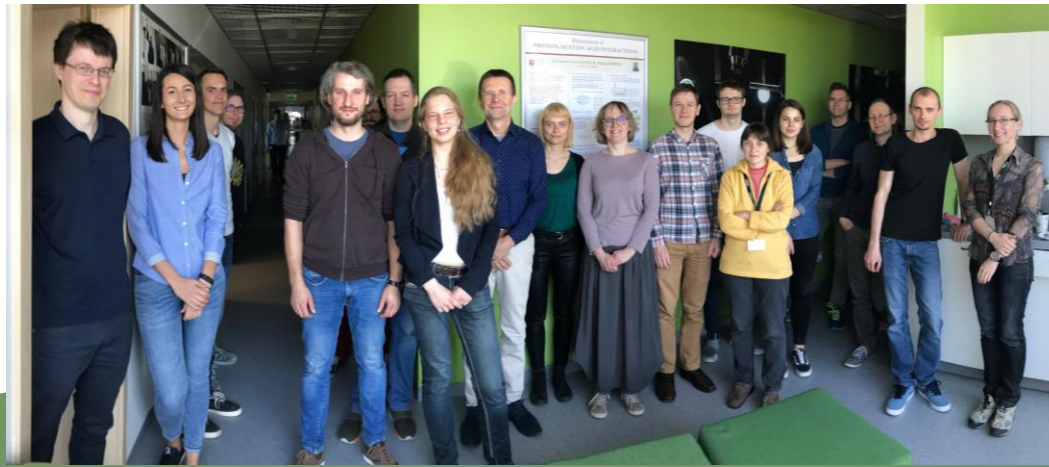
# Results and Conclusions

- ✓ Recombinant BREX proteins were expressed and purified;
- ✓ Methylation activity of BrxX was detected *in vitro*;
- ✓ BrxX methyltransferase unspecifically binds to DNA;
- ✓ The minimal complex for *in vivo* methylation activity comprises BrxBCXZ proteins;
- ✓ Expression of BrxZ protein alleviates toxicity of BrxL;
- ✓ BREX-resistant T7 phages carry mutation at the first BREX methylation site.

- Professor Virginijus Šikšnys
- Dr Tomas Šinkunas
- Giedre Tamulaitiene
- Inga Songailiene
- Elena Manakova

- Professor Konstantin Severinov
- Artem Isaev
- Yulia Gordeeva

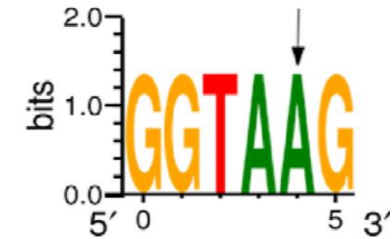
all my colleagues from Skoltech and Vilnius University Life Sciences Center



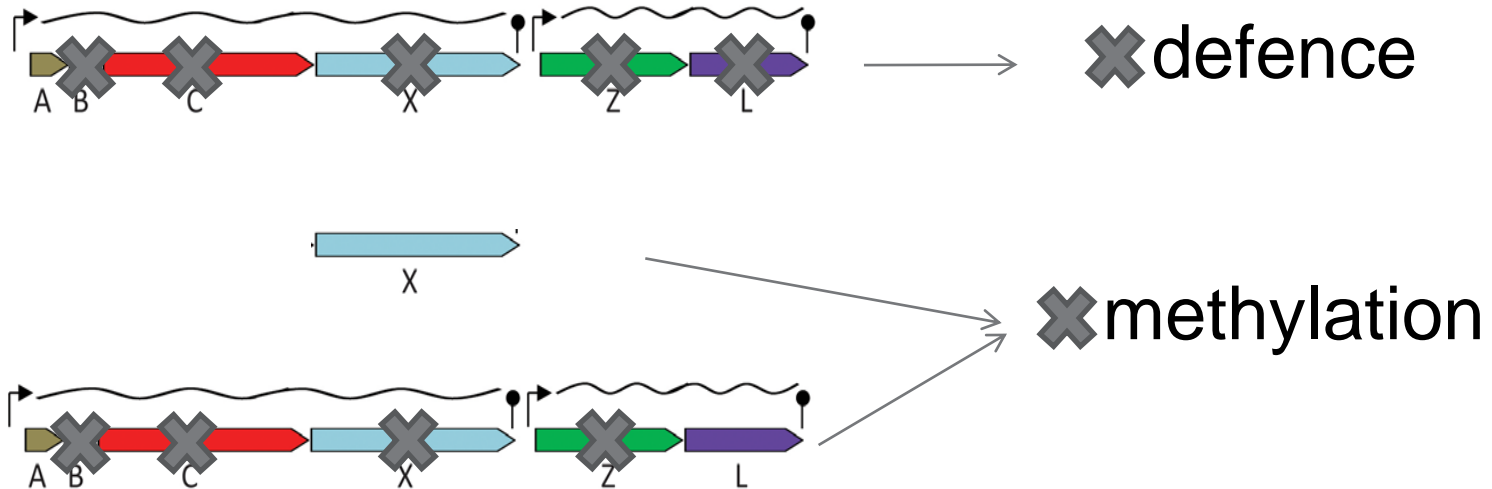


# Detection of the minimal BREX methylation complex *in vivo*

BREX system methylates the GGTAAG sites at the fifth adenosine residue



BREX site

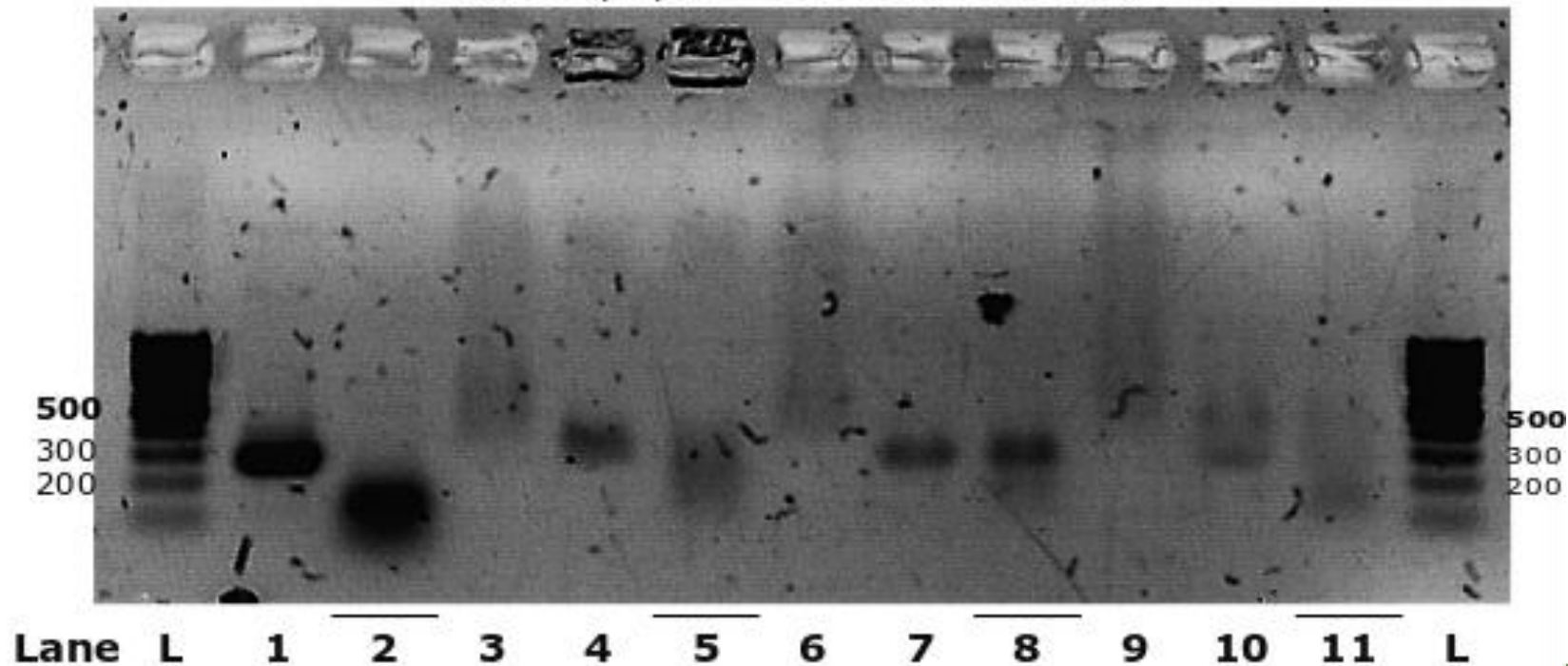


Based on (Gordeeva et al., *NAR*, 47, 2019)

# Interaction between the BrxX protein and the substrate containing multiple BREX sites

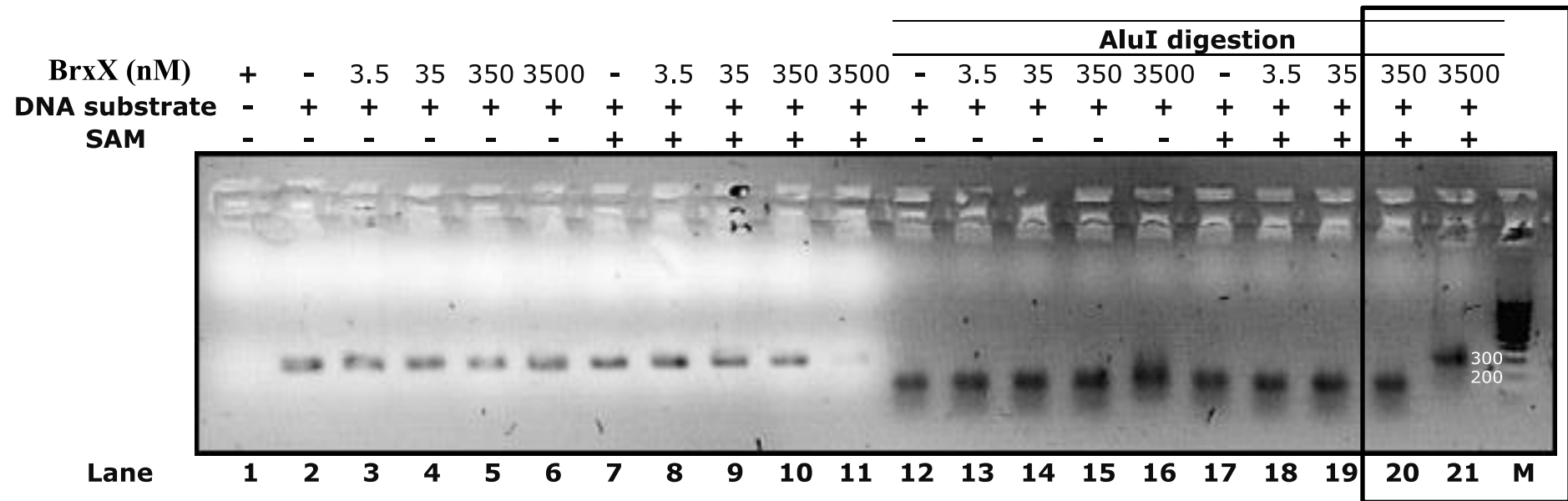
BrxX inactivation		Heating			Phenol			Proteinase K		
BrxX		-	-	+	+	+	+	+	+	+
DNA substrate	+	+	+	+	+	+	+	+	+	+
SAM	-	-	+	+	+	+	+	+	+	+
AluI	-	+	-	-	+	-	-	+	-	+

Lanes 3, 6, 9 – before inactivation

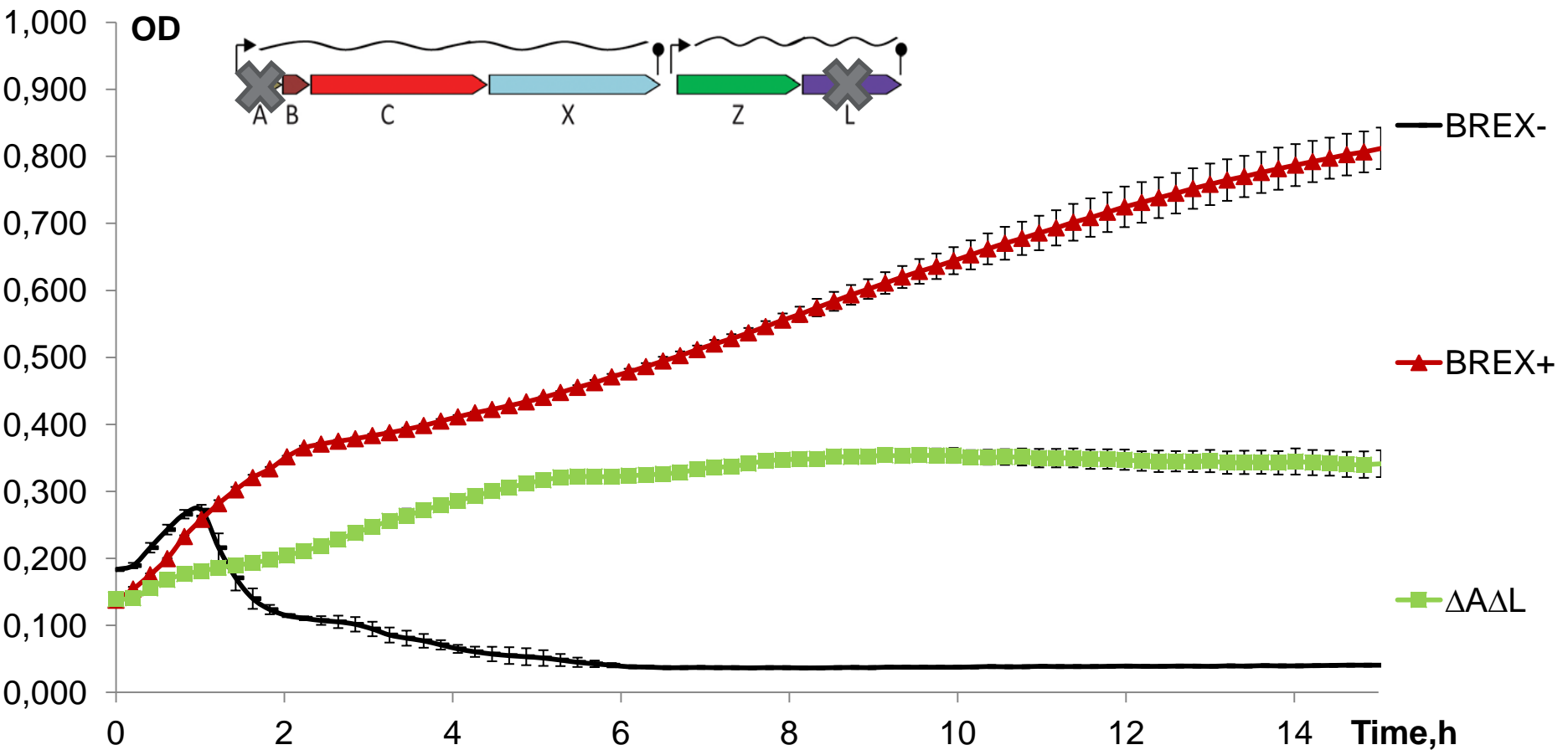


# BrxX *in vitro* methylation assay

As AluI restriction endonuclease is sensitive to methylation, we expect that substrate DNA modified with BrxX is left uncut

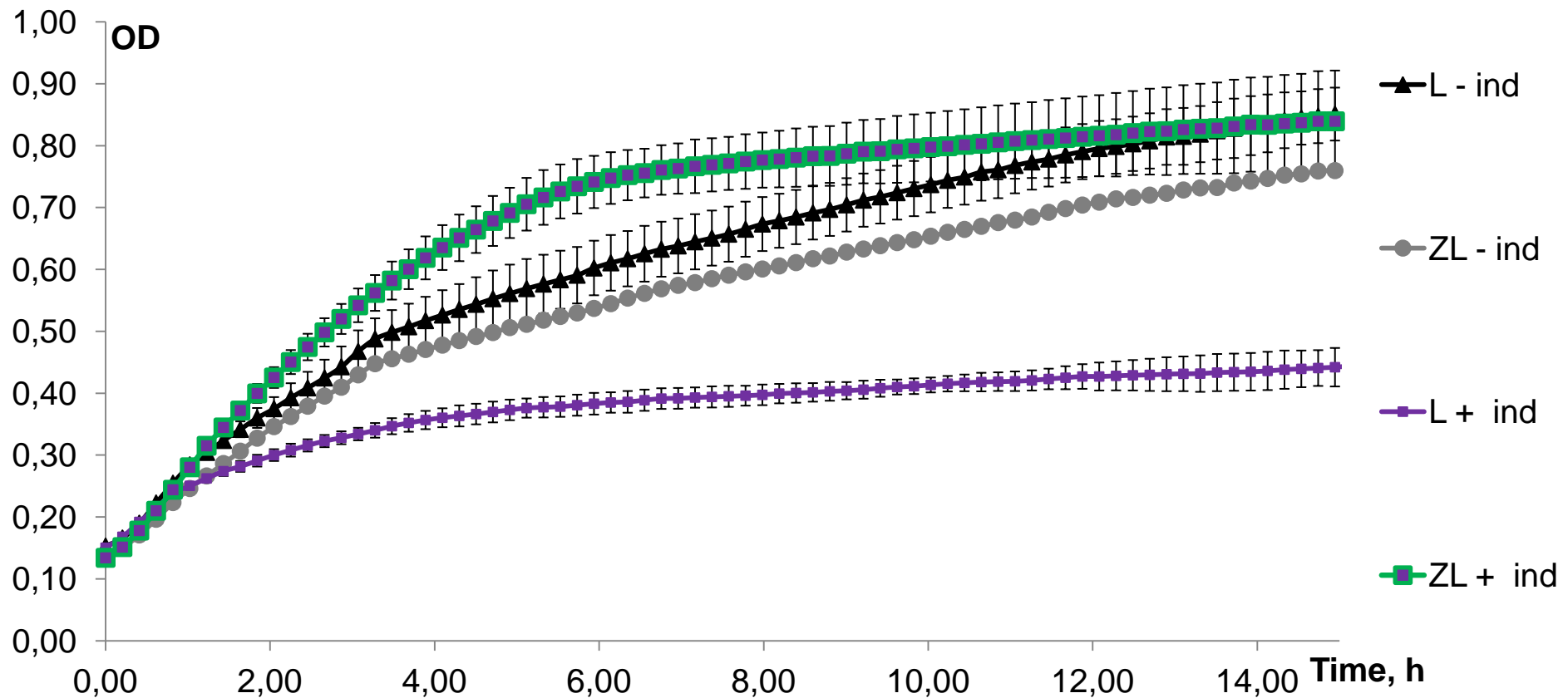


# Growth curves of BREX<sup>-</sup>, BREX<sup>+</sup> and $\Delta A\Delta L$ strains infected with T7



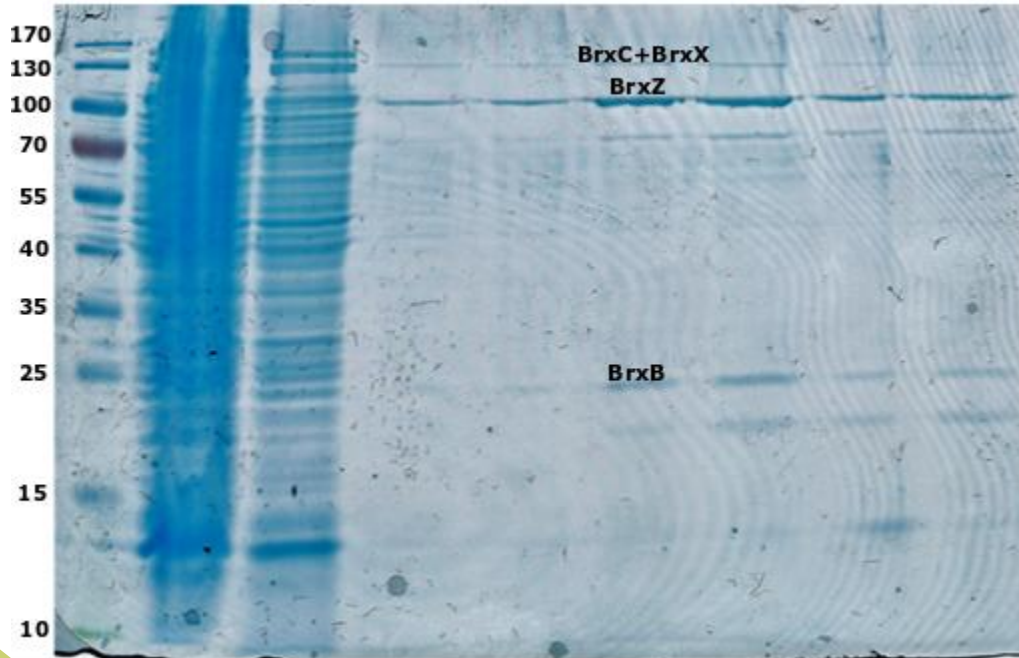
The expression of 4 BREX proteins provides low level of protection against T7 phage infection. MOI 0.001

# Growth curves of strains, expressing PglZ and BrxL proteins

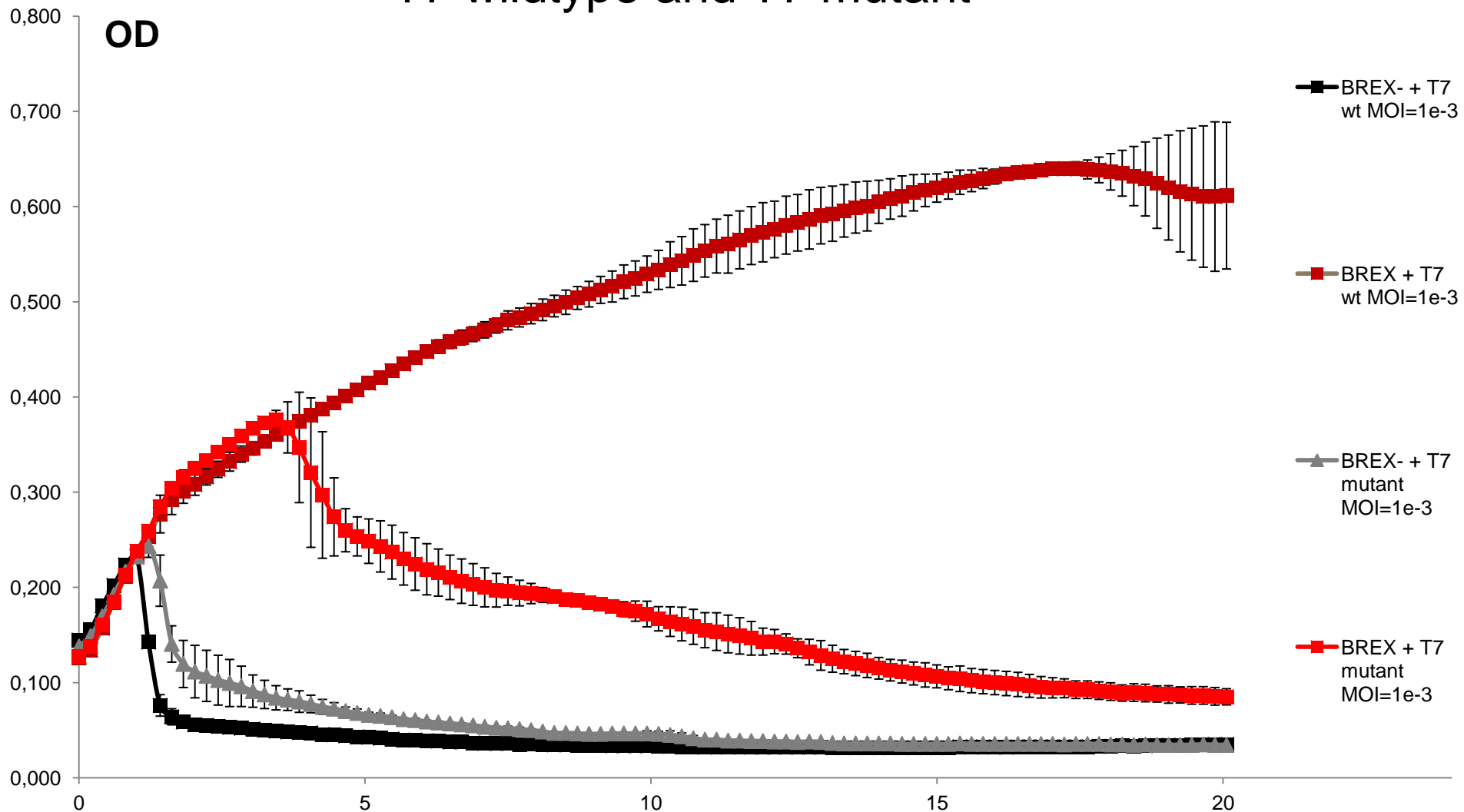


Co-expression of BrxL and BrxZ leads to higher OD comparing to control.

# Extraction of BCXZ complex



# Growth of BREX- and BREX+ cultures infected with T7 wildtype and T7 mutant



Several mutants carry a mutation at the first BREX site