



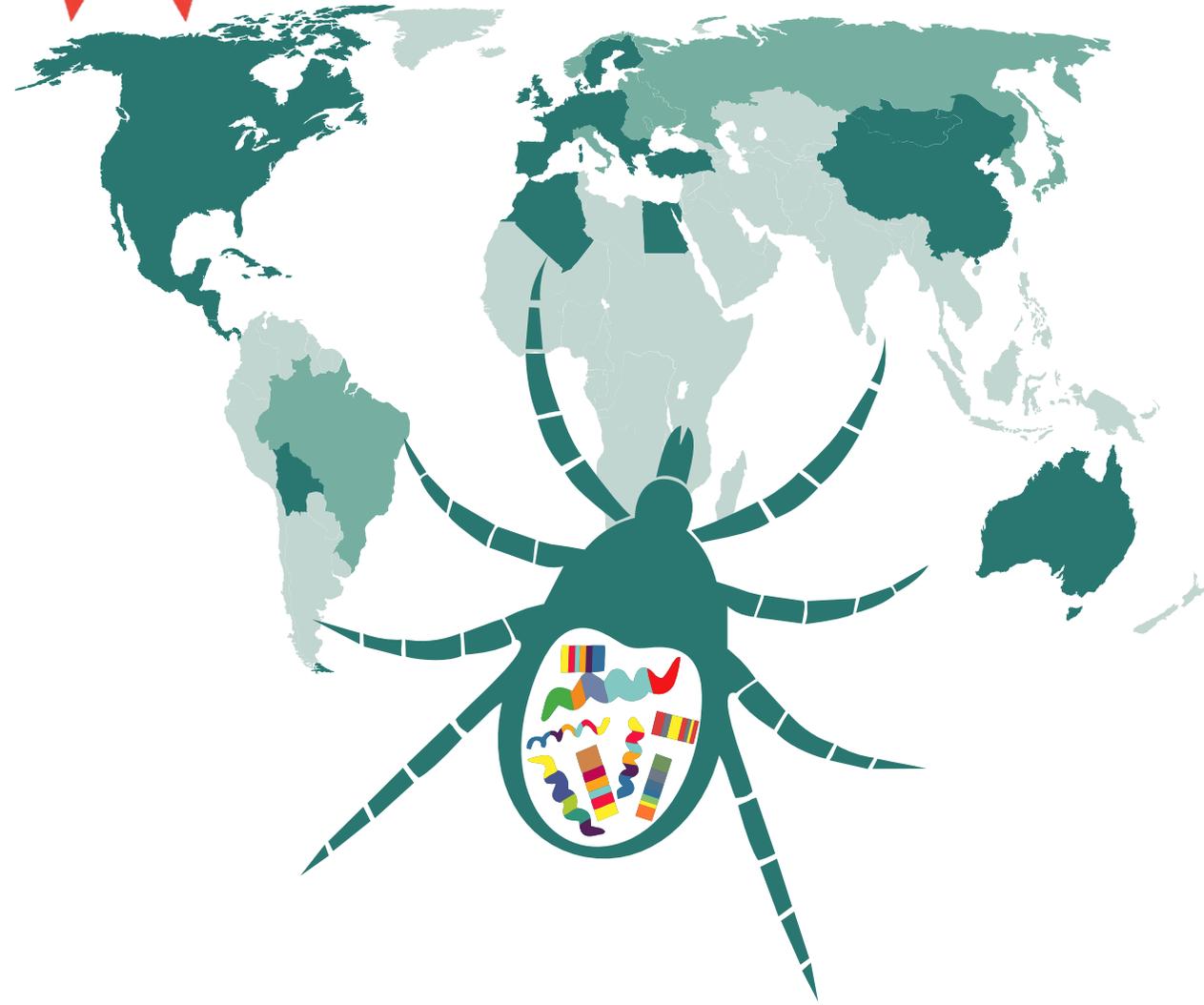
# Lyme Express

a portable biosensor for *Borrelia spp.* detection

# Project evolution: choosing the one



# Project description: stating the problem



Global problem: every year around 500,000 Lyme disease cases caused by tick-borne *Borrelia spp.* are reported worldwide

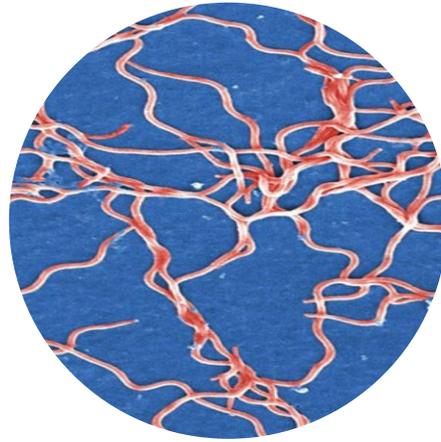
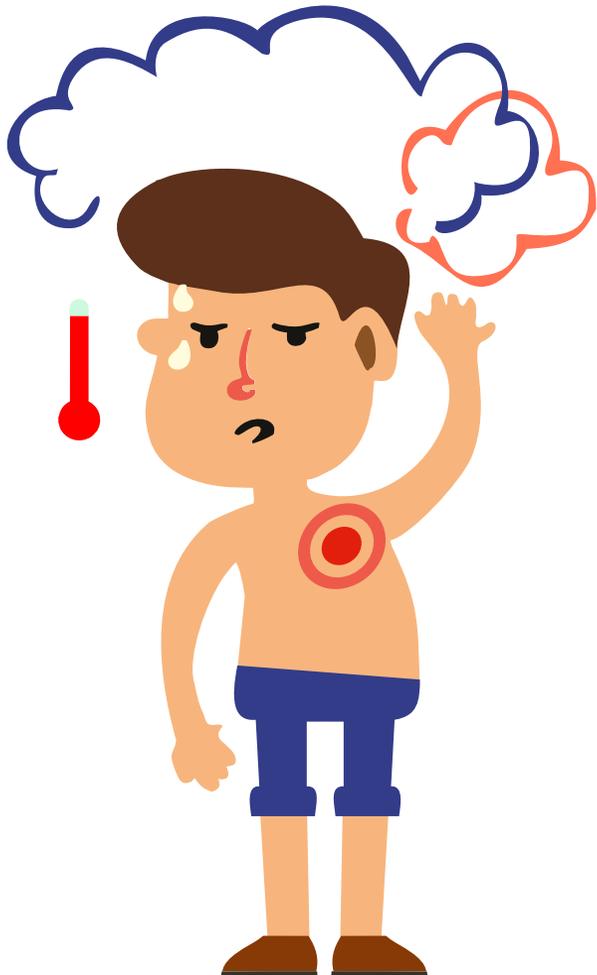


No commercial vaccine



No ways to detect the *Borrelia spp.* in the field

# Project description: what is Lyme?

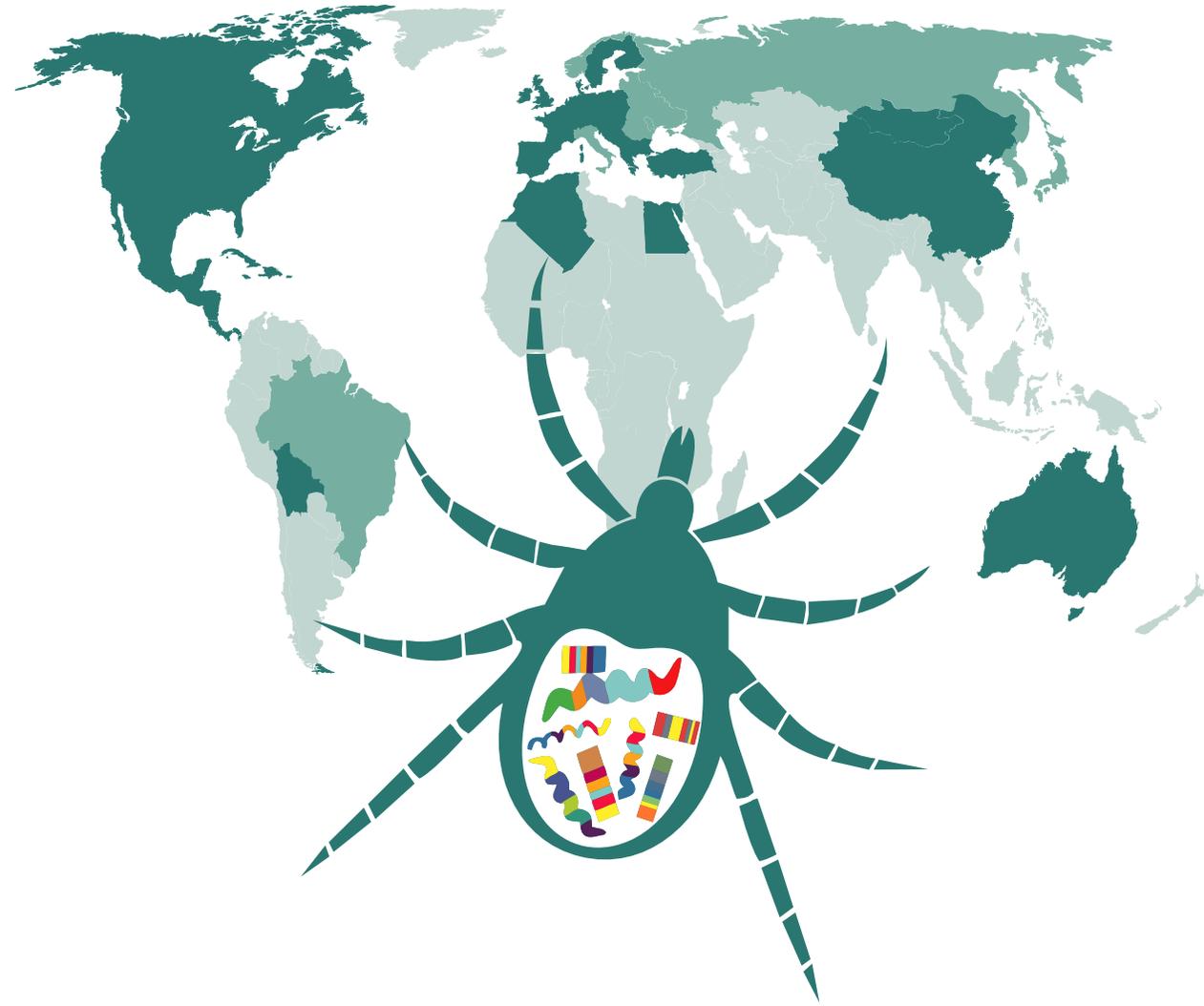


- *Borrelia burgdorferi* s.s.
- *Borrelia afzelii*
- *Borrelia miyamotoi*
- *Borrelia garinii*
- *Borrelia bavariensis*
- *Borrelia spielmanii*

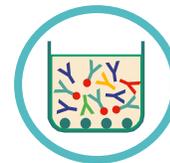


- *Ixodes scapularis*
- *Ixodes pacificus*
- *Ixodes persulcatus*
- *Ixodes ricinus*

# Project description: stating the problem



Diagnosis dilemma №1: no noticeable symptoms in many cases

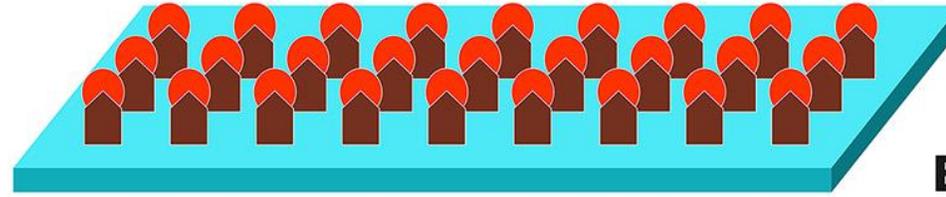


Diagnosis dilemma №2: serological tests can be performed ~10 days after the bite

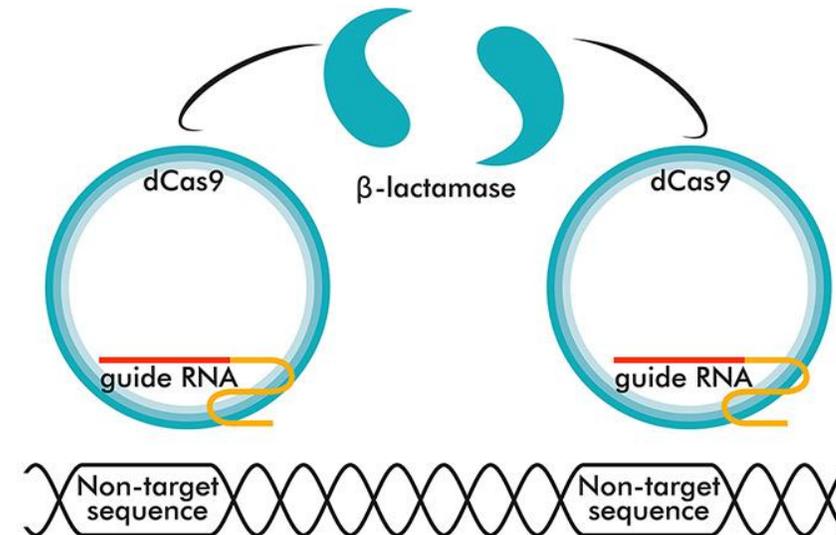
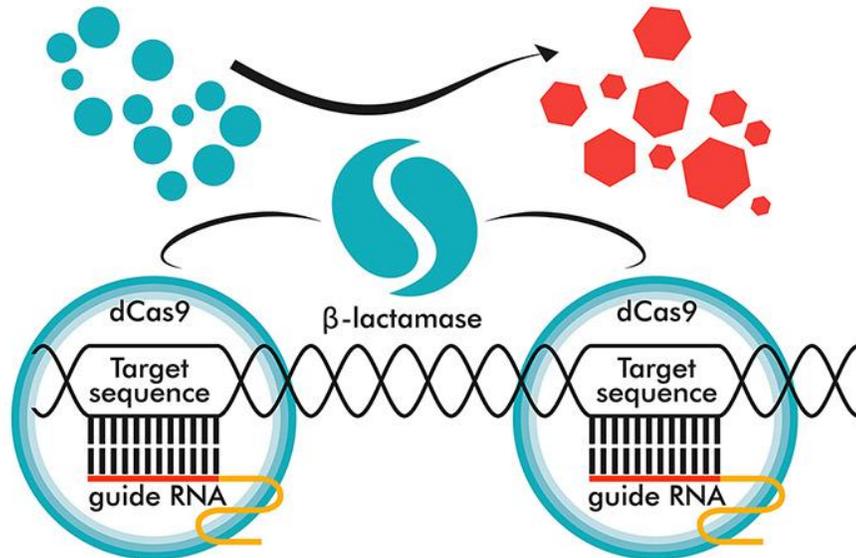
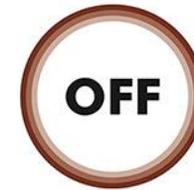
# Project description: overview



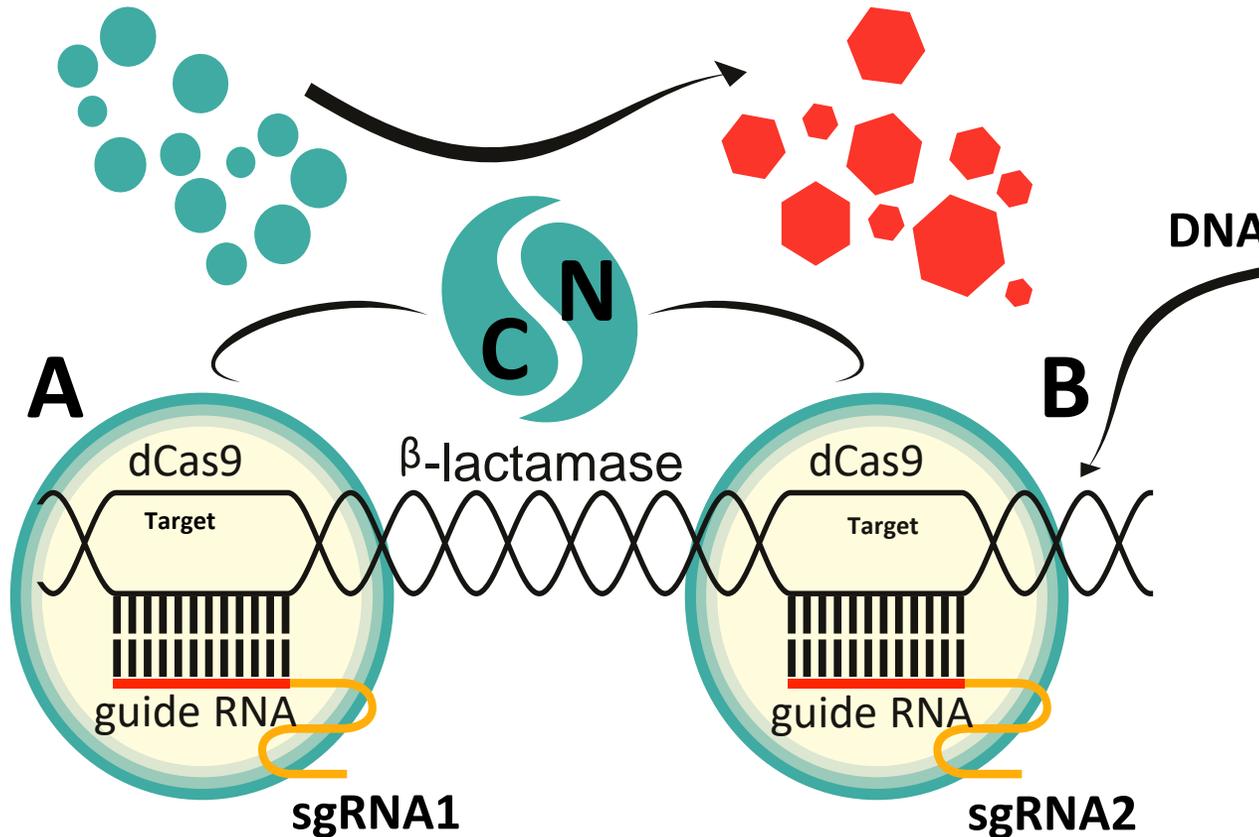
**HOMOGENIZATION**



**BIOSENSOR**



# Project description: main idea

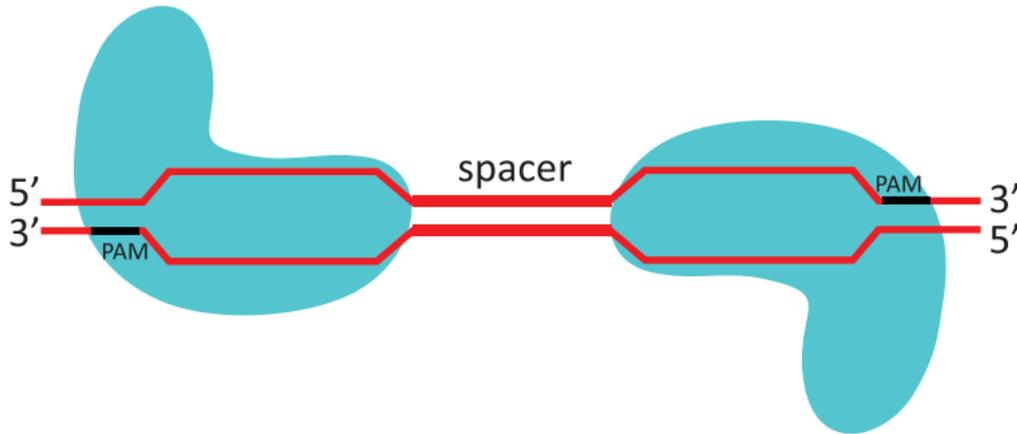


(A) dCas9 fused with C-terminal part of  $\beta$ -lactamase, forming a complex with sgRNA1

(B) dCas9 fused with N-terminal part of  $\beta$ -lactamase, forming a complex with sgRNA2

Combining dCas9 proteins from different organisms!

# Bioinformatics: modeling



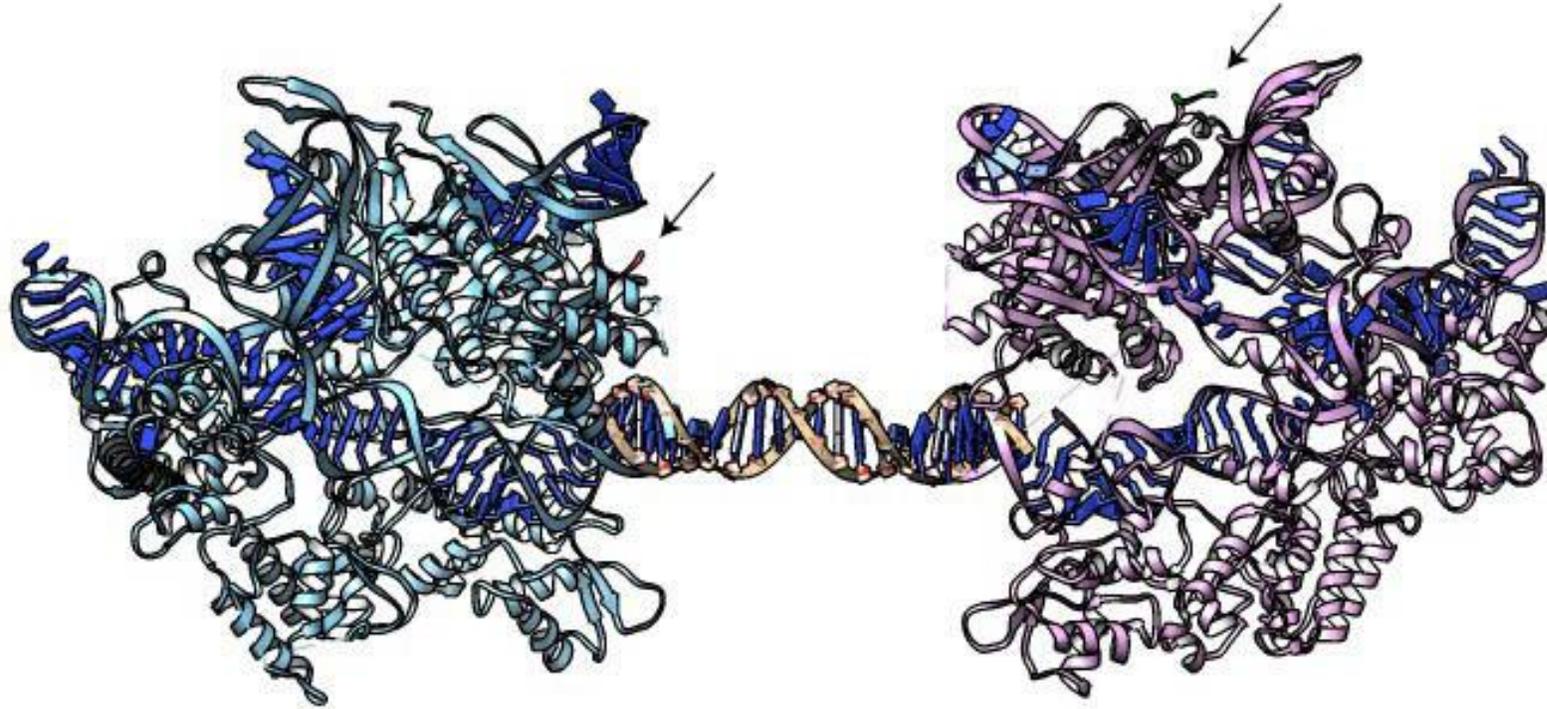
PAM-out

Cas <sup>1</sup>	Cas <sup>2</sup>	Length of spacer (between Cas <sup>1</sup> & Cas <sup>2</sup> )
Sp	Sp	22 bp
Sp	Sa	20-21 bp
Sa	Sa	20 bp

Sa = Streptococcus pyogenes

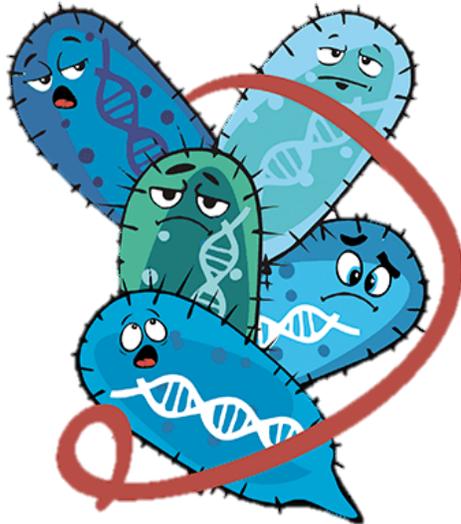
Sp = Staphylococcus aureus

# Bioinformatics: modeling



The model of two SpCas9 proteins binding to DNA

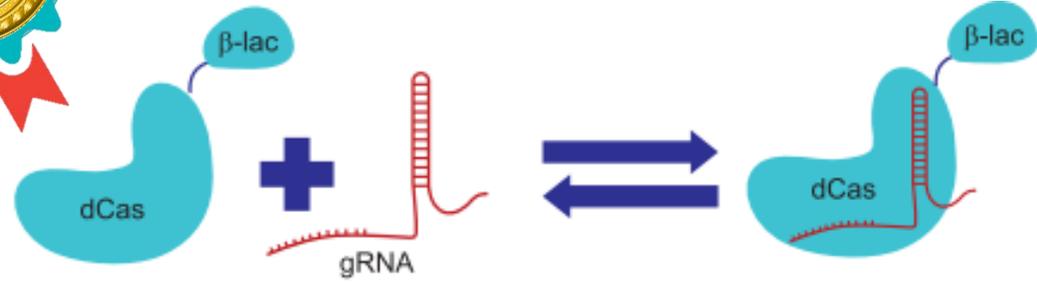
# Bioinformatics: guide RNA



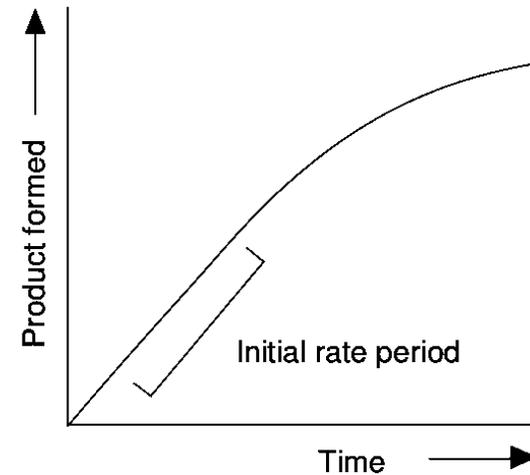
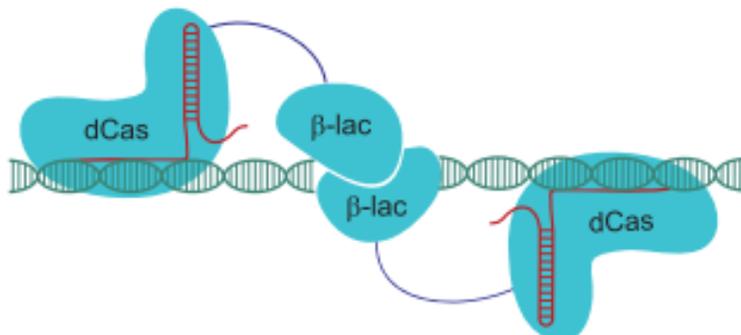
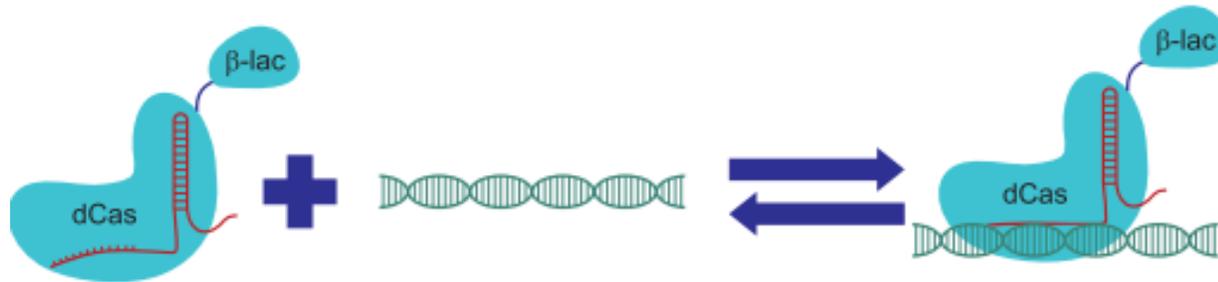
- *Borrelia burgdorferi s.s.*
- *Borrelia afzelii*
- *Borrelia garinii*
- *Borrelia bavariensis*

Product of gene	Number of targets
23S ribosomal RNA	8 (4 are unique)
16S ribosomal RNA	2
UDP-glucose pyrophosphorylase (pseudogene)	1
purine-binding chemotaxis protein	1
rod shape-determining protein MreC	1
penicillin-binding protein	1

# Modeling: kinetics

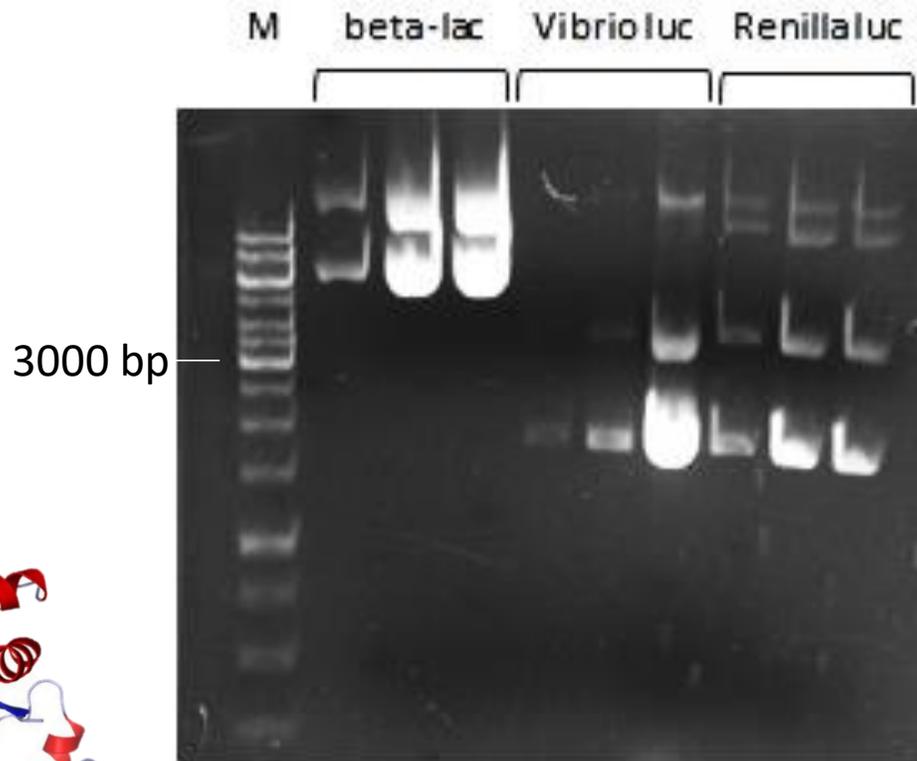


17,5  $\mu$ M of nitrocefin hydrolysis product for proper detection

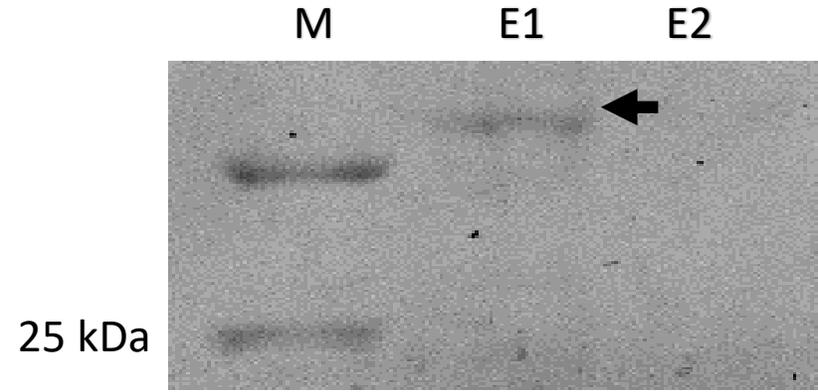


$$\frac{d[P]}{dt} = \frac{K_{cat}[E_0][S]}{K_m + [S]}$$

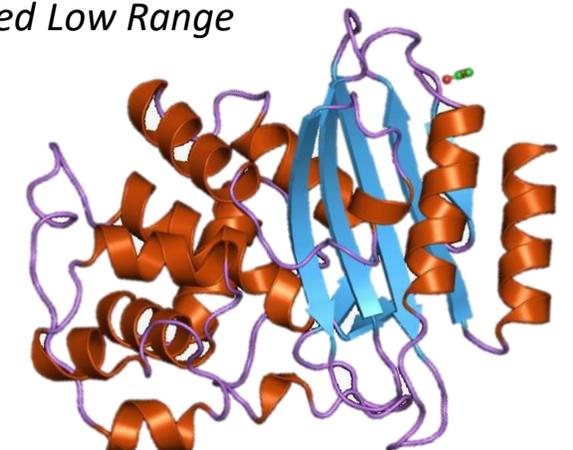
# Wet lab: choosing a reporter system



Agarose gel electrophoresis. Purification of plasmid DNA from Dh5a cells. *Beta-lac* - [Part: BBa K1189009](#), *Vibrio luc* - [Part: BBa K325909](#), *Renilla luc* - [Part: BBa J52008](#), M - GeneRuler 1 kb DNA Ladder



SDS-PAGE electrophoresis, Ni-NTA. E1, E2 – elution 1 & 2. *β-lactamase* with His-tag under IPTG-inducible promoter - [Part:BBa K1189007](#), M – PageRuler Unsgained Low Range

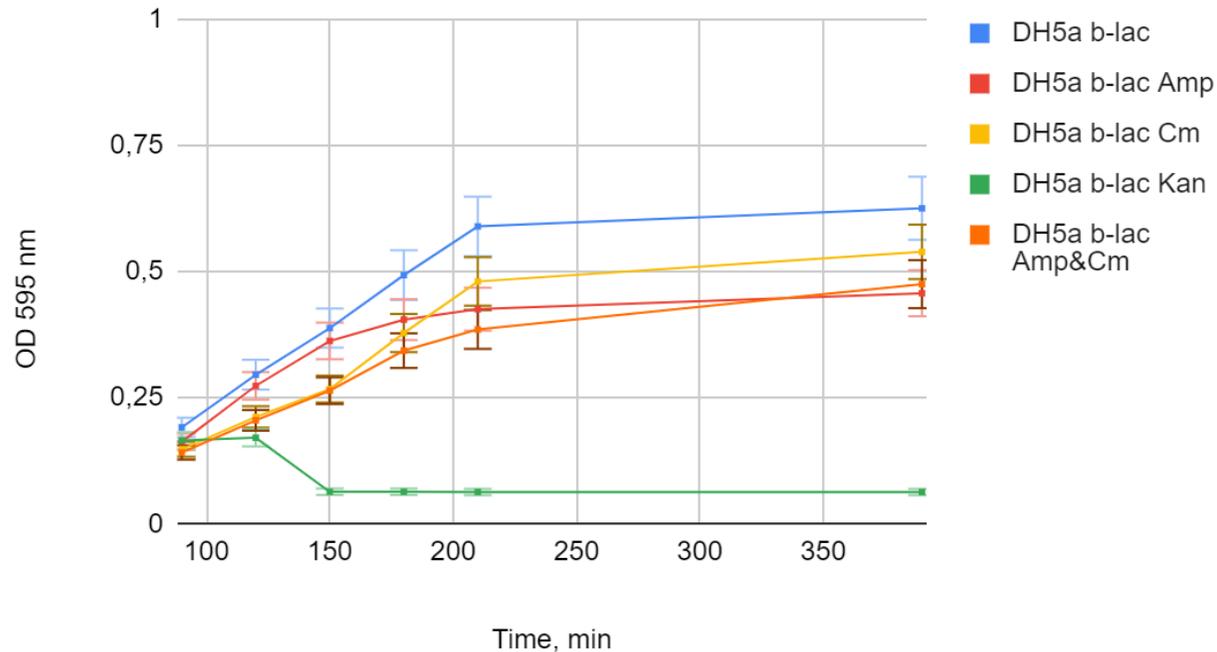


# Wet lab: characterization

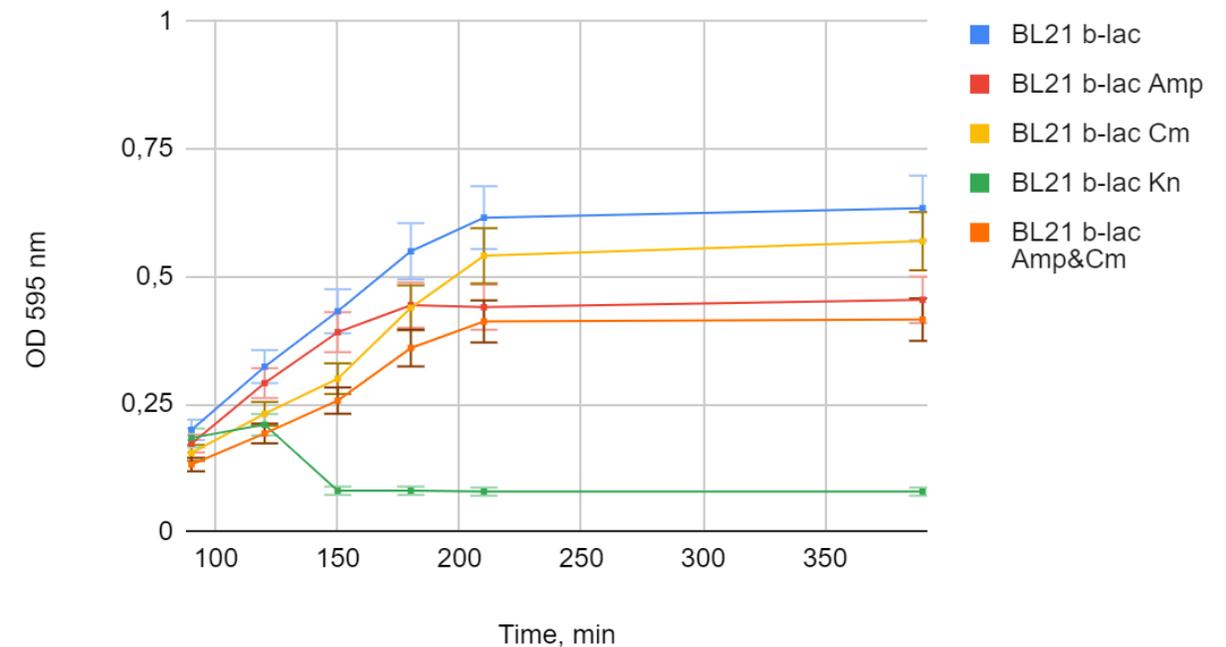


*Part:BBa\_K1189007*

Growth curve - antibiotic assay, DH5 $\alpha$



Growth curve - antibiotic assay, BL21



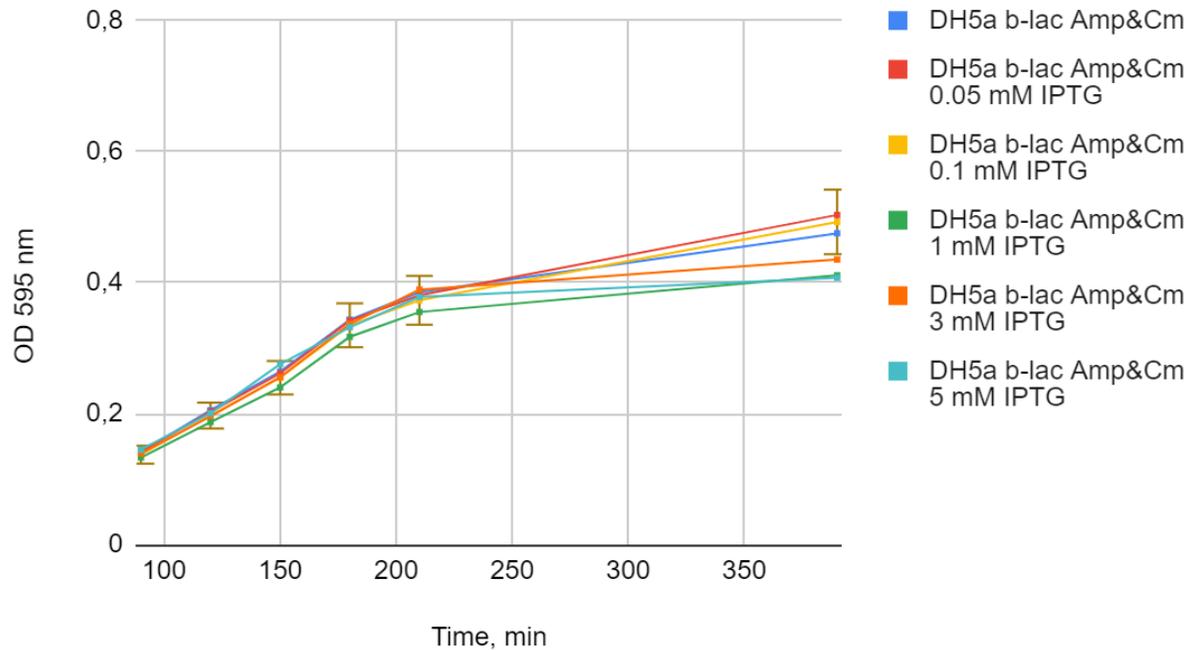
[http://parts.igem.org/Part:BBa\\_K1189007](http://parts.igem.org/Part:BBa_K1189007)

# Wet lab: characterization

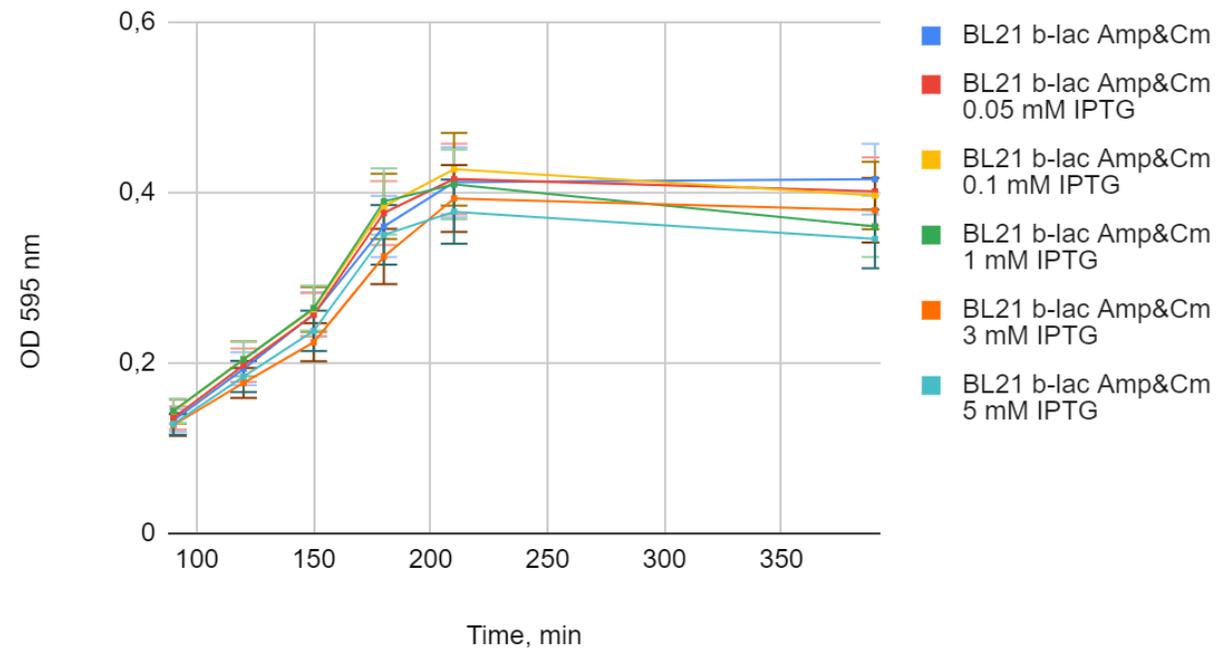


*Part:BBa\_K1189007*

Growth curve - Amp&Cm treated, IPTG assay, DH5α

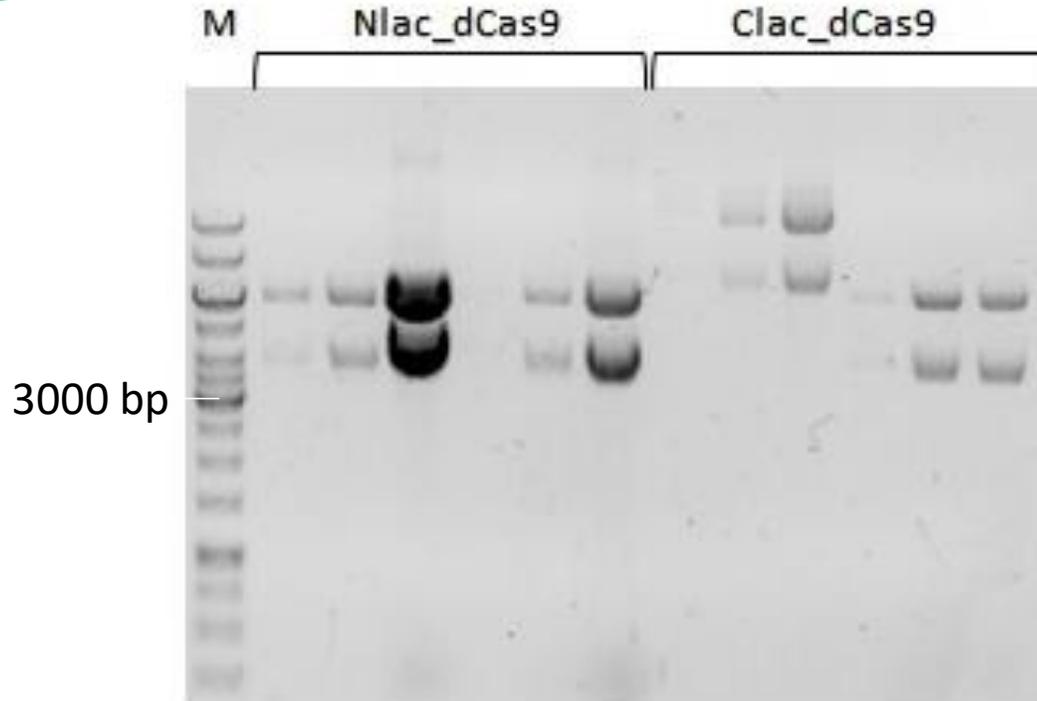


Growth curve - Amp&Cm treated, IPTG assay, BL21

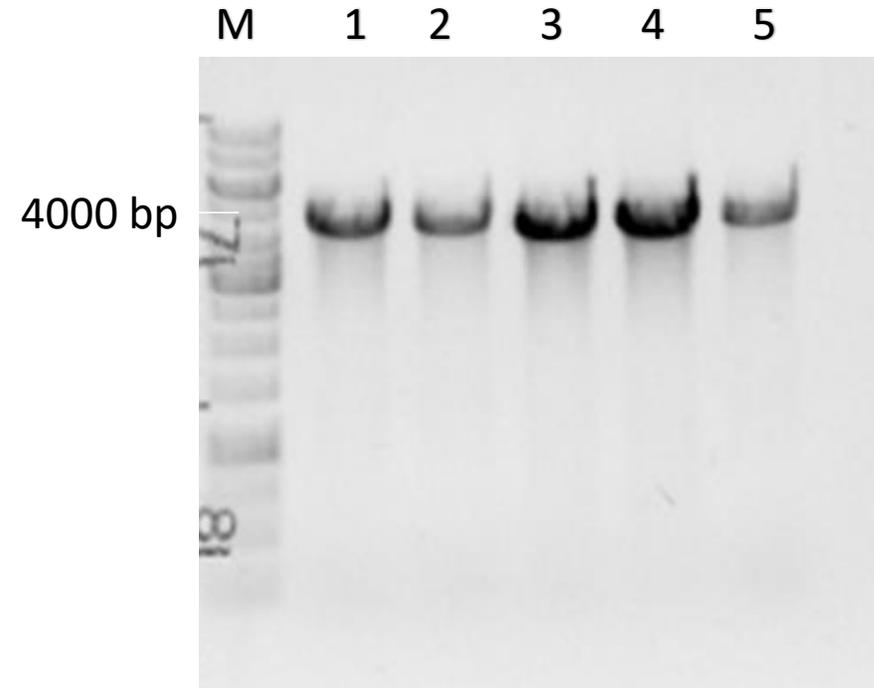


[http://parts.igem.org/Part:BBa\\_K1189007](http://parts.igem.org/Part:BBa_K1189007)

# Wet lab: essential parts



- Agarose gel electrophoresis. Purification of plasmid DNA from Dh5a cells. M- GeneRuler 1 kb DNA Ladder
- fusion protein dCas9 with the N-terminal domain of  $\beta$ -lactamase ([BBa\\_K1689013](#))
  - fusion protein dCas9 with the C-terminal domain of  $\beta$ -lactamase ([BBa\\_K1689014](#))



- Agarose gel electrophoresis. Purification of plasmid DNA from Dh5a cells. M - GeneRuler 1 kb DNA Ladder. 1-3 – PCR fragments, Nlac\_dCas9; 4-5 PCR fragments, Clac\_dCas9.

# Wet lab: contribution



Sequence alignment



Primer	Binding Sites	
uni_for	4 .. 24	↔
dCas_for	54 .. 89	↔
seq1	349 .. 376	↔
seq2	817 .. 848	↔
seq3	1379 .. 1411	↔
seq4	1808 .. 1843	↔
seq5	2340 .. 2367	↔
seq6	2856 .. 2891	↔
seq7	3385 .. 3414	↔
dCas_rev	4133 .. 4155	↔
C_rev	4435 .. 4458	↔
VR	4687 .. 4706	↔
VF2	6463 .. 6482	↔

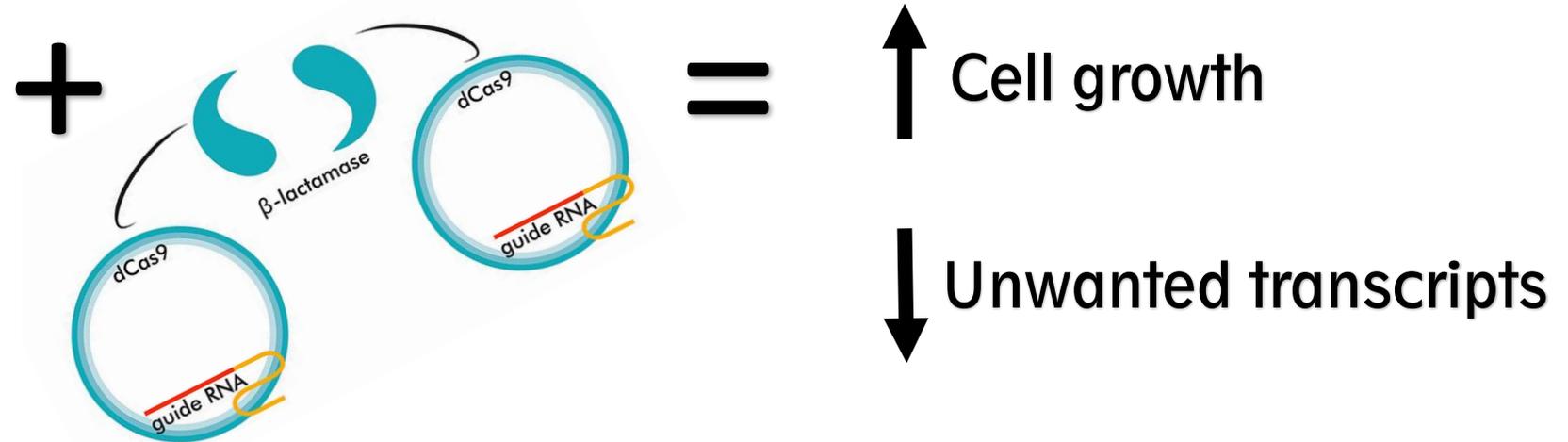
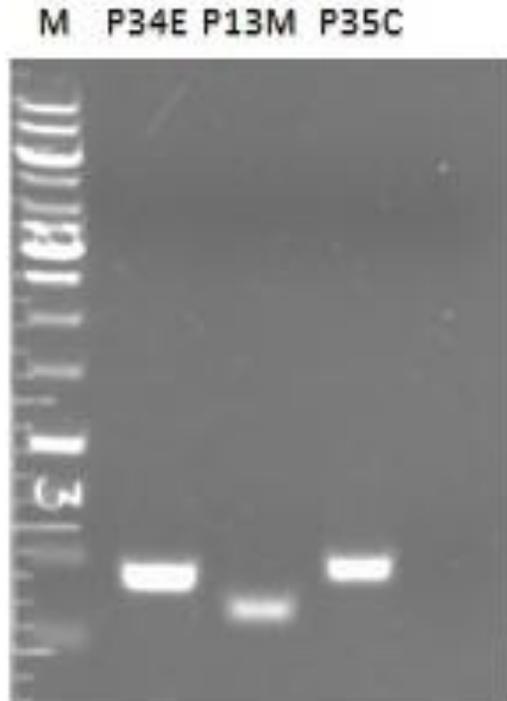
Primer design



Distribution	Sequencing
Spring 2019 Distribution	Confirmed
Spring 2018 Distribution	Long part
Spring 2017 Distribution	Long part

Clac\_dCas9 & Nlac\_dCas9  
sequence confirmed

# Wet lab: improvements



Agarose gel electrophoresis.

M - GeneRuler 1 kb DNA Ladder.

[http://parts.igem.org/Part:BBa\\_B0017](http://parts.igem.org/Part:BBa_B0017) = p34e,

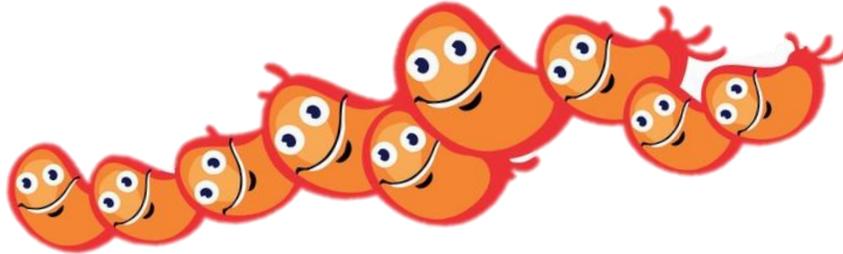
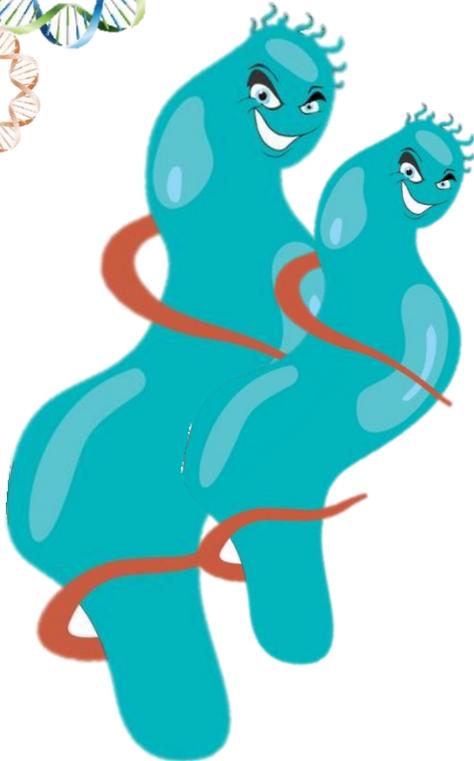
[http://parts.igem.org/Part:BBa\\_B0015](http://parts.igem.org/Part:BBa_B0015) = p35c,

[http://parts.igem.org/Part:BBa\\_K525998](http://parts.igem.org/Part:BBa_K525998) = p13m

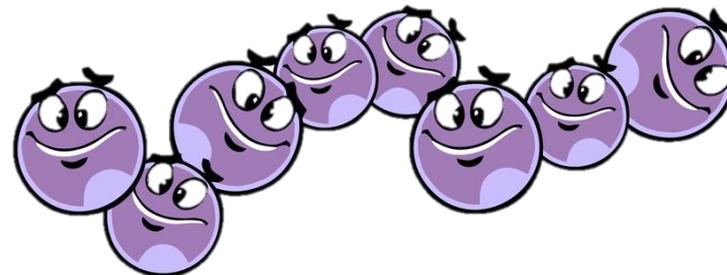
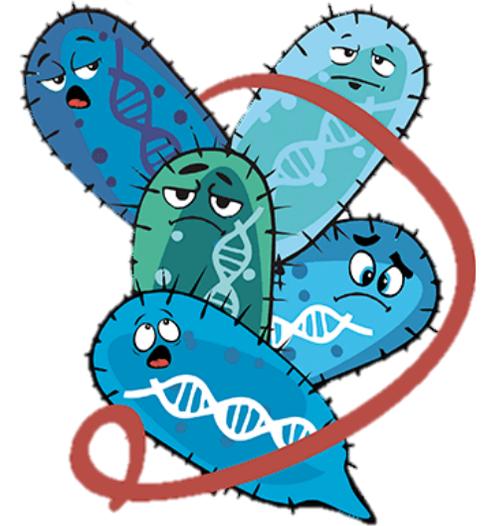
[http://parts.igem.org/Part:BBa\\_K3028000](http://parts.igem.org/Part:BBa_K3028000)

[http://parts.igem.org/Part:BBa\\_K3028001](http://parts.igem.org/Part:BBa_K3028001)

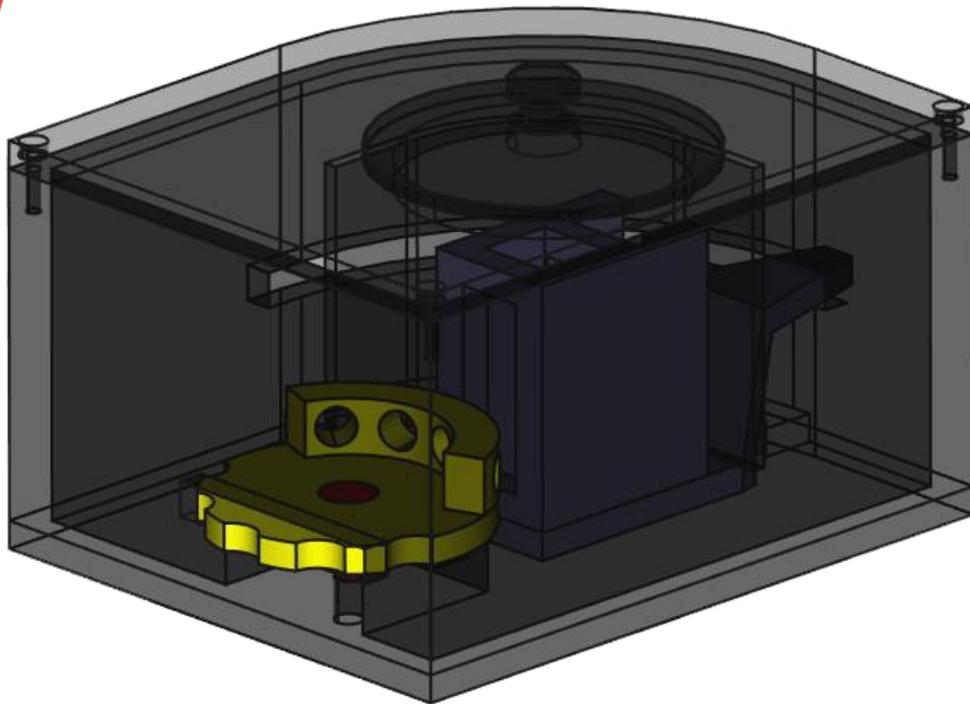
# Wet lab: CRISPR/Cas collection



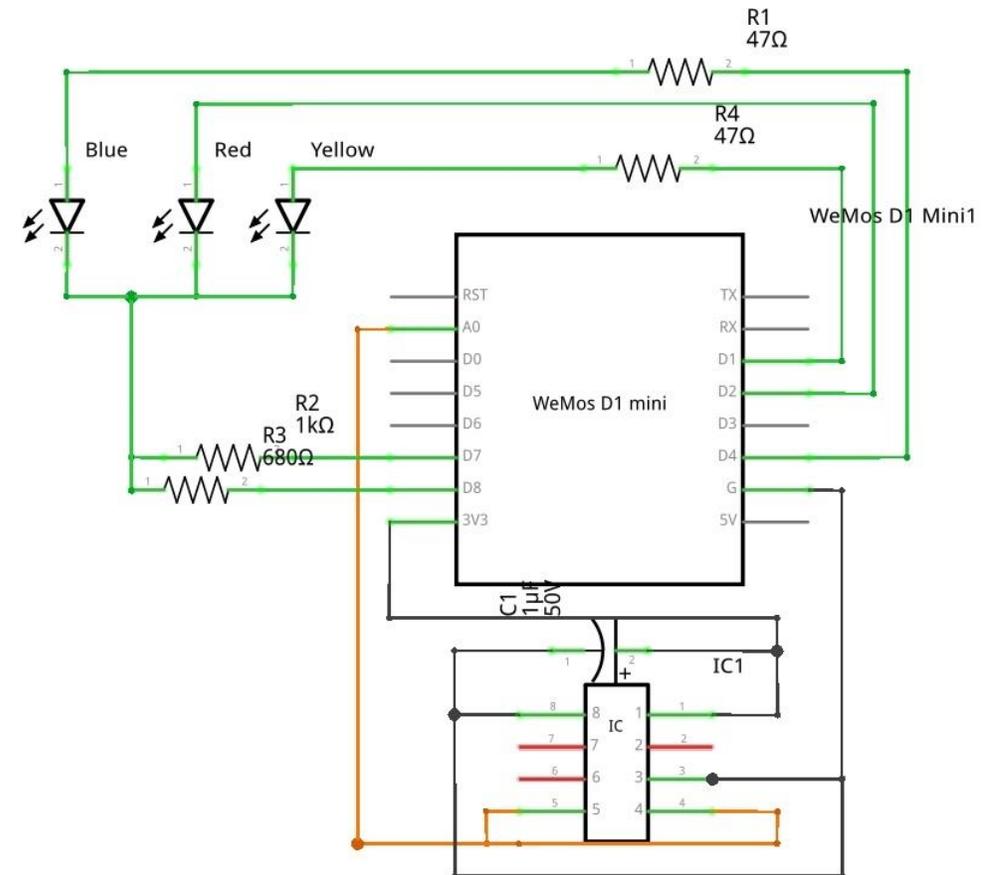
- Streptococcus pyogenes
- Staphylococcus aureus
- Streptococcus thermophiles
- Campylobacter jejuni
- Proteobacteria phylum



# Hardware: LymePhoton-M

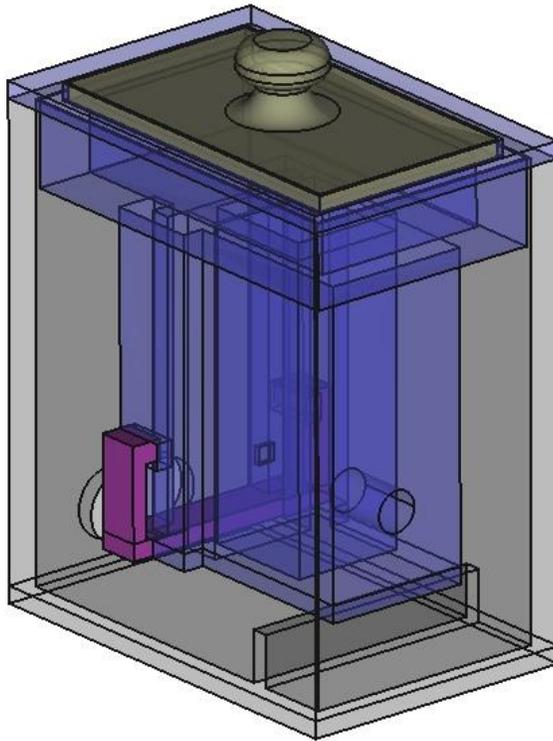


- $\lambda = 470 \text{ nm}, 590 \text{ nm}, 630 \text{ nm}$
- Two positions for the holder
- Subparts: ESP 8266, photo sensor OPT101, LEDs

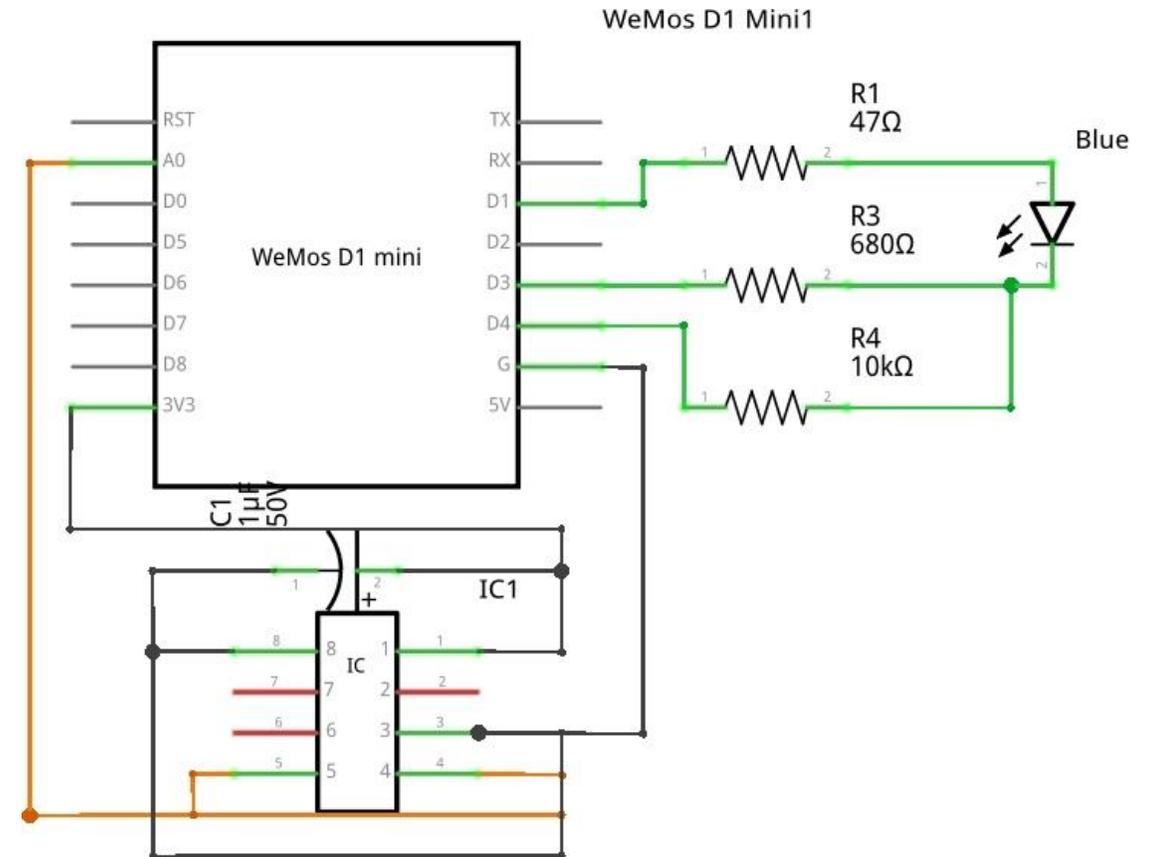


Electrical diagram of LymePhoton-M

# Hardware: LymePhoton-S



- $\lambda = 470 \text{ nm}$
- One position for the holder
- Subparts: ESP 8266, photo sensor OPT101, LED



Electrical diagram of LymePhoton-S

# Hardware: demonstration



# Collaborations: our survey among teams

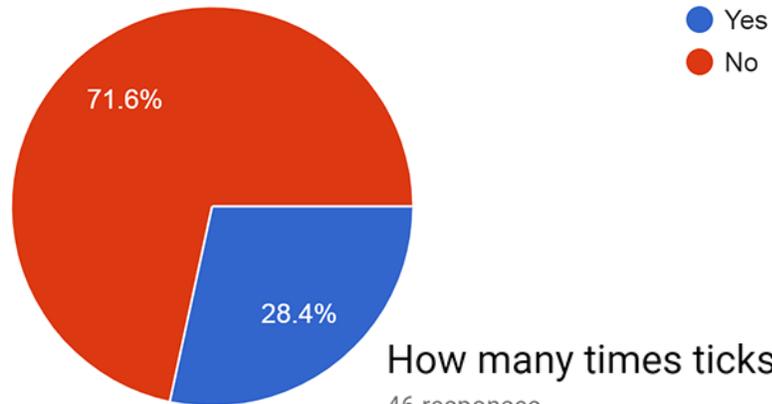


148 people from 15 countries have answered the questions!



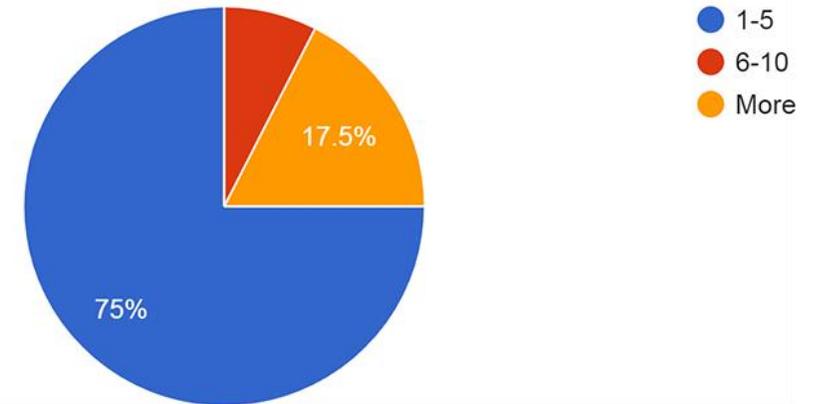
Have you ever been bitten by a tick?

148 responses



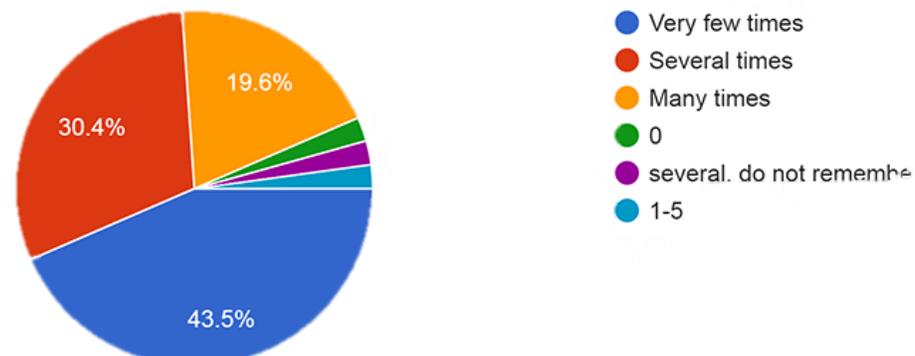
How many times have ticks bit you?

40 responses



How many times ticks have bitten your pet?

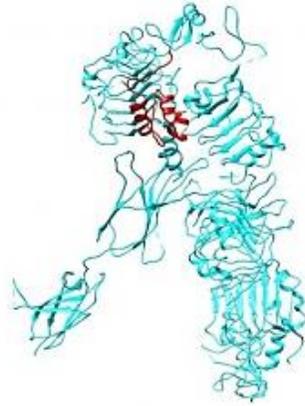
46 responses



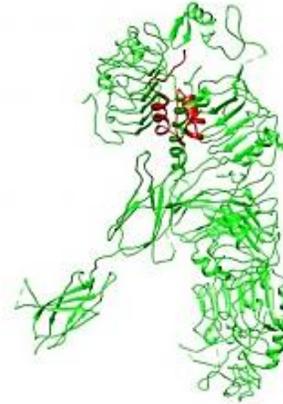
# Collaborations: smart and sporty



# Collaborations: ULaVerne, USA



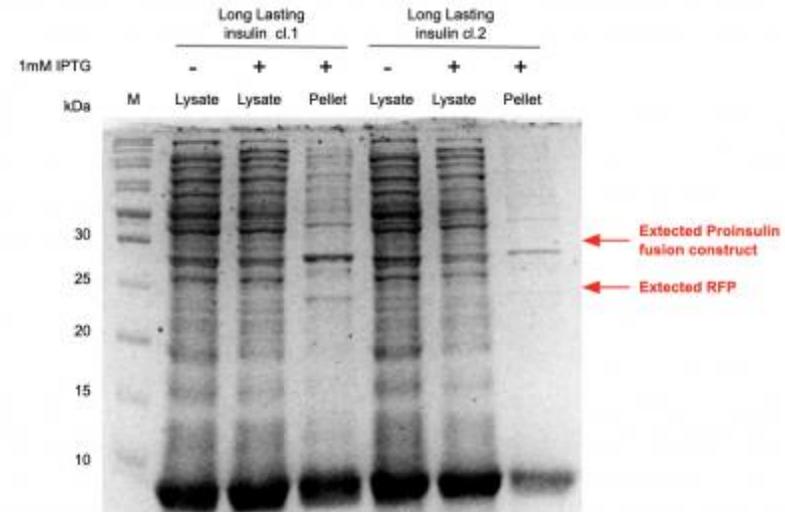
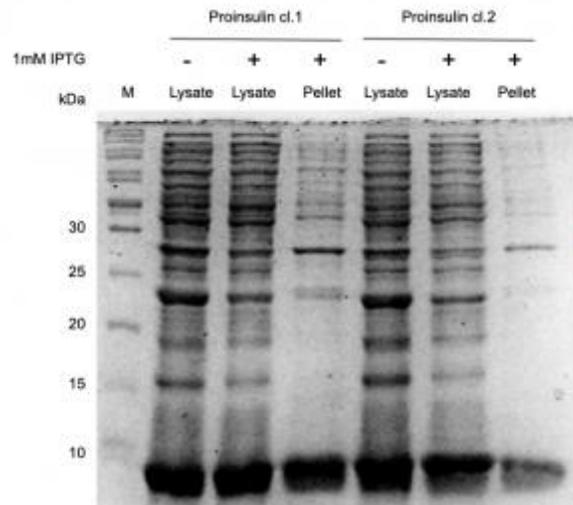
Native Insulin  
DG = 2.04839 kcal/mol



Proinsulin  
AsnA21Ala  
DG = 1.84606 kcal/mol



Single Chain Long Lasting Insulin  
ProB28Lys and LysB29Pro  
DG = 1.49372 kcal/mol



# Integrated Human Practice



- reaction time minimization
- sample preparation & homogenization =
- detection of multiple tick-borne infections at one time
- test our device on real samples

Nikolay I. Briko, Head of Epidemiology of Russia

# Human Practice: consulting the experts



## Biosensor development

Vladimir A. Gushchin,  
Federal Research Center of Epidemiology and Microbiology



## CRISPR/Cas & Bioinformatics

Sergey Shmakov,  
Skoltech & NCBI



## Entrepreneurship

Andrey Afanasiev,  
CEO & Cofounder at yRisk

# Integrated Human Practice



Alexandra Pianova,  
Deputy chief veterinarian of the  
Center Veterinary Clinic

«A LOOK FROM WITHIN»

# Education & Public Engagement



All-Russia Science Festival



Science Bar Hopping

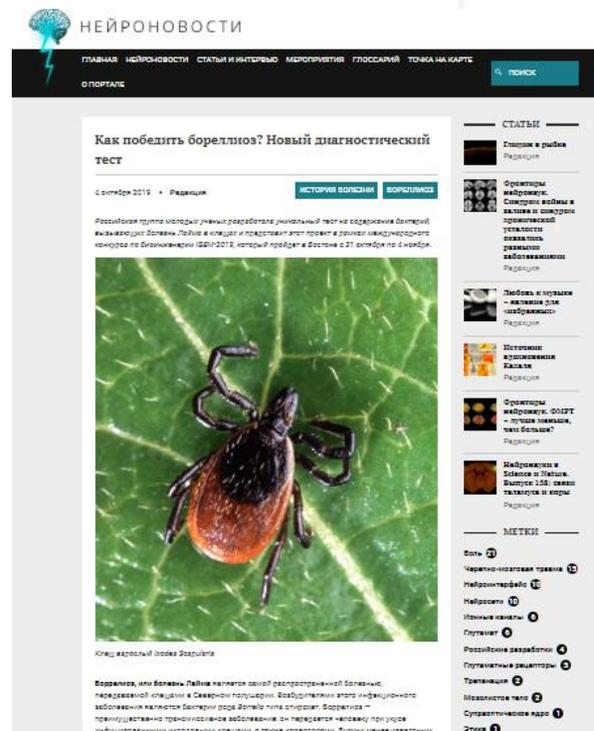
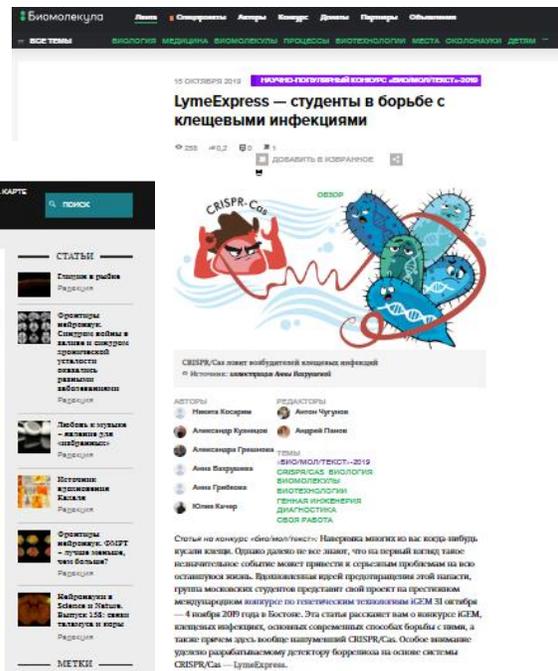


Lecture at The Boot Camp

# Education & Public Engagement



Article for the “Biomolecula” portal



Article for the “Neuronews” portal



Recording an educational podcast (in Russian)  
The podcast is available at: <https://medach.pro/post/2165>

# Human Practice during Jamboree



TEAM MOSCOW with James J. Collins at ILADS

# Attributions: supervisors & instructors



**Alexey Shaytan**

Primary Investigator (PI)



**Dmitry Karpov**

Secondary PI



**Grigory Glukhov**

Wet Lab Instructor



**Grigory Armeev**

Dry Lab Instructor



**Ekaterina Marilovtseva**

Wet Lab Instructor

# Attributions: advisors



**Mikhail P. Kirpichnikov**



**Nikolay I. Briko**



**Elena Krasilnikova**



**Vasily M. Studitsky**



**Alexey V. Feofanov**



**Olga S. Sokolova**



**Vera Bashmakova**

# Attributions: our team



**Julia Kacher**  
Student leader



**Petr Zaytsev**  
Student leader



**Max Bokov**  
SMM, PR



**Iunona Pospelova**  
Dry lab, SMM



**Nikolay Chechulin**  
Manager



**Anna Gribkova**  
Dry Lab, Human Practice



**Andrey Buynevich**  
Sponsorship, HumanPractice



**Irina Talyzina**  
Wet lab, Human Practice



**Alexandra Greshnova**  
Dry and Wet Lab



**Roman Novikov**  
Dry Lab



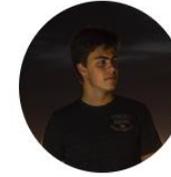
**Nikita Kosarim**  
Wet lab



**Nikolay Kristovsky**  
Dry lab and engineering



**Anna Vakhrusheva**  
Design and Wet lab



**Sergey Dumpis**  
Wet lab



**Alex Milenkin**  
Dry lab



**Alexander Kuznetsov**  
Wet lab



**Adil Kabylda**  
Dry lab



**Renat Vinnikov**  
Dry lab



**Pavel Vorobyev**  
Wet lab

# Sponsors



# Summary



- ✓ Characterization of  $\beta$ -lactamase (Part:BBa\_K1189007)
- ✓ Sequence confirmation (Part:BBa\_K1689013, BBa\_K1689014)

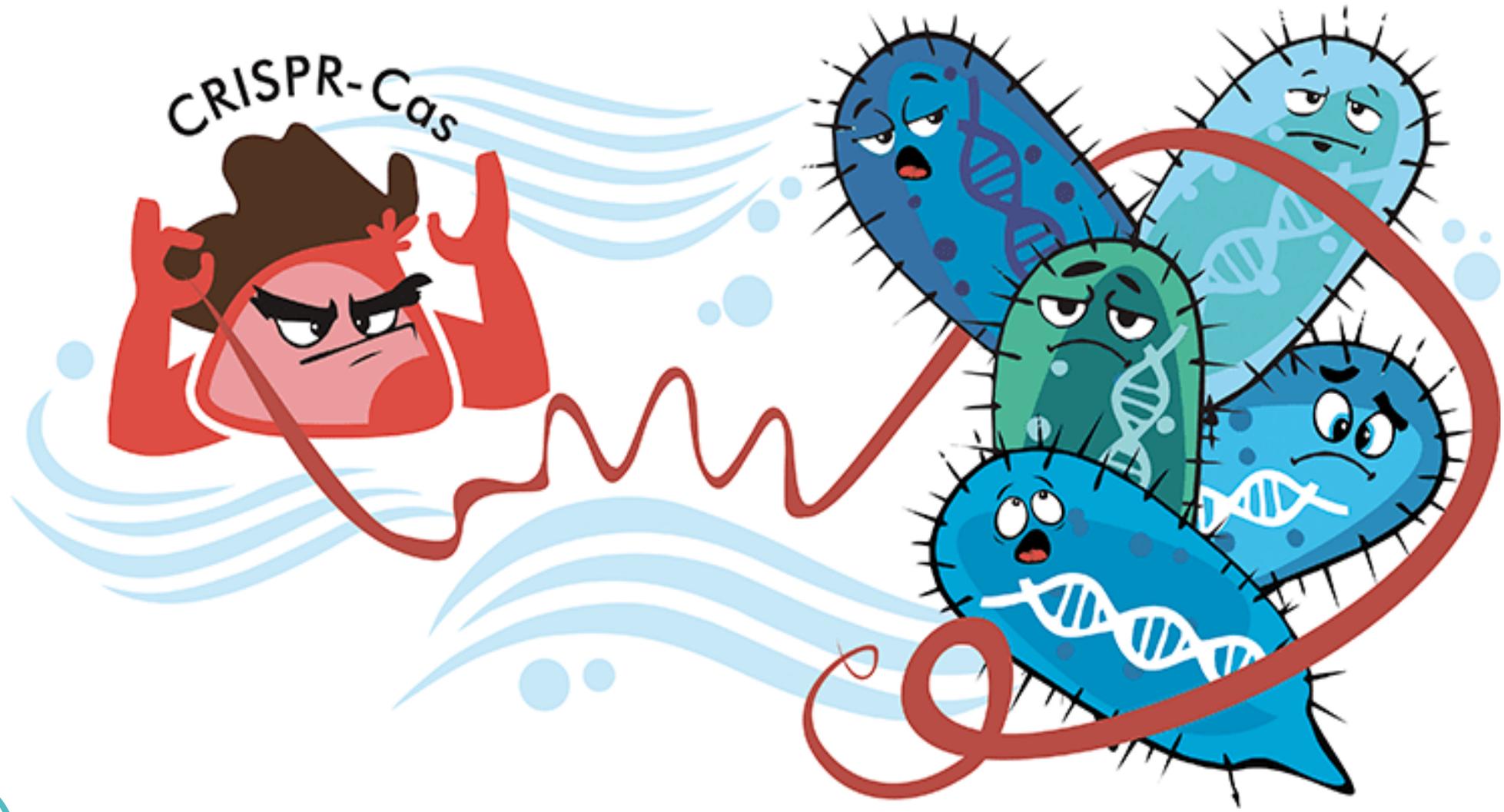


- ✓ Parts (Part:BBa\_K3028000&Part:BBa\_K3028001)
- ✓ Hardware development
- ✓ Collaborations
- ✓ Human Practice



- ✓ Integrated Human Practices
- ✓ Model of our project

# THANK YOU FOR ATTENTION! QUESTIONS?



[igemoscov@gmail.com](mailto:igemoscov@gmail.com)



[@igemoscov](https://www.facebook.com/igemoscov)



[@igem\\_moscow](https://www.instagram.com/igem_moscow)



# Bioinformatics: molecular dynamics



Conformational states of Cas9-sgRNA-DNA ternary complex in the presence of magnesium

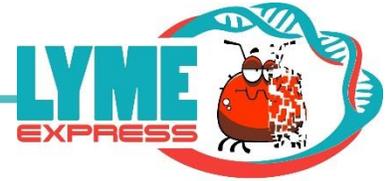
# Reporter protein choice



Enzyme	Organism	Mass, kDa	Split	Reaction & characteristics
Beta-lactamase	Escherichia coli	29	1-197 + 197-286	hydrolyze the beta-lactam bond in susceptible beta-lactam antibiotics (380 -> 500)
Renilla luciferase	from sea pansy (Renilla reniformis).	36	1-229 + 230-311 & 1-110 + 111-311	requires only coelenterazine and oxygen (blue light of 480nm)
Firefly luciferase	isolated from beetles (Photinus pyralis)	61	1-436 + 440-550	luciferin in the presence of oxygen, ATP and magnesium to produce light (greenish yellow light in the 550-570nm)
Vibrio luciferase	Photobacterium luminescens, Vibrio harveyi or Vibrio fischeri	77	luxAB + luxCDE	catalyzes the oxidation of reduced flavin mononucleotide (FMNH <sub>2</sub> ) and myristyl aldehyde to myristic acid and FMN, a reaction that liberates light at 490 nm
Gaussia luciferase	Marine copepod Gaussia princeps	20	1-92 + 93-168	emits light at a peak of 480 nm with a broad emission spectrum extending to 600 nm, reporter reaction using the coelenterazine substrate



# Comparison of the methods



PCR

ELISA

Microscopy

EXPRESS

	PCR	ELISA	Microscopy	EXPRESS
Sample	Tick, skin, blood, urine etc	Blood, lumbar puncture	Tick (hemolymph and intestines)	Tick, skin, blood, urine etc
Sensitivity	95% (from 1 pmol/ml)	40-55% on the early stage	low	100% (possible)
Specificity	100%, but it is decreased by Borrelia variability	92% to detect borrelia antibodies	Impossibility to identify the species	100% (possible)
Cost	from 8\$	from 10 \$	from 8\$	From 5\$
Time	from 25 min	10-15 days	to 10 min	Minutes
Features	Not unified or standardized	Placement to special laboratories	dark-field microscopy	