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FELLOWSHIP PROGRAM

High-throughput analysis of short DNA fragments generated *in vivo* in *Escherichia coli* and *Saccharomyces cerevisiae*

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Publications:

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- **Shiriaeva, A**., Fedorov, I., Vyhovskyi, D., and Severinov, K. (2020). Detection of CRISPR adaptation. Biochem. Soc. Trans. *48 (1)*, 257-269.
- Kurilovich, E., **Shiriaeva, A**., Metlitskaya, A., Morozova, N., Ivancic-Bace, I., Severinov, K., and Savitskaya, E. (2019). Genome Maintenance Proteins Modulate Autoimmunity Mediated Primed Adaptation by the Escherichia coli Type I-E CRISPR-Cas System. Genes *10*, 872.
- **Shiriaeva, A.A.,** Savitskaya, E., Datsenko, K.A., Vvedenskaya, I.O., Fedorova, I., Morozova, N., Metlitskaya, A., Sabantsev, A., Nickels, B.E., Severinov, K., et al. (2019). Detection of spacer precursors formed in vivo during primed CRISPR adaptation. Nat Commun *10*, 4603.
- Stepchenkova, E.I., **Shiriaeva, A.A.,** and Pavlov, Y.I. (2018). Deletion of the DEF1 gene does not confer UV-immutability but frequently leads to self-diploidization in yeast Saccharomyces cerevisiae. DNA Repair (Amst.) *70*, 49–54.
- Zhuk A.S., Zadorsky S.P., **Shiriaeva A.A.**, Kochenova O.V., Inge-Vechtomov S.G., Stepchenkova E.I. (2018). Identification of the kar1-1 mutation, leading to increase of cytoduction frequency and decrease of hybridization frequency in yeast Saccharomyces cerevisiae. Genetika (Rus). *54*, 18–21.
- Andreychuk Yu. V., **Shiryaeva A. A.**, Zhuk A. S., Stepchenkova E. I., Inge-Vechtomov S. G. (2017) Impact of prionization of the Sup35 protein [PSI+] on the frequency of genetic changes, accounted in the alpha-test in yeast *Saccharomyces cerevisiae*. Russian Journal of Genetics: Applied Research. *7(2)*, 172-174.

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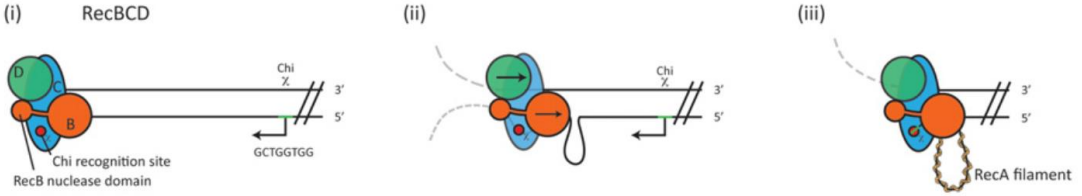
Brief overview of the research problem

DNA double-strand breaks (DSBs) are deleterious DNA lesions repaired by homologous recombination (HR) or nonhomologous end-joining (NHEJ). In bacteria *Escherichia coli*, DSBs are repaired through HR (reviewed in Dillingham and Kowalczykowski, 2008). In yeast *Saccharomyces cerevisiae* both pathways are involved in maintaining genome integrity (reviewed in Ito-Harashima and Yagi, 2017).

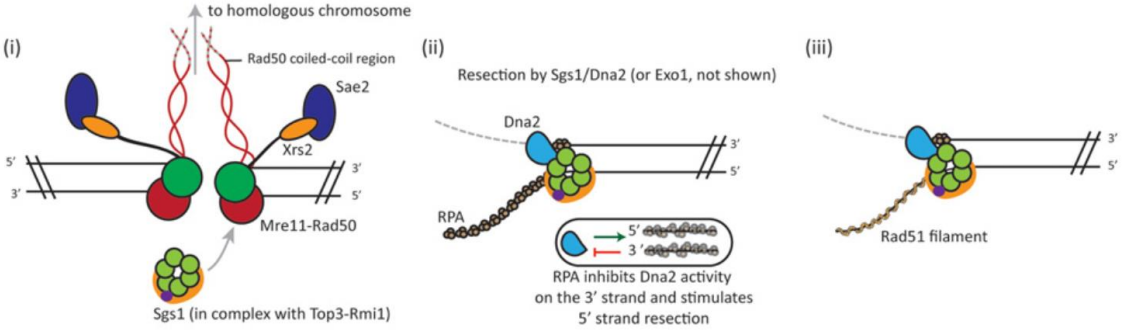
DNA end resection is the first step of HR in both prokaryotic and eukaryotic cells (reviewed in Blackwood et al., 2013). In brief, the 5'-terminated strand of a broken DNA molecule is resected to form a 3'-terminated single-strand tail. This tail is covered by recombinases RecA or Rad51 in prokaryotic and eukaryotic cells, respectively. The resulting nucleoprotein filaments search for homologous DNA sequences and serve as primers to initiate the synthesis of the lost DNA regions by DNA polymerases.

End resection in *E. coli* is performed by RecBCD helicase/nuclease. In *S. cerevisiae,* this process involves Mre11 endonuclease, ExoI 5'→3' exonuclease, and Dna2 helicase/nuclease. The activity of these nucleases was mostly characterized *in vitro* and depends on reaction conditions. The *in vivo* products of DNA end resection are not studied.

End resection in *E.coli*



End resection in *S. cerevisiae*



Blackwood et al., 2013, Biochemical Society Transactions

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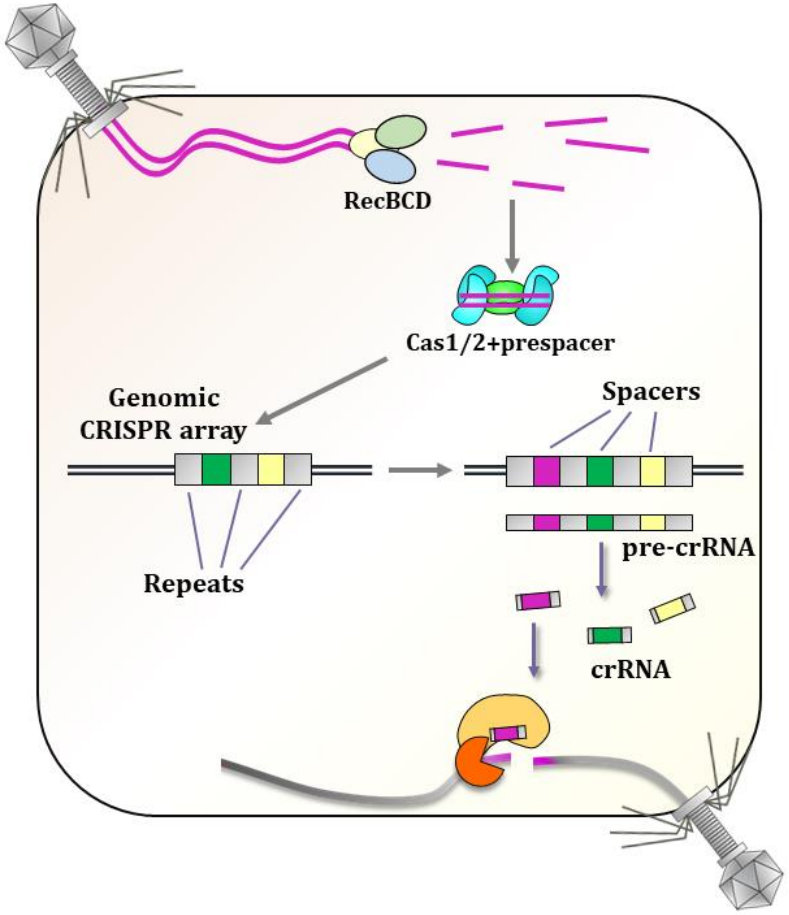
CRISPR-Cas systems are prokaryotic adaptive immunity systems (reviewed in Makarova 2019). A typical CRISPR-Cas system consists of a specific locus in a genome called CRISPR and several genes called *cas*. CRISPR loci are composed of DNA repeats separated by unique sequences called spacers. Upon phage infection, some cells incorporate pieces of viral DNA (prespacers) into the CRISPR array as new spacers. If a cell containing a bacteriophage-derived spacer faces the recurrent attack of the same virus, Cas proteins recognize a sequence in viral DNA identical to the spacer and cleave it inactivating the virus.

The mechanism of prespacer generation is not understood. Several studies suggest that RecBCD is involved in prespacer generation presumably by providing long spacer precursors to the Cas1-Cas2 complex, which integrates prespacers into the CRISPR array (Levy et al., 2015; Ivančić-Baće et al., 2015).



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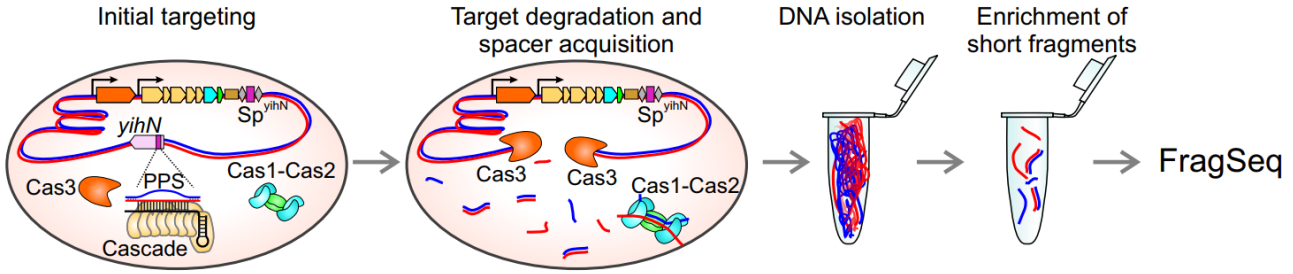
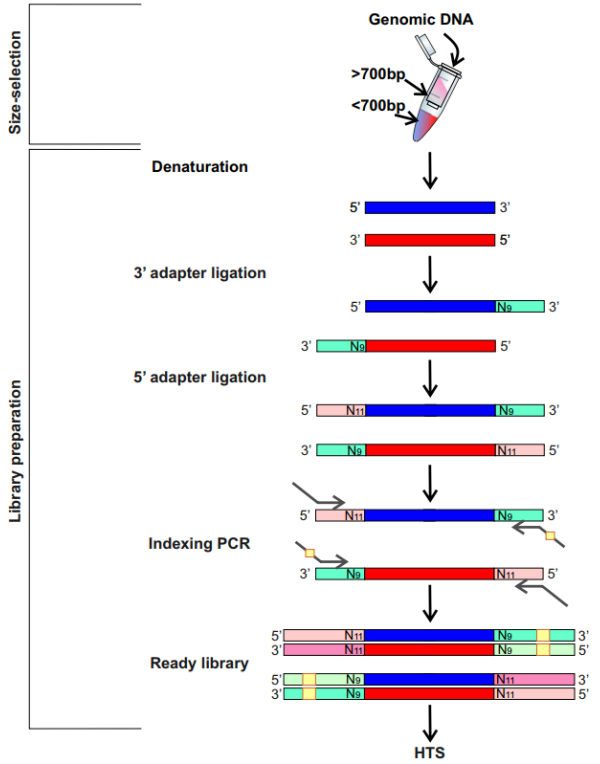


Brief overview of the research problem

We developed FragSeq, a method to analyze short DNA fragments generated *in vivo* using high-throughput sequencing (Shiriaeva et al., 2019). The method allows characterizing precise lengths of the fragments, a DNA strand where the fragments originate from, and associated sequence motifs. We applied this method to detect short-lived prespacers generated during CRISPR adaptation in the type I-E CRISPR-Cas system of *Escherichia coli*. We expect the same method can be applied to study other processes (for example, the resection of ends during DSB repair) in *E. coli* and other organisms.



Shiriaeva et al., 2019, Nature Communications

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Aims

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The goal of this study is to characterize short DNA fragments generated *in vivo* at various stages of DNA metabolism in prokaryotic and eukaryotic cells and identify proteins involved in their generation.

Specific Aims:

- To analyze short DNA fragments generated *in vivo* during double-strand break repair and termination of replication in *Escherichia coli*.
- To study *in vivo* the impact of DNA maintenance proteins into the generation of prespacers in the type I-E CRISPR-Cas system of *E. coli*.
- To analyze *in vivo* the resection of broken chromosomes and generation of DNA fragments during DNA double-strand break repair in a eukaryotic model organism - yeast *Saccharomyces cerevisiae*.

Background

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- Currently, I am a Ph.D. student in the laboratory of Dr. Severinov at Skolkovo Institute of Science and Technology where I study the type I CRISPR-Cas systems in *E. coli*.
- I am planning to perform the experiments on DNA repair of *S. cerevisiae* in the laboratory of Dr. Galkin at St. Petersburg Branch of Vavilov Institute of General Genetics, RAS.