Skoltech
Skolkovo Institute of Science and Technology

Thesis Changes Log

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PhD Program: Life Sciences

Title of Thesis: Escape mechanisms of mobile genetic elements against CRISPR-Cas system and diversity in microbial communities

Supervisor: Professor Konstantin Severinov

The thesis document includes the following changes in answer to the external review process.

1. Clarify in section 2.3.6 which kind of *E. coli* transformants were used in the transcriptome assays.

Page 42: Section 2.2.12 Transcriptome analysis was clarified by updating the sentence "*E. coli* strain KD263 overnight culture was diluted 100 times into 5 mL of LB." as follows: "CRISPR ON and CRISPR OFF colonies were preliminarily obtained by transformation using the plasmid pRSFG8 as described for the CRISPR interference assay in *section 2.2.2*. The obtained CRISPR ON and CRISPR OFF *E. coli* colonies were used for overnight cultivation in LB media supplemented with 50 µg/ml kanamycin, 1 mM arabinose and 1 mM IPTG for the CRISPR ON overnight culture, and in LB media with only 50 µg/ml kanamycin for the CRISPR OFF overnight culture. The CRISPR ON and CRISPR OFF overnight cultures were then diluted 100 times into 5 mL of LB."

Page 62: Section 2.3.6 was clarified by updating the sentence "To understand the mechanism providing survivability for the plasmid-free fraction, we additionally performed the transcriptome analysis of *E. coli* transformants after cultivation in LB media containing both kanamycin and inducers for CRISPR ON cells and kanamycin only for CRISPR OFF cells as control." as follows:
"To understand a potential mechanism providing survivability for the plasmid-free fraction of the CRISPR ON colony, we additionally performed transcriptome analysis of CRISPR ON and CRISPR OFF *E. coli* colonies obtained after transformation using the plasmid pRSFG8, as described in *section 2.2.12*. We randomly selected three CRISPR ON and three CRISPR OFF colonies and cultivated the selected colonies overnight in LB media containing both kanamycin and inducers for CRISPR ON cells, and kanamycin only for CRISPR OFF cells as a control. After overnight cultivation, we extracted RNA from the CRISPR ON and CRISPR OFF *E. coli* cells for sequencing, as described in *section 2.2.12*."

2. When referring to CRISPR-Cas types, sometimes the first letter of the word "type" is capitalized, while in other cases it is lower case. Please be consistent throughout the text.

Where is required the capitalized first letter of the word "type" was replaced with lower case on **pages 16 – 18, 21, 23 – 26**.

3. When referring to CRISPR-Cas subtypes, sometimes "subtype" is used before the subtype name, while in other cases "type" is used instead. Please be consistent throughout the text.

The word “subtypes” before the subtype name of CRIPRS-Cas systems were replaced with “types” on **pages 23 – 26.**

4. The list of abbreviations were updated as the following:

Page 10: “Cas – CRISPR Associated” is replaced with “Cas – CRISPR **a**ssociated”.
Page 11: The abbreviation “tracRNA – trans-acting CRISPR RNA” is replaced with “trac**r**RNA – trans-**activating** CRISPR RNA”.

5. The text for gene names in italics was checked:

Page 24: “*cas11*-like gene” were replaced “*cas11-like* gene”.
Page 27: “Cas genes” were replaced with “*cas* genes”.
Page 29: “acr genes”, “Acr-associated (Aca) genes” were replaced with “*acr* genes” and “Acr-associated (*aca*) genes”, respectively.

6. The doi is included in some references, but not in others. Please be consistent throughout the reference list.

The doi links were deleted from the references in the Bibliography so the number of pages was reduced from 175 to 174. The style of references from 297 up to 386 was updated by adding “,” and “.” after the names of authors for style consistency.

Reference 312 was updated from “Simon SA, Schmidt K, Griesdorn L, Soares AR, Bornemann TL V, Probst AJ. (2023). Dancing the Nanopore limbo–Nanopore metagenomics from small DNA quantities for bacterial genome reconstruction. *bioRxiv* 2022–2023.” on “Simon, S.A., Schmidt, K., Griesdorn, L., Soares, A.R., Bornemann, T.L.V., Probst, A.J. (2023). Dancing the Nanopore limbo–Nanopore metagenomics from small DNA quantities for bacterial genome reconstruction. *BMC Genomics* 24, 727.” because this article was accepted by *BMC Genomics*.

7. Page 130: The word “**that**” from the sentence “I suggest **that** the possibility of plasmids without genetic alterations to persist in the bacterial population under permanent CRISPR interference pressure” was deleted.

8. Page 131: The word “**but**” from the sentence “While not absolute, **but** an optimized CRISPR-Cas defense affords bacteria a window of opportunities to acquire beneficial MGEs that can provide a competitive edge under adverse conditions.” was deleted.

9. Clarifications related to the experiments with phage M13:

Page 43: The sentence “The replicative form of the M13mp18 genome phage bearing *g8* protospacer and *gfp* gene was provided by Aleksandra Strotskaya.” was updated as follows: “The M13mp18 replicative form bearing *g8* protospacer, **β-lactamase gene** and *gfp* gene was provided by Aleksandra Strotskaya.”

Page 63: The sentence “To test this hypothesis, the experiments with transformation of *E. coli* KD263 cells with M13 phages bearing the native g8 protospacer and a GFP fluorescent marker were performed.” was updated as follows “To test this hypothesis, the experiments with transformation of *E. coli* KD263 cells with M13 **replicative form** bearing the native g8 protospacer, **β-lactamase gene,** and a GFP fluorescent marker were performed.”