

Thesis Changes Log

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PhD Program: Life Sciences

Title of Thesis: RECOGNITION STRATEGIES OF TYPE I AND TYPE V BREX SYSTEMS

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

- The origin of the figures taken or modified from Gordeeva et al 2019 is indicated;
- Page 17 “most abundant” is deleted;
- Tables 4 and 5, Figures 4 and 14 are replaced;
- All “type” words are replaced by “Type”;
- “are thought to be” is placed in the last sentence p 28;
- “mutations in the PAM region or in the protospacers result in avoidance of CRISPR defense” is replaced with “mutations in the PAM region result in avoidance of CRISPR defense”;
- “effector modules” and “PLE” are defined on page 32 and “Cascade” on page 36;
- “Inoue tbuffer”, “...thus work...” and “Dh2 α ” are corrected;
- “efficiency of plating” is replaced with “efficiency of plaquing”;
- “for *ΔpyrF* strains” and “The plaques were counted in each drop of phage dilution.” were added at p 47;
- “E. coli” or “BREX^{Ec}” is added in the titles of subsections 2.9-2.12 and 3.3-3.6;
- New subsections “2.6 Knock-out of *brx* genes in *H. hispanica*” and “2.16. Restriction endonuclease activity assay” were added to Materials and methods;
- “Both pBTB-2 and pBREXAL plasmids transformed laboratory E. coli K12 strain BW25113 with equal efficiency” was changed to “A laboratory E. coli K12 strain BW25113, lacking endogenous *brx* genes, was transformed with pBTB-2 and pBREXAL plasmids with equal efficiency”;
- BREX+ MOI=0.001 is changed to BREX- MOI=0.001 at Figure 11d. “The lines for BREX+/- with no phage addition are masked by the line BREX+ MOI=0.001” is added to figure 11d description;
- “with Dam-methylated DNA” is added to the first sentence of the second paragraph on page 54;
- “No such modification was present in DNA of phages induced from BREX- cells.” was added to page 58;
- “Fig. 16c” was changed to “Fig. 16d” on page 60;
- “Such constructions are transcribed as a single mRNA and, thus, deletion of *brxA* destroys transcription and translation of downstream gene *brxB*” and “The failure to complete the system could be explained by *brx* gene regulation interruptions due to the usage of an inducible promoter for *brxC*.” and phrase “To tightly control the expression of *brx* genes” were added on page 62;
- Figure 20c. “linar” is changed to “linear”;
- Page 66. “Table 2” is replaced with “Fig. 21a”;
- “In PhD thesis of Dr. Artem Isaev it was shown that accumulation of dsDNA forms of M13 phage is inhibited in BREX+ cells.” and “The results can be explained by anti-BREX strategies used by phages.” were added to page 67;
- “there” is deleted from page 74;
- reference to Table 6 was added at the text;
- “which may help withstand phage predation with BREX modified DNA” was added to the last sentence of page 77;
- “The protein could inhibit host mechanisms while the system eliminates a phage. However, we postulate that BREX system does not work as *Abi* systems.” was added to page 78;
- “thus genomes of cells with deletions of individual methyltransferases only one *brx* motifs...” was replaced with “thus, in genomes of cells with deletions of individual methyltransferases only one *brx* motif...”;
- bibliography is corrected;

- “However, unlike R-M systems, pBREXAL plasmid expressing the BREX system and an empty pBTB-2 vector is transformed with equal efficiency.” was added to page 76;
- “while the host DNA remains unmodified and is not subject to restriction” is added to the first sentence of the last paragraph;
- “fluorescein isothiocyanate (FITC)-labeled casein as a substrate (Abcam’s Protease Activity Assay Kit) but” and “A leak of arabinose promoter in the absence of the inducer leads to a low level expression of the protein.” were added to the page 64;
- Figure 19 and Table 3 were corrected;
- “We calculated the number of reads covering cos sites and 150 bp from the left and the right from cos sites to the number of reads ending on cos sites.” was added to page 67;
- Appendix B was added;
- The accession number of BREX locus was added;
- “m4C” is added to page 71;
- “NucS and BrxU do not share sequence homology.” was added on page 64;