Summary about Experiment In-Vivo-Gene Editing by CRISPR

Lab workshop and the evaluation of experiments in total done by 20 international student teacher teams guided by mentors from Greece, Czech and Slovakia took us 3 days and showed the following results.

1. Step: Transformation

14 workgroups out of 20 had performed a perfect result. They edited successful the targeted *lac*-Z-gene coding for ß-galactosidase on the bacterial chromosome by the active CRISPR/Cas-complex inside the living cells in-vivo. Bacteria colonies of E. coli in approach D were existing on the nutrient and their color changed from wild type *lac*Z from blue to white.

6 Workgroups had no colonies in approach D. But other approaches A – C of the 6 workgroups showed expected results (see Fig. 1 and 2).

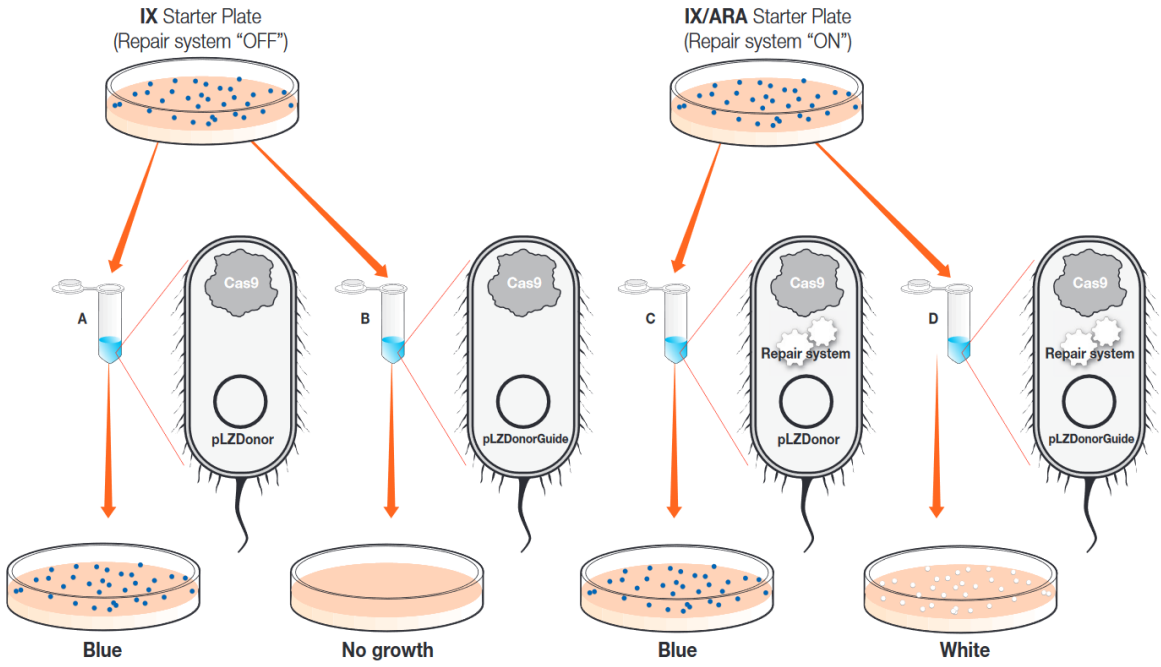
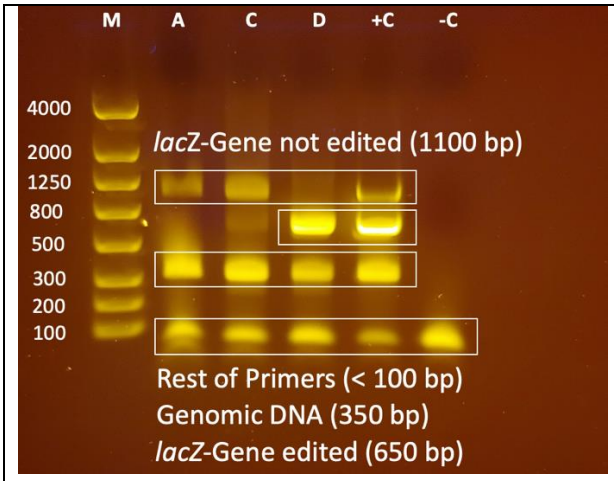


Fig. 1: Scheme of expected perfect results in the approaches A – D.

Fig. 2: Color of colonies in blue/white screening in the approaches A - D.

2. Step: Confirmation of *Lac*Z-Gene Status by Multiplex PCR

PCR amplification of isolated DNA out of blue colonies showed in gel-electrophoresis the length of wild type *lac*Z-fragment of not edited *lac*Z-gene 1100 bp (see Fig. 3).
PCR amplification of isolated DNA out of each investigated white colony of 14 workgroups showed in gel-electrophoresis the length of edited *lac*Z-gene fragment (650 bp).
Each investigated colony showed in gel-electrophoresis the control band for chromosomal DNA with the length of 350 bp (see Fig. 3).



Approach	*lac*Z-Status	Colonies
A	Not edited (1100 bp-Fragment)	blue
C	Not edited (1100 bp-Fragment)	blue
D	Edited (650 bp *lac*Z-Fragment)	white
+C	Positive Control	
-C	Negative Control	

Fig. 3: Band evaluation in gel-electrophoresis after multiplex PCR (M = marker; A, C and D approaches; +c = positive control; -c = negative control).