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In vitro CRISPR-mediated gamma globin gene activation in HEK293

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Abstract.

Blood disorders like B-thalassemia or sickle cell anaemia obligate patients to require constant blood transfusions. These, among others, make blood a highly demanded resource in the medical field. Blood has been obtained from in-vitro erythroid differentiation from embryonic stem cells or induced pluripotent stem cells, but this blood tends to produce embryonic haemoglobin.

In this project, we aim to use a CRISPR/Cas9 modified version and an aptamer approach to alter the expression of the β-globin locus upregulating the gamma globin gene to produce foetal haemoglobin. Guide vectors were created targeting previously known sequences in the gamma haemoglobin gene.
HEK cells were transfected with different combinations of CRISPR/Cas9 elements, as well as different combinations of aptamers/effectors, to test the amount of gene activation achieved. Gene expression analysis was performed through RTq/PCR.

It was found that HbG activation can be achieved to some extent, however, the levels of gamma haemoglobin attained are very low compared with the normal levels of HbG expressed in other cell lines like K562.
From the different grouping of vectors (dCas9 protein + aptamer/coating protein + activation effectors) tested, the combination of dCas9ES + PP7/PCP + VPH presented the best performance.

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