Construction of a light-induced Chimeric Antigen Receptor (CAR) expression system

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INTRODUCTION

The number of trials using genetically modified T cells to redirect cytotoxic activity through chimeric antigen receptors (CARs) has increased through the past decade. It has been known that one of the risks of this therapy is the recognition of the target antigen in healthy tissue, causing harm to the patient. We herein try to mitigate this "on-target, off-tumor" effect by controlling the expression of the CAR though a synthetic system independent of drugs. The use of a drug-free system is important in the context of a potential therapy, so that the treatment doesn't interfere with the organism other than through the activity of the CAR+ T cells over the tumor, and increase or decrease the CAR's sensibility for the antigen as needed, to avoid healthy tissue damage. In the system under development, we use a pair of fast light-responsive proteins to build a transcription factor exclusive for the CAR gene. Optogenetic-based systems control the expression and/or activation of different proteins and specific branches of a larger pathway, to have a desired output in response to specific light stimuli. By approaching the CAR gene expression as a synthetic biological circuit and using this expression mechanism, we should be capable of regulating where and when the CAR will be expressed.

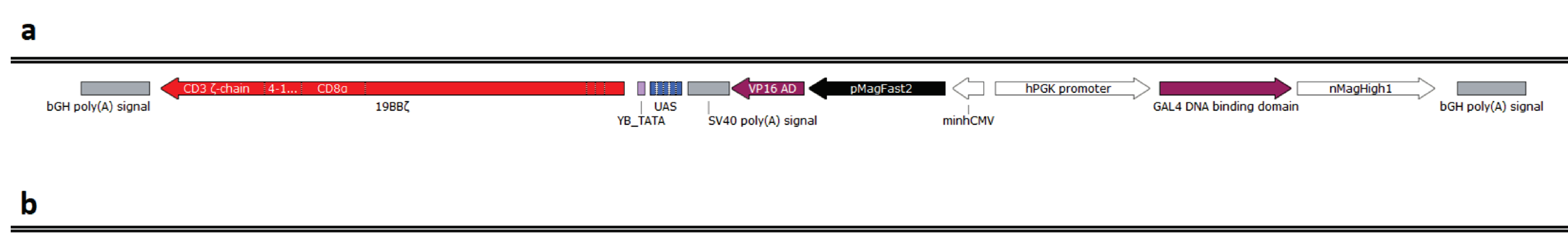

Figure 1: (a) System's basal structure, without incorporating regulatory elements for fine tuning. (b) System's struture used for validation and duty cycle curve setting.

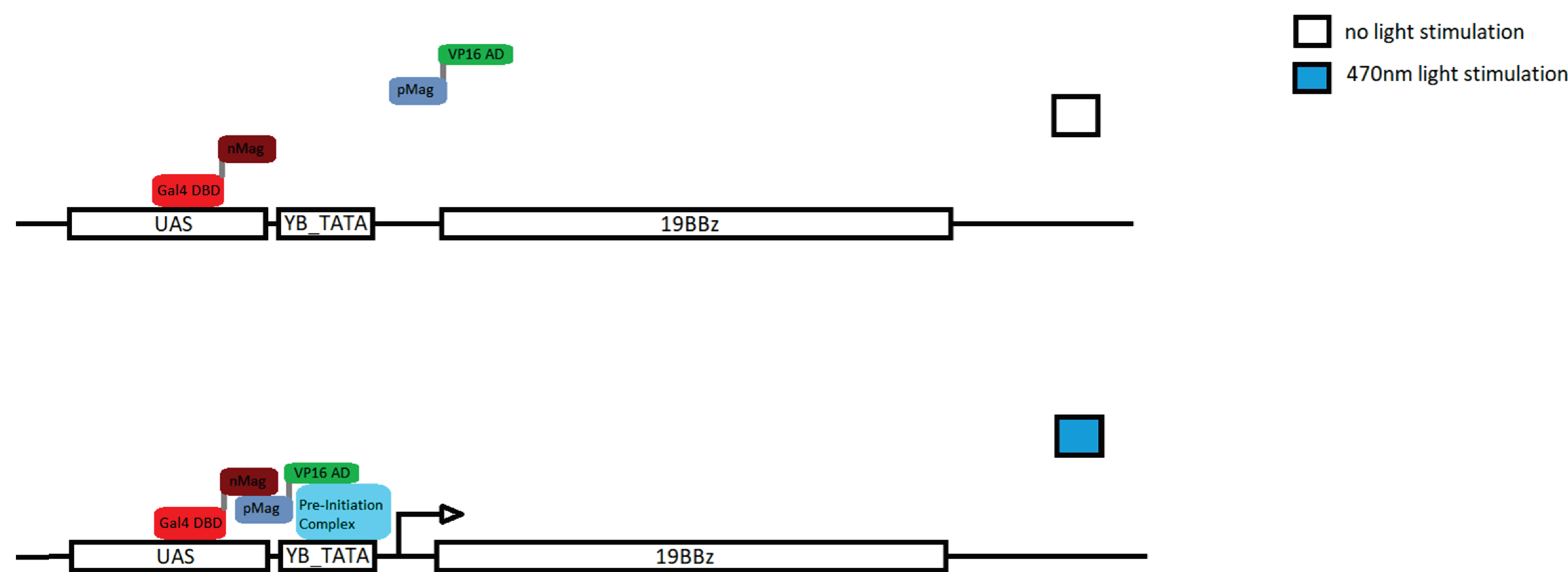

Figure 2: Mechanism of activation of the CAR gene. Once stimutaled with blue light, pMag and nMag interact, uniting the DNA binding Domain and Activating Domain in an heterodimer capable of recruiting transcription factors for the formation of the initiation complex.

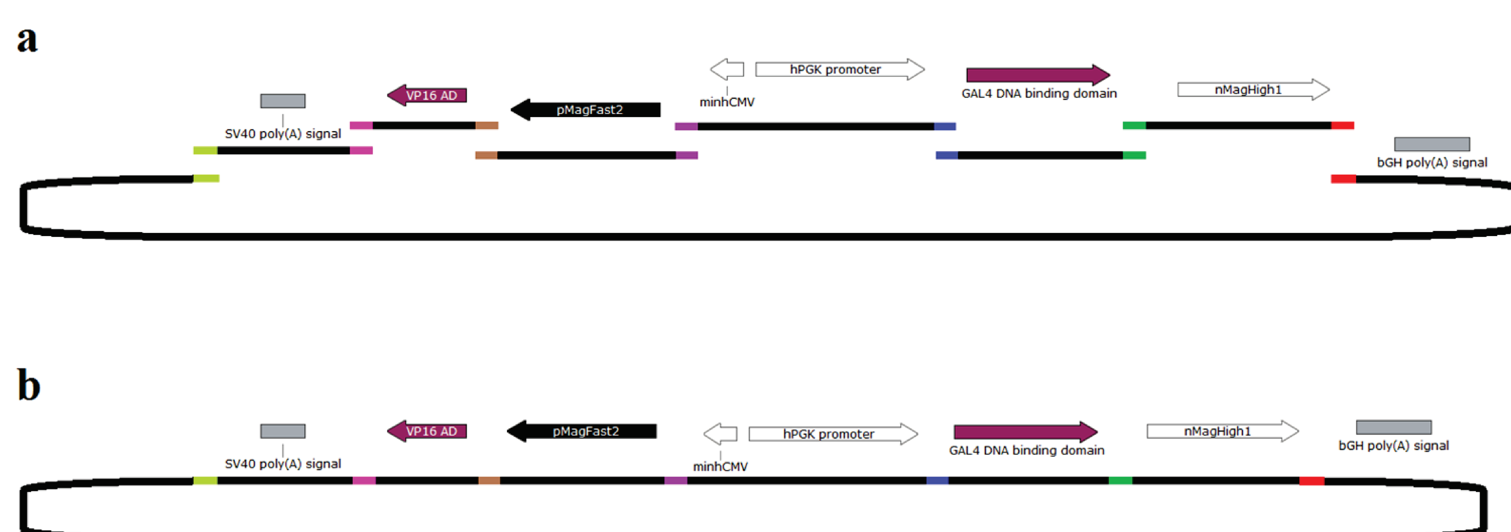

Figure 3: For the transcription factor cassete assemble, 6 amplicons plus the pT3 backbone will be cloned using Gibson Assembly method, going from (a) to (b) in a single cloning step.

MATERIALS AND METHODS

Plasmids enconding the Gal4 UAS, Gal4 DBD, VP16 AD, pMagFast2 and nMagHigh1 were acquired via AddGene. Except for Gal4 UAS, these sequences will be assembled in a plasmid containing a bidirectional promoter to generate the CAR's transcription factor gene. The YB_TATA core promoter was synthetized via Genscript and was assembled together with the CAR 19BBz coding sequence and Gal4 UAS to generate the CAR gene. The system activation will be validated by inducing GFP expression in conditions with or without light and under different light/dark periods. The photoresponse curve will be determined using GFP MFI under different light/dark periods. Once the photoresponse curve has been set, we will induce CAR expression and perform cytotoxicity assays of target leukemia cells using different light/dark periods to determinate the minimal period needed to induce cell cytotoxic activity.

Figure 4: Agarose gel of the PCR of nMagHigh1 and pMagFast2.
1→ Ladder
2→ Control of nMagHigh1(without template)*
3→ nMagHigh1
4→ nMagHigh1
5→ Control of pMagFast2 (without template)*
6→ pMagFast2
7→ pMagFast2

*dna fragment showing on the control conditions due to excess primer

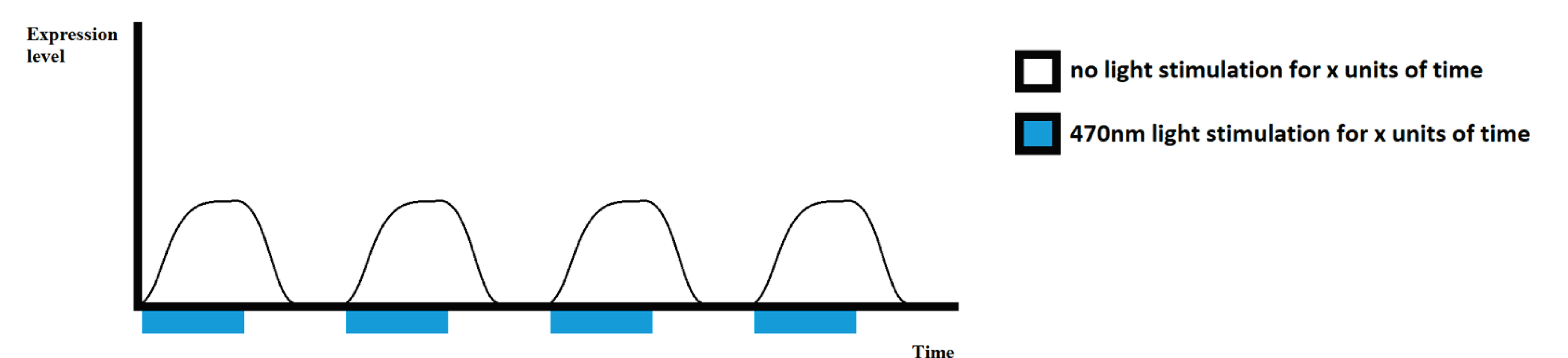

Figure 5: Theoretical expression level photoresponse curve of GFP or Myc-tagged-19BBz. This graphic is merely illustrative, based on the expected increase and decrease of controlled gene expression.

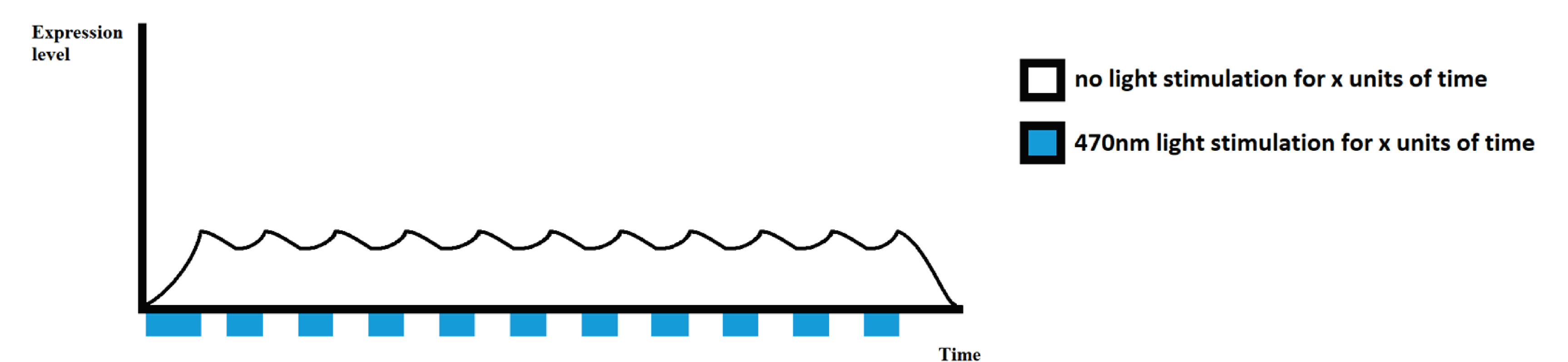

Figure 6: Theoretical expression level photoresponse curve, showing transgene expression stabilization on desired value due to duty cycle control. This graphic is merely illustrative, based on the expected increase and decrease of controlled gene expression.

RESULTS

We have built the light-responsive CAR gene construct and set the conditions for generating 7 of the 8 amplicons to be assembled into the final plasmid containing the inducible cassette.

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