Optimizing Long-term Gene Expression Using Chromatin Insulators in Stably Integrated Multi-gene Constructs

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Synthetic biology seeks to imbue living cells with complex genetic circuits that execute localized logical computations. These genetic circuits are usually composed of multiple genes expressed by multiple promoters. For these circuits to function in mammalian cells each promoter-gene pair or “expression unit” needs to be functionally independent. Eukaryotes use chromatin-binding sequences to shield or insulate adjacent expression units. I investigated which chromatin insulators enable sustained long-term expression of genes. Green Fluorescent Protein (GFP) was constitutively expressed using the promoter CAG. Three structurally identical plasmids containing the expression unit “CAG-GFP” were constructed. Each plasmid utilized a different type of chromatin insulator: chromatin insulator 2 (ci-2), chicken hypersensitive-site 4 (cHS4), and no chromatin insulator. The plasmids were then stably-integrated using CRISPR-Cas9 into HEK293T cells at the chromosome 19 genetic safe harbor locus, AAVS1. The percent of GFP-positive cells was measured weekly using fluorescence-activated cell sorting to analyze the GFP expression over time for each line of cells. Cells containing ci-2 maintained high GFP expression intensity and uniformity. Over the 10-week period, ci-2 cells maintained expression levels constant at ~99.5% cells GFP-positive. The percent of no insulator cells decreased to 87.3% at week 10. Cells containing cHS4 showed poor uniformity in GFP intensity and the percent of GFP-positive cells decreased to 61.7% at week 10. The chromatin insulator ci-2 is significantly more effective than the widely used cHS4. The use of ci-2 in multi-gene constructs should enable effective gene therapy and long-term studies of synthetic gene circuits in mammalian cells.