#42: Developing a Tet-On 3G and PiggyBac system for tunable and temporal gene expression in human pluripotent stem cells

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As highly proliferative cells with the potential to become any cell type, human pluripotent stem cells (hPSCs) provide an in vitro platform to study development and disease at the cellular and molecular level. The discovery of induced pluripotent stems cells (iPSCs) in 2006 opened new avenues for probing development and disease. With tight regulation and controlled expression of a gene in question, hPSCs provide an interesting clinical model towards understanding the relationship between genetic elements and disease progression or manifestation. An accurate, disease specific, cellular model could be used for high throughput drug screening. Additionally, patient derived iPSCs could provide an opportunity create personalized therapy and drug regimens. In order to achieve disease modeling that accurately incorporates the temporal expression of the genes involved in a specific disease, a strategy for tunable and temporal genetic regulation is needed. We are developing a system incorporating both Tet-on 3G and PiggyBac elements for tunable and temporal regulation of gene expression of hPSCs and their derivatives. We have fully integrated the plasmid system into the genome of hPSCs and will demonstrate tight temporal regulation with the addition and removal of doxycycline to the media. The expression level will be modulated with direct proportionality to the concentration of doxycycline administered, and expression will be maintained in differentiated cell types. This all-in-one system for gene expression regulation has extensive applications in medical genetics to further understanding of gene expression in developmental and disease models.