The Generation of Reporter Human Beta Cell Line and Pluripotent Stem Cells to Track Calcium Signaling and Insulin Secretion

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Purpose

Generate platforms, both human β-cell line and human iPS cell line based, to allow interrogation of beta cell activation by following calcium flux and secretion of luciferase a a surrogate for insulin secretion.

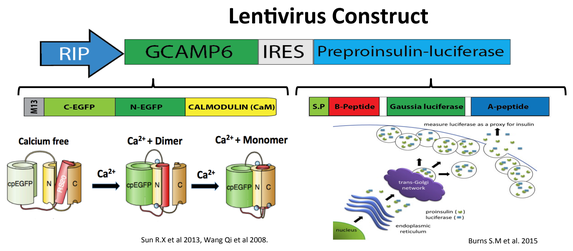
Background

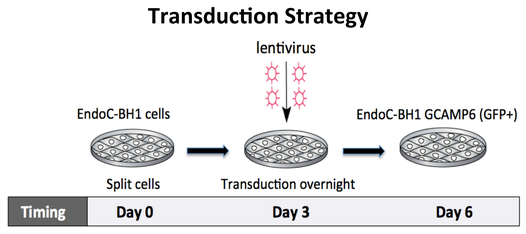
One challenge in the generation of a biomimetic human islet is to assay beta cell functionality in real time in a scalable manner. To follow beta cell functionality in such systems, we have generated a transgenic human beta cell line and iPS cell line. These lines express the calcium sensor GCAMP6 as well as a fusion protein between preproinsulin and luciferase. GCAMP6 expression allows interrogation of calcium flux via simple fluorescence intensity which does not require complex and expensive microscopy setups needed for dye based systems. In dye based systems it is also difficult to distinguish between beta and non-beta cells in mixed differentiation cultures which is avoided by using an *INS* locus knock-in strategy. The preproinsulin-luciferase fusion protein allows insulin secretion to be monitored by a simple and inexpensive luciferase assay that is more amenable to scaling. These tools will be invaluable to test beta cell functionality in various bioengineered devices.

Methods

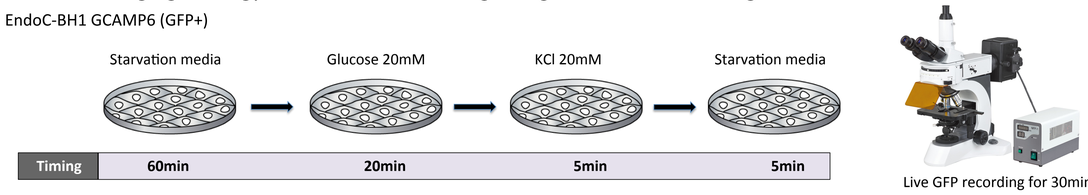
1. Generation of calcium and insulin secretion dual-reporter using the human β-cell line EndoC-BH1

A. Transduction of a lentivirus carrying GCAMP6 and Preproinsulin-luciferase transgenes.





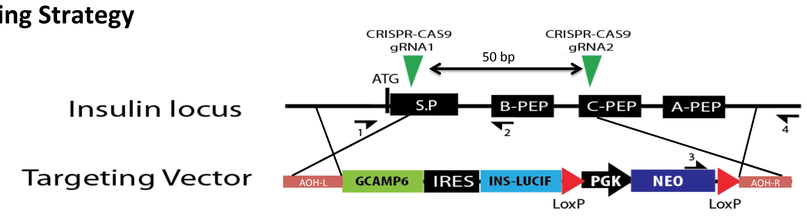
B. Live cell imaging strategy to examine calcium signaling in EndoC-BH1 transgenic line under different stimuli.



Live GFP recording for 30min

2. Generation of reporter human iPS cell line using the CRISPR-CAS9 nuclease system.

A. Targeting Strategy



Screening primers:
1 and 2 check wild type allele
3 and 4 check insertion targeting vector

B. Strategy for generation of an Insulin reporter cell line

Conditions CRISPR-CA9 transfection

CAS9 full cut

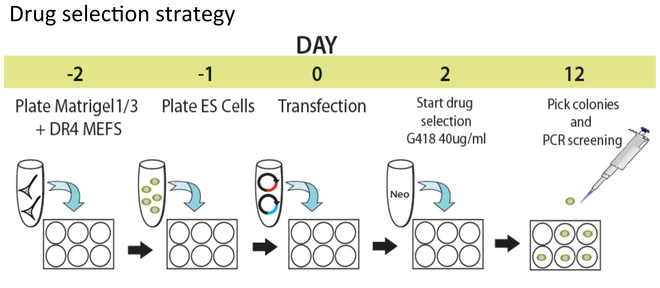
Condition	1	2	3
CAS9	0.1	0.5	1
gRNA	0.1	0.5	1
Targeting Vector	2.8	2	1

CAS9 Nickase

Condition	4	5	6
CAS9	0.1	0.5	0.75
gRNA1	0.1	0.5	0.75
gRNA2	0.1	0.5	0.75
Targeting Vector	2.7	1.5	0.75

DNA concentration in µg
Total DNA per well 3µg

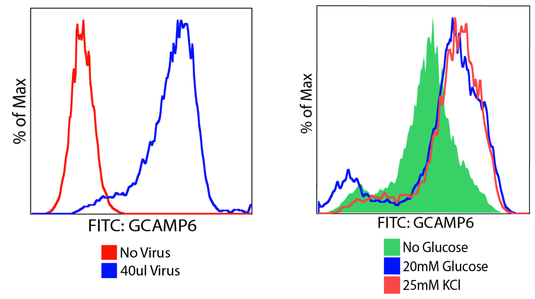
Drug selection strategy



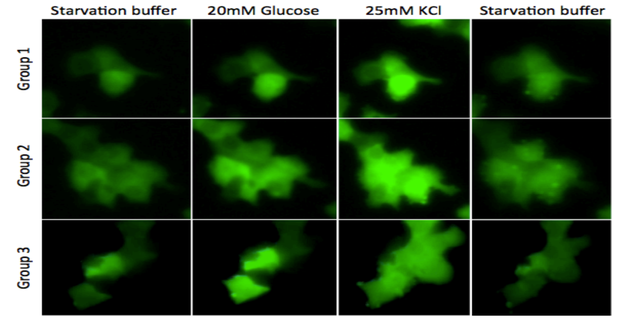
Results

1. Using RIP-GCAMP6-IRES-INSLUC transduced EndoC-βH1 allows to visualize calcium influx in-vitro upon different stimuli.

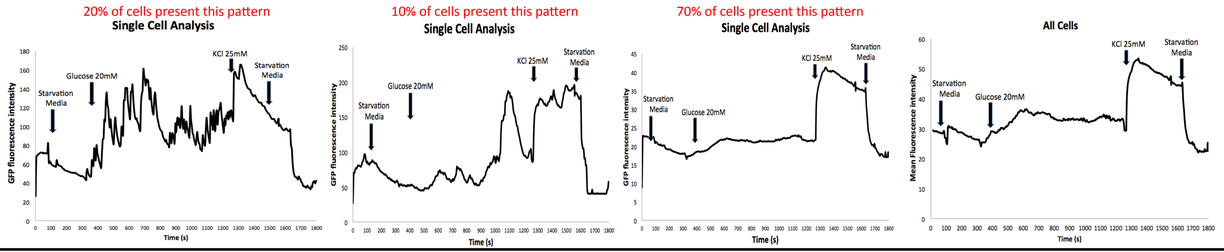
Lentivirus transduction in EndoC-BH1 cell line is highly efficient and GFP+ cells respond to stimuli.



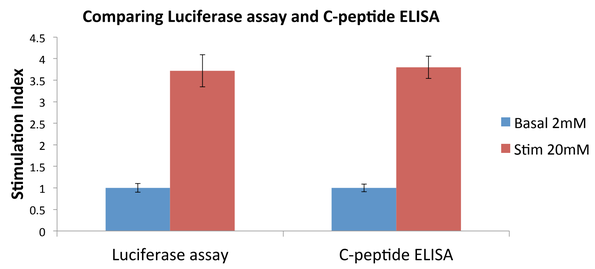
EndoC-BH1-GCAMP6 cells show changes in GFP intensity upon different stimuli

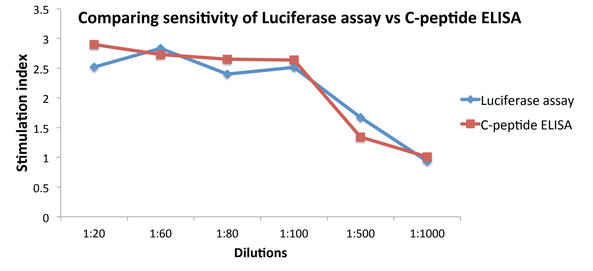


GFP intensity can be monitored in single cells over time.



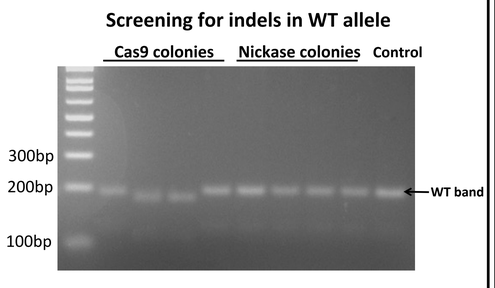
2. Using RIP-GCAMP6-IRES-INSLUC transduced EndoC-βH1 cells, luciferase can be detected in the media after glucose challenge with sensitivity comparable to the ultrasensitive c-peptide ELISA





3. Using the CRISPR-CAS9 system to target GCAMP6 and an INS-luciferase fusion protein into the human *INS* locus in a human iPS cell line.

	Condition	# of colonies obtained	# of colonies targeted	Targeting efficiency %	# of targeted clones with indels in non-targeted allele	% of targeted clones with indel in non-targeted allele
Cas9	1	12	4	33	1	25
	2	14	4	29	3	75
	3	14	5	36	3	60
Nickase	4	15	9	60	2	22
	5	11	4	36	1	25
	6	8	2	25	1	50



By testing both CAS9 Nickase and CAS9 and titrating down the amount of gRNA's and CAS9 used, we observed that the targeting efficiency is similar or better at low concentrations of CAS9/gRNA. Lower levels of CAS9/gRNA do decrease indel formation in the non-targeted allele .

Summary

- The use of a human β-cell line (EndoC-BH1) transduced with a lentivirus expressing GCAMP6 allows the real time analysis of calcium signaling upon stimulation with different stimuli.
- The Gaussia-Luciferase assay used to quantify the insulin-luciferase fusion protein accurately reflects insulin secretion and is highly sensitive, comparable to the quantification of c-peptide using the ultrahigh sensitivity ELISA.
- The use of CRISPR-CAS9 system allows the highly efficient generation of a human iPS cell line with both GCAMP6 and preproinsulin-luciferase fusion protein targeted into the endogenous insulin locus.