Smart Solutions in Drug Discovery: a faster route to assay reagents using cryopreserved cells and transient expression

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Introduction

The development of a fully validated clonal stable cell line as a screening reagent can take 6 to 12 months with extensive associated costs. Where appropriate, transiently transfected cell lines can significantly reduce this time from months to weeks. Transient transfection as a means of generating cell-based reagents has always been an option for any part of a screening campaign. However, this method used to be only suitable for generating just-in-time target expression and not sufficiently robust or logistically sustainable for a long-term screening campaign. The emergence of frozen cell methodology and subsequent decoupling of the transfection step from assay execution has enabled the use of transient expression systems for the rapid construction of cell-based screening reagents, potentially reducing the lead time from months to weeks[(1)]. In response to the increasing demand for transient cellular assays, the Cell Factory has developed a process for the large scale production of cryopreserved transiently transfected cell lines for use in drug discovery. Large scale production of cryopreserved transiently transfected cell lines offers the investigator:

- rapid reagent generation,
- convenience – 'off' the shelf' reagent,
- a single 'ready to go' batch of frozen cells for current and future requirements,
- potential to reduce batch to batch variation,
- flexibility – easing of scheduling,
- assays for difficult gene products.

Here we present data on the development of a large scale production process for the provision of two different cryopreserved transiently transfected cells lines. Included is identification of a suitable transfection reagent that is efficient, non-toxic and cost effective. This process has been validated using these two cell lines and two different cell based assays.

Experimental Methods

Stable Cell Lines

Frozen stocks of CHOK1 cells expressing the β2 adrenergic receptor and HeLa cells containing the GRE-luciferase reporter were prepared @ $1x10^7$ cells/1ml/vial and stored at -140°C.

Transfection reagent selection

A panel of 15 commercial transfection reagents were selected based on a review of the scientific literature, chemical class, protocol and reported performance. CHO, U-2OS and HEK293 cell lines were used in combination with a plasmid encoding EGFP, as a marker of transfection, and propidium iodide (PI) to assess cell viability. Samples were analysed by flow cytometry for expression of EGFP and propidium iodide staining. Successful outcomes were based on the following criteria:

- transfection efficiency
- cell viability
- breadth of performance across all cell lines tested

Transient assay development

CHOK1 cells were transfected with the plasmid encoding the β2 adrenergic receptor and HeLa cells transfected with the GRE-luciferase reporter gene plasmid using the optimised transfection conditions. For both assays, transfected cells were detached and seeded on to assay plates at $2x10^4$ in 100µl/well and incubated overnight at $37^0C/5\%$ CO_2. Remaining cells were cryopreserved at $1x10^7$ cells/1ml/vial. Frozen cells were thawed and diluted to $2x10^5$ cells/ml in pre-warmed growth medium and 100µl dispensed into replicate wells and incubated overnight at $37^0C/5\%$ CO_2.

Assay Development Workflow

24-well matrix - identify optimum transfection conditions

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Scale to T162cm^2 to confirm performance of assay

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Scale to Corning CellSTACK^{TM}, total area 3180cm^2 and confirm cell viability and performance of assay post cryopreservation

Results

Reagent Screen

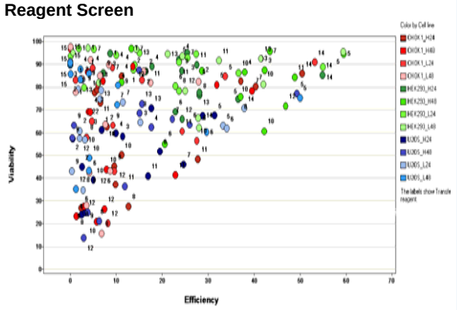


Figure 1. Summary of reagent screen data. Each point reflects the mean of three experiments and is colour coded by cell line, incubation time, and reagent concentration. Data points are labelled by reagent.

Transfection reagents that displayed moderate viability and efficiency (middle-to-top right of the plot) were selected for further evaluation, Figure 1. Reagents 5, 7 and 14 were chosen for a detailed study; increasing the number of variables such as cell line, concentration of transfection reagent and plasmid. A single reagent was identified as meeting the criteria outlined above. This reagent was used to develop two transient cell based assays.

CHO- β2 adrenergic receptor cAMP assay

Assay: Growth medium was replaced with 50µl agonist and all plates incubated at $37^0C/5\%$ CO_2 for 45mins. The cells were lysed by freeze thawing the plate at -80^{0}C, followed by detection of cAMP using the DiscoveR$_X$ HitHunter^{TM} cAMP II kit (code 90-0034-02).

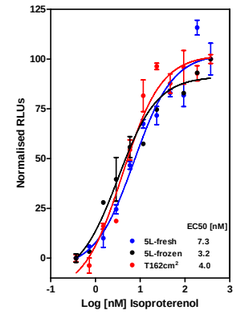
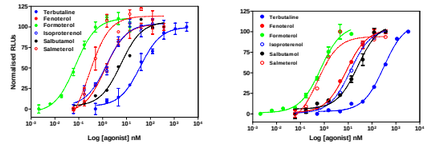


Figure 2. Assay response of CHOK1 cells transiently expressing the β2 adrenergic receptor. Cells were transfected in T162cm^2 flasks and CellSTACKs (5L).

Figure 2 illustrates the comparable performance between CHOK1 cells transfected in either T162cm^2 or CellSTACKs. EC_{50} values were similar for both formats, 7.3nM (CellSTACK) and 4nM (T162cm^2).
Cryopreservation of the cells prepared in CellSTACKs has not altered their response in the assay; 7.3nM (fresh) v 3nM (frozen).



3(a) Transient cell line 3(b) Stable cell line

Figure 3. Rank order of ligand potency in CHOK1 cells expressing the β2 adrenergic receptor either transiently, figure (3a), or stably, figure (3b).

Table 1 summarises the EC_{50} values for the data set presented in Figures (3a) and (3b). For the frozen transient system a shift in potency was observed for all ligands, however, the rank order was the same as that obtained with the frozen stable cell line.

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	Stable Cell line [nM]	Transient cell line [nM]
Terbutaline	311	37.7
Salbutamol	25	5.8
Isoproterenol	12.4	1.46
Fenoterol	8.3	1.28
Salmeterol	0.5	0.26
Formoterol	0.7	0.07

Table 1. Rank order of potency for a panel of β2 adrenergic receptor agonists.

	CHOK1 β2 assay	HeLa GRE-luc assay
Stable	0.79	0.59
Transient	0.44	0.54

Table 2. Z' determination for the stable and transient assays. CHOK1 cells were assayed with isoproterenol and HeLa GRE-luciferase with dexamethasone.

HeLa GRE-luciferase Receptor Gene Assay

Assay: Growth medium was replaced with either 100µl serum free medium (SFM) or agonist prepared in SFM. All plates were incubated at $37^0C/5\%$ CO_2 for 16 hours. Expression of the luciferase reporter was detected using the Promega Luciferase Assay system^{TM} (E1500).

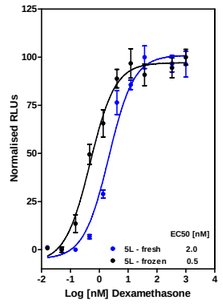
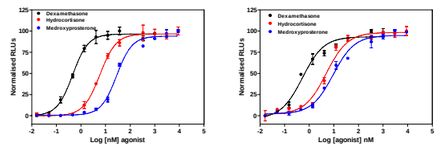


Figure 4. Shows the assay response of fresh and frozen HeLa cells transfected with GRE-luciferase plasmid.

Figure 4 illustrates the comparable performance of transfected HeLa cells assayed fresh (24 hours post transfection) and following cryopreservation. EC_{50} values were similar for both formats, 2.0nM (fresh) and 0.5nM (frozen). Cryopreservation of the transfected cells did not alter their response in the assay.



5(a) Transient cell line 5(b) Stable cell line

Figure 5. Rank order of ligand potency in HeLa cells transiently transfected, Figure (5a), or stably, Figure (5b) maintaining the GRE-luciferase reporter and treated with 2 glucocorticoid receptor agonists and 1 progesterone receptor agonist (medoxyprosterone).

	Stable Cells [nM]	Transient Cells [nM]
Medroxyprosterone	9.36	28.1
Hydrocortisone	4.92	5.84
Dexamethasone	0.53	0.46

Table 3. Rank order of potency for a panel of glucocorticoid and progesterone steroids.

Table 3 summarises the EC_{50} values for the data set presented in Figures (5a) and (5b). The rank order for the transient cell line was the same as that obtained with the stable cell line, highlighting the useful nature of the cryopreserved transient format.

	CHOK1 β2 assay	HeLa GRE-luc assay
Stable	89%	90%
Transient	95%	96%

Table 4. Post thaw viability for the cryopreserved stable and transient cell lines.

Data within tables 2 and 4 confirms that cryopreservation of transiently transfected cells is a viable option and those frozen cells can provide assays of an acceptable quality.

Summary

Figure 6. Illustrates the time lines required to provide transiently transfected cells for assay using either the 'just-in-time' or frozen cell approaches.

Separating the transfection step from the assay and combining with cryopreservation has the potential to increase productivity. The overall process can be reduced from 4 to 2 days, allowing several experiments to be completed in a single week.

Conclusions

- We have identified a transfection reagent that is compatible with the cryopreservation of transiently transfected cells.
- This reagent has been used to develop a scaleable process for the provision of cryopreserved transiently transfected cell lines.
- The reagent and process has been validated with two different cell based assays.
- Transiently transfected cell lines can be a useful reagent for use in drug discovery.

Reference

1. Cawkhill and Eaglestone Drug Discovery Today, 12, 19-20, pp820-825.

GE imagination at work

This poster was presented at the Society for Bimolecular Sciences SBS 15th Annual Conference & Exhibition, 26th – 30th April 2009, Lille, France.