Quality Tools and their Application in the Production of Cellular Reagents for Screening Assays.

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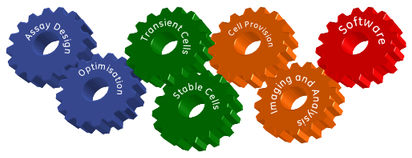
Introduction

The use of mammalian cell based assays in compound screening continues to grow. Over 50% of all screening assays (HTS and HCA) now utilise cells or cell derived reagents.

Adoption of assays employing cryopreserved cells and other cell based screening reagents in a 'ready to go format' is becoming increasingly common practice since it obviates the issues of batch variation, scheduling of cell production and capacity management arising from using 'fresh' cells.

To address these growing demands Cell Factory provides a number of assay ready cellular reagents and services.



Stable cell lines are key reagents in the drug discovery pipeline. We offer a comprehensive stable cell line generation service including molecular biology and functional validation in a variety of assay platforms (Figure 1).

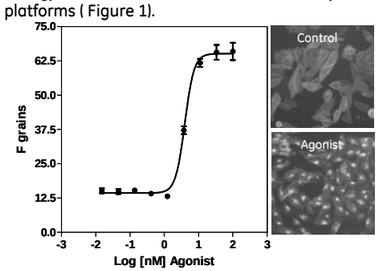


Figure 1. Pharmacological characterisation of a stable cell line generated for an HCA assay.

uHTS size batches of cryopreserved cells have been produced in our dedicated cell culture unit. A variety of culture techniques are used including microcarrier/stirred tank bioreactor and high density plasticware

Batches of cells are usually quality controlled in a functional assay and performance benchmarked against fresh cells or customer specifications (Figure 2).

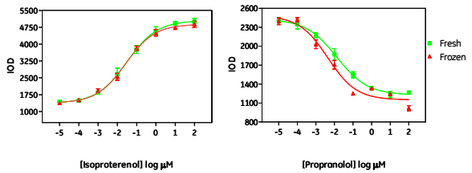


Figure 2. CHO cells expressing hβ2AR were expanded to 1x10[10] and cryopreserved. cAMP production was measured in response to agonist and antagonist. There were no differences in the assay performance of cryopreserved cells compared to fresh cells.

Transiently transfected cells in cryopreserved format are emerging as potential screening reagents (Figure 3).

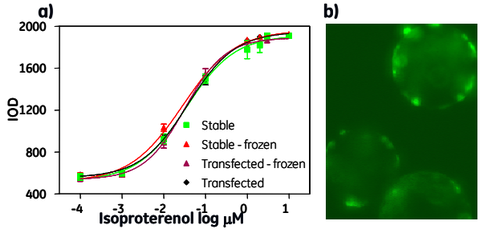


Figure 3. Transient transfection of cells (a) Stable and transient HEK cells expressing the hβ2AR were assayed for cAMP following stimulation with agonist in fresh and cryopreserved format. All formats produced similar assay performance and pharmacology.
(b) Optimisation of transfection on microcarriers (HEK cells transfected with FP tagged protein).

Cell membrane preparations from stable cell lines are frequently used for binding assays. We have therefore developed proprietary scaleable methods to enable production of these key reagents. (Table 1).

Culture Method	Membrane prep	Protein (pg/cell)	Optimum SPA membrane (µg/well)
Low density plastiware	Manual	22	10
High density plastic	Mechanical	29	2.5
Microcarrier/Bioreactor	Mechanical	28	2.5

Table 1. Scaled preparation of membranes from cells cultured on microcarriers and high density plastic-ware illustrating comparable protein yield and assay performance.

Application of Six Sigma.

In order to develop a robust framework for the production and validation of the Cell Factory reagents, we have adopted the use of a variety of six sigma tools. This approach ensures reproducibility and quality in the manufacture of different complex biological products.

Gauge R & R study

Essential to the production of functionally validated cells is a reliable assay measurement system. Gauge R & R is a six sigma tool that determines the total variation within a measurement system and is able to differentiate between error arising from the operator or from the instrument itself, allowing remedial action to be applied if necessary.

For the purpose of this six sigma project, we used a commercially available cAMP luminescence assay kit (which was appropriate for the cell culture optimisation) and measured assay output from a standard curve on 2 different instruments (Figure 4).

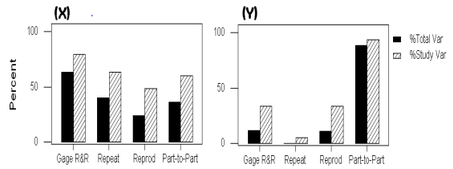


Figure 4. Multiple plates containing a cAMP 11 point standard curve (n=8) were prepared by one operator. 3 operators read all plates in triplicate on instruments X and Y. Data were analysed with MINITAB software.

The variation observed when different operators each measure the same plates repeatedly on different instruments is shown in Figure 4. Repeatability is the variation due to the instrument, whilst reproducibility is variation due to the operator. Gauge R&R is the sum of these totals, and part-to-part is the variation revealed by the measurement system between the parts measured, i.e. the plates in this instance.

Instrument X displays a total variation in the measurement system (Gauge R&R) of ~ 64%, which far exceeds the variation, observed between the plates. In contrast, instrument Y shows only ~ 11% total variation, which is acceptable (less than 10% variation is considered an excellent measurement system). Therefore instrument Y was selected as the instrument of choice for all future measurements.

Process capability

Confident that our measurement system Y was adequate for our needs, we then established current process baselines for a cell line in the appropriate functional validation assay. Analysis of the data with this tool enables us to set control limits for the process and to monitor the impact of any potential changes applied to our reagent production (Figure 5).

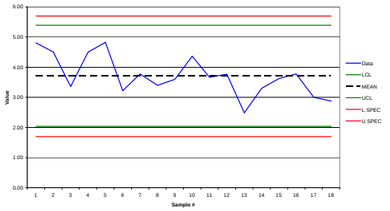


Figure 5. Process capability. Multiple operators (n=5) each set up a number of assay plates (n=4) with cryopreserved cells in agonist dose response format. The assay readout (cAMP) was read once on instrument Y (selected in the Gauge R & R). Data (agonist EC_{50}s) were analysed with MINITAB software.

The process capability experiments were set up to incorporate all potential variables (e.g. operators, assay day, assay kit, etc.). Analysis of the data (Figure 5) shows only random variations with no clustering or trending. We can therefore conclude that the process is performing normally, with no external special cause variation impacting on our system. Any future assay data obtained following process improvements should fit within the control limits for this process, indicating no adverse impact on the overall performance.

Process Improvement

DoE (Design of Experiments) enables assessment of the potential impact of changing multiple conditions simultaneously in a multiparameter process. An example factorial design (Figure 6) illustrates that 'full' is the preferred option as it covers all potential interactions.

Factors

Runs	2	3	4	5	6	7	8	9	10	11	12	13	14	15
4	Full	III												
8		Full	IV	III	III	III								
16			Full	V	IV	IV	IV	III	III	III	III	III	III	III
32				Full	VI	IV	IV	IV	IV	IV	IV	IV	IV	IV
64					Full	VII	V	IV	IV	IV	IV	IV	IV	IV
128						Full	VIII	VI	V	V	IV	IV	IV	IV

Figure 6. Typical factorial design. Available factorial designs, dependant on number of runs and number of factors under investigation.

We wanted to optimise the culture of a cell line and change 3 conditions (A, B and C) simultaneously. We therefore applied a full factorial DoE (Figure 7). This enabled a complete assessment to be conducted to compare existing conditions ('1') against changed conditions ('-1') and the potential impact on the process or cell functionality of changing one or multiple conditions simultaneously.

Assay data from the DoE were analysed (Figure 8). The first order interactions (Figure 8a) suggest optimal conditions are '1' (existing) for A and B and '-1' (changed) for C. The second order interactions (Figure 8b) show the impact that a combination of changed conditions has on the functionality of the cells. Although the second order interactions indicate BC/AC impact on the process, statistical analysis of the data shows these to be negligible and therefore only first order interactions would be considered. Clearly only conditions with no or beneficial effect on the process/functionality would be considered. These would be implemented for full characterisation and assessment in the process capability.

StdOrder	RunOrder	CenterPt	Blocks	Condition A	Condition B	Condition C
2	1	1	1	1	-1	-1
6	2	1	1	1	-1	1
1	3	1	1	-1	-1	-1
7	4	1	1	-1	1	1
8	5	1	1	1	1	1
3	6	1	1	-1	1	-1
4	7	1	1	1	1	-1
5	8	1	1	-1	-1	1

Figure 7. Full factorial DoE required to analyse the effects of changing 3 conditions – A, B &C. Cells were cultured following the DoE, cryopreserved and assayed for the production of cAMP in response to agonist.

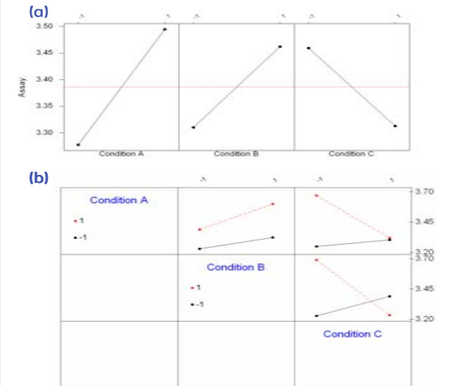


Figure 8. Shows the effect on cell line functionality generated by (a) changing individual conditions (first order interactions) and (b) changing multiple conditions simultaneously (second order interactions) in the culture process.

Summary

- Cell Factory is a custom service providing reagents for all aspects of cellular screening assays.
- These services include production and full functional validation of key reagents such as stable and transient cell lines, screen size batches of cryopreserved cells and scaled membrane preparations.
- We employ a number of six sigma tools to ensure robust production optimisation and manufacture of these complex biological reagents.

GE imagination at work

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