FairJourneyBio
THE ANTIBODY COMPANY

CASE STUDY

Development of custom reporter cell lines

Engineered highly stable and sensitive reporter cell lines to enable robust evaluation of therapeutic antibodies designed to block ligand binding and downstream signaling through dual-chain receptors and GPCRs in animal health.

Mário Soares, PhD, Group leader Cell Engineering and António Barroso, PhD, Director Cell Sciences
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FairJourneyBio

CASE STUDY

CUSTOM REPORTER CELL LINES • ANIMAL HEALTH • BLOCKING ANTIBODIES

Reporter Cell Line Engineering for Therapeutic Antibody Evaluation in Animal Health

GPCR and Dual-chain receptor

CRE-LUC AND STAT-LUC REPORTER CELL LINES

Max fold = 36

TARGET: DUAL-CHAIN

Max fold = 15

TARGET: GPCR

IC50 ≈ 0.24 nM

TARGET: DUAL-CHAIN

IC50 ≈ 6.9 nM

TARGET: GPCR

≤ 3.5 months

FROM VECTOR DESIGN TO IND-READY REPORTER CELL LINES

BACKGROUND

Supporting regulatory needs in both human and animal health with custom-made, highly robust reporter cell lines

The therapeutic antibody market has transformed the treatment landscape across multiple disease areas, including oncology and autoimmune disorders. While antibody-based therapeutics are well established in human medicine, biologics for veterinary applications have only recently begun to gain significant traction, driven by increasing demand for targeted therapies in companion animals.

As regulatory expectations for biologics continue to evolve, robust reporter cell lines are essential throughout therapeutic development, supporting target validation, antibody screening, potency as-sessment, dose-response characterization, and lot release testing. In contrast to human cell lines, the information and tools required for the development of companion animal reporter cell lines is often limited or unavailable.

As a result, custom vector design and overall development strategies become critical factors for success. In this case study, we have developed complex custom reporter cell lines designed to support IND-enabling studies and validate lead therapeutic antibodies against the ligands of two different companion animals' target classes: a dual-chain receptor and a GPCR (Figure 1). Dual-chain receptors and GPCRs, many of which are conserved across mammals, repre-sent attractive therapeutic targets due to their central roles in regulating disease-associated pro-cesses.

Reporter cell line development for these receptor classes can be challenging, particularly for GPCRs, due to complex signaling profiles, difficulties in achieving stable receptor expression, main-taining cell fitness, and generating assays with a high dynamic range. The main objective here was to generate stable and sensitive reporter systems capable of reliably quantifying ligand-induced path-way activation and accurately measuring antibody-mediated inhibitory potency through IC50 deter-mination.

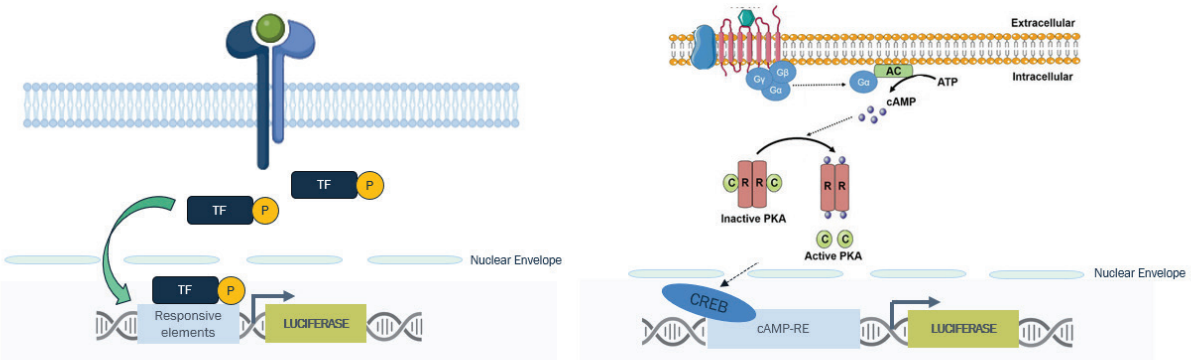

Figure 1. Representative scheme of both signalling pathways explored. Ligand binding to either a dual-chain receptor or GPCR initiates intracellular signalling cascades leading to activation of specific transcription fac-tors. For dual-chain receptors, receptor dimerization induces kinase activation and downstream transcription factor phosphorylation, enabling nuclear translocation and binding to STAT responsive elements. For GPCRs, ligand binding activates the Gs/cAMP/PKA pathway, resulting in CREB phosphorylation and binding to CRE regulatory elements. In both pathways, transcription factor activation drives firefly luciferase reporter gene expression.

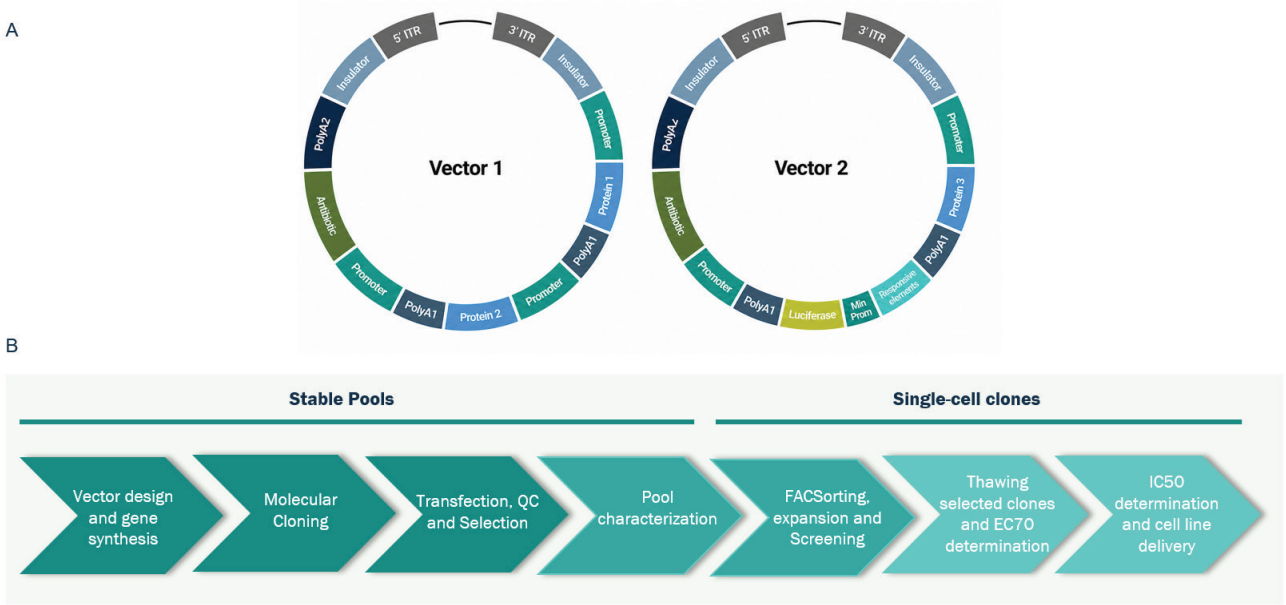

Figure 2. Vector design and project workflow. A) Two vectors were generated for each reporter cell line. Image shows an example of vector design for one of the projects where three different proteins were overexpressed, using independent promoters, and luciferase gene under the control of respective custom designed regulatory elements. B) Reporter cell line generation workflow used in the generation of these reporter cell lines.

RESULTS

Finding the best single-cell clone – Fold inductions of > 17 achieved

Stable pools were single-cell sorted by FACS using tagged ligands and detecting with fluorophore-conjugated anti-tag antibodies. This strategy allowed to sort high-expressing clones. Clonality and clonal growth was assessed using a cell imager, and once the necessary confluency was reached, clones were expanded and screened via a luciferase assay (Figure 3).

Based on data collected during stable pool generation, it was decided to stimulate the dual-chain receptor clones with two different concentrations whereas one single ligand concentration was sufficient to distinguish the different clones for the GPCR. Our overall strategy allowed to generate and find highly responsive clonal cell lines. Three top clones were selected based on fold induction and one vial from each was thawed to perform final clone characterization and determine the EC70 for each ligand (Figure 4).

A sub-saturating concentration ranging from EC50 to EC80 is often selected, particularly important when antibodies bind the ligands and not the receptor, to maximize signal while avoiding excess ligand that can impact IC50 determinations. EC70 concentrations were selected based on client feedback and given that high fold inductions were observed at these concentrations allowing for a sensitive assay to be performed.

The ligand for the dual-chain receptor had a calculated EC70 of 0.81 nM (1C5), 0.48 nM (4F6) and 0.29 nM (3E10). Ligand for GPCR had a calculated EC70 of 92.08 nM (1F2), 147.80 nM (3C10) and 367.20 nM (3G8). These concentrations were used for IC50 determination of lead antibodies.

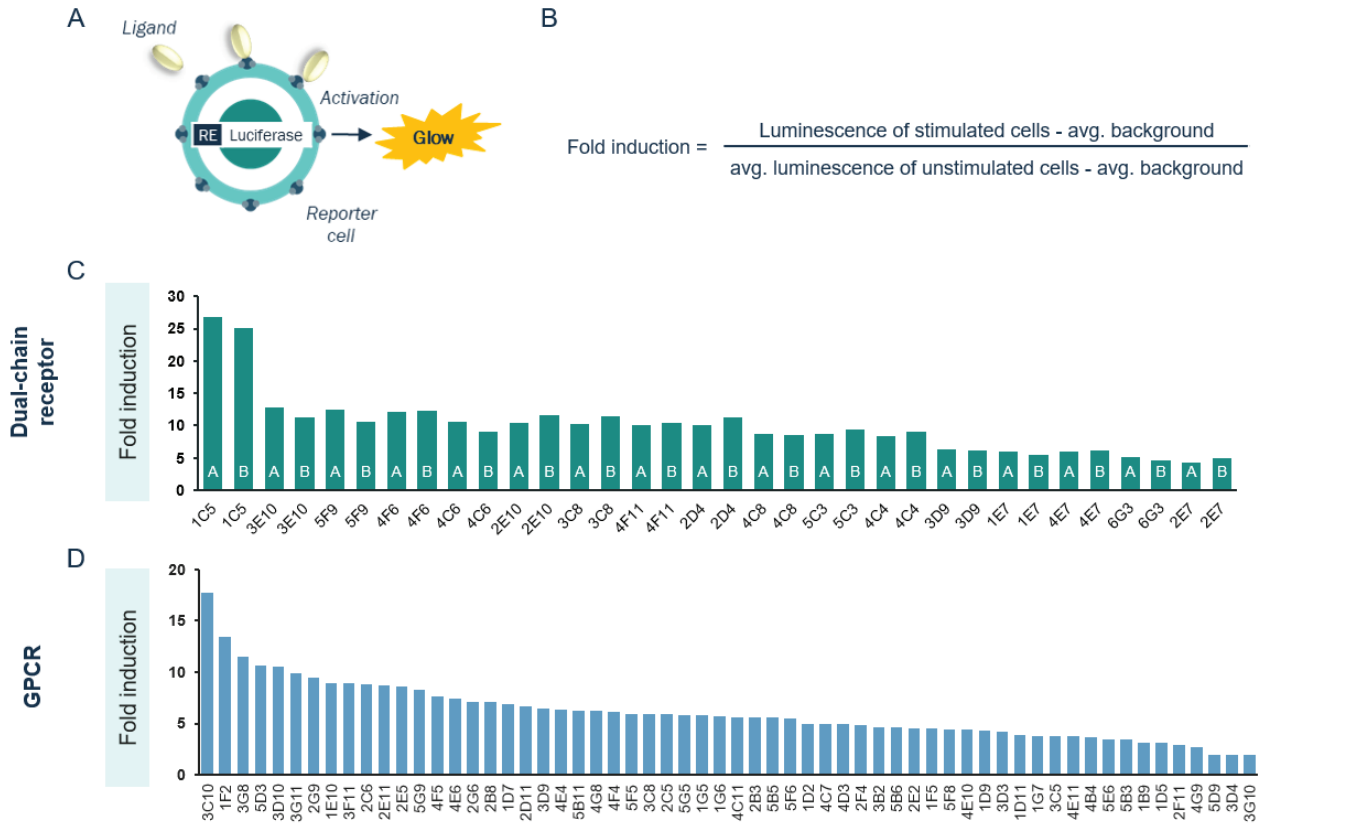

Figure 3. A) Upon ligand binding, respective pathway is activated leading to luciferase transcription. When lysing the cells and mixing luciferase with its substrate, a bioluminescence reaction occurs leading to the production of light which is measured using a luminometer. B) Formula used for fold induction calculation. C) Single-cell clones overexpressing the dual-chain receptor were screened by performing a luciferase assay incubat-ing with ligand at two different concentrations (depicted as "A" or "B"). D) Single-cell clones overexpressing the GPCR were screened by performing a luciferase assay incubating with a single ligand concentration. Clones were ranked by fold induction.

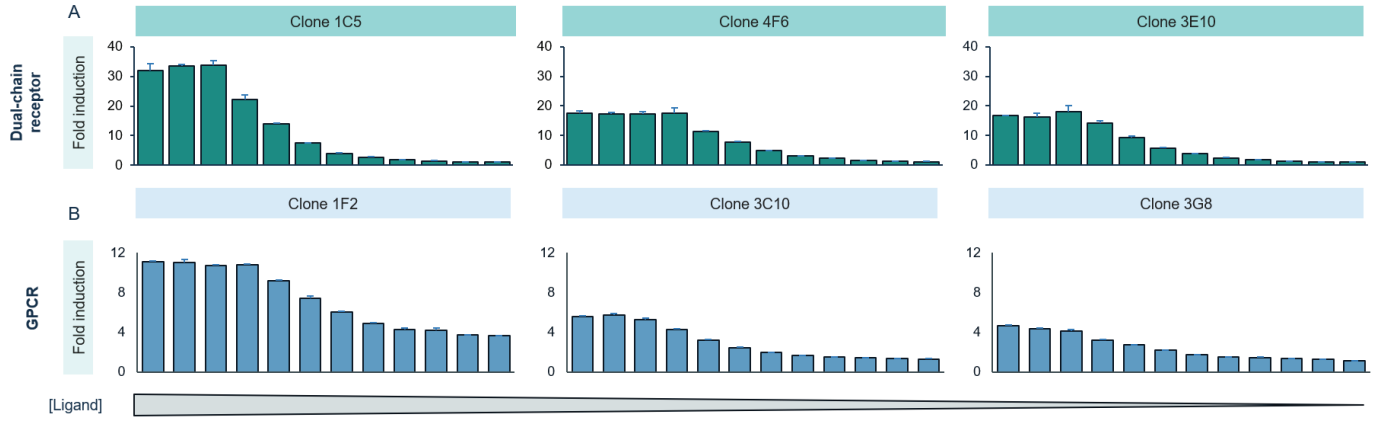

Figure 4. Ligands used for dual-chain receptor (A) or GPCR (B) reporter cell lines were titrated to determine their EC70 concentration.

Generated clonal reporter cell lines allow robust IC50 determination of lead antibodies

Lead antibodies were functionally characterized using the generated clonal reporter cell lines by performing 3-fold titrations starting at 200 nM (Figure 5). Calculated IC50 for the dual-chain recep-tor were similar between clones whereas for the GPCR, IC50 varied from 6.9 and 11.3 nM to 34 nM (Table 1). All clones were shared with client but clones 1C5 and 1F2 were suggested to be used in future IND-enabling studies.

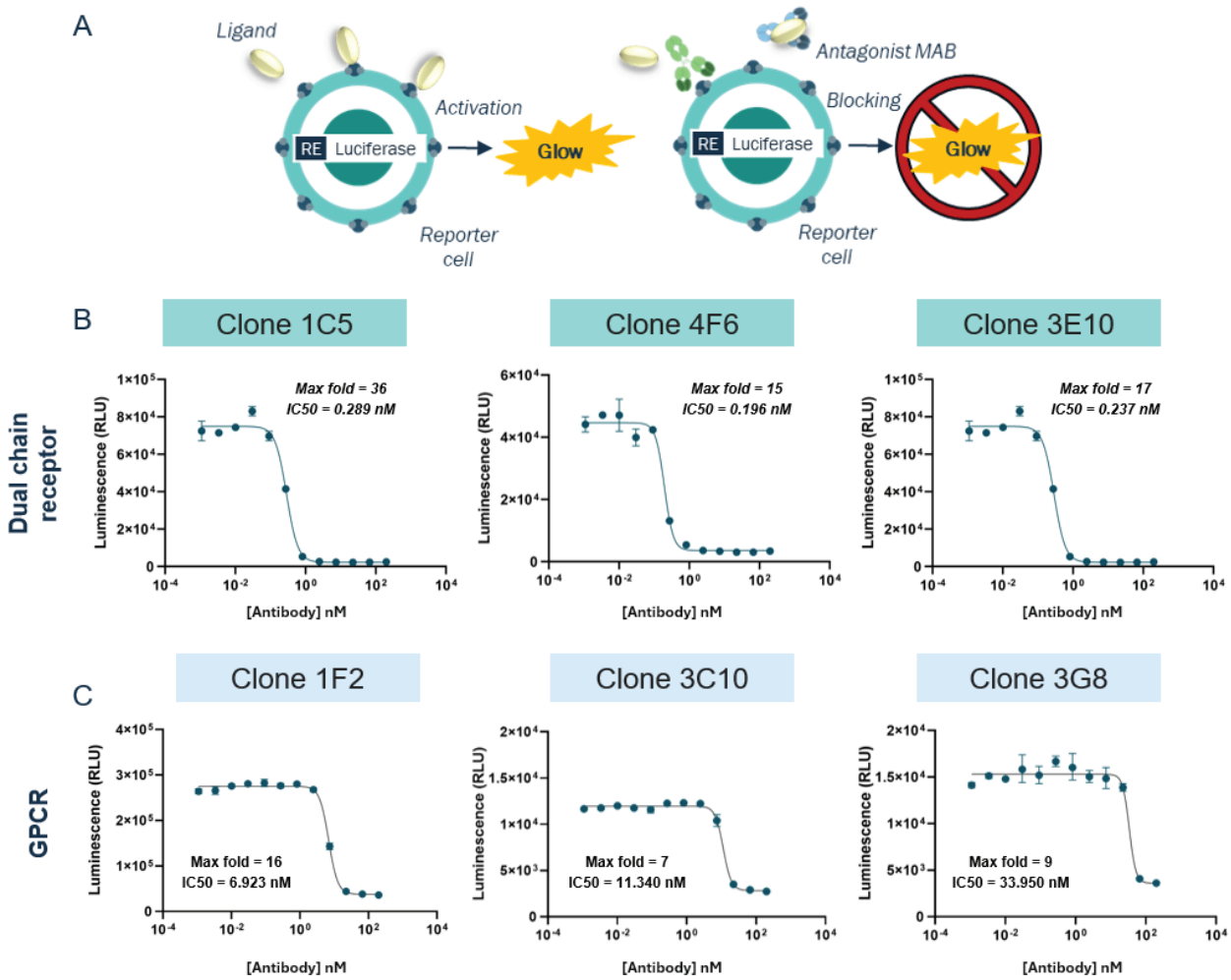

Figure 5. A) Schematic representation of blocking luciferase assay. When the antibody binds to the ligand in a way that blocks its ability to activate intracellu-lar signalling upon receptor binding, luciferase is not transcribed and thus a reduction in bioluminescence signal is detected. B) Lead antibodies were titrated using either the dual-chain receptor reporter cell lines or the GPCR reporter cell lines to determine IC50.

Table 1 - Reporter cell lines profile

Target	Clone ID	Max Fold induction	EC70 (nM)	IC50 (nM)
Dual-Chain Receptor	1C5	36	0.81	0.29
	4F6	15	0.48	0.20
	3E10	17	0.29	0.24
GPCR	1F2	16	92.1	6.92
	3C10	7	147.8	11.34
	3G8	9	367.2	33.95

DISCUSSION

The importance of developing sensitive reporter cell lines earlier on to assess both human and non-human therapeutic antibodies

Achieving smooth approval of investigational therapeutic antibodies by regulatory agencies often requires a thorough characterization of the molecule's mechanism of action. These regulatory pre-requisites have been extending to animal therapeutics. Reporter cell lines have become an indispen-sable tool for functional characterization from early R&D to IND-enabling studies, potency testing and lot release at later stages.

We have developed two non-human animal reporter cell lines for two complex targets in less than 3.5 months from vector design to clone delivery with full characterization and IC50 calculated for lead molecules. This was achieved by combining the use of a transposase technol-ogy with custom vector generation, optimized protocols and adapted strategies to select high-performing single-cell clones.

With the generated reporter cell lines, sensitive and robust functional assays were performed which allowed us to observe the full blocking potential of the two tested lead antibodies with the one against the dual-chain receptor ligand having a sub-nanomolar IC50 whereas the antibody against the GPCR ligand showing a two-digit nanomolar IC50.

BOTTOM LINE

Knowing the Mechanism Of Action (MOA) of therapeutic antibodies has become almost indispensable in modern biologics development, both for humans and non-human animals. Sensitive and robust reporter cell lines are critical tools to evaluate MOA and their successful generation depends on optimized vector design, selection of appropriate technologies, and optimized reagents and protocols. In this case study, two customized reporter cell lines, not commercially available and with scarce supporting literature were successfully developed through a strategic and expertise-driven approach. The resulting assays and protocols were robustly validated, trans-ferred to the client, and enabled the generation of lead antibody data to support future IND submissions.

Successful track record

>50
Overexpressing cell lines

>40
Reporter cell lines

3
Knock-out cell lines

High diversity of targets generated

- Lymphocyte receptors
- Enzymes
- Growth factors
- Immune checkpoints
- Activation differentiation markets
- Death receptors
- Cytokyne interleukins
- Hormone receptors
- Adhesion molecules
- Transmembrane transporter proteins

Experience with major transcriptional regulatory factors

- NF-xB
- NFAT
- AP-1
- STAT1/3/5 family
- SMADs
- CREB
- SRE/SRF/TCF
- C/EBP-ATF2
- RBPJ

Cell line portfolio

>30
Cell lines available of different backgrounds for target engineering with easy access to much more

Own your cells

Engineered cells can be transferred to you and used for research purposes, with no fees associated

Discuss your programme with our team:

info@fjbio.com | fjbio.com
Porto · Cambridge · San Diego

www.fjbio.com
info@fjbio.com