TECHNICAL BRIEF

User-Friendly, No-Maintenance Reporter Assays - The Technology and Utility of Reverse Transfection

Bill Ho, Ph.D.
Cayman Chemical Company

Key Features

- Reverse transfection reporter assays offer an alternative and advantageous method over conventional transfection and eliminate the need to produce/maintain stable cell lines
- With reverse transfection, multiple assays can be performed in parallel on cells with identical lineage
- Reverse transfection assay technology is suitable for multiplexing
- Custom assays can be built in 6-8 weeks
- Reverse transfection plates have a long shelf life
- Ready for assay within 24-48 hours

Reverse Transfection Assay Overview

In a reverse transfection assay, cells are added to a multi-well tissue culture plate that has been pre-coated with transfection complexes containing DNA and an optimized mixture of lipids and proteins. Furthermore, with a one-day shorter protocol, cells are plated at higher densities (ranging from 20,000-80,000/well in culture media), which is approximately twice the number of cells recommended for solution transfection methods. This reduces preparation time for the end user (especially if using frozen, assay-ready cells) in comparison to solution transfection where cells are plated and maintained the day before transfection. While the conventional solution transfection technique remains the principal method for many cell types, reverse transfection methods offer experimental efficiency and convenience advantages such as: additional reagents are not needed for the transfection process as all are contained on the pre-coated plate, multiple assays can be performed in parallel on cells of identical lineage since cells from the same passage can be used, and stable cell lines do not need to be maintained.

Cayman has developed a series of reverse transfection reporter assays that exploit these advantages. In these assays, 96-well plates are coated with a proprietary transfection complex that contains a secreted alkaline phosphatase (SEAP) reporter gene construct under the control of a specific promoter sequence. Adherent cells, supplied by the user, are applied directly to the plate and allowed to grow in the pre-coated wells. Using this method, well-to-well consistency and uptake of DNA complexes by the cells are significantly increased compared to solution-phase transfection. This enhances transfection efficiency for both single plasmid and multiple plasmid (co-transfection) experiments.

SEAP Reporter Gene Construct Design

Each reporter construct contains either a consensus sequence for a transcription factor regulatory element or key genomic fragments that have been mapped to be important for induction. These reporters are supplemented with optimal levels of expression constructs for cell surface receptor(s), G protein(s), and/or transcription factor(s)/nuclear receptor(s) and then applied to the bottom of a multi-well plate in a mixture of proprietary proteins and lipids necessary for cell attachment and transfection.

For example, a cytochrome P450 (CYP)3A4-SEAP reporter can be assembled using human CYP3A4 promoter sequences including the upstream xenobiotic-responsive enhancer module (XREM; **Figure 1**). Hepatocyte nuclear factor 4 (HNF4) and pregnane X receptor-responsive element (PXRE) are the most critical regulatory sequences for the induction of the CYP3A4 gene. Therefore, expression constructs for PXR and HNF4α are included in the reverse transfection complex to compensate for insufficient endogenous PXR expression and variable levels of HNF4α in most immortalized hepatic cell lines including HepG2 and its derivatives (*e.g.*, C3A cells).

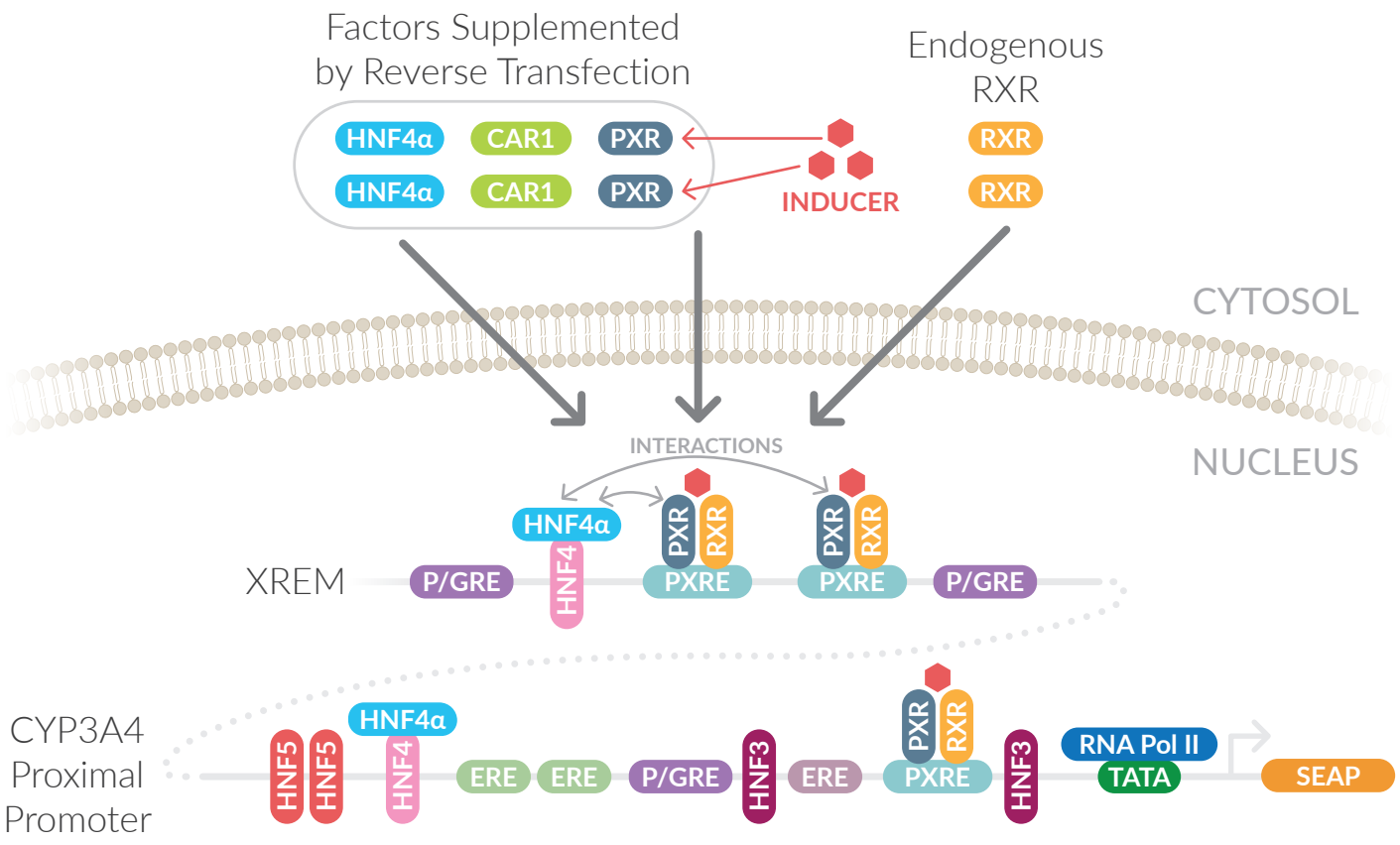

Figure 1. CYP3A4 reverse transfection complex includes a SEAP reporter with upstream XREM and supplemental HNF4α, CAR1, and PXR.

Binding of test compounds to the appropriate nuclear receptor, PXR in this example, leads to the interaction between the activated receptor and the responsive element on the reporter construct in the nucleus. The expression of the SEAP reporter gene is then triggered by interactions among various transcription factors (HNF4α and PXR/RXR) and RNA polymerase.

Reverse Transfection Assay Workflow

Reverse transfection plates are supplied with transfection complexes immobilized on multi-well plates (**Figure 2**). In the CYP3A4 example, the transfection complex contains mixtures of DNA encoding an optimal balance of SEAP reporter constructs and supplemental nuclear receptors CAR1, PXR, and HNF4α.

When ready to use, plates are brought to room temperature then freshly passaged cells are added directly to the multi-well plates at a requisite density per well in stripped media. (This example uses 40,000-50,000 HepG2/C3A cells per well in MEM with 10% Dextran-Charcoal treated fetal bovine serum and penicillin/streptomycin). The plates are incubated for 24-48 hours to allow for receptor gene expression. Next, test compounds are added in fresh media. The plates are then incubated in the presence of various test compounds for 24-96 hours. Then, the conditioned media is collected onto white assay plates (supplied in kit) at desired time points. Test compounds that promote binding of the nuclear receptors/transcription factors to the CYP3A4 promoter will activate expression of SEAP, which is secreted into the cell culture medium. Collected samples should be heat inactivated at 65°C for 30 minutes to eliminate endogenous alkaline phosphatase activity (SEAP is thermally stable). The collected samples

can then be stored at -20°C if not assayed immediately. After the samples are brought to room temperature, a luminescence-based, alkaline phosphatase substrate is added to the wells and SEAP converts the substrate into a luminescent product. After 10-20 minutes, the plates are read on a plate reader capable of detecting a luminescent signal.

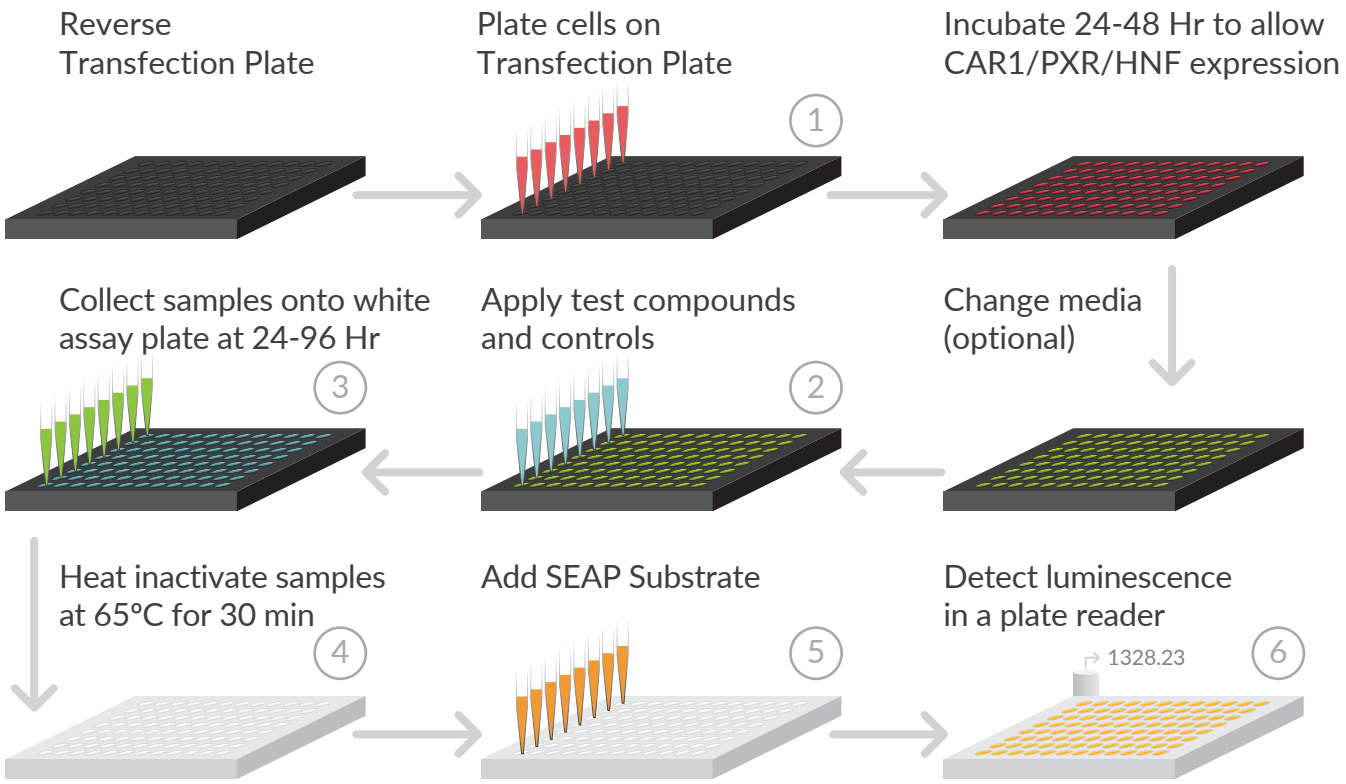

Figure 2. Reverse transfection assay workflow

Available from Cayman

Cayman's series of CYP450 Induction Reporter Assay Kits represent a novel method to evaluate CYP450 induction by drug candidates. You can read about the application of this technology in the application note titled: Tracking the induction of five Cytochrome P450 (CYP450) Enzymes using a Platform of Parallel Reporter Assays. It demonstrates how reverse transfection techniques lead to superior co-transfection efficiency and reproducibility of the CYP450 induction reporter assay. It also highlights the flexibility of this platform and its utility for rapid assessment of compounds in CYP450 gene induction by enabling the evaluation of the activation of different transacting factors at multiple time points.

Cayman has also applied this reverse transfection technology to receptor reporter assays for examining the activation of various G protein-coupled receptors. These kits are simple to use and can be easily adapted to high-throughput screening. Custom reporter assay development is also available by contacting contractresearch@caymanchem.com.

Visit www.caymanchem.com for a complete list of reverse transfection assays.