Color-Switch CRE reporter stable cell line manual

Catalog Number	Product Name / Description	Amount
SC018-Bsd	**CRE reporter cell line (Bsd):** HEK293-**loxP-GFP- RFP (Bsd).** Cell line expresses "LoxP-GFP-stop-LoxP-RFP" cassette with **Blasticidin** antibiotic marker.	1 vial of cells (1.0 ml, 2 x 10^6 cells) in 80% DMEM, 10% FBS, 10% DMSO
SC018-Neo	**CRE reporter cell line:** HEK293-**loxP-GFP- RFP (Neo).** Cell line expresses "LoxP-GFP-stop-LoxP-RFP" cassette with **Neomycin** antibiotic marker.	1 vial of cells (1.0 ml, 2 x 10^6 cells) in 80% DMEM, 10% FBS, 10% DMSO
SC018-Puro	**CRE reporter cell line:** HEK293-**loxP-GFP- RFP (Puro).** Cell line expresses "LoxP-GFP-stop-LoxP-RFP" cassette with **Puromycin** antibiotic marker.	1 vial of cells (1.0 ml, 2 x 10^6 cells) in 80% DMEM, 10% FBS, 10% DMSO

Storage: Liquid Nitrogen;

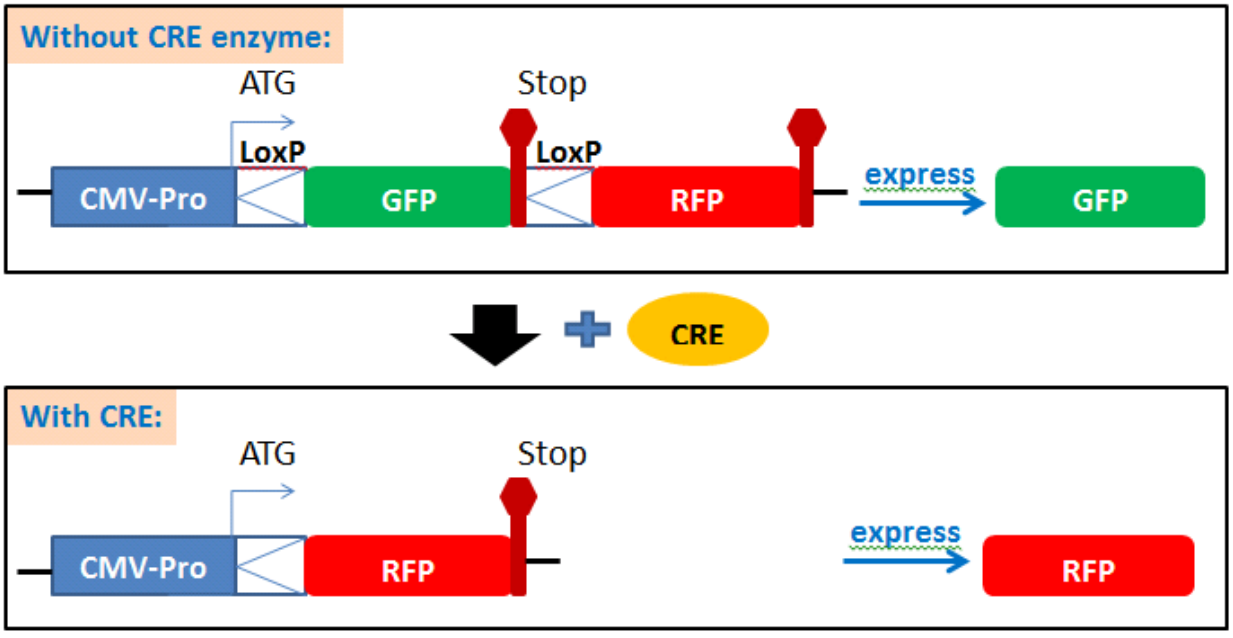
Product Description

CRE recombinase, from bacteriophage P1, catalyzes recombination between 34 base pair target sequences, named Lox sites. CRE-Lox recombination is a special type of site-specific recombination, and widely used to delete loxP-flanked chromosomal DNA sequences at high efficiency *in vivo*. By inserting a "LoxP-flanked expression target" into host's genome, we can control this target expression with the help of CRE recombinase. In other words, the target's expression occurs before CRE enzyme applied. And after CRE enzyme is applied, CRE deletes the LoxP flanked target segment and stops the target expression. In the mean time, as desirable, this genomic alternation can activate the 2nd target at downstream of the deleted segment. The CRE recombination provides an excellent tool for conditional gene targeting in transgenic animal models to link genotypes (alterations in genomic DNA) to the biological outcomes (phenotypes).

CRE-LoxP recombination depends upon an effective CRE enzyme delivery *in vivo*. Amsbio provides **pre-made CRE expression lentiviral particles** which are engineered with a NLS (nuclear localization signal) element, providing high

efficiency delivery of the expressed CRE protein into cell nucleus where the recombination occurs.

In order to monitor and to confirm the CRE-LoxP recombination event, there are also three **CRE reporter cell lines** (SC018 stable cell lines). Those stable cell lines are derived from HEK293 cell [Note: Amsbio provides the pre-made **CRE reporting lentivirus** (CAT#: **LVP460**) needed to make this reporter cell line in your desired host cell types]. The HEK293 cell line is a cell line established from primary embryonic human kidney transformed with sheared human adenovirus type 5 DNA. The expressed E1A adenovirus gene allows these cells to produce very high levels of protein. The CRE reporter cell line's genome has integrated a CRE recombination response, "**Color-Switch segment**" (see DNA insertion scheme below). Each cell line has also integrated an antibiotic selection marker under a RSV promoter (not showed in the scheme below), thus each cell line has a specific antibiotic marker, Blasticidin (Bsd), Puromycin (puro) or Neomycin (Neo) for selection purpose when applicable.



How it works:

The CRE reporter cell lines are used to monitor or confirm the efficiency of CRE recombination *in vivo*. It is a great method and easy tool to verify the performance of your CRE enzyme (your CRE expression plasmids, or pre-made CRE expression lentivirus, or purified CRE enzyme) in *in vivo* conditions. It is also a control test to verify your CRE-loxP based system.

The cell line demonstrates strong GFP fluorescent signal in normal culture condition as the constitutive CMV promoter drives the high GFP expression. The downstream RFP ORF is not expressed because of the stop codon after the GFP ORF*. Once the CRE protein is present in nucleus, the CRE excises / deletes the DNA fragment between two loxP sites, which removes the stop codon (see the image above). As a result, the RFP ORF is then expressed under the CMV promoter, and the cell line switches to RFP fluorescent**. The RFP signal can be easily monitored via fluorescent cell sorting (for the ratio between GFP and RFP cells), or visualized under microscope, or measured the fluorescent intensity by a meter or reader with RFP filter set.

Note:

***:** Like any mammalian pol II promoter, the CMV promoter could seek any possible ORFs after the designed stop codon, and may express the far end ORF (the RFP in this case), which is considered the basal RFP signal or leaking RFP signal here. But the selected stable cell has negligible RFP signal before CRE recombination event.

****:** The CRE can not be delivered into all cells, therefore, there are still GFP signal only cells even after the CRE is applied. Also, because the stable cell line has integrated multiple copies of LoxP-GFP-LoxP-RFP cassettes and the CRE recombination is not possible at 100% rate for all sites in each cell, therefore, there are many cells that demonstrate both GFP and RFP signal after addition of CRE enzyme. The main observation here is to see the dramatically increase of RFP positive cells which reflects the CRE recombination rate.

Application protocol (for reference only):

1. Culture the cell in complete medium (see **Culture procedures** below):
   All three CRE reporting cell lines (**SC018-Bsd, SC018-Neo and SC018-Puro**) normally express strong GFP signal in normal culture conditions without any treatment. Seed cells into 24-well plate at appropriate cell density (e.g. 1 - 2 $x10^5$ cells/per well), incubate 37°C, 5% CO2 overnight;

2. CRE enzyme delivery:
   One the 2nd day when the cell density is at 50% - 75% confluent, deliver CRE protein using your favorite procedures which could be carried out as follows:
   a. Simply add 50ul of pre-made CRE expression lentiviral particles.
   b. Transfect your CRE expression plasmid using a transfection reagent (like LF2k or Fugene).
   c. Add purified nuclear membrane penetrating CRE protein directly into cell culture.

(**Note:** remember to set the negative control wells without adding CRE, and you may also set the positive control wells by adding 50ul of NLS-CRE expression lentiviral particles, Cat#: **LVP297**, **LVP336**, **LVP339** and others).

3. CRE recombination reaction detection:
   The RFP signal will gradually appear and peak at 48 hours or longer (dependent upon CRE delivery methods) after the CRE delivery. The RFP/GFP cell population ratio or the RFP signal intensity reflects the CRE-LoxP recombination efficiency (rate). You can sort the cell by FACS machine, other meters, or visualize the RFP positive cell under fluorescent signal.

Culture procedures

1. Thaw the frozen vial of cells quickly in a 37°C water bath (1-3min), decontaminate the outside of the vial with 70% ethanol.
2. Transfer the entire content of the vial into a T75 flask containing 15 ml of pre-warmed complete medium. Incubate the cells overnight in a 37°C incubator, 5% CO2.
3. The following day, replace the medium with 15 ml of pre-warmed, complete medium (**Optional:** add 10 µg/ml blasticidin in medium for SC018-Bsd cell, or 5 µg/ml of puromycin for SC018-Puro cells or 25 µg/ml of neomycin for SC018-Neo cells).
4. Incubate the cells and monitor cell density.
5. Pass cells (1:10 dilution) when the culture reaches 80-90% confluency.
6. For storage freeze cells at a density of 3 x 10^6 cells/ml using 90% complete medium with 10% DMSO.

Complete medium

D-MEM (high glucose)
2mM L-glutamine
10% Fetal Bovine Serum (FBS)
0.1 mM MEM Non-Essential Amino Acids (NEAA)
1% Pen-strep

Quality Control:

Each vial contains more than 2 x 10^6 cells with >95% viability before freezing. Cells are free of bacteria, viruses, mycoplasma.

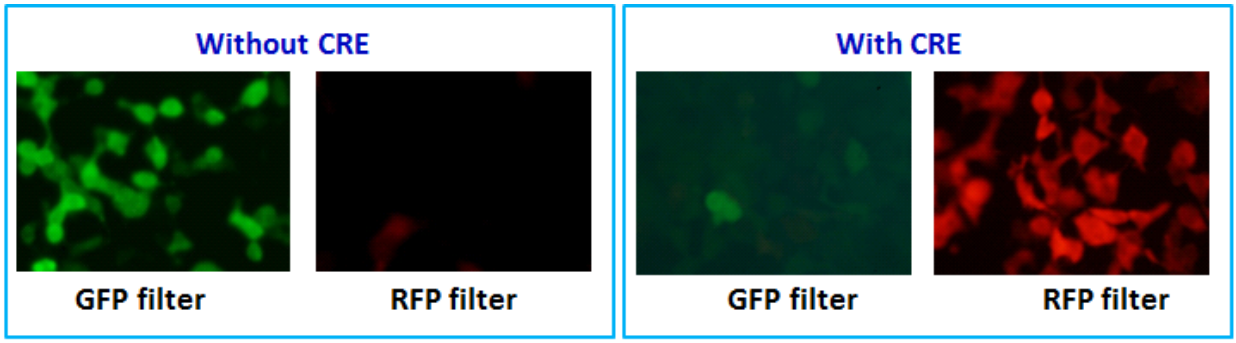
Warranty and Terms:

1. This product is warranted to perform as described when used in accordance with this manual. Amsbio's sole remedy for breach of warranty should be, at the option of Amsbio, to repair or replace the product. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY.

2. The delivered cell line is intended to be used for non-commercial purposes only. A separate license is required for any commercial use, including the use of this cell line for production purposes by any commercial entity.

3. This product is for research use only. This product is sold to the each specific buyer, and is prohibited to be transferred or passed to other parties.

Sample images of CRE-loxP recombination detection:



Left panel / without CRE: CRE reporter cell line (Cat#: **SC018-Bsd**) was cultured in complete media. Images were taken under microscope with GFP filter set (Ex 490nm/Em 525nm) and RFP filter set (Ex 545nm/Em 620nm).
Right panel / with CRE: CRE reporter cell line (Cat#: **SC018-Bsd**) was cultured in complete media in 24-well plate. 50µl of CRE expression lentiviral particle (Cat#: **LVP339)** was added into the cells in one well. Images were taken at ~ 72 hours after the addition of CRE expression lentivirus.

References:

1. Sauer, B. (1987) "Functional expression of the Cre-Lox site-specific recombination system in the yeast *Saccharomyces cerevisiae*", *Mol Cell Biol* **7**: 2087-2096
2. Stanislaw J. Kaczmarczyk and Jeffrey E. Green. Nucleic Acids Res. 2001 June 15; 29(12): e56.