# Introduction to controls and normalization for 3'UTR GoClones

The SwitchGear LightSwitch Luciferase Assay System's 3' UTR GoClone controls can be used to normalize luminescence values across multiple experimental conditions. Normalization is particularly beneficial for studies that focus on the presence or absence of a stimulus or specific/non-specific siRNAs or miRNAs.

SwitchGear offers the following controls for 3'UTR GoClone assay normalization (see the LightSwitch Assay Luciferase System "Getting Started with Your 3'UTR GoClone Reporter Assays" guide for more details).

- Empty 3'UTR vector: The empty 3'UTR vector contains a constitutive promoter (found on all UTR constructs) and the luciferase gene (RenSP). This construct may also serve as a positive control for the transfection because the constitutive promoter is highly active in most cell types.
- Housekeeping gene 3'UTR vectors: Housekeeping control constructs contain the 3'UTRs for common housekeeping genes cloned downstream of the luciferase reporter.
- Random 3'UTR control vectors: Random control constructs contain non-conserved, non-genic, and non-repetitive human genomic fragments.

## An example experimental normalization

Table 1 below shows sample luminescence values for three experimental 3' UTR GoClone constructs (UTR1, UTR2, UTR3) and three control 3' UTR GoClone constructs (UTR_R1, ACTB, and 3UTR_EMPTY) under two conditions. Let us suppose that we expect Condition 2 to specifically interact with the experimental UTRs to reduce luminescence and that we do not expect specific interaction from Condition 2 with the control constructs.

| Table 1 | Experimental 3' UTRs | | | Controls | | |
|---|---|---|---|---|---|---|
| | UTR1 | UTR2 | UTR3 | UTR_R1 | ACTB | 3UTR_EMPTY |
| Condition 1 | 46.6 | 20.7 | 27.5 | 20.5 | 68.6 | 78.3 |
| Condition 2 | 13.3 | 4.3 | 16.7 | 18.1 | 64.1 | 69.3 |

In Table 1, we observe slight decrease in control construct activity under Condition 2 that likely reflect non-UTR-specific effects of that treatment. So we would like to normalize the luminescence across the two experimental conditions. The first step is to calculate the average signal for each construct in the two conditions, as shown in Table 2.

| Table 2 | Controls | | |
|---|---|---|---|
| | UTR_R1 | ACTB | 3UTR_EMPTY |
| Condition 1 | 20.5 | 68.6 | 78.3 |
| Condition 2 | 18.1 | 64.1 | 69.3 |
| Average | 19.3 | 66.4 | 73.8 |

Next we will calculate the ratio of each construct's luminescence to the average as shown in Table 3.

| Table 3 | Controls | | |
|---|---|---|---|
| | UTR_R1 | ACTB | 3UTR_EMPTY |
| Condition1/ avg signal | 20.5/19.3 | 68.6/66.4 | 78.3/73.8 |
| Condition1 ratio | 1.06 | 1.03 | 1.06 |
| Condition2/ avg signal | 18.1/19.3 | 64.1/66.4 | 69.3/73.8 |
| Condition2 ratio | 0.94 | 0.97 | 0.94 |

To determine the value that will be used for normalization, take the average of the ratios for each condition (Table 4). If you have used more than four control constructs, you may want to drop the highest and lowest ratio observed for each condition and then take the average of the remaining ratios to prevent normalization being skewed by outliers.

**Table 4**

| | Controls | | | |
|---|---|---|---|---|
| | UTR_R1 | ACTB | 3UTR_EMPTY | CONTROL AVERAGE |
| Condition 1 ratio | 1.06 | 1.03 | 1.06 | 1.05 |
| Condition 2 ratio | 0.94 | 0.97 | 0.94 | 0.95 |

The final step in normalization is to divide the original luminescence values by the average control ratio for each condition (Table 5). With respect to the original data, the normalization would look as follows.

**Table 5**

| | Experimental 3' UTRs | | | Controls | | |
|---|---|---|---|---|---|---|
| | UTR1 | UTR2 | UTR3 | UTR_R1 | ACTB | 3UTR_EMPTY |
| Condition 1/avg ratio | 46.6/1.05 | 20.7/1.05 | 27.5/1.05 | 20.5/1.05 | 68.6/1.05 | 78.3/1.05 |
| Condition 1 normailzed | 44.3 | 19.7 | 26.1 | 19.5 | 65.2 | 74.3 |
| Condition 2/avg ratio | 13.3/0.95 | 4.3/0.95 | 16.7/0.95 | 18.1/0.95 | 64.1/0.95 | 69.3/0.95 |
| Condition 2 normalized | 14.0 | 4.5 | 17.6 | 19.1 | 67.6 | 73.2 |

## Normalization results

The results of normalization can be seen below. In these types of experiments, it is helpful to view the data as a ratio/format. In this case, we have taken the ratio of Condition 2 to Condition 1. For the control constructs, all three demonstrated ratios of less than 1 as raw values. After normalization, these values were closer to 1, as expected.