Active Motif Epigenetic Services
ChIP-qPCR Data Normalization

After ChIP has been performed, qPCR reactions are set up in triplicate for each ChIP sample. Each qPCR plate also contains input DNA and a standard curve for normalization. Normalized data is expressed as Binding Events Detected per 1000 Cells.

Standard curve

1. Each qPCR plate contains a standard curve generated with known amounts of genomic DNA and a primer pair with an efficiency that is set to 1.

2. The ChIP sample values are then normalized in a way that takes into account the efficiency of each primer pair, the amount of chromatin in the reaction and the resuspension volume of the ChIP DNA (see below).

Primer efficiency ratio

Primer efficiency ratios are determined for each primer set used in the experiment. The primer efficiency ratio is calculated by dividing the average input value by the expected copy number of the input. The average input value is the average of the 3 qPCR values that are generated by the primer set when 12.5 ng of input DNA (unprecipitated genomic DNA) are amplified. The expected copy number is calculated based on 12.5 ng of input and assuming 6.6 pg of DNA per cell:

$$\frac{\text{12,500 pg input DNA}}{\text{6.6 pg DNA per cell}} * \text{2 DNA copies per cell} = \text{3,788 copies}$$

Data normalization

Binding Events Detected per 1000 Cells are calculated as follows:

The average of the 3 qPCR values generated by the primer set when each ChIP sample is amplified in triplicate is calculated. This value represents the number of copies in the 5 µl of the ChIP sample that was amplified.

To calculate the number of copies in the entire ChIP, the above value is multiplied by the resuspension volume and then is divided by 5.

To normalize the values per 1000 cells, the value is then multiplied by the ratio of 1000 / cell equivalents in the ChIP.

Finally, the value is divided by the primer efficiency ratio.

The complete formula for normalization is:

$$\frac{\text{average qPCR value} * \left(\frac{\text{resuspension volume}}{5}\right) * \left(\frac{1000}{\text{cell equivalents in ChIP}}\right)}{\text{primer efficiency ratio}}$$