ChIP PCR and analysis with an example:

1. A typical reaction plate contains reactions for standard curves and for immunoprecipitated samples. Each treatment (or genotype here) should has its own input and standard curve.

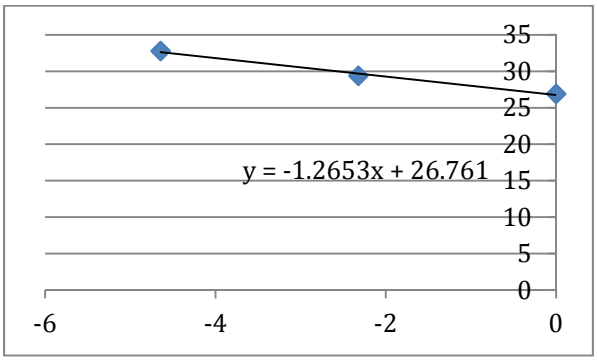
Sample Name	Detector Name	Ct
bc H3K4	Rex1_Ex1_2	29.162222
bc H3K4	Rex1_Ex1_2	29.270094
bc H3K4	Rex1_Ex1_2	29.443207
bc IgG	Rex1_Ex1_2	Undetermined
bc IgG	Rex1_Ex1_2	Undetermined
bc IgG	Rex1_Ex1_2	Undetermined
bc input 1:125	Rex1_Ex1_2	31.062077
bc input 1:25	Rex1_Ex1_2	28.420832
bc input 1:5	Rex1_Ex1_2	25.732788
bc input 1:625	Rex1_Ex1_2	34.675995
WT H3K4	Rex1_Ex1_2	28.829643
WT H3K4	Rex1_Ex1_2	28.921722
WT H3K4	Rex1_Ex1_2	28.930042
WT IgG	Rex1_Ex1_2	Undetermined
WT IgG	Rex1_Ex1_2	Undetermined
WT IgG	Rex1_Ex1_2	Undetermined
WT input 1:125	Rex1_Ex1_2	32.79188
WT input 1:25	Rex1_Ex1_2	29.388535
WT input 1:5	Rex1_Ex1_2	26.915913
WT input 1:625	Rex1_Ex1_2	Undetermined

2. Calculate standard curves (yellow) of each locus (PCR primer sets) using the input
    a. Do serial dilution for the input. Pick one of the dilutions below that covers the Ct for real (immunoprecipiated) experimental results
        i. 1:5, 1:25, 1:125, 1:625
        ii. 1:10, 1:100, 1:1000, 1:10000
    b. Perform qPCR (duplicate if needed) to get the strand curve
    c. Make the table with the format below. Then make the standard curve to retrieve the equation of the standard curve

Dilution	Concentration	log2(Concentration)	WT Ct
1:5	1	0	26.915913
1:25	0.2	-2.321928095	29.388535
1:125	0.04	-4.64385619	32.79188

- 1:625 was removed because the Ct is too high
- Notice that the dilution and concentration are inversely correlated.

- The concentration is arbitrary. But it's easier to set the first dilution as 1.
- The regression equation:



3. Based on the standard curve, Y=Ct and X=log2(concentration). So if you know the Ct (Y) of immunoprecipitated samples, you can use the equation to get X, as well as the concentration relatively to the input.

	Ct (Y)	X = (Ct-26.761)/(-1.2653)	Concentration (=2^X)
WT H3K4	28.829643	-1.634903185	0.321992015
WT H3K4	28.921722	-1.70767565	0.30615292
WT H3K4	28.930042	-1.714251166	0.304760712

4. From the last column you can get the % of input. In this experiment, immunoprecipitated samples were diluted 1:5 for the qPCR reaction, same as the concentration of the first serial dilution of the input. So the concentration (2^X) value equals to the ratio to the input. After calculating each value, the mean and the STD can also be derived:

Concentration	Percentage (%)	Mean	Std
0.321992015	32.19920146	31.09685489	0.957194644
0.30615292	30.61529204		
0.304760712	30.47607116		

5. The last coefficient to consider is how much lysate you took as the input. For example, if you take 100 μl from 2000μl lysate (the final lysate you put antibodies for IP) for making the input. Then the actual percentage will be divided by 20 since you only use 1/20 to compare with the DNAs IP-ed from the whole sample.