Quality Assessment of MeDIP

- **DNA Concentration:**

  DNA concentration can be determined by NanoDrop ND-1000 reading of A_{260}.

  A_{260} X dilution factor X 50 = ug DNA / ml

- **DNA Purity:**

  DNA purity can be determined by NanoDrop ND-1000 readings of A_{260}:A_{280} and A_{260}:A_{230} ratios.

- **Specificity of MeDIP:**

  Specificity of methylated DNA by immunoprecipitation can be assessed using Real-time quantitative PCR for highly methylated and unmethylated genes.

1. Setup qPCR assay for assessment of MeDIP Quality:

qPCR Assay	Input DNA	MeDIP DNA	Mock IP (Non-Immune serum)
Positive Control Primer set*	√	√	√

* Positive Control Primer set: Primers design according to specific methylated site

2. MeDIP -qPCR Data Analysis (ΔΔCt method):
   a) Normalize each MeDIP DNA fractions' Ct value to the Input DNA fraction Ct value for the same qPCR Assay (ΔCt) to account for chromatin sample preparation differences. Calculate the % Input for each MeDIP fraction:

   $\%Input = 2^{(CtInput - Ct\ MeDIP)} \times Fd \times 100\%$

   Here, Fd is Input dilution factor.

   For example, if 100ul sonicated sample is used for MeDIP and 20ul sonicated sample is used as Input, Fd =1/5.

   b) Adjust the normalized MeDIP fraction Ct value for the normalized background (mock IP) fraction Ct value.

   ΔΔCt [MeDIP /mock IP] = ΔCt [normalized MeDIP] - ΔCt [normalized mock IP]

3. Assessment of MeDIP Quality:
   a) The Input DNA Ct value should be less than 30.
   b) The % Input for the mock IP DNA fraction should be less than 0.01%.
   c) The MeDIP DNA Ct value should be at least one cycle less than the mock IP DNA Ct value (Ct [mock] – Ct [MeDIP] > 1.0) to be considered quantitatively above the background signal (noise) for the sample.