High Background observed in negative control

Possible Causes	What can you do?
Inadequate washing	Use a more stringent washing buffer. Try to use a high salt washing buffer or increase the number of washes.
Non specific binding to Protein A,G or L	Include a pre-clear step by incubating lysate with Protein A/G/L agarose beads.
Too much DNA template added to the PCR reaction, or too many cycles of amplification	Add less DNA template or reduce the number of cycles of amplification. Alternatively, real-time PCR can be used for the detection of ChIPed DNA products.
Buffers may be contaminated	Use freshly prepared lysis or wash buffers.

Positive signal seen in no template control

Possible Causes	What can you do?
PCR reagent might be contaminated	Prepare new solutions from stock

Low/No Signal

Possible Causes	What can you do?
Not enough cells/chromatin	Add enough chromatin for each IP experiment. We suggest using at least 25 μg of chromatin for each IP.
Incorrect Protein A/G/L used	Make sure that the Protein A/G/L beads are capable of binding to the antibody subclass being used.
Cross-linking process too long	Over Cross-linking with formaldehyde might mask the antibody binding sites and reduce antibody binding ability. It is advisable to optimize the cross-linking steps by using different concentration of formaldehyde or reducing cross-linking time.
Not enough antibody	Titre antibody amount used for each IP to determine the optimal condition. Up to 10ug of antibody can be used for each IP experiment.
Washes too stringent	Reduce the number of washes. Reduce salt concentration in the wash buffer.
Antibody not capable of immunoprecipitation	Try a different antibody. Try polyclonal antibody if monoclonal antibody does not work well.
The chromatin size might be too small	Make sure that the shearing condition is not too harsh which might results in fragments of DNA smaller than what the primers are able to amplify.
Incomplete elution from the Protein A/G/L beads	Incubate beads in elution buffer at 65°C with frequent mixing.