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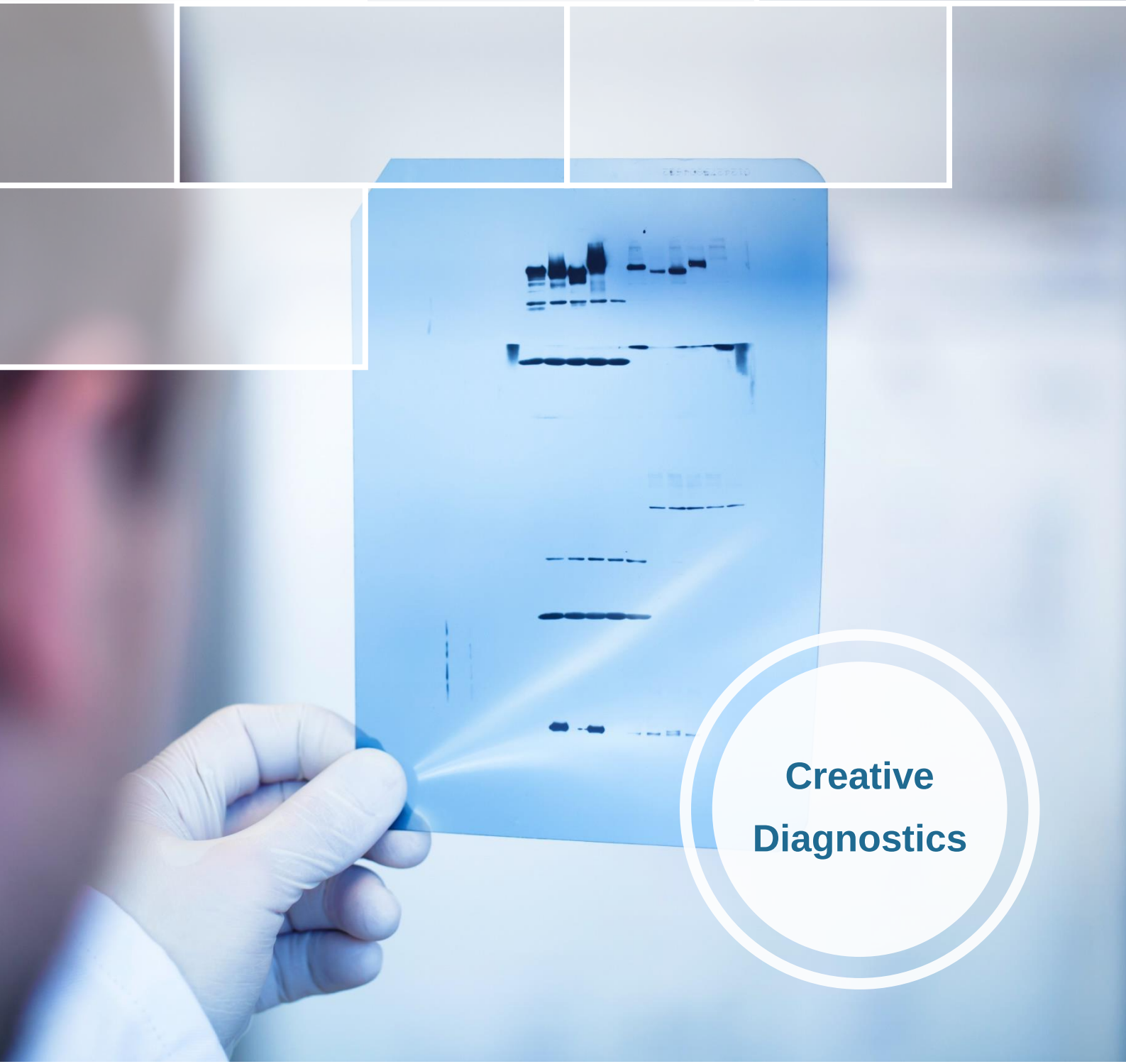
ChIP TROUBLESHOOTING TIPS



ChIP troubleshooting tips

Solve your ChIP problems with these troubleshooting tips, covering common causes of low resolution and high background, no/low signal, multiple bands and problems with PCR amplification on immunoprecipitated DNA.

Problems Index

low resolution and high background

- High background across large regions

1. Chromatin insufficiently digested and fragments too large can lead to increased background and lower resolution. The following are possible causes.

a) Too many cells or not enough micrococcal nuclease was added to the chromatin digestion

Weigh tissue or count a separate plate of cells prior to cross-linking to determine accurate cell number. Add less tissue or cells, or more micrococcal nuclease to the chromatin digest.

b) Inadequate sonication

Firstly, inspect and ensure that the sonicator probe is not in contact with the tube wall. Secondly, increase the number of sonication steps properly, but **avoid increasing the time (or the power) of each step**, as this may overheat the sample and lead to the loss of antigenicity. Different cell types may have different optimal DNA fragmentation. Determine appropriate sonication times to get your optimal DNA fragmentation.

c) Tissue or cells may have been over cross-linked. Cross-linking for longer than 10 min may inhibit digestion of chromatin.

Perform a time course at a fixed formaldehyde concentration. Shorten the time of cross-linking to 10 min or less.

2. Too much or not enough chromatin added to the IP reaction. Alternatively, too much antibody added to the IP reaction.

For optimal ChIP results, add 5 to 10 µg of chromatin and 10 µL of histone H3 antibody to each IP reaction. Reduce the amount of normal rabbit IgG to 1 µL per IP.

3. Excessive incubation of antibody/beads with chromatin.

Reduce the duration of incubations.

4. Insufficient washes

Increase the number and/or stringency of the washes after immunoprecipitation.

- High background in non-specific antibody controls

1. Non-specific binding to Protein A or G beads

Include a pre-clearing step, whereby the lysed sample is mixed with beads alone for 1 hr and removed prior to adding the antibody.

2. The ChIP buffers may be contaminated

Prepare fresh lysis and wash solutions.

3. Some Protein A or G beads give high background

Find a suitable supplier that provides the cleanest results with low background in the non-specific control.

➢ No/low signal

- Antibody problems

1. Antibody did not work

Use ChIP-grade antibodies and, if possible, test antibody efficiency by different techniques (immunofluorescence, ChIP-western blot, qChIP) using experimental conditions as close as possible to those used in the PAT-ChIP assay.

2. The antibody is working but the signal is weak

a) Try a different type of ChIP. For example, if you are performing N-ChIP try X-ChIP.

b) Seek a different location. It may be that the antigen is present but not on the section of the genome that you are looking at.

c) Specific antibody binding may be removed by too stringent washing buffer. Do not use higher than 500 mM NaCl in the washing buffers.

d) Use a different antibody if one is available.

e) The epitope may be masked in X-ChIP. Further optimize the cross-linkage time course.

3. Scanty antibody used in the immunoprecipitation

Usually, 3-5 µg of antibody in the first instance is recommended, but the quantity could be increased to 10 µg if no signal is observed.

4. No antibody enrichment at region of interest

The epitope is not found at the particular locus of interest. Use an appropriate positive control antibody to confirm the preservation of nucleosomes at particular genomic loci. What antibody controls should be choose depends on the locus you are studying. Histone H3 (tri methyl K4) is a popular positive control used in ChIP when chipping active genes. Negative control choose an antibody that recognizes a non-chromatin epitope such as an anti-GFP antibody.

5. Using a monoclonal antibody for X-ChIP

The epitope may have been masked during cross-linking thus preventing epitope recognition. Polyclonal antibody within a number of antibodies can recognize mutiple epitopes, whereas monoclonal antibodies recognize only a single epitope. A **polyclonal antibody will be preferable in X-ChIP** as there is an increased chance of immunoprecipitating the protein of interest.

- Sample problems

1. Chromatin was excessively fragmented

Do not sonicate chromatin to a fragment size of less than 500 bp. Sonication to a smaller size can displace nucleosomes as intranucleosomal DNA becomes digested. If performing N-ChIP enzymatic digestion is generally sufficient to fragment chromatin.

2. Improper cross-linking time

Cross-linking is a time-critical procedure.

On the one hand, excessive cross-linking can lead to a reduction in antigen availability, say, epitopes may be masked or changed, affecting the ability of the antibody to bind the antigen. Excessive cross-linking can also make it difficult to fragment chromatin. On the other hand, an insufficient cross-linking may produce false negatives.
Ensure always perform a time-course experiment to optimize cross-linking conditions.

3. The amount of starting material not enough

We would suggest using 25 µg of chromatin per immunoprecipitation.

4. Cells are not effectively lysed

Using RIPA buffer to lyse cells.

- Other possible problems

1. Unsuitable ChIP type was chosen

X-ChIP may be more suitable when analyzing proteins that have either a weaker DNA affinity or are a long way from DNA. Cross-linking may be required to stop proteins dissociating from the DNA. Histones are tightly associated therefore N-ChIP can be performed when studying histones.

2. The wrong antibody affinity beads were used

Protein A and G are bacterial proteins that bind various classes of immunoglobulins with varying affinities. Use an affinity matrix that will bind your antibody of interest. We would suggest using a mix of Protein A and Protein G that have been coupled to sepharose.

➢ Problems with PCR amplification on immunoprecipitated DNA

1. High signal in all samples after PCR, including no template control Real-time PCR solutions may be contaminated. Prepare new solutions from stocks.
2. PCR signal is weaker than expected or no DNA amplification in samples

a) Inadequate primer result in PCR amplified region spanning nucleosome-free region. Design new primers and decrease length of amplicon to less than 150 bp. Test primer pair conditions using different dilutions of genomic. Using standard/input DNA to confirm that the primers are working well.

b) Not enough DNA added to the PCR reaction or PCR conditions are not optimal.

Add more DNA to the PCR reaction or increase the number of amplification cycles.

3. Excessive variations in qPCR data between replicates

a) Variable amounts of protein G–Sepharose between samples

Ensure that beads are well suspended while pipetting.

b) Insufficient amount of DNA template

High Ct values increase variations between replicates; reduce dilution of DNA templates.

c) Ethanol-precipitated DNA is not fully redissolved

Heat the sample at 37 °C until complete solubilization.

4. No product in the positive control histone H3-IP RPL30 PCR reaction.

a) Not enough chromatin or antibody added to the IP reaction or IP incubation time is too short.

Be sure to add 5 to 10 µg of chromatin and 10 µl of antibody to each IP reaction and incubate with antibody overnight and an additional 2 hr after adding Protein G beads.

b) Incomplete elution of chromatin from Protein G beads.

Elution of chromatin from Protein G beads is optimal at 65°C with frequent mixing to keep beads suspended in solution.

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