



DNA Methylation Detection Using the ChIP-GLAS System

This short protocol is designed for the use of the ChIP-GLAS system for the detection of methylated DNA promoters. This protocol replaced steps 1 and 2 of the standard ChIP-GLAS protocol as described in the ChIP-GLAS manual.

Additional materials needed:

In addition to the materials needed to process ChIP-GLAS **Protein G sepharose** or **protein G/A sepharose** is required to immunoprecipitate the anti-5-methylcytosine monoclonal antibody.

A: Purification genomic DNA from sample

5-20 μg of genomic DNA can be purified from either cultured cells or tissue using the Qiagen genomic DNA purification kit (QIAGEN Genomic-tip 20/G, Cat#: 10223). Please note that 10^6 cells will yield approximately 5 μg of DNA. Elute the sample in a 50 μl volume.

B: Digestion of DNA:

Digestion of 1 μg genomic DNA could be carried out using NlaIII enzyme. (New England Biolabs, Cat# R0125S) for 3 hours. Purify the DNA in 50 μl elution buffer using the Qiagen PCR purification Kit [QIAquick PCR Purification Kit (50), Cat# 28104].

C: Immunoprecipitation of methylated DNA using Anti-5-Methylcytosine antibody (Aviva Systems Biology, Cat. No. AVAMM99021)

1. Divide 50 μl genomic DNA samples into 2 tubes (25 μl /each). One will be labeled as total input DNA control. Label the other as test sample.
2. For test sample tube, adjust the volume to 150 μl with PBS buffer, then add 5 μg anti-5-methylcystosine antibody (Cat. No. AVAMM99021) to each test sample tube only. Incubate at 4°C overnight or 37°C for 2 hours on a rotating platform.
3. Add 40 μl Protein G/A or Protein G sepharose beads, rotating for 1 h at room temperature.
4. Washing step: transfer sample into 5 ml PBS buffer in 15 ml tube and spin down beads at 2000 X g for 4 min and discard the supernatants. Repeat the washing step 2 more times.

5. Finally, washing sample once with 1 ml TE buffer.
6. Add 50 μ l Elution Buffer (from ChIP-GLAS kit), and vortex briefly to resuspend the beads. Incubate at 65°C for 15 min. Vortex briefly every 2 min during the incubation. Spin 30 sec at maximum speed in a microcentrifuge and transfer the supernatant to a new tube. Repeat elution twice and pool the supernatants. Discard the beads.
7. Purify the DNA with the Qiagen PCR purification kit, and elute the DNA in 50 μ l water.
8. Samples can be stored at – 80°C or the experiment can proceed to step 3, biotinylation, in the ChIP-GLAS protocol.