

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 replaces 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 for the ChIP-GLAS assay, **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 (gDNA) 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 ~5 µg of DNA. Elute the sample in a 50µl volume.

B: Digestion of DNA:

Digest 5 µg gDNA with NlaIII for 3 hours. (New England Biolabs, Cat. # R0125S). Purify the DNA using the Qiagen PCR purification Kit, (QIAquick PCR Purification Kit (50), Cat. # 28104). Elute DNA in 50 µl elution buffer.

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

Important note regarding starting amounts: Before immunoprecipitation, a portion of the gDNA sample must be set aside (stored at -20°C). This sample will serve as the “total input DNA” in the Biotinylation reaction (ChIP-GLAS step 3). If possible, measure the DNA concentration of the 50 µl eluted sample from part B (above) and reserve 0.5 µg of input gDNA per Biotinylation reaction. Alternatively, divide the 50 µl sample into two tubes of 25 µl each (label them ‘IP’ and ‘input’) and use 5ul input per Biotinylation reaction. Both methods strive to achieve a final ratio of ~ 1:5 input DNA : IP'd DNA for the GLAS reaction.

1. For the immunoprecipitated sample, adjust the volume to 150 µl with PBS buffer + 0.05% Triton X-100, pH 7.0, then add 5 µg anti-5-methylcytosine 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.

2. Add 40 μ l Protein G/A or Protein G sepharose beads, rotate for 1 hour at room temperature.
3. Washing step: transfer sample into 5 ml PBS buffer in 15 ml tube, spin down beads at 2000 X g for 4 min and discard the supernatant. Repeat this wash step 2 more times.
4. Finally, wash sample once with 1 ml TE buffer.
5. 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.
6. Purify the DNA with a Qiagen PCR purification kit, and elute the DNA in a final volume of 20 μ l H₂O.
7. Proceed to ChIP-GLAS step 3, DNA Biotinylation, in the ChIP-GLAS protocol (posted below). Alternatively samples can be stored at – 80°C for later use.

Important: For the “**Biotinylation of DNA sample**” step, below (ChIP-GLAS Step 3), adjust the volume of the desired amount of “total input DNA” to 18 μ l with H₂O.

ChIP-GLAS Step 3. Biotinylation of DNA Sample

Procedure:

1. Biotinylation of DNA samples is set up in a PCR tube as follows:

IP DNA or total input DNA	18 μ l
λ DNA (100ng/ μ l)	1 μ l
PHOTOPROBE [®] Biotin.	1 μ l

Continue procedure as outlined in the ChIP-GLAS manual.