

**The anti-CMS technique for genome-wide mapping of 5-hydroxymethylcytosine.**

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**Public Summary:**

5-Hydroxymethylcytosine (5hmC) is a recently discovered base in the mammalian genome, produced upon oxidation of 5-methylcytosine (5mC) in a process catalyzed by TET proteins. The biological functions of 5hmC and further oxidation products of 5mC are under intense investigation, as they are likely intermediates in DNA demethylation pathways. Here we describe a novel protocol to profile 5hmC at a genome-wide scale. This approach is based on sodium bisulfite-mediated conversion of 5hmC to cytosine-5-methylenesulfonate (CMS); CMS-containing DNA fragments are then immunoprecipitated using a CMS-specific antiserum. The anti-CMS technique is highly specific with a low background, and is much less dependent on 5hmC density than anti-5hmC immunoprecipitation (IP). Moreover, it does not enrich for CA and CT repeats, as noted for 5hmC DNA IP using antibodies to 5hmC. The anti-CMS protocol takes 3 d to complete.

**Scientific Abstract:**

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