
Chemistry | Medical Science | Technology Offer

# Direct Programmable Detection of Epigenetic Cytosine Modifications in DNA using TALEs

## Field of Application

Epigenetic modifications at the 5-position of cytosine in DNA provide important clues for diseases such as neurological disorders and a range of cancers. For this reason, a simple and reliable method to differentiate unmodified from modified cytosine fractions (such as 5-methylcytosine ($^{5m}$C) and 5-hydroxymethylcytosine ($^{5hm}$C) is invaluable for the diagnosis and therapy of tumors amongst other diseases. Using TALEs (transcription-activator-like effectors), the present invention makes it possible to directly determine the status and level of epigenetic cytosine modifications at user-defined sequences with high resolution.

## State of the Art

Until now, a great variety of methods for the recognition of cytosine modifications have been in use, such as the bisulfite conversion or antibody-based methods (e.g. (h)MeDIP). These methods do not offer inherent, programmable sequence selectivity. They furthermore require harsh conditions and are hard to optimize (bisulfite) or only produce qualitative information at low resolution ((h)MeDIP).

## Innovation

Scientists at the University of Constance have now developed a method which allows the direct detection, i.e. without prior chemical modification of the DNA sample, of the epigenetic modification status in the 5-position of cytosine (such as $^{5m}$C and $^{5hm}$C) in any user defined sequence.
To achieve this, the inventors make use of the characteristics of TALEs (transcription-activator-like effectors) which allows the recognition of double-stranded DNA with freely selectable sequences. Due to the high modularity and flexibility of TALEs, they can be constructed in such a way that they bind to a specific target DNA sequence. The recognition of the target sequence is achieved by means of two amino acid residues per module (repeat variable di-residue or RVD).
The inventors have programmed TALEs in such a way as to ensure that they bind in a sequence-specific manner to a region of a DNA that contains the cytosine residue of interest. The cytosine modification status influences the binding affinity of specific RVDs so that $^{5m}$C and $^{5hm}$C can be detected. DNA polymerase reactions are used as assay read-out.
In this way, not only the status, but also the level of $^{5m}$C or $^{5hm}$C modification can be analyzed.

## Advantages

- ✓ Direct and bisulfit-free detection of cytosine-5 modifications in DNA molecules
- ✓ Recognition at user-defined genomic locations by means of an inherent programmable sequence selectivity
- ✓ Simple, direct detection method with high resolution
- ✓ Possibility of determining quantitatively the degree of modification
- ✓ Can be combined with a multitude of detection methods
- ✓ Detection both in vivo and in vitro possible

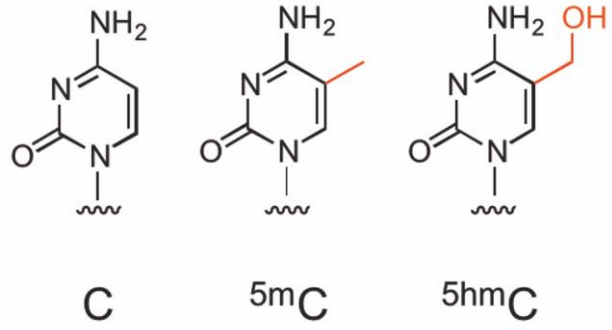


Figure: cytosine-5 modifications.

## Technology Transfer

The Technologie-Lizenz-Büro GmbH is responsible for the commercialisation of this technology and is now offering suitable enterprises licenses for the use of this technology.

## Patent Portfolio

Patents granted in DE, CH, FR and GB; pending in US.

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