Bioelectroanalytical technologies for determining global and regional methylations in nucleic acids

Brief description

The developed technologies allow the individual or simultaneous determination of the main methylated bases in DNA (5-methylcytosine, 5-mC; 5-hydroxymethylcytosine, 5-hmC; 5-formylcytosine, 5-fC; and 5-carboxyl-cytosine, 5-caC) and RNA (N6-methyladenosine, m6A), both globally and regionally, in a fast, reliable and simple manner.

How does it work?

The technologies are based on the implementation of affinity formats and enzymatic labelling with the enzyme horseradish peroxidase (HRP) on commercial magnetic microparticles (MBs) and on amperometric transduction on disposable screen-printed electrodes.

For regional determination, the target methylated DNA or RNA is selectively captured on MBs modified with a synthetic biotinylated DNA probe and is detected using an antibody selective to the target methylated base that is enzymatically labelled with HRP (Protein A-polyHRP80).

For global determination, direct competitive immunoassay formats are used on immunoconjugates in which the DNA or RNA methylated target competes for the limited binding sites of the antibodies immobilized on the MBs with a synthetic biotinylated DNA or RNA oligomer that carries a single methylated base and is labelled with the enzyme HRP (streptavidin-HRP conjugate).

In all cases, the modified MBs are magnetically captured on the surface of disposable electrodes (or electrode arrays) and amperometric transduction is performed using the HRP/hydroquinone (HQ)/H_2O_2 system, obtaining a variation in cathodic current that can be related to the concentration of the target methylated base.

What problem does it solve?

The results obtained confirm excellent performance for reliable determinations in human cells and biopsies of different nature (solid and liquid). The promising potential of these bioplatforms has also been confirmed for:

- discriminating between healthy individuals and those affected by cancer by determining the global level of 5-mC and 5-hmC in genomic DNA extracted from tissues or 5-mC at a regional level directly in serum samples;
- evaluating the metastatic capacities of cancer cells and identifying tumor tissues and their cancer stage by simultaneously analyzing the total content of a miRNA and its methylated fraction (Figure 1);
- confirming complete tumor resection after surgery and identifying different types of cancer by globally interrogating 5mC, 5-hmC, 5-fC and 5-caC in genomic DNA of tissues;
- characterize the aggressiveness of tumor cells by detecting m6A globally in total RNA extracts without the need for enrichment or fragmentation.

In determinations in human cells and tissues, the amount of genomic DNA or cellular RNA used per determination is between 10 and 100 ng.

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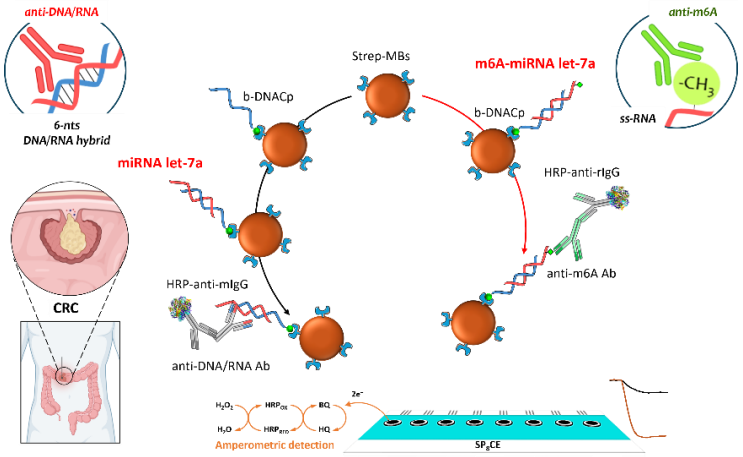

Figure 1. *Bioelectroanalytical technology to simultaneously determine the total content of a miRNA and its methylated fraction.*

What future products will it develop?

The incorporation into clinical routine of these bioelectroanalytical technologies, which provide results consistent with those provided by conventional technologies (RT-PCR), would entail important advantages in terms of simplicity, speed, cost, portability and multiplexing, reliability for early diagnosis and prognosis of prevalent diseases and improvement in the efficiency of therapy and the patients' survival and quality of life.

Competitive advantages compared to other research

The competitive advantages of the developed technologies include:

- Easily transferable to individual or multiple determination of other epimarks of interest.
- Simple implementation in point-of-care (POC) diagnostic devices.
- Sensitivity at a single base level without the need to use nucleic acid amplification steps.
- Providing quantitative results in less than 2 hours and at a very affordable cost (< 2 €/determination).

Where has it been developed?

These bioplatforms have been developed in the Electroanalysis and Electrochemical (Bio)sensors Group of the Faculty of Chemical Sciences of the Complutense University of Madrid (GEBE-UCM Group, Reference 910319) and in collaboration with researchers and clinicians from the Carlos III Health Institute (ProteoFun-ISCIII Group).

And moreover…

Website with additional information: https://gebeucm.wordpress.com/.

Researcher in charge

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