

Methods and constructs for locating and profiling single cells in a biological sample

FIELD OF THE INVENTION

The present invention generally relates to preparation of biological samples for next generation sequencing. Particularly, the invention utilizes a random combination of microbeads with microscopically detectable or otherwise visible labels coupled to sequence barcode for spatial labelling and identification of mRNA or DNA products originating from a single position and for locating said single position in the sample slide.

BACKGROUND OF THE INVENTION

In the complex cellular environment, cells of a tissue often display multiple phenotypes, which is reflected in RNA expression profiles of the cells. In the prior art, RNA expression profiling has been applied to single cells in suspensions, typically utilizing microwells or emulsion droplets where the single cells are encapsulated together with microbeads coated with unique cell barcode sequence and poly-T which capture the poly-A mRNA of the lysing cell, labelling the 3' end of the mRNA with the cell barcode sequence in reverse transcription reaction and cDNA synthesis (see e.g. Macosko et al 2015 and WO2016040476 by the Broad Institute Inc.). After sequencing the mRNA transcripts derived from same original cell can be grouped together based on the cell barcode, and separate gene expression profiles of all cells generated. The current cell barcoding beads, however, are targeted only to cells in suspension.

To understand the tissue structure, cell functions and cell-to-cell interactions within the tissues, there is a need to link RNA expression data with the cell image and spatial information gained from examination of cells or tissue sections with single cell resolution and high throughput manner. WO2021046232 discloses a system based on optically readable barcodes for characterization of molecular interactions of single cells. The system is relying on the use of oligonucleotide probes with multiple fluorescent labels. Improved methods are still in need.

SUMMARY

The present disclosure as defined in the appended claims is based on a novel design of microbeads with visible labels coupled with label specific barcodes that make it possible to link the obtained mRNA or DNA sequencing data to spatial coordinates of the cells in a biological sample profiled for RNA expression.

One aspect of the disclosure is to provide construct for detecting nucleic acid targets, said construct comprising a bead, preferably a magnetic bead, wherein said bead comprises one or several visually detectable features and/or said bead is coupled to one or several visually detectable entities, and wherein said bead comprises an oligonucleotide, and said oligonucleotide comprises a) an amplification handle sequence, b) a barcode sequence specific to one or several of said visually detectable features and/or entities and c) a 3' terminal capture or anchor sequence, and wherein said oligonucleotide is arranged to be detachable from said bead. Preferably, said terminal capture or anchor sequence comprises a poly-A sequence for hybridizing to a complementary poly-T sequence on commercially available cell barcoding beads.

Alternatively, also the cell barcoding sequence can be placed on the same construct with visible labels.

In another aspect, the present disclosure provides a composition comprising a construct as described herein. Another object of the disclosure is to provide a composition comprising several constructs as defined above preferably with a mixture of distinct visually detectable features or entities.

Another aspect of the disclosure is to provide a well plate or chip comprising the composition according to the present disclosure loaded to the wells of said plate or chip.

Another aspect of the disclosure is to provide a kit comprising the well plate or chip as defined above and a computer readable entity comprising a file of a photograph or scan of said plate or chip with the composition of the present disclosure loaded to the wells of said plate or chip.

Another aspect of the disclosure is to provide a use of said construct or composition for identifying desired nucleic acid targets or mRNA sequencing products originating from a single cell or position in biological sample.

Another aspect of disclosure is to provide a method for profiling biological samples on a single cell level, the method comprising the steps of:

- loading the construct or the composition of the present disclosure with a biological sample together or sequentially in any order to a substrate comprising wells,
- photographing or scanning the wells in said substrate with a microscope, preferably a light microscope or a fluorescence microscope, before and/or after loading the biological sample to said substrate; and