



US 20210301336A1

(19) **United States**

(12) **Patent Application Publication**
BASHKIROV et al.

(10) **Pub. No.: US 2021/0301336 A1**

(43) **Pub. Date: Sep. 30, 2021**

(54) **METHOD FOR LABEL-FREE SINGLE-MOLECULE DNA SEQUENCING AND DEVICE FOR IMPLEMENTING SAME**

(30) **Foreign Application Priority Data**

Dec. 26, 2017 (RU) 2017135756

(71) Applicant: **LIMITED LIABILITY COMPANY "GAMMA-DNA", Moscow (RU)**

Publication Classification

(72) Inventors: **VLADIMIR IVANOVICH BASHKIROV, Moscow (RU); ANTON VLADIMIROVICH GRIGORIEV, Saratov (RU); MIKHAIL ALEXANDROVICH GUTOROV, Moscow (RU); EDUARD ANATOLIEVICH ILICHEV, Moscow (RU); VLADIMIR VLADIMIROVICH KOLESOV, Moscow (RU); KONSTANTIN VALERIEVICH KRUTOVSKY, Göttingen (DE); ALEXEY OLEGOVICH MANTUROV, Saratov (RU); ELENA KIMOVNA BELOGLAZKINA, Moscow (RU); MIKHAIL MIKHAILOVICH GUTOROV, Moscow (RU)**

(51) **Int. Cl.**
C12Q 1/6869 (2006.01)
B01L 3/00 (2006.01)
(52) **U.S. Cl.**
CPC *C12Q 1/6869* (2013.01); *B01L 2300/1833* (2013.01); *B01L 2300/0645* (2013.01); *B01L 3/5027* (2013.01)

(73) Assignee: **LIMITED LIABILITY COMPANY "GAMMA-DNA", Moscow (RU)**

(57) **ABSTRACT**

A method and a device for determining a nucleotide sequence are proposed. The method comprises immobilizing circularized fragments of a nucleic acid and a polymerase on a sensor surface and adding a mixture of unlabeled nucleotides onto the sensor surface. Moreover, in the mixture added, the nucleotides of each type are present in their own concentration, which differs from the concentrations of the other three types of nucleotides. The time intervals between each of the charge separation events are determined and the registration steps for each nucleotide are repeated, regardless of the type of nucleotides. The nucleotide sequence of a nucleic acid molecule is determined by the analysis of the time intervals between each of the charge separation events registered, which result from the insertion, facilitated by the polymerase, of said unlabeled nucleotides into the growing nucleic acid chain. The device comprises a matrix having a plurality of sensor cells, and a digital-analog circuit, a microfluidic apparatus for feeding working solutions to the sensors, and data processing and display means.

(21) Appl. No.: **17/316,416**

(22) Filed: **May 10, 2021**

Related U.S. Application Data

(63) Continuation-in-part of application No. 16/484,461, filed on Aug. 8, 2019, now Pat. No. 11,034,998, filed as application No. PCT/RU2018/000202 on Mar. 29, 2018.

Specification includes a Sequence Listing.

