

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
24 August 2023 (24.08.2023)



(10) International Publication Number
WO 2023/158821 A2

(51) International Patent Classification:
C12Q 1/6806 (2018.01) *G06V 20/69* (2022.01)

(21) International Application Number:
PCT/US2023/013339

(22) International Filing Date:
17 February 2023 (17.02.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/311,319 17 February 2022 (17.02.2022) US
63/424,891 12 November 2022 (12.11.2022) US

(71) Applicant: **VIZGEN, INC.** [US/US]; 61 Moulton Street,
Cambridge, MA 02138 (US).

(72) Inventors: **HE, Jiang**; C/o Vizgen, Inc., 61 Moulton Street,
Cambridge, MA 02138 (US). **HE, Lizhi**; C/o Vizgen, Inc.,
61 Moulton Street, Cambridge, MA 02138 (US). **GHOSH,**
Arpan; C/o Vizgen, Inc., 61 Moulton Street, Cambridge,
MA 02138 (US).

(74) Agent: **VARMA, Anita** et al.; White & Case LLP, 75 State
Street, Boston, MA 02109 (US).

(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE,
KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU,
LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG,
NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS,
RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH,

(54) Title: METHODS OF ANCHORING FRAGMENTED NUCLEIC ACID TARGETS IN A POLYMER MATRIX FOR IMAGING

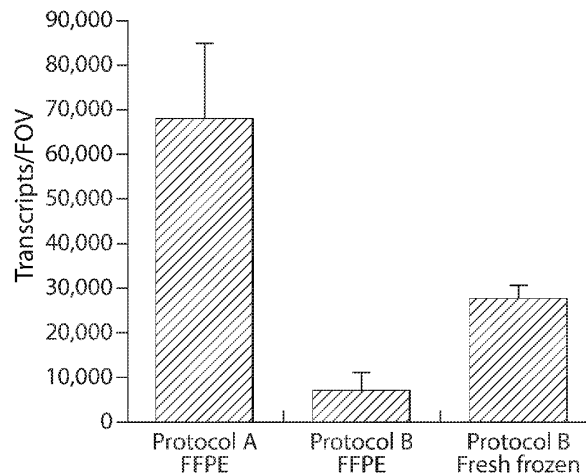


Fig. 5

(57) **Abstract:** The present disclosure is generally directed to methods for anchoring nucleic acid in a matrix and subsequently imaging nucleic acid targets (e.g. RNA transcript molecules) within tissue samples, for example, formalin- fixed paraffin-embedded (FFPE) tissue sections wherein the nucleic acid may be fragmented. For example, a method of anchoring target nucleic acid within a matrix and clearing non-target cellular components is provided herein, and the method includes contacting a tissue sample with at least two anchoring agents, wherein the first anchoring agent forms a covalent bond with the target nucleic acid and the second anchoring agent contains an oligonucleotide that hybridizes with the target nucleic acid; embedding the sample in a polymer matrix wherein the first and second anchoring agents each form a covalent bond with the polymer matrix; and, clearing the non-target cellular components from the polymer matrix wherein the target nucleic acid remains anchored in the polymer matrix to form a matrix anchored target nucleic acid sample. Additional steps include contacting the anchored target nucleic acid sample with one or more primary oligonucleotide

[Continued on next page]

TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS,
ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

probes that hybridize to the target nucleic acids and a plurality of secondary nucleic acid probes containing a fluorescent label and a recognition sequence that hybridizes to a sequence of the primary nucleic acid probe and imaging the target nucleic acids.