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(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).

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

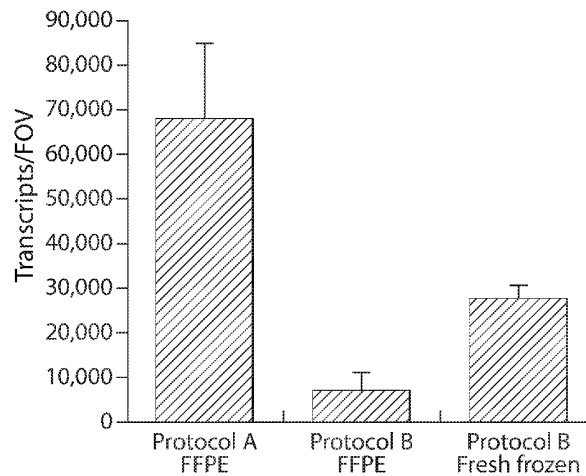


Fig. 5

(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

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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.