

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:

PCT/DK2011/050136

(22) International Filing Date:

28 April 2011 (28.04.2011)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/343,596

30 April 2010 (30.04.2010)

US

(71) Applicant (for all designated States except US): **EXIQON A/S** [—/DK]; Skelstedet 16, DK-2950 Vedbæk (DK).

(72) Inventors; and

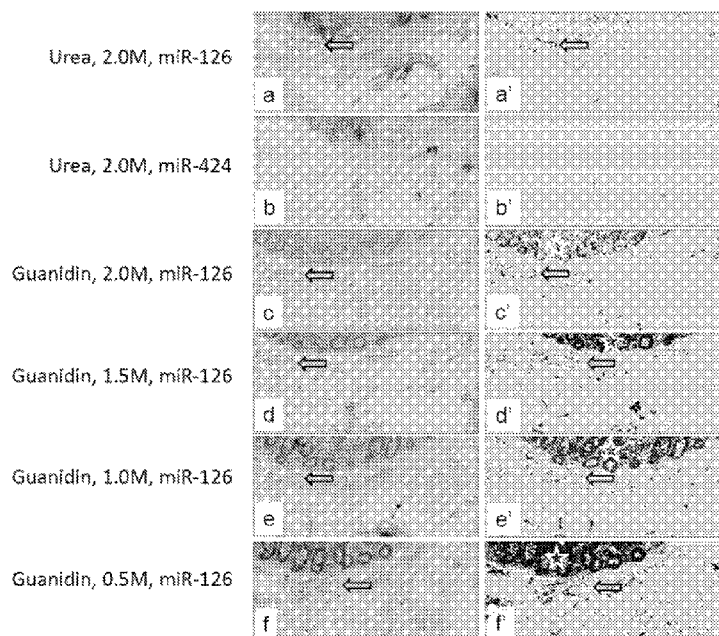
(75) Inventors/Applicants (for US only): **NIELSEN, Boye, Schnack** [DK/DK]; Hillerødsgade 28, 4. tv, DK-2200 Copenhagen N (DK). **JØRGENSEN, Stine** [DK/DK]; Charlotte Munchs Vej 35, 2. tv, DK-2400 Copenhagen NV (DK). **SKOUV, Jan** [DK/DK]; Stokholmsvej 55,DK-3060 Espergærde (DK). **BAKER, Adam** [GB/DK]; Vietofte 29, DK-2950 Vedbæk (DK). **MØLLER, Søren** [DK/DK]; Ved Furesøen 9, DK-2840 Holte (DK).(74) Common Representative: **EXIQON A/S**; Skelstedet 16, Att.: Jan Skouv, DK-2950 Vedbæk (DK).

(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, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

[Continued on next page]

(54) Title: IN SITU HYBRIDIZATION METHOD AND BUFFER

**Fig. 6**

(57) Abstract: An improved method of in situ hybridization which relies on an improved formulation of the in situ hybridization buffer is described. In at least some formulations the buffer are non-toxic. The combination of Locked Nucleic Acid (LNA) comprising ISH probes and the improved ISH buffer are useful for detection of small non-coding RNA as well as in the manufacturing of ISH kits directed to the detection of such small non-coding RNA. Further disclosed is a method of semi-quantitative ISH and demonstration of the semi-quantitative ISHs diagnostic potential.



EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

Published:

- *with international search report (Art. 21(3))*