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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
09 July 2020 (09.07.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/141143 A1

(51) International Patent Classification:
C12Q 1/6806 (2018.01)

(21) International Application Number:
PCT/EP2019/087048

(22) International Filing Date:
26 December 2019 (26.12.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
19305007.7 03 January 2019 (03.01.2019) EP

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(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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, 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, 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, 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, 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:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2020/141143 A1

(54) Title: ONE POT SYNTHESIS OF SETS OF OLIGONUCLEOTIDES

(57) Abstract: The invention is directed to methods for synthesizing a plurality of oligonucleotides in the same reaction vessel, and in some embodiments, using the synthesized oligonucleotides in an oligonucleotide-based assay in such reaction vessel. In some embodiments, methods of the invention are implemented by steps of (a) providing a plurality of different initiators attached to one or more supports, each different initiator having a terminal nucleotide with a different 3'-O-blocking group; (b) for each different initiator, synthesizing a polynucleotide by repeated cycles of template-free enzymatic additions of 3'-O-blocked nucleoside triphosphates, wherein the blocking group of the 3'-O-blocked nucleoside triphosphate is removable under deblocking conditions orthogonal to the deblocking conditions for removing blocking groups of the other initiators; and (c) releasing the oligonucleotides from the polynucleotides and the one or more solid supports.