

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

(19) World Intellectual Property
Organization
International Bureau



(10) International Publication Number
WO 2023/228174 A1

(43) International Publication Date
30 November 2023 (30.11.2023)

(51) International Patent Classification:

C12Q 1/6806 (2018.01) C12Q 1/6886 (2018.01)

(21) International Application Number:

PCT/IL2023/050518

(22) International Filing Date:

22 May 2023 (22.05.2023)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

293202 22 May 2022 (22.05.2022) IL

(71) Applicant: **NUCLEIX LTD.** [IL/IL]; 3 Pekeris St.,
7670203 Rehovot (IL).

(72) Inventors: **FRUMKIN, Danny**; 1 Carmel Street, 7630501
Rehovot (IL). **KNIRSH, Revital**; 48 Granit Street, 4854145
Rosh HaAyin (IL).

(74) Agent: **WEBB, Cynthia** et al.; WEBB & CO., P.O. BOX
4177, 7414003 Ness Ziona (IL).

(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, MU, 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,
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, SC, 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).

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— with international search report (Art. 21(3))

(54) Title: USEFUL COMBINATIONS OF RESTRICTION ENZYMES

(57) Abstract: Various compositions and methods are disclosed in which a plurality of restriction enzymes, including methylation-sensitive and/or methylation-dependent restriction enzymes, are used for the analysis of cfDNA. Useful combinations can be inactivated by heating to 65°C, ideally for longer than 15 minutes. Restriction digestion of cfDNA can occur for 11 hours or less. Digestion may be followed by amplification and/or sequencing steps.



WO 2023/228174 A1