

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

(19) World Intellectual Property
Organization

International Bureau

(43) International Publication Date
06 April 2023 (06.04.2023)



(10) International Publication Number
WO 2023/056344 A1

(51) International Patent Classification:

C12N 15/34 (2006.01) C12N 9/10 (2006.01)
C12N 15/54 (2006.01) C12N 15/52 (2006.01)

(21) International Application Number:

PCT/US2022/077242

(22) International Filing Date:

29 September 2022 (29.09.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/249,819 29 September 2021 (29.09.2021) US

(72) Inventor; and

(71) Applicant: CHEN, Cheng-Yao [US/CN]; No. 101, Sec. 2, Kuang-Fu Rd., East Dist., Hsinchu City, 30013 (TW).

(72) Inventors: CHENG, Yi-Wen; No. 101, Sec. 2, Kuang-Fu Rd., East Dist., Hsinchu City, 30013 (TW). HUNG, Yu Ting; No. 101, Sec. 2, Kuang-Fu Rd., East Dist., Hsinchu City, 30013 (TW). CHEN, Wen-Ting; No. 101, Sec. 2, Kuang-Fu Rd., East Dist., Hsinchu City, 30013 (TW).

(74) Agent: MUNCY, Joe McKinney; Muncy, Geissler, Olds & Lowe, P.C., 125 S. Royal St., Alexandria, VA 22314 (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, 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, 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, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, 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,

(54) Title: NUCLEIC ACID POLYMERASE VARIANTS, KITS AND METHODS FOR TEMPLATE-INDEPENDENT RNA SYNTHESIS

	20	40	60	80					
Tgo					1				
KodI					1				
9 th					1				
Pfu					1				
Vent					1				
Mma					1				
Msa					1				
hPOLD	MDGRRPGPG	PVPPKRRAG	GLWDDDDAPR	PSGF.....EEDLA	LMEDMEAEH.....LL 50				
SoxPOLD	MSKKSL.....	PMVDPVKI.....	DDSDTPO.....	EKKIKKQSI	DHGVQSEPVSTFETIPSDSF	RKVNQGFKA	KDTLGMGTQL 71		
Pfu	WELKYPW.....				7				
Sso	WTGQLTLDI	PSGKPAKSEQ	NT.....		22				
Pae	MFEL.....				4				
Eco	MAQA.....				4				
R999	WRE.....				3				
T4	WKE.....				3				
Phi29	WKE.....				2				
Consensus	M.....				2				
	100	120	140	160					
Tgo					20				
KodI					20				
9 th					20				
Pfu					20				
Vent					20				
Mma					20				
Msa					20				
hPOLD	DEEEELQSG	VLEGVADGV	PPSAIDPRWL	RPTPPALDPQ	TEPLIFQGLE	LDHYVGPAGP	VPGGPPPSRG	SVPLRFAFGV 130	
SoxPOLD	ESTFEGELSG	MEHMDADGEE	NDLSPERKK	LPTD.....FDPS	LYDISQGIQD	AE.....	CS	VLNGIKDENT	STVVRFFGV 142
Pfu									
Sso									
Pae									
Eco									
R999									
T4									
Phi29									
Consensus									

FIG.1

(57) Abstract: Provided herein relates to nucleic acid polymerase variants and kits including the same, where the nucleic acid polymerase variant has an improved function and activity of performing template-independent nucleic acids synthesis using ribonucleotides (rNTPs) in a thermotolerant manner.

[Continued on next page]

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))*
- *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))*