

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
10 February 2022 (10.02.2022)



(10) International Publication Number
WO 2022/029142 A1

(51) International Patent Classification:

C07K 14/44 (2006.01) C12N 9/12 (2006.01)
C12N 9/10 (2006.01) C12N 9/14 (2006.01)

(21) International Application Number:

PCT/EP2021/071698

(22) International Filing Date:

03 August 2021 (03.08.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

20305899.5 04 August 2020 (04.08.2020) EP

(71) Applicant: **EUKARYS** [FR/FR]; PÉPINIÈRE GENO-
POLE-CAMPUS 3, 4 RUE PIERRE FONTAINE, 91058
ÉVRY-COURCOURONNES CEDEX (FR).

(72) Inventor: **JAIS, Philippe**; 58 avenue du Bas-Meudon,
92130 ISSY-LES-MOULINEAUX (FR).

(74) Agent: **BRINGER IP**; 1 Place du Président Wilson, 31000
TOULOUSE (FR).

(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, IT, 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.

(54) Title: ARTIFICIAL EUKARYOTIC EXPRESSION SYSTEM WITH ENHANCED PERFORMANCES

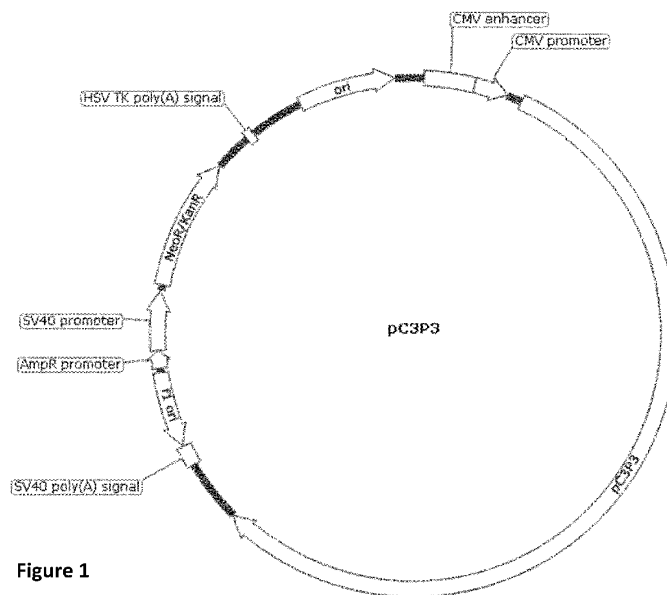


Figure 1

(57) Abstract: The present invention concerns a method for expressing a recombinant DNA molecule in a eukaryotic host cell, comprising the steps of: (a) expressing or introducing at least one chimeric protein, in said host cell, wherein said chimeric protein comprises: (i) at least one catalytic domain of a capping enzyme, in particular selected in the group consisting of cap-0 canonical capping enzymes, cap-0 non-canonical capping enzymes, cap-1 capping enzymes and cap-2 capping enzymes; and (ii) at least one catalytic domain of a DNA-dependent RNA polymerase, in particular a bacteriophage DNA-dependent RNA polymerase, (b) constitutively or transiently downregulating the phosphorylation level of subunit α of translation initiation factor eIF2 (eIF2 α) in said host cell. The invention also concerns an isolated nucleic acid molecule or a set of nucleic acid molecules, comprising or consisting of (1) at least one nucleic acid sequence encoding a chimeric protein comprising at least one catalytic domain of a capping enzyme; and at least one

[Continued on next page]

(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))*
- *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))*

catalytic domain of a DNA-dependent RNA polymerase; and (2) at least one nucleic acid sequence downregulating the phosphorylation level of eIF2 α in a eukaryotic host cell or encoding a polypeptide downregulating said phosphorylation level; and (3) optionally, at least one nucleic acid sequence encoding a poly(A) polymerase, as well as vectors, kits and cells comprising said nucleic acid molecule or set, and different uses and applications thereof.