

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
12 August 2021 (12.08.2021)



(10) International Publication Number
WO 2021/158817 A1

(51) International Patent Classification:

C12N 15/81 (2006.01) C12N 1/16 (2006.01)
C12P 21/02 (2006.01) A23J 3/20 (2006.01)
A23J 1/18 (2006.01)

(21) International Application Number:

PCT/US2021/016658

(22) International Filing Date:

04 February 2021 (04.02.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/970,052 04 February 2020 (04.02.2020) US

(71) Applicant: **CLARA FOODS CO.** [US/US]; 1 Tower Place, Suite 800, South San Francisco, California 94080 (US).

(72) Inventors: **IVEY, Frank Douglas**; 1 Tower Place, Suite 800, South San Francisco, California 94080 (US). **KREPS, Joel Andrew**; 1 Tower Place, Suite 800, South San Francisco, California 94080 (US). **TINDELL, Charles Albert**; 1 Tower Place, Suite 800, South San Francisco, California 94080 (US). **ZHONG, Weixi**; 1 Tower Place, Suite 800, South San Francisco, California 94080 (US).

(74) Agent: **SHARMA, Swati**; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, California 94304 (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, 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.

(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))

(54) Title: SYSTEMS AND METHODS FOR HIGH YIELDING RECOMBINANT MICROORGANISMS AND USES THEREOF

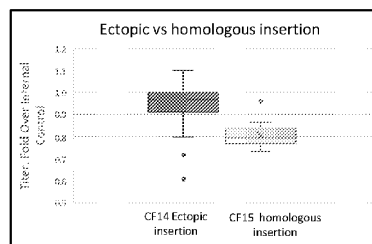


FIG. 1A

(57) Abstract: Provided are systems and methods for high-yield production of recombinant proteins in engineered microorganisms. Also provided is an engineered host cell for expressing a heterologous protein, said engineering host cell may comprise at least three different expression cassettes integrated into the genome of the engineered host cell wherein; a first expression cassette may comprise a first promoter operably linked to a heterologous gene sequence encoding the heterologous protein; a second expression cassette may comprise a second promoter operably linked to a heterologous gene sequence encoding the heterologous protein; a third expression cassette may comprise a third promoter operably linked to a helper factor sequence.



WO 2021/158817 A1