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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 August 2003 (28.08.2003)

PCT

(10) International Publication Number
WO 03/070880 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US03/01800

(22) International Filing Date: 22 January 2003 (22.01.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/057,582 23 January 2002 (23.01.2002) US

(71) Applicant *(for all designated States except US)*: **WISCONSIN ALUMNI RESEARCH FOUNDATION** [US/US]; 614 Walnut Street, P.O. Box 7365, Madison, WI 53707-7365 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BLATTNER, Frederick, R.** [/]; 1547 Jefferson Street, Madison, WI 53711 (US). **POSFAI, Gyorgy** [/]; Majus 1 u. 74, H-6727 Szeged (HU). **HERRING, Christopher, D.** [/]; 32 Oakbridge Court, Madison, WI 53717 (US). **PLUNKETT, Guy** [/]; 1613 Gilbert Road, Madison, WI 53711 (US). **GLASNER, Jeremy, D.** [/]; 1102 East Johnson Street, Madison, WI 53706 (US). **TWOSE, Trevor, Martin** [/]; 1506 Chandler Street, Madison, WI 53711 (US).

(74) Agent: **SEAY, Nicholas, J.**; Quarles & Brady LLP, P.O. Box 2113, Madison, WI 53701-21113 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/070880 A2

(54) Title: BACTERIA WITH REDUCED GENOME

(57) Abstract: The present invention provides a bacterium having a genome that is genetically engineered to be at least 2 to about 20% smaller than the genome of its native parent strain. A bacterium with a smaller genome can produce a commercial product more efficiently. The present invention also provides methods for deleting genes and other DNA sequences from a bacterial genome. The methods provide precise deletions and seldom introduces mutations to the genomic DNA sequences around the deletion sites. Thus, the methods can be used to generate a series of deletions in a bacterium without increasing the possibility of undesired homologous recombination within the genome. In addition, some of the methods provided by the present invention can also be used for replacing a region of a bacterial genome with a desired DNA sequence.