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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 March 2005 (17.03.2005)

PCT

(10) International Publication Number
WO 2005/024000 A1

(51) International Patent Classification[7]: C12N 1/21, 15/72, C07K 16/00, C12P 21/02

(21) International Application Number: PCT/GB2004/003747

(22) International Filing Date: 2 September 2004 (02.09.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0321100.0 9 September 2003 (09.09.2003) GB

(71) Applicant *(for all designated States except US)*: **CELLTECH R & D LIMITED** [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GLOVER, David, John** [GB/GB]; Celltech R & D Limited, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **SEHDEV, Mukesh** [GB/GB]; Celltech R & D Limited, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **REEKS, Dominic, Gambier** [GB/GB]; Celltech R & D Limited, 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

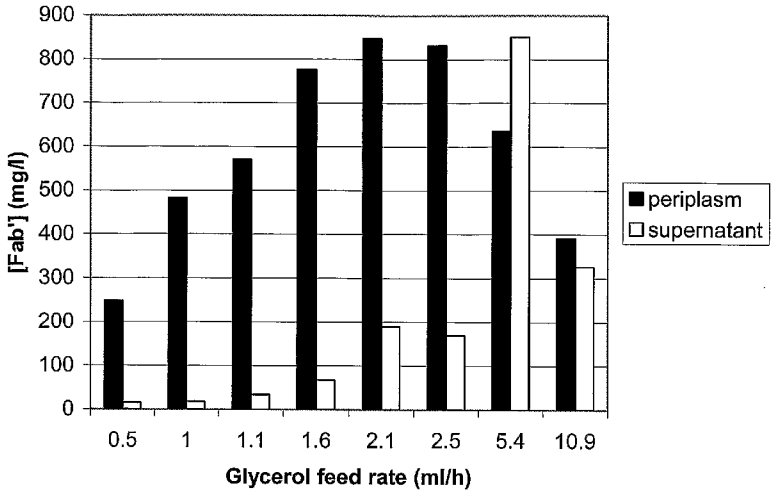
(74) Agent: **THOMPSON, John**; Celltech R & D Limited, 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, 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, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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

[Continued on next page]

(54) Title: METHODS FOR PRODUCING RECOMBINANT PROTEINS



(57) Abstract: The present invention provides a method for controlling the partitioning of a recombinant protein between the supernatant and the periplasm in *E.coli* host cell cultures wherein expression of the recombinant protein by said cells is under the control of an inducible system, which method comprises: a) providing an *E.coli* host cell culture b) changing the growth rate of the*E.coli* host cells c) inducing expression of the recombinant protein wherein steps (b) and (c) can be performed in any order or simultaneously; and subsequently d) determining the yield of recombinant protein in the culture supernatant and the *E.coli* host cell periplasm e) comparing the yield determined in step (d) with the yield determined when at least one other growth rate has been used in step (b) f) selecting a growth rate from the comparison made in step (e) in which the partitioning of the recombinant protein between the supernatant and the periplasm is most suited to the primary recovery of the recombinant protein.

WO 2005/024000 A1

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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.