



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/09, C12P 19/34		A1	(11) International Publication Number: WO 98/42832
			(43) International Publication Date: 1 October 1998 (01.10.98)
(21) International Application Number: PCT/US98/05956 (22) International Filing Date: 25 March 1998 (25.03.98) (30) Priority Data: 60/041,666 25 March 1997 (25.03.97) US 60/045,211 30 April 1997 (30.04.97) US 60/046,256 12 May 1997 (12.05.97) US 08/905,359 4 August 1997 (04.08.97) US (71) Applicant (for all designated States except US): CALIFORNIA INSTITUTE OF TECHNOLOGY [US/US]; 1201 E. California Boulevard, Pasadena, CA 91125 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): ARNOLD, Frances, H. [US/US]; 629 S. Grand Avenue, Pasadena, CA 91106 (US). SHAO, Zhixin [CN/CN]; 110 S. Michigan Avenue #7, Pasadena, CA 91106 (US). AFFHOLTER, Joseph, A. [US/US]; 823 E. Sanford Road, Midland, MI 48642 (US). ZHAO, Huimin [CN/CN]; 1324 Cordova Street, Pasadena, CA 91106 (US). GIVER, Lorraine, J. [US/US]; 140 S. Catalina Avenue #4, Pasadena, CA 91106 (US).			(74) Agents: OLDENKAMP, David, J. et al.; Oppenheimer Wolff & Donnelly LLP, Suite 3800, 2029 Century Park East, Los Angeles, CA 90067 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: RECOMBINATION OF POLYNUCLEOTIDE SEQUENCES USING RANDOM OR DEFINED PRIMERS (57) Abstract A method for <i>in vitro</i> mutagenesis and recombination of polynucleotide sequences based on polymerase-catalyzed extension of primer oligonucleotides is disclosed. The method involves priming template polynucleotide(s) with random-sequences or defined-sequence primers to generate a pool of short DNA fragments with a low level of point mutations. The DNA fragments are subjected to denaturation followed by annealing and further enzyme-catalyzed DNA polymerization. This procedure is repeated a sufficient number of times to produce full-length genes which comprise mutants of the original template polynucleotides. These genes can be further amplified by the polymerase chain reaction and cloned into a vector for expression of the encoded proteins.			