

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
5 July 2001 (05.07.2001)

PCT

(10) International Publication Number  
WO 01/48229 A2

(51) International Patent Classification<sup>7</sup>: C12N 15/81, 5/10

(21) International Application Number: PCT/EP00/13350

(22) International Filing Date:  
22 December 2000 (22.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
99204557.5 27 December 1999 (27.12.1999) EP

(71) Applicant (for all designated States except US):  
VLAAMS INTERUNIVERSITAIR INSTITUUT  
VOOR BIOTECHNOLOGIE VZW [BE/BE]; Rijviss-  
chestraat 120, B-9052 Zwijnaarde (BE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): CALLEWAERT,  
Nico, Luc, Marc [BE/BE]; Ketelbuiserstraat 99,  
B-8810 Lichtervelde (BE). CONTRERAS, Roland,

Henry [BE/BE]; Molenstraat 53, B-9820 Merelbeke  
(BE). LAROY, Wouter [BE/BE]; Kerselaarslaan 43,  
B-9800 Deinze (BE). MARTINET, Wim [BE/BE];  
Archimedeslaan 42, B-2650 Edegem (BE).

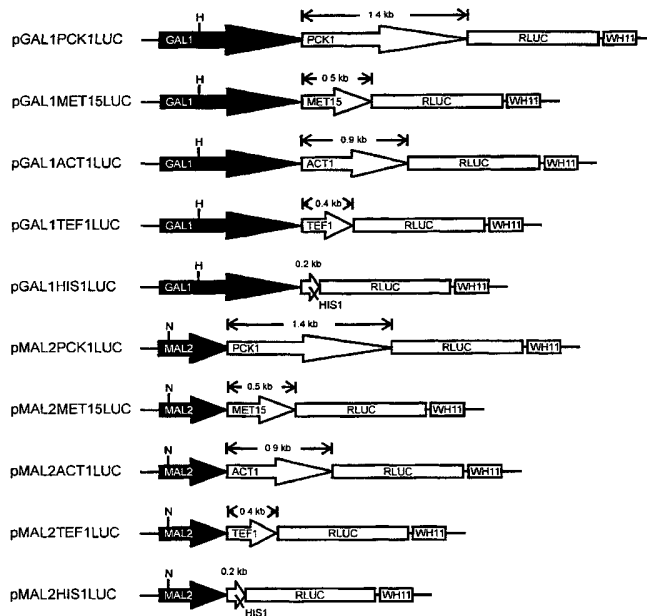
(74) Common Representative: VLAAMS INTERUNIVER-  
SITAIR INSTITUUT VOOR BIOTECHNOLOGIE  
VZW; Rijvisschestraat 120, B-9052 Zwijnaarde (BE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, 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, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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

[Continued on next page]

(54) Title: CONDITIONAL GENE SILENCING IN EUKARYOTES BY PROMOTER INSERTION



(57) Abstract: The *Renilla reniformis* luciferase (*RLUC*) gene was used as a bioluminescent reporter to investigate promoter silencing in the opportunistic fungus *Candida albicans*. Five expression modules, containing the *RLUC* coding sequence under transcriptional control of a yeast promoter, were cloned downstream of the strong inducible *GAL1* or *MAL2* promoter of *C. albicans*. After transformation and growth under inducing conditions, luciferase activity was inhibited up to 96 % as a result of upstream transcription initiation and elongated transcript formation. Similar silencing results were obtained in *P. pastoris*, by placing the constitutive *OCH1* promoter behind the inducible *AOX1* promoter. Repression of the *OCH1p* after induction of the *AOX1* promoter results in a change in glycosylation pattern.



WO 01/48229 A2