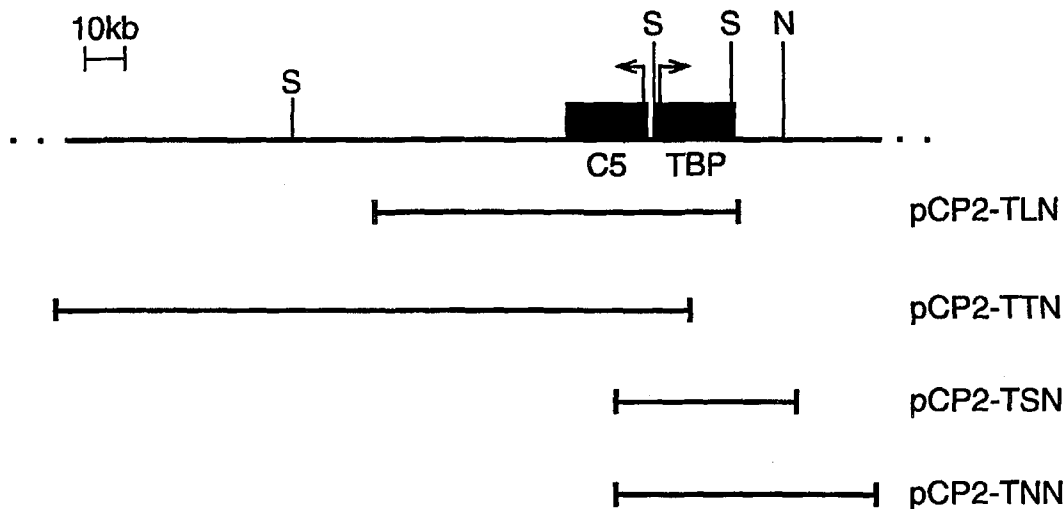




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁷ : C12N 15/85, 5/10, C12Q 1/68, A61K 48/00, C12N 15/00, A01K 67/027, C12N 15/11</p>	<p>A2</p>	<p>(11) International Publication Number: WO 00/05393 (43) International Publication Date: 3 February 2000 (03.02.00)</p>
<p>(21) International Application Number: PCT/GB99/02357 (22) International Filing Date: 21 July 1999 (21.07.99) (30) Priority Data: 9815879.3 21 July 1998 (21.07.98) GB 60/107,688 9 November 1998 (09.11.98) US 9906712.6 23 March 1999 (23.03.99) GB 60/127,410 1 April 1999 (01.04.99) US 9909494.8 23 April 1999 (23.04.99) GB 60/134,016 12 May 1999 (12.05.99) US (71) Applicant (for all designated States except US): COBRA THERAPEUTICS LIMITED [GB/GB]; The Science Park, University of Keele, Keele ST5 5SP (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): ANTONIOU, Michael [GB/GB]; Dept. of Experimental Pathology, UMDS, Guy's Hospital, London Bridge, London SE1 9RT (GB). CROMBIE, Robert [GB/GB]; Cobra Therapeutics Limited, The Science Park, University of Keele, Keele ST5 5SP (GB).</p>	<p>(74) Agents: HALLYBONE, Huw, George et al.; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, 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, 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, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, 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), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published Without international search report and to be republished upon receipt of that report.</p>	

(54) Title: A POLYNUCLEOTIDE COMPRISING A UBIQUITOUS CHROMATIN OPENING ELEMENT (UCOE)



(57) Abstract
The present invention relates to a polynucleotide comprising a ubiquitous chromatin opening element (UCOE) which is not derived from an LCR. The present invention also relates to a vector comprising the polynucleotide sequence, a host cell comprising the vector, use of the polynucleotide, vector or host cell in therapy and in an assay, and a method of identifying UCOEs. The UCOE opens chromatin or maintains chromatin in an open state and facilitates reproducible expression of an operably-linked gene in cells of at least two different tissue types.

Claims

1. A polynucleotide comprising a UCOE, which opens chromatin or maintains chromatin in an open state and facilitates reproducible expression of an operably-linked gene in cells of at least two different tissue types, wherein the polynucleotide is not derived from a locus control region.
5
2. The polynucleotide of claim 1 which facilitates reproducible expression of an operably-linked gene non-tissue specifically.
10
3. The polynucleotide of claim 1, which facilitates reproducible expression of an operably-linked gene in all tissue types where active gene expression occurs.
4. The polynucleotide of any one of the previous claims which facilitates expression of an operably-linked gene at a physiological level.
15
5. The polynucleotide of any one of the previous claims wherein the UCOE comprises an extended methylation-free, CpG-island.
- 20 6. The polynucleotide of any one of the previous claims wherein the UCOE is derived from a sequence that in its natural endogenous position is associated with a ubiquitously expressed gene.
7. The polynucleotide of any one of the previous claims wherein the UCOE comprises dual or bi-directional promoters that transcribe divergently.
25
8. The polynucleotide of any one of the previous claims wherein the UCOE is a 44kb DNA fragment spanning the human TATA binding protein (TBP) gene and 12kb each of the 5' and 3' flanking sequence, or a functional homologue or fragment thereof.
30

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 March 2002 (28.03.2002)

PCT

(10) International Publication Number
WO 02/24930 A2

- (51) International Patent Classification⁷: C12N 15/64 (74) Agent: HARRISON GODDARD FOOTE; Belgrave Hall, Belgrave Street, Leeds LS2 8DD (GB).
- (21) International Application Number: PCT/GB01/04210
- (22) International Filing Date: 20 September 2001 (20.09.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
0022995.5 20 September 2000 (20.09.2000) GB
60/252,048 20 November 2000 (20.11.2000) US
- (71) Applicant (for all designated States except US): COBRA THERAPEUTICS LTD [GB/GB]; Stephenson Building, Keele University Science Park, Keele, staffordshire ST5 5SP (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): ANTONIOU, Michael [GB/GB]; Stephenson Building, Keele University Science Park, Keele, staffordshire ST5 5SP (GB). CROMBIE, Robert [GB/GB]; Stephenson Building, Keele University Science Park, Keele, staffordshire ST5 5SP (GB).
- (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, PH, 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, 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 02/24930 A2

(54) Title: POLYNUCLEOTIDE

(57) Abstract: The present invention relates to a polynucleotide comprising a ubiquitous chromatin opening element (UCOE) that does not occur in nature. The present invention also relates to a vector comprising the polynucleotide sequence, a host cell comprising the vector and use of the polynucleotide, vector or host cell in therapy, or for in vitro protein expression applications.

CLAIMS

1. A polynucleotide comprising a UCOE, which opens chromatin or maintains chromatin in an open state and facilitates reproducible expression of an operably-linked gene in cells of at least two different tissue types, wherein the nucleotide
5 sequence of the UCOE does not occur in nature.
2. The polynucleotide of claim 1, which facilitates reproducible expression of an operably-linked gene non-tissue specifically.
- 10 3. The polynucleotide of claim 1, which facilitates reproducible expression of an operably-linked gene in all tissue types where active gene expression occurs.
4. The polynucleotide of any one of the preceding claims which facilitates expression of an operably-linked gene at a physiological level.
- 15 5. The polynucleotide of any one of the preceding claims wherein the UCOE comprises an extended methylation-free, CpG-island.
6. The polynucleotide of any one of the preceding claims wherein the UCOE
20 comprises one or more naturally-occurring sequences associated with the control of gene expression.
7. The polynucleotide of any one of the preceding claims wherein the UCOE comprises one or more naturally-occurring promoters.
- 25 8. The polynucleotide of any one of the preceding claims wherein the UCOE comprises dual or bi-directional promoters that transcribe divergently.
9. The polynucleotide of any one of the preceding claims wherein the UCOE
30 comprises the human β -actin CpG island/ promoter region or a fragment thereof.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 August 2004 (12.08.2004)

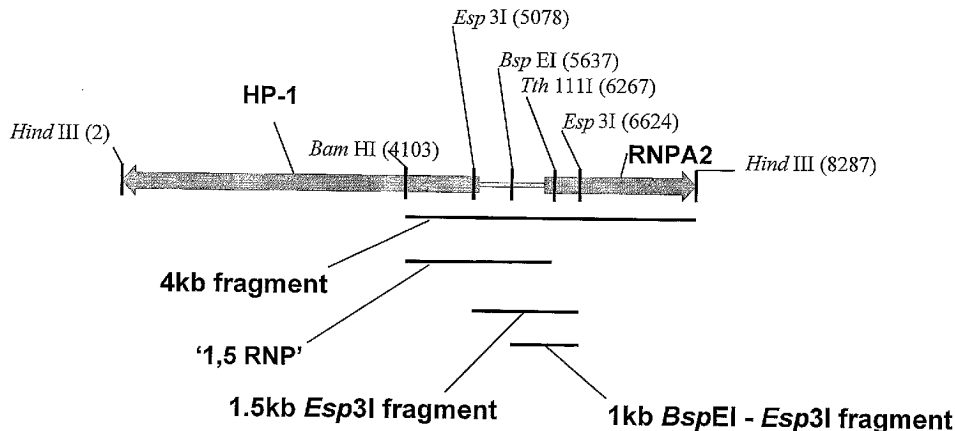
PCT

(10) International Publication Number
WO 2004/067701 A2

- (51) International Patent Classification⁷: C12N
- (21) International Application Number: PCT/GB2004/000387
- (22) International Filing Date: 2 February 2004 (02.02.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
0302330.6 1 February 2003 (01.02.2003) GB
0308503.2 12 April 2003 (12.04.2003) GB
0320824.6 5 September 2003 (05.09.2003) GB
- (71) Applicant (for all designated States except US): ML LABORATORIES PLC [GB/GB]; 17 Hanover Square, London W1S 1HU (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): WILLIAMS, Steven, Geraint [GB/GB]; ML Laboratories plc, Research Division, Stephenson Building, The Science Park, Keele ST5 5SP (GB). CROMBIE, Robert, Lachlan [GB/GB]; ML Laboratories plc, Research Division, Stephenson Building, The Science Park, Keele ST5 5SP (GB). LIPINSKI, Kai, Stefan [GB/GB]; ML Laboratories PLC, Stephenson Building, Reaserch Division, The Science Park, Keele, Keele ST5 5SP (GB). IRVINE, Alistair, Simpson [GB/GB]; ML Laboratories PLC, Stephenson Building, Reaserch Division, The Science Park, Keele, Keele ST5 5SP (GB).
- (74) Agents: GODDARD, David, John et al.; Harrison Goddard Foote, Orlando House, 11c Compstall Road, Marple Bridge, Stockport SK6 5HH (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, 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, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Declaration under Rule 4.17:**
— of inventorship (Rule 4.17(iv)) for US only
- Published:**
— without international search report and to be republished upon receipt of that report

[Continued on next page]

(54) Title: IMPROVED EXPRESSION ELEMENTS



(57) Abstract: The invention relates to improved genetic elements providing high levels of expression of operably-linked genes in a variety of tissues. In particular, fragments of unmethylated, CpG islands of less than 2kb are shown to provide enhanced transgene expression and have advantages in terms of vector construction and cloning capacity.

WO 2004/067701 A2

CLAIMS

1. An isolated polynucleotide comprising
 - a) an extended methylation-free CpG island;
 - 5 b) an expressible open reading frame, operably linked to said extended methylation-free CpG island;
 - c) a promoter, operably-linked to said open reading frame, wherein said promoter is not naturally linked to said CpG island;characterised in that said CpG island is not more than 2kb in size and wherein
10 reproducible expression of said open reading frame is obtained in two or more tissue types.
2. The polynucleotide of claim 1, wherein said CpG island comprises a fragment of
15 the HP-1/hnRNPA2 locus.
3. The polynucleotide of claim 2, wherein the fragment is of the human HP-1/hnRNPA2 locus.
4. A polynucleotide according to any of claims 1 to 3, comprising a fragment of an
20 HP-1/ hnRNP A2 locus of no more than 1.6kb.
5. The polynucleotide according to claim 4, comprising an *Esp3I* restriction fragment.
- 25 6. The polynucleotide according to claim 5, comprising the sequence of Figure 2, nucleotides 977 to 2522 (SEQ ID NO: 1) or a functional homologue thereof.
7. A polynucleotide according to any of claims 1 to 6, comprising a fragment of an
30 HP-1/ hnRNP A2 locus of no more than 1 kb.
8. The polynucleotide according to claim 7, comprising a *BspE1 – Esp3I* restriction fragment.