

CLAIMS

1. A purified and isolated DNA sequence having protein production increasing activity, characterized in that said DNA sequence comprises a) at least one bent
5 DNA element, wherein the bent DNA element contains at least 33% of dinucleotide TA and/or at least 33% of dinucleotide AT on a stretch of 100 contiguous base pairs, b) at least one binding site for a DNA binding protein, c) and that it is a MAR nucleotide sequence with the sequence SEQ IDS No 27, a sequence complimentary thereof, or
10 a sequence having at least 90% identity with said sequence SEQ IDS No 27.
2. The purified and isolated sequence of claims 1, characterized in that said DNA binding protein is a transcription factor.
- 15 3. The purified and isolated sequence of claim 2, characterized in that the transcription factor is selected from the group comprising the polyQpolyP domain proteins.
4. The purified and isolated sequence of claim 2, characterized in that the
20 transcription factor is selected from the group comprising SATB1, NMP4, MEF2, S8, DLX1, FREAC7, BRN2, GATA 1/3, TATA, Bright, MSX, AP1, C/EBP, CREBP1, FOX, Freac7, HFH1, HNF3alpha, Nkx25, POU3F2, Pit1, TTF1, XFD1, AR, C/EBPgamma, Cdc5, FOXD3, HFH3, HNF3 beta, MRF2, Oct1, POU6F1, SRF, V\$MTATA_B, XFD2, Bach2, CDP CR3, Cdx2, FOXJ2, HFL, HP1, Myc, PBX, Pax3,
25 TEF, VBP, XFD3, Brn2, COMP1, Evl, FOXP3, GATA4, HFN1, Lhx3, NKX3A, POU1F1, Pax6, TFIIA and Vmw65 or a combination of two or more of these transcription factors.
5. The use of a purified and isolated DNA sequence comprising a first isolated
30 matrix attachment region (MAR) nucleotide sequence which is a MAR nucleotide sequence selected from the group comprising:
 - a purified and isolated DNA sequence of any of claims 1 to 4,
 - the sequence SEQ IDS No 27,

or a sequence complementary thereof, for increasing protein production activity in a eukaryotic host cell.

5 6. The use of the purified and isolated DNA sequence of claim 5, characterized in that said purified and isolated DNA sequence further comprises a promoter operably linked to a gene of interest.

10 7. The use of the purified and isolated DNA sequence of claims 5 or 6, characterized in that said purified and isolated DNA sequence further comprises at least a second isolated matrix attachment region (MAR) nucleotide sequence which is a MAR nucleotide sequence selected from the group comprising:

- a purified and isolated DNA sequence of any of claims 1 to 4,
- the sequence SEQ IDS No 27,

15 or a sequence complementary thereof, for increasing protein production activity in a eukaryotic host cell.

20 8. The use of the purified and isolated DNA sequence of claim 7, characterized in that said first and at least second MAR sequences are located at both the 5' and the 3' ends of the sequence containing the promoter and the gene of interest.

9. The use of the purified and isolated DNA sequence of claim 7, characterized in that said first and/or at least second MAR sequences are located on a sequence distinct from the one containing the promoter and the gene of interest.

25 10. The use of the purified and isolated DNA sequence of any of claims 5 to 9, characterized in that said purified and isolated DNA sequence is in the form of a linear DNA sequence as vector.

30 11. A method for transfecting a eukaryotic host cell, said method comprising a) introducing into said eukaryotic host cell at least one purified DNA sequence comprising at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence of any of claims 1 to 4, b) subjecting within a defined time said transfected eukaryotic host cell to at least one additional transfection step with at least one purified DNA sequence comprising at least one DNA sequence of interest

and/or with at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence or other chromatin modifying elements, and c) selecting said transfected eukaryotic host cell.

5 12. The method of claim 11, characterized in that said DNA sequence of interest is a gene of interest coding for a protein operably linked to a promoter.

10 13. The method of claims 11 or 12, characterized in that the selected transfected eukaryotic host cells are high protein producer cells with a production rate of at least 10 pg per cell per day.

14. The method of any of claims 11 to 13, characterized in that the MAR nucleotide sequence is selected from the group comprising:
15 - a purified and isolated DNA sequence of any of claims 1 to 4,
- the sequence SEQ IDS No 27,
or a sequence complementary thereof.

15. The method of any of claims 11 to 14, characterized in that the MAR nucleotide is a purified and isolated sequence according to any of claims 1 to 4, or
20 a sequence complementary thereof.

16. The method of any of claims 11 to 15, characterized in that the defined time corresponds to intervals related to the cell division cycle.

25 17. The method of claim 16, characterized in that the defined time is the moment the host cell just has entered into a second cell division cycle.

18. A method for transfecting a eukaryotic host cell, said method comprising co-transfecting into said eukaryotic host cell at least one first purified and isolated DNA
30 sequence comprising at least one DNA sequence of interest, and a second and isolated purified DNA comprising at least one MAR nucleotide selected from the group comprising:

- a purified and isolated DNA sequence of any of claims 1 to 4,
 - the sequence SEQ IDS No 27,
- or a sequence complementary thereof.

5 19. A process for the production of a protein wherein a) a eukaryotic host cell transfected according to any of claims 11 to 18 is cultured in a culture medium under conditions suitable for expression of said protein and b) said protein is recovered.

10 20. A eukaryotic host cell transfected according to any one of claims 11 to 18.

21. A cell transfection mixture or kit comprising at least one DNA sequence according to any of claims 1 to 4.

15 22. A transgenic non-human organism characterized in that at least some of its cells have stably incorporated at least one DNA sequence of any of claims 1 to 4.

23. A transgenic non-human organism characterized in that its genome has stably incorporated at least one DNA sequence of any of claims 1 to 4.

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24. The transgenic non-human organism of claims 22 or 23 characterized in that some of its cells have been transfected according to the method of any of claims 11 to 18.

25 25. A eukaryotic host cell transfected with at least one DNA sequence according to any of claims 1 to 4.

The Claims Defining the Invention are as Follows:

1. A purified and isolated DNA sequence having protein production increasing activity comprising:

- 5 a) at least one bent DNA element, and
 b) at least one binding site for a DNA binding protein,

wherein said DNA sequence is a MAR nucleotide with a sequence selected from the following:

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- (i) SEQ ID NO:27,
(ii) a sequence complementary to SEQ ID NO:27,
(iii) a sequence at least 96.3% identical to SEQ ID NO:27.

15 2. The purified and isolated DNA sequence of claim 1 wherein the bent DNA element contains at least 10% of dinucleotide TA, and/or at least 12% of dinucleotide AT on a stretch of 100 contiguous base pairs.

20 3. The purified and isolated DNA sequence of claim 2 wherein the bent DNA element contains at least 33% of dinucleotide TA, and/or at least 33% of dinucleotide AT on a stretch of 100 contiguous base pairs.

4. The purified and isolated sequence of any one of claims 1 to 3, wherein said DNA binding protein is a transcription factor.

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5. The purified and isolated sequence of claim 4, wherein the transcription factor is selected from the group comprising the polyQpolyP domain proteins.

30 6. The purified and isolated sequence of claim 4, wherein the transcription factor is selected from the group comprising: SATB1, NMP4, MEF2, S8, DLX1, FREAC7, BRN2, GATA 1/3, TATA, Bright, MSX, AP1, C/EBP, CREBP1, FOX, Freac7, HFH1, HNF3alpha, Nkx25, POU3F2, Pit1, TTF1, XFD1, AR,

C/EBPgamma, Cdc5, FOXD3, HFH3, HNF3 beta, MRF2, Oct1, POU6F1, SRF, V\$MTATA_B, XFD2, Bach2, CDP CR3, Cdx2, FOXJ2, HFL, HP1, Myc, PBX, Pax3, TEF, VBP, XFD3, Brn2, COMP1, Evf1, FOXP3, GATA4, HFN1, Lhx3, NKX3A, POU1F1, Pax6, TFI IA and Vmw65 or a combination of two or more of these transcription factors.

7. A synthetic MAR sequence comprising natural MAR elements and/or fragments according to one of claims 1 to 6 assembled between linker sequences.

8. The synthetic MAR sequence of claim 7, wherein the linker sequences are BglII-BamHI linker.

9. The use of a purified and isolated DNA sequence comprising a first isolated matrix attachment region (MAR) nucleotide sequence selected from the group comprising:

- (i) a purified and isolated DNA sequence of any one of claims 1 to 6,
- (ii) the sequence of SEQ ID NO:27,
- (iii) a synthetic MAR sequence of claims 7 or 8,
- (iv) a sequence complementary to any one of (i) to (iii),
- (v) a sequence at least 96.3% identical to SEQ ID NO:27

for increasing protein production activity in a eukaryotic host cell.

10. The use of the purified and isolated DNA sequence of claim 9, wherein said purified and isolated DNA sequence further comprises a promoter operably linked to a gene of interest.

11. The use of the purified and isolated DNA sequence of claims 9 or 10, wherein said purified and isolated DNA sequence further comprises at least a second isolated matrix attachment region (MAR) nucleotide sequence which is a MAR nucleotide sequence selected from the group comprising:

- (i) a purified and isolated DNA sequence of any one of claims 1 to 6,
- (ii) the sequence of SEQ ID NO:27,
- (iii) a synthetic MAR sequence of claims 7 or 8,
- 5 (iv) a sequence complementary to any one of (i) to (iii),
- (v) a sequence at least 96.3% identical to SEQ ID NO:27,

for increasing protein production activity in a eukaryotic host cell.

10 12. The use of the purified and isolated DNA sequence of claim 11, wherein said first and at least second MAR sequences are located at both the 5' and the 3' ends of the sequence containing the promoter and the gene of interest.

15 13. The use of the purified and isolated DNA sequence of claim 11, wherein said first and or at least second MAR sequences are located on a sequence distinct from the one containing the promoter and the gene of interest.

20 14. The use of the purified and isolated DNA sequence of any of claims 9 to 13, wherein said purified and isolated DNA sequence is in the form of a linear DNA sequence as vector.

15. A method for transfecting a eukaryotic host cell, said method comprising the steps of:

- 25 a) introducing into said eukaryotic host cell at least one purified DNA sequence comprising at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence according to any one of the previous claims,
- b) subjecting within a defined time said transfected eukaryotic host cell to at
30 least one additional transfection step with at least one purified DNA sequence comprising at least one DNA sequence of interest and/or with at least one purified and isolated DNA sequence consisting of a MAR nucleotide sequence or other chromatin modifying elements, and

c) selecting said transfected eukaryotic host cell.

16. The method of claim 15, wherein said DNA sequence of interest is a gene of interest coding for a protein operably linked to a promoter.

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17. The method of claims 15 or 16, wherein the selected transfected eukaryotic host cells are high protein producer cells with a production rate of at least 10 pg per cell per day.

10 18. The method of any one of claims 15 to 17, wherein the MAR nucleotide sequence is selected from the group comprising:

- (i) a purified and isolated DNA sequence of any one of claims 1 to 6,
- (ii) the sequence of SEQ ID NO:27,
- 15 (iii) a synthetic MAR sequence of claims 7 or 8,
- (iv) a sequence complementary to any one of (i) to (iii),
- (v) a sequence at least 96.3% identical to SEQ ID NO:27.,

19. The method of any one of claims 15 to 17, wherein the MAR nucleotide is
20 selected from the following:

- (i) a purified and isolated sequence according to any one of claims 1 to 6,
- (ii) a sequence complementary to (i),
- 25 (iii) a sequence at least 96.3% identical to SEQ ID NO:27.,

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20. The method of any one of claims 15 to 19, wherein the defined time corresponds to intervals related to the cell division cycle.

21. The method of claim 20, wherein the defined time is the moment the host cell
30 has just entered into a second cell division cycle.

22. A method for transfecting a eukaryotic host cell, said method comprising co-transfecting into said eukaryotic host cell at least one first purified and isolated

DNA sequence comprising at least one DNA sequence of interest, and a second and isolated purified DNA comprising at least one MAR nucleotide selected from the group comprising:

- 5 (i) a purified and isolated DNA sequence of any one of claims 1 to 6,
- (ii) the sequence of SEQ ID NO:27,
- (iii) a synthetic MAR sequence of claims 7 or 8,
- (iv) a sequence complementary to any one of (i) to (iii),
- (v) a sequence at least 96.3% identical to SEQ ID NO:27.,

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23. A process for the production of a protein comprising the steps:

- a) culturing in a culture medium under conditions suitable for expression of
 said protein a eukaryotic host cell transfected according to any one of
- 15 claims 15 to 22, and
- b) recovering said protein.

24. A eukaryotic host cell transfected according to any one of claims 15 to 22.

20 25. A cell transfection mixture or kit comprising at least one purified and isolated
 DNA sequence according to any one of claims 1 to 8.

26. A non-human transgenic organism wherein at least some of its cells have
 stably incorporated at least one DNA sequence of any one of claims 1 to 8.

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27. A non-human transgenic organism wherein its genome has stably incorporated
 at least one DNA sequence of any one of claims 1 to 8.

28. The non-human transgenic organism of claims 26 and 27 wherein some of its
30 cells have been transfected according to the method of any one of claims 15 to
 22.

29. A eukaryotic host cell transfected with at least one DNA sequence of any one of claims 1 to 8.