Claims

1. A nucleotide construct for use in a method of screening multiple putative gene regulatory elements (pGRE) in parallel, comprising nucleotide blocks, in sequence PRIMREG1-pGRE-PRIMREG2-GENE, or GENE-PRIMREG1-pGRE-PRIMREG2
   wherein:
   PRIMREG1 is either a primer recognition sequence 1, or a FLEx cassette comprising a primer recognition sequence 1.
   pGRE is a putative gene regulatory element,
   PRIMREG2 is either a primer recognition sequence 2, or a FLEx cassette comprising a primer recognition sequence 2,
   GENE comprises nucleotide blocks IRES and SSR, wherein
      IRES is either an internal ribosome entry site or a 2A self-cleaving peptide, and
      SSR is a site specific recombinase gene;
   wherein at least one of PRIMREG1 and PRIMREG2 is a FLEx cassette comprising a primer recognition sequence.

2. A vector comprising a nucleotide sequence for use in a method of screening multiple putative gene regulatory element (pGRE) in parallel, comprising the nucleotide blocks, in sequence PRIMREG1-pGRE- PRIMREG2-GENE, or GENE-PRIMREG1-pGRE-PRIMREG2
   wherein:
   PRIMREG1 is either a primer recognition sequence 1, or a FLEx cassette comprising a primer recognition sequence 1.
   pGRE is a putative gene regulatory element,
   PRIMREG2 is either a primer recognition sequence 2, or a FLEx cassette comprising a primer recognition sequence 2,
   GENE comprises nucleotide blocks IRES and SSR, wherein
      IRES is either an internal ribosome entry site or a 2A self-cleaving peptide, and
      SSR is a site specific recombinase gene;

   wherein at least one of PRIMREG1 and PRIMREG2 is a FLEx cassette comprising a primer recognition sequence.

3. The nucleotide construct according to claim 1 or the viral vector according to claim 2 wherein GENE further comprises nucleotide block REPSEQ, wherein REPSEQ is a reporter gene, such that GENE comprises the nucleotide blocks REPSEQ, IRES and SSR.

4. A method of screening multiple putative gene regulatory elements (pGREs) in parallel comprising the steps:
   a. generating or having generated a population of vectors comprising a nucleotide construct comprising nucleotide blocks, in sequence PRIMREG1-pGRE- PRIMREG2-GENE, or GENE-PRIMREG1-pGRE-PRIMREG2
      wherein:
      PRIMREG1 is either a primer recognition sequence 1, or a FLEx cassette comprising a primer recognition sequence 1.
      pGRE is a putative gene regulatory element,
      PRIMREG2 is either a primer recognition sequence 2, or a FLEx cassette comprising a primer recognition sequence 2,
      GENE comprises nucleotide blocks REPSEQ, IRES and SSR, wherein

REPSEQ is a reporter gene,
IRES is either an internal ribosome entry site or a 2A self-cleaving peptide, and
SSR is a site specific recombinase gene;
wherein at least one of PRIMREG1 and PRIMREG2 is a FLEx cassette comprising a primer recognition sequence;

b. contacting target cells with the population of vectors under conditions suitable for transduction of the target cells;
c. isolating DNA from the transduced target cells;
d. selectively amplifying active and/or inactive pGREs using PCR; and
e. determining the sequence of active and/or inactive pGREs.

5. A method of identifying a novel active putative gene regulatory element (pGRE) comprising the steps:
    a. generating or having generated a population of vectors comprising a nucleotide construct comprising nucleotide blocks, in sequence PRIMREG1-pGRE- PRIMREG2-GENE, or GENE-PRIMREG1-pGRE-PRIMREG2
    wherein:
    PRIMREG1 is either a primer recognition sequence 1, or a FLEx cassette comprising a primer recognition sequence 1.
    pGRE is a putative gene regulatory element,
    PRIMREG2 is either a primer recognition sequence 2, or a FLEx cassette comprising a primer recognition sequence 2,
    GENE comprises nucleotide blocks REPSEQ, IRES and SSR, wherein
    REPSEQ is a reporter gene,
    IRES is either an internal ribosome entry site or a 2A self-cleaving peptide, and
    SSR is a site specific recombinase gene;
    wherein at least one of PRIMREG1 and PRIMREG2 is a FLEx cassette comprising a primer recognition sequence;
    b. contacting target cells with the population of vectors under conditions suitable for transduction of the target cells;
    c. isolating DNA from the transduced target cells expressing the reporter gene;
    d. selectively amplifying active pGREs using PCR; and
    e. determining the sequence of active pGREs.

6. The vector according to claim 2 or claim 3, or the method according to claim 4 or claim 5 wherein the vector is a viral vector.

7. The vector or method according to claim 6, wherein the viral vector is an AAV vector.

8. The construct, vector or method according to any preceding claim wherein the FLEx cassette comprises, in sequence, ABC1-ABC2-PRSEQ-1CBA-2CBA wherein:
    - ABC1 is a recognition site for the recombinase protein encoded by the SSR block,
    - ABC2 is a different recognition site for the recombinase protein encoded by the SSR block,
    - PRSEQ is either primer recognition sequence 1 or primer recognition sequence 2,
    - 1CBA is inverted ABC1, and
    - 2CBA is inverted ABC2.

9. The construct, vector or method according to claim 8 wherein:
    - ABC1 is Lox1,
    - ABC2 is Lox2,

- SSR is cre,
- 1CBA is 1xoL, and
- 1CBA is 2xoL.

10. The construct, vector or method according to claim 8 wherein:
    - ABC1 is FRT1,
    - ABC2 is FRT2,
    - SSR is Flp,
    - 1CBA is 1TRF, and
    - 1CBA is 2TRF.

11. The construct, vector or method according to claim 8 wherein:
    - ABC1 is Rox1,
    - ABC2 is Rox2,
    - SSR is dre,
    - 1CBA is 1xoR, and
    - 1CBA is 2xoR.

12. The construct, vector or method according to any preceding claim wherein the nucleotide construct has a 5' ITR1 block and a 3' ITR2 block.

13. The method according to any one of claims 4 to 12 wherein both PRIMREG1 and PRIMREG2 are FLEx cassettes, and the target cells are capable of expressing a site specific recombinase which is different to the one encoded by the SSR block of the nucleotide construct.