

CLAIMS

1. An isolated mammalian host cell comprising a heterologous gene of interest (GOI) chromosomally integrated at a target site within an intergenic region between a pair of adjacent essential genes, wherein each of the adjacent essential genes of said pair has a transcription start site (TSS) and the distance between the TSSs is at least 80 nucleotides and less than 20.000 nucleotides (nt).
2. The host cell of claim 1, wherein the length of the intergenic region is less than 20.000 nt.
3. The host cell of claim 1 or 2, wherein the length of the intergenic region is less than 5.000 nt.
4. The host cell of any one of claims 1 to 3, wherein the intergenic region comprises or consists of the nucleic acid sequence which is at least 90% identical to any one of SEQ ID NO:2-21.
5. The host cell of any one of claims 1 to 4, wherein the essential genes of said pair are oriented such that their promoters face in opposite directions.
6. The host cell of any one of claims 1 to 5, wherein the GOI is comprised in a heterologous expression cassette integrated at the target site.
7. The host cell of any one of claims 1 to 6, which is
 - a) a hematopoietic stem cell, an embryonic stem cell, a pluripotent stem cell, an induced pluripotent stem cell, or an endothelium endothelial cell; or
 - b) of a cell type selected from the group consisting of a Natural Killer (NK) cell, a microglia cell, a macrophage, or a T cell, such as a cytotoxic T lymphocyte (CTL), a regulatory T cell, or a T helper cell.
8. The cell of claim 7, which is an immune effector cell comprising a chimeric antigen receptor (CAR), wherein the GOI encodes the CAR expressed by said immune effector cell.

9. A preparation comprising a population of mammalian host cells, wherein at least 1% of the cells are host cells of any one of claims 1 to 8.

5 10. An *in vitro* host cell culture of a host cell of any one of claims 1 to 9.

11. A method of producing a gene product encoded by a gene of interest (GOI), by transforming a host cell to chromosomally integrate the GOI within an intergenic region between a pair of adjacent essential genes, and culturing the transformed host
10 cell under conditions to express said GOI, wherein each of the adjacent essential genes of said pair has a transcription start site (TSS) and the distance between the TSSs is at least 80 nt and less than 20.000 nt.

12. A method of modifying a mammalian cell by site-directed chromosomal
15 integration of a heterologous gene of interest (GOI) within an intergenic region between a pair of adjacent essential genes, wherein each of the adjacent essential genes of said pair has a transcription start site (TSS) and the distance between the TSSs is at least 80 nt and less than 20.000 nt.

20 13. The method of claim 11 or 12, which employs any one of a programmable nuclease, preferably a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a CRISPR endonuclease or an Argonaute protein for chromosomal integration.

25 14. A host cell of any one of claims 1 to 8, or the preparation of claim 9, for medical use.

15. The host cell of any one of claims 1 to 8, or the preparation of claim 9, for use according to claim 14, wherein a subject is treated by expressing said GOI *in vivo*.

30 16. An *ex vivo* use of the host cell of any one of claims 1 to 8 in a method of producing induced pluripotent stem cells, antibody production, and/or production of a protein of interest.

17. A method for producing a chimeric antigen receptor (CAR) microglia or macrophage, comprising:

(a) obtaining a microglia or macrophage from a mammalian subject, preferably a human microglia or macrophage; and

5 (b) modifying the microglia or macrophage by one or more genetic modifications to chromosomally integrate a heterologous gene of interest (GOI) at a target site within an intergenic region between a pair of adjacent essential genes, wherein the GOI encodes a chimeric antigen receptor (CAR) and wherein each of the adjacent essential genes of said pair has a transcription start site (TSS) and the distance between the TSSs
10 is at least 80 nt and less than 20.000 nt.