[0005] There is a need in the art for means for achieving a stable integration and/or high level of gene expression of heterologous polynucleotide in mammalian cells. The present disclosure addresses this and other needs.

SUMMARY

[0005a] In one aspect, provided is an *ex vivo* or *in vitro* method for stable integration and expression of a heterologous polynucleotide in a host cell, comprising inserting the heterologous polynucleotide into the genome of the host cell at a native chromosomal site located at or close to positions 130-131 of SEQ ID NO: 1 in CHO cells or a position corresponding to the positions 130-131 of SEQ ID NO: 1 in other mammalian cells in the ankyrin 2 gene (Ank2).

[0006] Also disclosed~~In one aspect, provided~~ are methods for stable integration and expression of a heterologous polynucleotide in a host cell. The methods involve inserting the heterologous polynucleotide into the genome of the host cell at a native chromosomal site located within or adjacent to a gene selected from the group consisting of ankyrin 2 gene (Ank2), cleavage and polyadenylation specific factor 4 gene (Cpsf4), C-Mos gene and Nephrocystin-1/Mal gene. In some methods, insertion of the heterologous polynucleotide into the host genome is mediated by homologous recombination or by a hybrid recombinase. In some methods, the host cell is a mammalian cell, e.g., a Chinese hamster ovary (CHO) cell. In some of these methods, the native chromosomal insertion site is at or close to positions 130-131 of SEQ ID NO:1 for the Ank2 gene, positions 629-630 of SEQ ID NO:2 for the Cpsf4 gene, positions 272-273 of SEQ ID NO:3 for the C-Mos gene, or positions 239-240 of SEQ ID NO:4 for the Nephrocystin-1/Mal gene. In some methods, the heterologous polynucleotide to be integrated into the host genome can encode a polypeptide, e.g., a therapeutic protein or an industrial protein.

[0006a] In another aspect, provided is a recombinant polynucleotide for stably integrating a heterologous polynucleotide sequence into the genome of a mammalian cell, comprising:

a first homology arm, the heterologous polynucleotide sequence, and a second homology arm;

wherein the first and second homology arms are substantially identical to the 5'- and 3'- sequences, respectively, that flank a native chromosomal insertion site located at or close to positions 130-131 of SEQ ID NO: 1 in CHO cells or a position corresponding to positions 130-131 of SEQ ID NO: 1 in other mammalian cells in the ankyrin 2 gene (Ank2).

[0007] Also disclosed~~In a related aspect, provided~~ are recombinant or engineered polynucleotides for stably integrating a heterologous polynucleotide sequence into the genome of a mammalian cell. The recombinant polynucleotides typically contain a first homology arm, the heterologous polynucleotide sequence, and a second homology arm. The first and second homology arms are substantially identical to the 5'- and 3'- sequences, respectively, that flank a native chromosomal insertion site located within or adjacent to a gene selected from the group consisting of ankyrin 2 gene (Ank2), cleavage and polyadenylation specific factor 4 gene (Cpsf4), C-Mos gene and Nephrocystin-1/Mal gene. Typically, the native chromosomal insertion site is capable of supporting stable integration of a foreign gene. In some methods, the heterologous polynucleotide sequence encodes a polypeptide, e.g., a therapeutic protein or an industrial protein. In some other methods, the heterologous polynucleotide sequence comprises a site-specific recombination sequence (chromosomal landing pad). For example, the site-specific recombination sequence can be a recognition sequence recognized by a phage integrase, such as the attP site or the attB site recognized by phiC-31 phage integrase.

In some methods, the host mammalian cell is a Chinese hamster ovary (CHO) cell. In these methods, the native chromosomal insertion site can be located at or close to positions 130-131 of SEQ ID NO:1 for the Ank2 gene, positions 629-630 of SEQ ID NO:2 for the Cpsf4 gene, positions 272-273 of SEQ ID NO:3 for the C-Mos gene, or positions 239-240 of SEQ ID NO:4 for the Nephrocystin-1/Mal gene. In related embodiments, vectors containing the recombinant or engineered polynucleotides are also provided in the invention.

[0008] In another aspect, provided is an engineered mammalian cell, comprising a heterologous polynucleotide which is stably integrated into its genome at one or more native chromosomal insertion sites located at or close to positions 130-131 of SEQ ID NO: 1 in CHO cells or a position corresponding to positions 130-131 of SEQ ID NO: 1 in other mammalian cells in the ankyrin 2 gene (Ank2). Also disclosed are engineered mammalian cells. The cells harbor a heterologous polynucleotide that is stably integrated into its genome at one or more native chromosomal insertion sites located within or adjacent to a gene selected from the group consisting of ankyrin 2 gene (Ank2), cleavage and polyadenylation specific factor 4 gene (Cpsf4), C-Mos gene and Nephrocystin-1/Mal gene. Typically, the chosen native chromosomal insertion site supports stable integration of a foreign gene. In some of the methods, the heterologous polynucleotide encodes a polypeptide, e.g., a therapeutic protein or an industrial protein. In some other methods, the heterologous polynucleotide contains a site-specific recombination sequence (chromosomal landing pad). For example, the site-specific recombination sequence can be a recognition sequence recognized by a phage integrase, such as the attP site or the attB site recognized by phiC-31 phage integrase. Some preferred embodiments are directed to recombinant or engineered Chinese hamster ovary (CHO) cells. In these embodiments, the heterologous polynucleotide can be preferably integrated at or close to positions 130-131 of SEQ ID NO:1 for the Ank2 gene, positions 629-630 of SEQ ID NO:2 for the Cpsf4 gene, positions 272-273 of SEQ ID NO:3 for the C-Mos gene, or positions 239-240 of SEQ ID NO:4 for the Nephrocystin-1/Mal gene.

[0009] In still another related aspect, provided is an *ex vivo* or *in vitro* method for stably integrating a heterologous polynucleotide into the genome of a mammalian cell, comprising:

(a) inserting a site-specific recombination sequence into the genome of the cell, wherein the insertion is at a native chromosomal insertion site located at or close to positions 130-131 of SEQ ID NO: 1 in CHO cells or a position corresponding to positions 130-131 of SEQ ID NO: 1 in other mammalian cells in ankyrin 2 gene (Ank2);

(b) integrating by homologous recombination the heterologous polynucleotide into the genome of the cell at the inserted site-specific recombination sequence.

[0009a] Also disclosed are methods for stably integrating a heterologous polynucleotide into the genome of a mammalian cell. These methods entail (a) inserting a site-specific recombination sequence into the genome of the cell, wherein the insertion is at a native chromosomal insertion site located within or adjacent to a gene selected from the group consisting of ankyrin 2 gene (Ank2), cleavage and polyadenylation specific factor 4 gene (Cpsf4), C-Mos gene and Nephrocystin-1/Mal gene; and (b) integrating by homologous recombination the heterologous polynucleotide into the genome of the cell at the inserted site-specific recombination sequence. The native chromosomal insertion site chosen for the methods typically supports stable integration of a foreign gene. In some methods, the site-specific recombination sequence is a first recognition sequence recognized by a phage integrase, e.g., the attP site or the attB site of phiC-31 phage integrase. In these