[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

[0006] In one example, 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 one aspect, the present disclosure refers to an *ex vivo* method for stable integration and expression of a heterologous polynucleotide in a Chinese Hamster Ovary (CHO) 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 239-240 of SEQ ID NO:4 for Nephrocystin-1/Mal gene.

[0007] In a related example, 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 examples, vectors containing the recombinant or engineered polynucleotides are also provided in the disclosure.

[0008] In another example, provided 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 examples are directed to recombinant or engineered Chinese hamster ovary (CHO) cells. In these examples, 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 example, provided 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

methods, the heterologous polynucleotide is usually attached to a second recognition sequence of the phage integrase which is cognate to the first recognition sequence, e.g., the attB site or the attP site recognized by the phage integrase. In some methods, the employed mammalian host cell is a Chinese hamster ovary (CHO) cell. In these methods, the site-specific recombination sequence can be preferably inserted into the genome 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 contains a target polypeptide-encoding sequence that is operably linked to a promoter sequence. Typically, integration of the heterologous polynucleotide into the host genome occurs in the presence of the phage integrase. In some of these methods, the phage integrase can be expressed from a vector introduced into the cell.

[0009a] In another aspect, the present disclosure refers to an *ex vivo* method for stably integrating a heterologous polynucleotide into the genome of a CHO 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 239-240 of SEQ ID NO: 4 for 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.

[0010] A further understanding of the nature and advantages of the present disclosure may be realized by reference to the remaining portions of the specification and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Figure 1 is a diagram showing the structure of the plasmid used for random integration into CHO genome for identifying native chromosomal insertion sites that support strong transcription activities. The plasmid contains a sv40 promoter driven EGFP expression cassette. The BamHI site is between EGFP and Hygromycin resistence gene (for linearization before stable integration).

[0012] Figure 2 illustrates the plasmid used for introducing an attP site into identified native chromosomal insertion sites in CHO genome. The attP site is in the 5' of Neo gene. The left-homology arm is cloned 5'of attP and Neo gene; the right-homology arm is cloned at 3' of Neo gene. The HSV-TK gene is used for negative selection. The sequences shown are the double strand sequences of the attP and attB sites recognized by phi-C31 phage integrase: attP (SEQ ID NO:5 and SEQ ID NO:6), attB (SEQ ID NO:7 and SEQ ID NO:8).

[0013] Figure 3 shows a native chromosomal insertion site and flanking sequences in the ankyrin 2 (Ank2) gene of CHO cell genome (SEQ ID NO:1).

[0014] Figure 4 shows a native chromosomal insertion site and flanking sequences in the cleavage and polyadenylation specific factor 4 (Cpsf4) gene of CHO cell genome (SEQ ID NO:2).

WHAT IS CLAIMED IS:

1. An *ex vivo* method for stable integration and expression of a heterologous polynucleotide in a Chinese Hamster Ovary (CHO) 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 239-240 of SEQ ID NO:4 for Nephrocystin-1/Mal gene.

2. The method of claim 1, wherein insertion of the heterologous polynucleotide is mediated by homologous recombination or by a hybrid recombinase.

3. The method of claim 1, wherein the heterologous polynucleotide encodes a polypeptide.

4. The method of claim 3, wherein the polypeptide is a therapeutic protein or an industrial protein.

5. An *ex vivo* method for stably integrating a heterologous polynucleotide into the genome of a CHO 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 239-240 of SEQ ID NO:4 for 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.

6. The method of claim 5, wherein the native chromosomal insertion site supports stable integration of a foreign gene.

7. The method of claim 5, wherein the site-specific recombination sequence is a first recognition sequence recognized by a phage integrase.

8. The method of claim 7, wherein the phage integrase is phiC-31 integrase.

9. The method of claim 7, wherein the first recognition sequence is an attP site or an attB site.

10. The method of claim 7, wherein the heterologous polynucleotide is attached to a second recognition sequence of the phage integrase which is cognate to the first recognition sequence.

11. The method of claim 10, wherein the second recognition sequence is an attB site or an attP site.

12. The method of claim 7, wherein the integration occurs in the presence of the phage integrase.

13. The method of claim 12, wherein the phage integrase is expressed from a vector introduced into the cell.

14. The method of claim 5, wherein the heterologous polynucleotide comprises a target polypeptide-encoding sequence that is operably linked to a promoter sequence.