

What is claim is:

1. A mammalian cell comprising a first Stable Integration Site located in a Genomic Safe Harbor and a second Stable Integration Site that is not located in the Genomic Safe Harbor, wherein the first Stable Integration Site comprises a first reporter gene encoding a first reporter protein and the second Stable Integration Site comprises a second reporter gene encoding a second reporter protein, wherein the first reporter protein and the second reporter protein are different.
2. The mammalian cell according to claim 1, wherein the first and second Stable Integration Sites comprise recombinase recognition sites.
3. The mammalian cell according to claim 1, wherein the mammalian cell is a human cell.
4. The mammalian cell according to claim 3, wherein the human cell is a Human Amniotic Epithelial Cell.
5. The mammalian cell according to claim 3, wherein the human cell is a HEK293 Cell.
6. The mammalian cell according to claim 1, wherein the mammalian cell is a CHO cell.
7. The mammalian cell according to claim 1, wherein the second Stable Integration Site is located in a second Genomic Safe Harbor that is different from the first Genomic Safe Harbor.
8. The mammalian cell according to claim 1, wherein the second Stable Integration Site is located in a region that is not a Genomic Safe Harbor.

9. A mammalian cell comprising a first Stable Integration Site located in a Genomic Safe Harbor and a second Stable Integration Site that is not located in the Genomic Safe Harbor, wherein the first Stable Integration Site comprises first polynucleotide encoding a first protein and the second Stable Integration Site comprises a second polynucleotide encoding a second protein.

10. The mammalian cell according to claim 9, wherein the second Stable Integration Site is located in a second Genomic Safe Harbor that is different from the first Genomic Safe Harbor.

11. The mammalian cell according to claim 9, wherein the second Stable Integration Site is located in a region that is not a Genomic Safe Harbor.

12. The mammalian cell according to claim 9, wherein the mammalian cell is a human cell.

13. The mammalian cell according to claim 9, wherein the mammalian cell is a CHO cell.

14. A mammalian cell comprising a first Stable Integration Site located in a Genomic Safe Harbor and a second Stable Integration Site that is not located in the Genomic Safe Harbor, wherein the first Stable Integration Site comprises a polynucleotide encoding a first reporter gene encoding a first reporter protein and the second Stable Integration Site comprises a polynucleotide encoding Cas9 and a polynucleotide encoding a second reporter gene encoding a second reporter protein, wherein the first reporter protein and the second reporter protein are different.

15. The mammalian cell according to claim 14, wherein the first and second Stable Integration Sites comprise recombinase recognition sites .

16. The mammalian cell according to claim 14, wherein the mammalian cell is a Human Amniotic Epithelial Cell.

17. The mammalian cell according to claim 14, wherein the mammalian cell is a HEK293 Cell.

18. The mammalian cell according to claim 14, wherein the mammalian cell is a BHK Cell.

19. The mammalian cell according to claim 14, wherein the mammalian cell is a CHO Cell.

20. A method for making at least one protein of interest, comprising:

- (a) providing a mammalian cell comprising a first Stable Integration Site located in a first Genomic Safe Harbor and a second Stable Integration Site that is not located in the first Genomic Safe Harbor, wherein the first Stable Integration Site comprises a first reporter gene encoding a first reporter protein and the second Stable Integration Site comprises a second reporter gene encoding a second reporter protein, wherein the first reporter protein and the second reporter protein are different, and wherein the first and second Stable Integration Sites comprise recombinase recognition sites;
- (b) introducing a polynucleotide encoding the protein of interest into a Stable Integration Site by recombinase mediated cassette exchange, and
- (c) culturing the mammalian cell under conditions that allow expression of the polynucleotide encoding the polynucleotide of interest.

21. The method according to claim 20, wherein the mammalian cell is a Human Amniotic Epithelial Cell.

22. The method according to claim 20, wherein the mammalian cell is a HEK293 cell.

23. The method according to claim 20, wherein the mammalian cell is a CHO cell.

24. The method according to claim 20, wherein the second Stable Integration Site is located in a second Genomic Safe Harbor that is different from the first Genomic Safe Harbor.

25. A mammalian cell comprising a modified genome, wherein the genome is modified by insertion of at least three DNA cassettes within different regions of the genome, wherein the modified genome comprises

(1) a first deoxyribonucleic acid sequence that is at least 90% identical to at least one selected from the group consisting of SEQ ID NOS: 1 and 2 prior to modification;

(2) a second deoxyribonucleic acid sequence that is at least 90% identical to at least one selected from the group consisting of SEQ ID NOS: 5 to 10 prior to modification; and

(3) a third deoxyribonucleic acid sequence that is at least 90% identical to at least one selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 12 prior to modification,

wherein the first deoxyribonucleic acid sequence is modified by insertion of a first DNA cassette, the second deoxyribonucleic acid sequence is modified by

insertion of a second DNA cassette, and the third deoxyribonucleic acid sequence is modified by insertion of a third DNA cassette.

26. The mammalian cell according to claim 25, wherein

- (1) the first deoxyribonucleic acid sequence is at least 95% identical to at least one selected from the group consisting of SEQ ID NOS: 1 and 2 prior to modification;
- (2) the second deoxyribonucleic acid sequence is at least 95% identical to at least one selected from the group consisting of SEQ ID NOS: 5 to 10 prior to modification; and
- (3) the third deoxyribonucleic acid sequence is at least 95% identical to at least one selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 12 prior to modification.

27. The mammalian cell according to claim 25, wherein

- (1) the first deoxyribonucleic acid sequence is at least 98% identical to at least one selected from the group consisting of SEQ ID NOS: 1 and 2 prior to modification;
- (2) the second deoxyribonucleic acid sequence is at least 98% identical to at least one selected from the group consisting of SEQ ID NOS: 5 to 10 prior to modification; and
- (3) the third deoxyribonucleic acid sequence is at least 98% identical to at least one selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 12 prior to modification.

28. The mammalian cell according to claim 25, wherein

- (1) the first deoxyribonucleic acid sequence is at least 99% identical to at least one selected from the group consisting of SEQ ID NOS: 1 and 2 prior to modification;

(2) the second deoxyribonucleic acid sequence is at least 99% identical to at least one selected from the group consisting of SEQ ID NOS: 5 to 10 prior to modification; and

(3) the third deoxyribonucleic acid sequence is at least 99% identical to at least one selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 12 prior to modification.

29. The mammalian cell according to claim 25, wherein

(a) the first DNA cassette comprises a promoter and at least one selected from the group consisting of a selectable marker gene and a reporter gene;

(b) the second DNA cassette comprises a promoter and at least one selected from the group consisting of a selectable marker gene and a reporter gene; and

(c) the third DNA cassette comprises a promoter and at least one selected from the group consisting of a selectable marker gene and a reporter gene.

30. The mammalian cell according to claim 29, wherein

(a) the first DNA cassette comprises a promoter, a selectable marker gene and a reporter gene;

(b) the second DNA cassette comprises a promoter, a selectable marker gene and a reporter gene; and

(c) the third DNA cassette comprises a promoter, a selectable marker gene and a reporter gene.

31. The mammalian cell according to claim 29, wherein the first deoxyribonucleic acid sequence comprises a Stable Integration Site.

32. The mammalian cell according to claim 31, wherein a gene of interest is inserted into the Stable Integration Site.

33. The mammalian cell according to claim 31, wherein the gene of interest encodes a polypeptide of interest selected from the group consisting of antibodies, antibody chains, receptors, Fc-containing proteins, trap proteins, enzymes, factors, repressors, activators, ligands, reporter proteins, selection proteins, protein hormones, protein toxins, structural proteins, storage proteins, transport proteins, neurotransmitters and contractile proteins.

34. The mammalian cell of claim 25, wherein the mammalian cell is a human cell and the first deoxyribonucleic acid sequence is at least 90% identical to SEQ ID NO: 1.

35. The mammalian cell of claim 25, wherein the mammalian cell is a CHO cell and the first deoxyribonucleic acid sequence is at least 90% identical to SEQ ID NO: 2.

36. The mammalian cell of claim 35, wherein the first deoxyribonucleic acid sequence comprises a Stable Integration Site produced using a guide sequence selected from the group consisting of SEQ ID NOS: 13 to 419.

37. The mammalian cell of claim 35, wherein the first deoxyribonucleic acid sequence comprises a Stable Integration Site produced by using a guide sequence that are complementary to target sequences in SEQ ID NO:2 at nucleotide position ranges selected from the group consisting of: (a) 1 to 2000; (b) 2001 to 4000; (c) 4001 to 6000; (d) 6001 to 8000; (e) 8001 to 10,000; (f) 10,001 to 12,000; (g) 12,001 to 14,000; (h) 14,001 to 16,000; (i) 16,001 to 18,000; (j) 18,001 to 20,000; (k) 20,001 to 22,000; (l) 22,001 to 24,000; (m) 24,001 to 26,000; (n) 26,001 to 28,000; (o)

28,001 to 30,000; (p) 30,001 to 32,000; (q) 32,001 to 34,000; (r) 34,001 to 36,000; (s) 36,001 to 38,000; (t) 38,001 to 40,000; (u) 40,001 to 42,000; and (v) 42,001 to 44,232.

38. A mammalian cell comprising a modified genome, wherein the modified genome comprises a deoxyribonucleic acid sequence comprising an AAVS1-like region modified by insertion of at least one DNA cassette, and wherein a guide sequence selected from the group consisting of SEQ ID NOS: 13 to 419 that is complementary to a sense or antisense strand of the AAVS1-like region.

39. The mammalian cell according to claim 38, further comprising a second deoxyribonucleic acid sequence that is at least 90% identical to at least one selected from the group consisting of SEQ ID NOS: 5 to 10 prior to modification; and a third deoxyribonucleic acid sequence that is at least 90% identical to at least one selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 12 prior to modification,

wherein the first deoxyribonucleic acid sequence is modified by insertion of a first DNA cassette, the second deoxyribonucleic acid sequence is modified by insertion of a second DNA cassette, and the third deoxyribonucleic acid sequence is modified by insertion of a third DNA cassette.

40. The mammalian cell of claim 39, wherein the second deoxyribonucleic acid sequence is at least 90% to 99%, 95% to 99% or 98% to 99% identical to at least one selected from the group consisting of one selected from the group

consisting of SEQ ID NOS: 5 to 10 prior to modification; and the third deoxyribonucleic acid sequence is at least 90% to 99%, 95% to 99% or 98% to 99% identical to at least one selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 12 prior to modification.

41. The mammalian cell of claim 38, wherein the first deoxyribonucleic acid sequence comprises a Stable Integration Site produced by using a guide sequence that is complementary to at least one target sequence having at least 50%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or (s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

42. The mammalian cell of claim 41, wherein the first deoxyribonucleic acid sequence comprises a Stable Integration Site produced by using a guide sequence that is complementary to at least one target sequence having at least 75%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or

(s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

43. The mammalian cell of claim 42, wherein the first deoxyribonucleic acid sequence comprises a Stable Integration Site produced by using a guide sequence that is complementary to at least one target sequence having at least 85%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or (s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

44. The mammalian cell of claim 43, wherein the first deoxyribonucleic acid sequence comprises a Stable Integration Site produced by using a guide sequence that is complementary to at least one target sequence having at least 90%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or (s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

45. The mammalian cell of claim 44, wherein the first deoxyribonucleic acid sequence comprises a Stable Integration Site produced by using a guide sequence that is complementary to at least one target sequence having at least 95%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or (s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

46. The mammalian cell of claim 45, wherein the first deoxyribonucleic acid sequence comprises a Stable Integration Site produced by using a guide sequence that is complementary to at least one target sequence having at least 98%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or (s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

47. A mammalian cell comprising a modified genome,

wherein the modified genome comprises a Stable Integration Site in a AAVS1-like region, wherein the Stable Integration Site is produced by using a guide sequence that is complementary to at least one target sequence having at least 50%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or (s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

48. A mammalian cell of claim 47, wherein the modified genome comprises a Stable Integration Site in a AAVS1-like region, wherein the Stable Integration Site is produced by using a guide sequence that is complementary to at least one target sequence having at least 75%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or (s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

49. A mammalian cell of claim 48, wherein the modified genome comprises a Stable Integration Site in a AAVS1-like region, wherein the Stable Integration Site

is produced by using a guide sequence that is complementary to at least one target sequence having at least 85%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or (s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

50. A mammalian cell of claim 49, wherein the modified genome comprises a Stable Integration Site in a AAVS1-like region, wherein the Stable Integration Site is produced by using a guide sequence that is complementary to at least one target sequence having at least 90%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or (s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

51. A mammalian cell of claim 50, wherein the modified genome comprises a Stable Integration Site in a AAVS1-like region, wherein the Stable Integration Site is produced by using a guide sequence that is complementary to at least one target sequence having at least 95%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or (s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

52. A mammalian cell of claim 51, wherein the modified genome comprises a Stable Integration Site in a AAVS1-like region, wherein the Stable Integration Site is produced by using a guide sequence that is complementary to at least one target sequence having at least 98%-99% identity to SEQ ID NO:2 at nucleotide positions:

(a) 1 to 2000; or (b) 2001 to 4000; or (c) 4001 to 6000; or (d) 6001 to 8000; or (e) 8001 to 10,000; or (f) 10,001 to 12,000; or (g) 12,001 to 14,000; or (h) 14,001 to 16,000; or (i) 16,001 to 18,000; or (j) 18,001 to 20,000; or (k) 20,001 to 22,000; or (l) 22,001 to 24,000; or (m) 24,001 to 26,000; or (n) 26,001 to 28,000; or (o) 28,001 to 30,000; or (p) 30,001 to 32,000; or (q) 32,001 to 34,000; or (r) 34,001 to 36,000; or (s) 36,001 to 38,000; or (t) 38,001 to 40,000; or (u) 40,001 to 42,000; or (v) 42,001 to 44,232.

53. A mammalian cell according to claim 47, further comprising a second deoxyribonucleic acid sequence that is at least 90% identical to at least one selected from the group consisting of SEQ ID NOS: 5 to 10 prior to modification; and

a third deoxyribonucleic acid sequence that is at least 90% identical to at least one selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 12 prior to modification,

wherein the first deoxyribonucleic acid sequence is modified by insertion of a first DNA cassette, the second deoxyribonucleic acid sequence is modified by insertion of a second DNA cassette, and the third deoxyribonucleic acid sequence is modified by insertion of a third DNA cassette.

54. The mammalian cell of claim 53, wherein the second deoxyribonucleic acid sequence at least is 90% to 99%, 95% to 99% or 98% to 99% identical to at least one selected from the group consisting of one selected from the group consisting of SEQ ID NOS: 5 to 10 prior to modification; and the third deoxyribonucleic acid sequence is at least 90% to 99%, 95% to 99% or 98% to 99% identical to at least one selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 12 prior to modification.

55. A method of producing protein, wherein the method comprises the steps of:

- (1) culturing mammalian cells according to claim 47; and
- (2) harvesting the protein.