WHAT IS CLAIMED:

1. An expression system comprising:

(a) a host cell in which the endogenous gene encoding an auxotrophic selectable marker protein has been removed from the genome of the host cell; and

(b) an integration vector comprising:

(1) a nucleic acid having an open reading frame (ORF) encoding a function that is complementary to the function of the endogenous gene encoding the auxotrophic selectable marker protein and which is operably linked to a weak promoter, an attenuated endogenous or heterologous promoter, a cryptic promoter, a truncated endogenous or heterologous promoter, or no promoter;

(2) a nucleic acid having an insertion site for the insertion of one or more expression cassettes comprising a nucleic acid encoding peptides, proteins, and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by homologous recombination.

2. The expression system of claim 2 wherein the auxotrophic selectable marker protein is encoded by a gene selected from the group consisting of *ADE, URA*, and *LYS*.

3. The expression system of claim 2 wherein the auxotrophic selectable marker protein is encoded by the *ADE1* gene.

4. The expression system of claim 2 wherein the auxotrophic selectable marker protein is encoded by the *ADE2* gene.

5. The expression system of claim 2, wherein the integration vector comprises multiple insertion sites for the insertion of one or more expression cassettes encoding the one or more heterologous peptides or proteins.

6. The expression system of claim 2, wherein the integration vector comprises more than one expression cassette.

7. The expression system of claim 6, wherein the integration vector comprises little or no homologous DNA sequence between the expression cassettes.

8. The expression system of claim 6, wherein the integration vector comprises a first expression cassette encoding a light chain of a monoclonal antibody and a second expression cassette encoding a heavy chain of a monoclonal antibody.

9. The expression system of claim 2, wherein the host cell is a lower eukaryote.

10. The expression system of claim 9, wherein the host cell is from a species selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia minuta* (*Ogataea minuta*, *Pichia lindneri*), *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia* sp., *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Hansenula polymorpha*, *Kluyveromyces* sp., *Kluyveromyces lactis*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium* sp., *Fusarium gramineum*, *Fusarium venenatum*, *Physcomitrella patens*, and *Neurospora crassa*.

11. The expression system of claim 2, wherein the host cell is *Pichia pastoris*.

12. The expression system of claim 11, wherein the *Pichia pastoris* cell has been modified to be capable of producing glycoproteins having hybrid or complex *N*-glycans.

13. A method for expressing a recombinant peptide, proteins, and/or nucleic acid of interest in a host cell comprising:

(a) providing the host cell in which the endogenous gene encoding an auxotrophic selectable marker protein has been removed from the genome of the host cell; and

(a) transforming the host cell with an integration vector comprising:

(1) a nucleic acid having an open reading frame (ORF) encoding a function that is complementary to the function of the endogenous gene encoding the auxotrophic selectable marker protein and which is operably linked to a weak promoter, an attenuated endogenous or heterologous promoter, a cryptic promoter, a truncated endogenous or heterologous promoter, or no promoter;

(2) a nucleic acid having one or more expression cassettes comprising a nucleic acid encoding peptides, proteins, and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by homologous recombination,

wherein the transformed host cell produces the recombinant peptide, protein and/or nucleic acid of interest.

14. The method of claim 14 wherein the auxotrophic marker is encoded by a gene selected from the group consisting of *ADE, URA*, and *LYS*.

15. The method of claim 14 wherein the auxotrophic marker protein is encoded by the *ADE1* gene

16. The method of claim 14 wherein the auxotrophic marker protein is encoded by the *ADE2* gene.

17. The method of claim 13, wherein the recombinant vector comprises multiple insertion sites for the insertion of one or more expression cassettes encoding the one or more heterologous peptides or proteins.

18. The method of claim 13, wherein the recombinant vector comprises more than one expression cassette.

19. The method of claim 18, wherein the recombinant vector comprises little or no homologous DNA sequence between the expression cassettes.

20. The method of claim 18, wherein the recombinant vector comprises a first expression cassette encoding a light chain of a monoclonal antibody and a second expression cassette encoding a heavy chain of a monoclonal antibody.

21. The method of claim 13, wherein the host cell is a lower eukaryote.

22. The method of claim 21, wherein the host cell is from a species selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia minuta* (*Ogataea minuta*, *Pichia lindneri*), *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia* sp., *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Hansenula polymorpha*, *Kluyveromyces* sp., *Kluyveromyces lactis*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium* sp., *Fusarium gramineum*, *Fusarium venenatum*, *Physcomitrella patens*, and *Neurospora crassa*.

23. The method of claim 13, wherein the host cell is *Pichia pastoris*.

24. The method of claim 23, wherein the *Pichia pastoris* cell has been modified to be capable of producing glycoproteins having hybrid or complex *N*-glycans.

25. An isolated nucleic acid comprising the *ADE2* gene of *Pichia pastoris*.

26. The nucleic acid of Claim 25 wherein the nucleic acid comprises the open reading frame that encodes the Ade2p protein.

27. The nucleic acid of Claim 26 wherein the nucleic acid has a nucleotide sequence with 95% identity to the nucleic acid sequence shown in SEQ ID NO:60 from nucleotide 127 to nucleotide 1,815.

28. A vector comprising the nucleic acid of Claim 27.

29. A *Pichia pastoris* cell comprising a deletion or disruption of the endogenous *ADE2* gene, or any other modification, rendering the *ADE2* gene or Ade2p non-functional.

30. The *Pichia pastoris* of Claim 29 wherein a vector comprising an *ADE2* open reading frame is integrated into a location in the *Pichia pastoris* genome other than the location where the endogenous *ADE2* gene had been located.

31. An isolated polypeptide comprising an amino acid sequence with 95% identity to the amino acid sequence shown in SEQ ID NO:61.

32. An expression system comprising:

(a) a *Pichia pastoris* host cell in which the endogenous *ADE2* gene encoding Ade2p has been removed from the genome of the host cell; and

(b) an integration vector comprising:

(1) a nucleic acid encoding the Ade2p;

(2) a nucleic acid having an insertion site for the insertion of one or more expression cassettes comprising a nucleic acid encoding one or more heterologous peptides, proteins, and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by homologous recombination.

33. A method for producing a recombinant *Pichia pastoris* host cell that expresses a heterologous peptide, proteins, and/or nucleic acid of interest comprising:

(a) providing the host cell in which the endogenous *ADE2* gene encoding an Ade2p has been removed from the genome of the host cell; and

(a) transforming the host cell with an integration vector comprising:

(1) a nucleic acid encoding the Ade2p;

(2) a nucleic acid having one or more expression cassettes comprising a nucleic acid encoding one or more heterologous peptides, proteins, and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by homologous recombination,

wherein the transformed host cell produces the recombinant peptide, protein, and/or nucleic acid of interest.

WHAT IS CLAIMED:

1. An expression system comprising:

(a) a *Pichia* host cell in which the *ADE2* gene represented by SEQ ID NO: 60 has been removed from the genome of the host cell; wherein the cell is auxotrophic for adenine; and

(b) an integration vector comprising:

(1) a nucleic acid having an open reading frame encoding a function that is complementary to the function of the endogenous *ADE2* gene operably linked to a promoter or no promoter wherein the nucleic acid consists of the nucleic acid sequence shown in SEQ ID NO: 60 from nucleotide 127 to nucleotide 1,815;

(2) a nucleic acid having an insertion site for the insertion of one or more expression cassettes comprising a nucleic acid encoding peptides, proteins, and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single-crossover homologous recombination; wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.

2. An expression system comprising:

(a) a *Pichia* host cell in which the *ADE1* gene has been removed from the genome of the host cell; wherein the cell is auxotrophic for adenine; and

(b) an integration vector comprising:

(1) A nucleic acid having an open reading frame encoding a function that is complementary to the function of the endogenous *ADE1* gene operably linked to a promoter or no promoter;

(2) a nucleic acid having an insertion site for the insertion of one or more expression cassettes comprising a nucleic acid encoding peptides, proteins, and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single-crossover homologous recombination; wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.

3. An expression system of claim 2, comprising:

(a) a *Pichia* host cell in which the *ADE1* gene represented by SEQ ID NO: 56 has been removed from the genome of the host cell; wherein the cell is auxotrophic for adenine; and

(b) an integration vector comprising:

(1) a nucleic acid having an open reading frame encoding a function that is complementary to the function of the endogenous *ADE1* gene operably linked to a promoter or no promoter wherein the nucleic acid consists of the nucleic acid sequence shown in SEQ ID NO: 56;

(2) a nucleic acid having an insertion site for the insertion of one or more expression cassettes comprising a nucleic acid encoding peptides, proteins, and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single-crossover homologous recombination; wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.

4. The expression system of any one of claims 1 to 3, wherein the integration vector comprises multiple insertion sites for the insertion of one or more expression cassettes encoding the one or more heterologous peptides or proteins.

5. The expression system of any one of claims 1 to 3, wherein the integration vector comprises more than one expression cassette.

6. The expression system of claim 5, wherein the integration vector comprises little or no homologous DNA sequence between the expression cassettes.

7. The expression system of claim 5, wherein the integration vector comprises a first expression cassette encoding a light chain of a monoclonal antibody and a second expression cassette encoding a heavy chain of a monoclonal antibody.

8. The expression system of any one of claims 1 to 3, wherein the *Pichia* host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia minuta* (*Ogataea minuta*, *Pichia lindneri*), *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, and *Pichia methanolica*.

9. The expression system of claim 8, wherein the *Pichia* host cell is *Pichia pastoris*.

10. The expression system of claim 9, wherein the *Pichia pastoris* cell has been modified to be capable of producing glycoproteins having hybrid or complex N-glycans.

11. A method for expressing a recombinant peptide, proteins, and/or nucleic acid of interest in a *Pichia* host cell comprising:

(a) providing the host cell in which the endogenous *ADE2* gene represented by SEQ ID NO: 60 has been removed from the genome of the host cell; wherein the host cell is auxotrophic for adenine; and

(b) transforming the host cell with an integration vector comprising:

(1) a nucleic acid having an open reading frame encoding a function that is complementary to the function of the endogenous *ADE2* gene operably linked to a promoter or no promoter wherein the nucleic acid consists of the nucleic acid sequence shown in SEQ ID NO: 60 from nucleotide 127 to nucleotide 1,815;

(2) a nucleic acid having one or more expression cassettes comprising a nucleic acid encoding peptides, proteins, and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single crossover homologous recombination, wherein the transformed host cell produces the recombinant peptide, protein and/or nucleic acid of interest; wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.

12. A method for expressing a recombinant peptide, protein and/or nucleic acid of interest in a *Pichia* host cell comprising:

(a) providing the host cell in which the endogenous *ADE1* gene has been removed from the genome of the host cell, wherein the host cell is auxotrophic for adenine; and

(b) transfom1ing the host cell with an integration vector comprising:

(1) a nucleic acid having an open reading frame encoding a function that is complementary to the function of the endogenous *ADE1* gene operably linked to a promoter or no promoter;

(2) a nucleic acid having one or more expression cassettes comprising a nucleic acid encoding peptides, proteins, and/or functional nucleic acids of interest,

and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single crossover homologous recombination, wherein the

transformed host cell produces the recombinant peptide, protein and/or nucleic acid of interest; wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.

13. The method of claim 12, comprising:

a) providing the host cell in which the endogenous *ADE1* gene represented by SEQ ID NO: 56 has been removed from the genome of the host cell, wherein the host cell is auxotrophic for adenine; and

(b) transforming the host cell with an integration vector comprising:

(1) a nucleic acid having an open reading frame encoding a function that is complementary to the function of the endogenous *ADE1* gene operably linked to a promoter or no promoter wherein the nucleic acid consists of the nucleic acid sequence shown in SEQ ID NO: 56;

(2 ) a nucleic acid having one or more expression cassettes comprising a nucleic acid encoding peptides, proteins, and/or functional nucleic acids of interest,

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single crossover homologous recombination,

wherein the transformed host cell produces the recombinant peptide, protein and/or nucleic acid of interest; and wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.

14. The method of any one of claims 11 to 13, wherein the recombinant vector comprises multiple insertion sites for the insertion of one or more expression cassettes encoding the one or more heterologous peptides or proteins.

15. The method of any one of claims 11-13, wherein the recombinant vector comprises more than one expression cassette.

16. The method of claim 15, wherein the recombinant vector comprises little or no homologous DNA sequence between the expression cassettes.

17. The method of claim 15, wherein the recombinant vector comprises a first expression cassette encoding a light chain of a monoclonal antibody and a second expression cassette encoding a heavy chain of a monoclonal antibody.

18. The method of any one of claims 11 to 13, wherein the *Pichia* host cell is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia minuta* (*Ogataea minuta*, *Pichia lindneri*), *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, and *Pichia methanolica*.

19. The method of claim 18, wherein the *Pichia* is *Pichia pastoris*.

20. The method of claim 19, wherein the *Pichia pastoris* cell has been modified to be capable of producing glycoproteins having hybrid or complex N-glycans.

21. A *Pichia pastoris* cell prototrophic for adenine comprising a deletion or disruption of the endogenous *ADE2* gene, or any other modification, rendering the *ADE2* gene or Ade2p non-functional and more than one copy of a vector comprising an *ADE2* open reading frame operably linked to a promoter or no promoter integrated by roll-in single-crossover homologous recombination into a location in the *Pichia pastoris* genome other than the location where the endogenous *ADE2* gene had been located.

22. An expression system comprising:

(a) a *Pichia pastoris* host cell in which the endogenous *ADE2* gene encoding Ade2p represented by SEQ ID N0:60 has been removed from the genome of the host cell; wherein said cell is auxotrophic for adenine; and

(b) an integration vector comprising:

(1) a nucleic acid encoding the Ade2p protein having the amino acid sequence set forth in SEQ ID NO: 61;

(2) a nucleic acid having an insertion site for the insertion of one or more expression cassettes comprising a nucleic acid encoding one or more heterologous peptides, proteins, and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single-crossover homologous recombination; wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.

23. An expression system comprising:

(a) a *Pichia pastoris* host cell in which the endogenous *ADE1* gene encoding Ade1p has been removed from the genome of the host cell; wherein said cell is auxotrophic for adenine; and

(b) an integration vector comprising:

(1) a nucleic acid encoding the Ade1p;

(2) a nucleic acid having an insertion site for the insertion of one or more expression cassettes comprising a nucleic acid encoding one or more heterologous peptides, proteins. and/or functional nucleic acids of interest and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single-crossover homologous recombination; wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.

24. The expression system of claim 23, comprising:

(a) a *Pichia pastoris* host cell in which the endogenous *ADE1* gene encoding Ade1p represented by SEQ ID NO:56 has been removed from the genome of the host cell; wherein said cell is auxotrophic for adenine; and

(b) an integration vector comprising:

(1) a nucleic acid encoding the Ade1 protein having the amino acid sequence set forth in SEQ ID NO: 57;

(2) a nucleic acid having an insertion site for the insertion of one or more expression cassettes comprising a nucleic acid encoding one or more heterologous peptides, proteins and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single-crossover homologous recombination; wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.

25. A method for producing a recombinant *Pichia pastoris* host cell that expresses a heterologous peptide, proteins, and/or nucleic acid of interest comprising:

(a) providing the host cell in which the endogenous *ADE2* gene encoding Ade2p represented by SEQ ID NO: 60 has been removed from the genome of the host cell; wherein said cell is auxotrophic for adenine; and

(b) transforming the host cell with an integration vector comprising:

(1) a nucleic acid encoding the Ade2p protein having the amino acid sequence set forth in SEQ ID NO: 61;

(2) a nucleic acid having one or more expression cassettes comprising a nucleic acid encoding one or more heterologous peptides, proteins, and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single-crossover homologous recombination, wherein the transformed host cell produces the recombinant peptide, protein, and/or nucleic acid of interest; wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.

26. A method for producing a recombinant *Pichia pastoris* host cell that expresses a heterologous peptide, proteins, and/or nucleic acid of interest comprising:

(a) providing the host cell in which the endogenous *ADE1* gene encoding Ade1p has been removed from the genome of the host cell; wherein said cell is auxotrophic for adenine; and

(b) transforming the host cell with an integration vector comprising:

(1) a nucleic acid encoding the Ade1p;

(2) a nucleic acid having one or more expression cassettes comprising a nucleic acid encoding one or more heterologous peptides, proteins and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single-crossover homologous recombination, wherein the transformed host cell produces the recombinant peptide, protein, and/or nucleic acid of interest; wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.

27. The method of claim 26, comprising:

(a) providing the host cell in which the endogenous *ADE1* gene encoding Ade1p represented by SEQ ID NO:56 has been removed from the genome of the host cell; wherein said cell is auxotrophic for adenine; and

(b) transforming the host cell with an integration vector comprising:

(1) a nucleic acid encoding the Ade1p protein having the amino acid sequence set forth in SEQ 1D NO: 57;

(2) a nucleic acid having one or more expression cassettes comprising a nucleic acid encoding one or more heterologous peptides, proteins, and/or functional nucleic acids of interest, and

(3) a targeting nucleic acid that directs insertion of the integration vector into a particular location of the genome of the host cell by roll-in single-crossover homologous recombination, wherein the transfom1ed host cell produces the recombinant peptide, protein, and/or nucleic acid of interest; wherein the auxotrophy of said host cell is not fully complemented by the nucleic acid having the open reading frame when the nucleic acid is in the host cell.