AOX1 promoters described herein can be used for efficient expression of operably linked coding sequences in *Pichia*, for example.

In one example, provided herein is a nucleic acid construct comprising a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element includes a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28. In one aspect, the present disclosure provides a nucleic acid construct comprising a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element comprises a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28.

Implementations can have one or more of the following features. The first alcohol oxidase promoter element can include a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 673-729 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 678-724 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 683-719 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 688-714 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include two or more mutations at nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include three or more mutations at nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include four or more mutations at nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include five or more mutations at nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28.

In another example, provided herein is a nucleic acid construct comprising a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element can include one or more mutations at a nucleotide position selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28. In another aspect, the present disclosure provides a nucleic acid construct comprising a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element comprises one or more mutations at a nucleotide position selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28.

Implementations can include one or more of the following features. The first alcohol oxidase promoter element can include two or more mutations at nucleotide positions selected

from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include three or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include four or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include five or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include one or more mutations at a nucleotide position selected from the group consisting of nucleotide positions corresponding to T688, A696, T702, A712, and T714 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include two or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to T688, A696, T702, A712, and T714 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include three or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to T688, A696, T702, A712, and T714 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include four or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to T688, A696, T702, A712, and T714 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include mutations at nucleotide positions corresponding to T688, A696, T702, A712, and T714 relative to SEQ ID NO: 28.

In another example, provided herein is a nucleic acid construct comprising a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element can include one or more mutations selected from the group consisting of mutations corresponding to T146C,

C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. In one aspect, the present disclosure provides a nucleic acid construct comprising a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element comprises one or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28.

Implementations can include one or more of the following features. The first alcohol oxidase promoter element can include two or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include three or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include four or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include five or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include one or more mutations selected from the group consisting of T688C, A696T, T702C, A712G, and T714G relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include two or more mutations selected from the group consisting of T688C, A696T, T702C, A712G, and T714G relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include three or more mutations selected from the group consisting of T688C, A696T, T702C, A712G, and T714G relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include four or more mutations selected from the group consisting of T688C, A696T, T702C, A712G, and T714G relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include the mutations T688C, A696T, T702C, A712G, and T714G relative to SEQ ID NO: 28.

Implementations of any of the nucleic acid constructs described herein can have one or more of the following features. The first alcohol oxidase promoter element can be an alcohol oxidase 1 promoter element. The first alcohol oxidase promoter element can have at least 90%

sequence identity to SEQ ID NO: 28. The first alcohol oxidase promoter element can have at least 95% sequence identity to SEQ ID NO: 28. The nucleic acid construct can further include a nucleotide sequence encoding a first protein, wherein the nucleotide sequence encoding the first protein is operably linked to the first alcohol oxidase promoter element. The first protein can be exogenous to a methylotrophic yeast cell. The first protein can be heterologous to a methylotrophic yeast cell. The first protein can be selected from the group consisting of an antibody or fragment thereof, an enzyme, a regulatory protein, a peptide hormone, a blood clotting protein, a cytokine, a cytokine inhibitor, and a heme-binding protein. The first protein can be a heme-binding protein. The heme-binding protein can be selected from the group consisting of a globin, a cytochrome, a cytochrome c oxidase, a ligninase, a catalase, and a peroxidase. The heme-binding protein can be selected from the group consisting of an androglobin, a chlorocruorin, a cytoglobin, an erythrocruorin, a flavohemoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a histoglobin, a leghemoglobin, a myoglobin, a neuroglobin, a non-symbiotic hemoglobin, a protoglobin, and a truncated hemoglobin. The heme-binding protein can be a non-symbiotic hemoglobin. The heme-binding protein can be a leghemoglobin. The heme-binding protein can include an amino acid sequence having at least 90% sequence identity to the amino acid sequence of any of SEQ ID NOs: 1-27. The first alcohol oxidase promoter element can include a recognition sequence for a transcription factor.

In one aspect, the present disclosure provides a cell comprising a first nucleic acid construct, wherein the first nucleic acid construct is any one of the nucleic acid construct described herein. In another example, also provided herein is a methylotrophic yeast cell comprising a first nucleic acid construct, wherein the first nucleic acid construct is any nucleic acid construct described herein.

Implementations can have one or more of the following features. The methylotrophic yeast cell can be a *Pichia* cell, a *Candida* cell, a *Hansenula* cell, or a *Torulopsis* cell. The methylotrophic yeast cell can be a *Pichia methanolica* cell, a *Pichia pastoris* cell, a *Candida boidinii* cell, or a *Hansenula polymorpha* cell. The methylotrophic yeast cell can be a *Pichia pastoris* cell. The methylotrophic yeast cell can further include a second nucleic acid construct including a nucleotide sequence encoding a second protein, wherein the nucleotide sequence encoding the second protein is operably linked to the first alcohol oxidase promoter element or to a second promoter element. The nucleotide sequence encoding the second protein can be operably linked to a second promoter element that has the same sequence as the first alcohol

oxidase promoter element. The second protein can be a transcription factor. The nucleotide sequence encoding the second protein can be operably linked to a second promoter element that can include a recognition sequence for the transcription factor. The first alcohol oxidase promoter element can include a recognition sequence for the transcription factor. The second protein can be a protein involved in heme biosynthesis. The protein involved in heme biosynthesis can be selected from the group consisting of aminolevulinic acid synthase (ALAS), δ-aminolevulinic acid dehydratase (ALAD), porphogilinogen deaminase (PBGD), uroporphyrinogen III synthase (UPG3S), uroporphyrinogen III decarboxylase (UPG3D), coprotoporphyrinogen oxidase (COPROX), protoporphyrinogen IX oxidase (PROTOX), and ferrochelatase (FC).

In another aspect, the present disclosure provides a method of producing a product in a cell comprising expressing a nucleic acid construct comprising a nucleotide sequence operably linked to a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element comprises a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28. In another example, provided herein is method of producing a protein in a methylotrophic yeast cell including expressing a nucleic acid construct including a nucleotide sequence encoding a first protein operably linked to a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element includes a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28.

Implementations can include one or more of the following features. The first alcohol oxidase promoter element can include a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 673-729 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 678-724 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 683-719 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 688-714 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include two or more mutations at nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include three or more mutations at nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include four or more mutations at nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28. The first

alcohol oxidase promoter element can include five or more mutations at nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28.

In yet another aspect, the present disclosure provides a method of producing a product in a cell comprising expressing a nucleic acid construct comprising a nucleotide sequence operably linked to a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element comprises one or more mutations at a nucleotide position selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28. In another example, also provided herein is a method of producing a protein in a methylotrophic yeast cell including expressing a nucleic acid construct including a nucleotide sequence encoding a first protein operably linked to a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element includes one or more mutations at a nucleotide position selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28.

Implementations can include one or more of the following features. The first alcohol oxidase promoter element can include two or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include three or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include four or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include five or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include one or more mutations at a nucleotide position selected from the group consisting of nucleotide positions corresponding to of T688, A696, T702, A712, and T714 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include two or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to of T688, A696, T702, A712, and T714 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include three or more mutations at nucleotide

positions selected from the group consisting of nucleotide positions corresponding to of T688, A696, T702, A712, and T714 relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include four or more mutations at nucleotide positions selected from the group consisting of nucleotide positions corresponding to of T688, A696, T702, A712, and T714 as compared to SEQ ID NO: 28. The first alcohol oxidase promoter element can include mutations at nucleotide positions corresponding to T688, A696, T702, A712, and T714 as compared to SEQ ID NO: 28.

In one aspect, the present disclosure provides a method of producing a product in a cell comprising expressing a nucleic acid construct comprising a nucleotide sequence operably linked to a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element includes one or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. In another example, provided herein is a method of producing a protein in a methylotrophic yeast cell including expressing a nucleic acid construct including a nucleotide sequence encoding a first protein operably linked to a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element includes one or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28.

Implementations can include one or more of the following features. The first alcohol oxidase promoter element can include two or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include three or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include four or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include five or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. The first alcohol oxidase promoter element can include two or more mutations selected from

same sequence as the first alcohol oxidase promoter element. The second protein can be a transcription factor. The nucleotide sequence encoding the second protein can be operably linked to a second promoter element that can include a recognition sequence for the transcription factor. The first alcohol oxidase promoter element can include a recognition sequence for the transcription factor. The second protein can be a protein involved in heme biosynthesis. The protein involved in heme biosynthesis can be selected from the group consisting of ALAS, ALAD, PBGD, UPG3S, UPG3D, COPROX, PROTOX, and FC. The method can be carried out in the absence of added methanol.

In another example, provided herein is a *Pichia pastoris* cell including a nucleic acid construct comprising a nucleotide sequence encoding a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element includes one or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. In some examples, the one or more mutations can be selected from the group consisting of mutations corresponding to T688C, A696T, T702C, A712G, and T714G relative to SEQ ID NO: 28.

In another example, also provided herein is a method of producing leghemoglobin, the method including expressing a nucleic acid construct including a nucleotide sequence encoding leghemoglobin operably linked to a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element includes one or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. In some examples, the method can be carried out in the absence of added methanol. In some examples, the one or more mutations can be selected from the group consisting of mutations corresponding to T688C, A696T, T702C, A712G, and T714G relative to SEQ ID NO: 28.

In another example, provided herein is a *Pichia pastoris* cell including a first nucleic acid construct including a nucleotide sequence with at least 90% sequence identity to SEQ ID NO: 28, wherein the first nucleic acid construct includes one or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G,

T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28. In some examples, the one or more mutations can be selected from the group consisting of mutations corresponding to T688C, A696T, T702C, A712G, and T714G relative to SEQ ID NO: 28.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more examples of the disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the disclosure will be apparent from the description and drawings, and from the claims. The word "comprising" in the claims may be replaced by "consisting essentially of" or with "consisting of," according to standard practice in patent law.

DESCRIPTION OF THE DRAWINGS

Figure 1 provides the sequences of exemplary heme-binding proteins (SEQ ID NOs: 1-27).

Figure 2 provides the sequences of pAOX1 wild-type and mutant sequences (SEQ ID NOs: 28-29).

Figure 3 is an image showing growth of pMx0414 transformants on YPD medium.

Figure 4 is a graph plotting the relative expression of GFP in strains MxY0270 and MxY0279 under different growth conditions.

Figure 5 is a comparison of portions of the sequence of MxG0038 and MxG0220.

Figure 6 is a graph plotting the relative expression of GFP in strains MxY0964, MxY965, and MxY1039.

Figure 7 provides the sequences of SEQ ID NOs: 30-37.

WHAT IS CLAIMED IS:

1. A nucleic acid construct comprising a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element comprises a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28.

2. A nucleic acid construct comprising a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element comprises one or more mutations at a nucleotide position selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28.

3. The nucleic acid construct of any one of claims 1-2, wherein the first alcohol oxidase promoter element comprises one or more mutations at a nucleotide position selected from the group consisting of nucleotide positions corresponding to T688, A696, T702, A712, and T714 relative to SEQ ID NO: 28.

4. The nucleic acid construct of any one of claims 1-3, wherein the first alcohol oxidase promoter element comprises mutations at nucleotide positions corresponding to T688, A696, T702, A712, and T714 relative to SEQ ID NO: 28.

5. A nucleic acid construct comprising a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element comprises one or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28.

6. The nucleic acid construct of any one of claims 1-5, wherein the first alcohol oxidase promoter element comprises one or more mutations selected from the group consisting of T688C, A696T, T702C, A712G, and T714G relative to SEQ ID NO: 28.

7. The nucleic acid construct of any one of claims 1-5, wherein the first alcohol oxidase promoter element comprises the mutations T688C, A696T, T702C, A712G, and T714G relative to SEQ ID NO: 28.

8. The nucleic acid construct of any one of claims 1-7, wherein the first alcohol oxidase promoter element is an alcohol oxidase 1 (AOX1) promoter element.

9. The nucleic acid construct of any one of claims 1-8, wherein the first alcohol oxidase promoter element has at least 90% sequence identity to SEQ ID NO: 28.

10. The nucleic acid construct of any one of claims 1-9, further comprising a nucleotide sequence, wherein the nucleotide sequence is operably linked to the first alcohol oxidase promoter element.

11. The nucleic acid construct of claim 10, wherein the nucleotide sequence encodes a first protein.

12. The nucleic acid construct of claim 11, wherein the first protein is selected from the group consisting of an antibody or fragment thereof, an enzyme, a regulatory protein, a peptide hormone, a blood clotting protein, a cytokine, a cytokine inhibitor, and a heme-binding protein.

13. The nucleic acid construct of any one of claims 11-12, wherein the first protein is a heme-binding protein.

14. The nucleic acid construct of claim 13, wherein the heme-binding protein comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of any of SEQ ID NOs: 1-27.

15. A cell comprising a first nucleic acid construct, wherein the first nucleic acid construct is the nucleic acid construct of any one of claims 1-14.

16. The cell of claim 15, further comprising a second nucleic acid construct comprising a nucleotide sequence, wherein the nucleotide sequence is operably linked to the first alcohol oxidase promoter element or to a second promoter element.

17. A method of producing a product in a cell comprising:

expressing a nucleic acid construct comprising a nucleotide sequence operably linked to a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element comprises a mutation at one or more nucleotide positions corresponding to any of nucleotide positions 668-734 relative to SEQ ID NO: 28.

18. A method of producing a product in a cell comprising:

expressing a nucleic acid construct comprising a nucleotide sequence operably linked to a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element comprises one or more mutations at a nucleotide position selected from the group consisting of nucleotide positions corresponding to T146, C154, T303, T426, A433, A435, T530, C572, T596, T617, T688, A696, T702, A709, A712, T714, A790, A841, and T862 relative to SEQ ID NO: 28.

19. The method of any one of claims 17-18, wherein the first alcohol oxidase promoter element includes one or more mutations at a nucleotide position selected from the group consisting of nucleotide positions corresponding to of T688, A696, T702, A712, and T714 relative to SEQ ID NO: 28.

20. A method of producing a product in a cell comprising:

expressing a nucleic acid construct comprising a nucleotide sequence operably linked to a first alcohol oxidase promoter element, wherein the first alcohol oxidase promoter element includes one or more mutations selected from the group consisting of mutations corresponding to T146C, C154T, T303C, T426A, A433T, A435G, T530A, C572T, T596C, T617C, T688C, A696T, T702C, A709G, A712G, T714G, A790G, A841T, and T862A relative to SEQ ID NO: 28.

21. The method of any one of claims 17-20, wherein the first alcohol oxidase promoter element is an alcohol oxidase 1 (AOX1) promoter element.

22. The method of any one of claims 17-21, wherein the nucleotide sequence operably linked to the first alcohol oxidase promoter element encodes a first protein.

23. The method of claim 22, wherein the first protein is selected from the group consisting of an antibody or fragment thereof, an enzyme, a regulatory protein, a peptide hormone, a blood clotting protein, a cytokine, and a heme-binding protein.

24. The method of any one of claims 22-23, wherein the first protein is a heme-binding protein.

25. The method of any one of claims 17-24, wherein the method is carried out in the absence of added methanol.