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(54) **HERBICIDE TOLERANCE GENES AND METHODS OF USE THEREOF**

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(52) **U.S. Cl.**
CPC **C12N 15/8274** (2013.01); **C12N 9/0071** (2013.01); **A01N 57/00** (2013.01); **C12Y 114/11033** (2013.01)

(21) Appl. No.: **14/871,768**

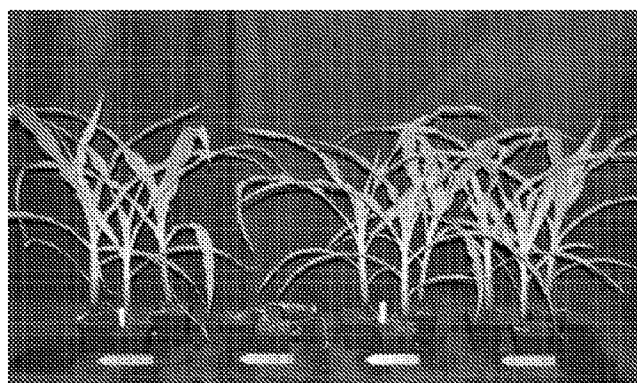
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(60) Provisional application No. 62/064,343, filed on Oct. 15, 2014.

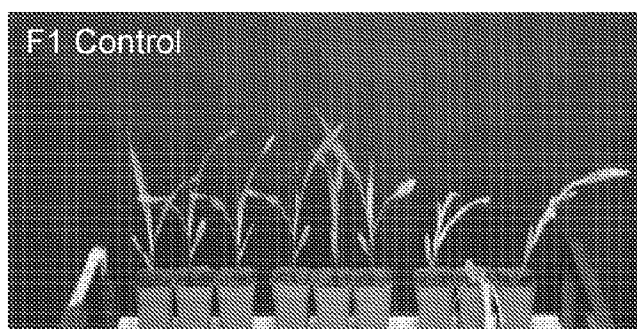
(57) **ABSTRACT**

Polypeptides and recombinant DNA molecules useful for conferring tolerance to AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides are provided in the present invention, as well as herbicide tolerant transgenic plants, seeds, cells, and plant parts containing the recombinant DNA molecules, as well as methods of using the same.



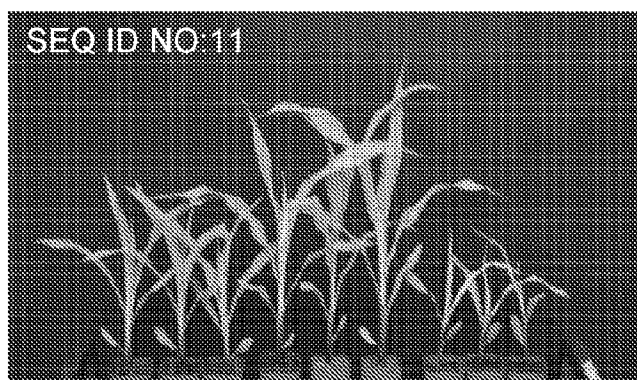
Untreated Quizalofop Untreated Quizalofop
LH244 SEQ ID NO:11

FIG. 1A



20C/20C 28C/20C 38C/30C

FIG. 1B



20C/20C 28C/20C 38C/30C

FIG. 1C

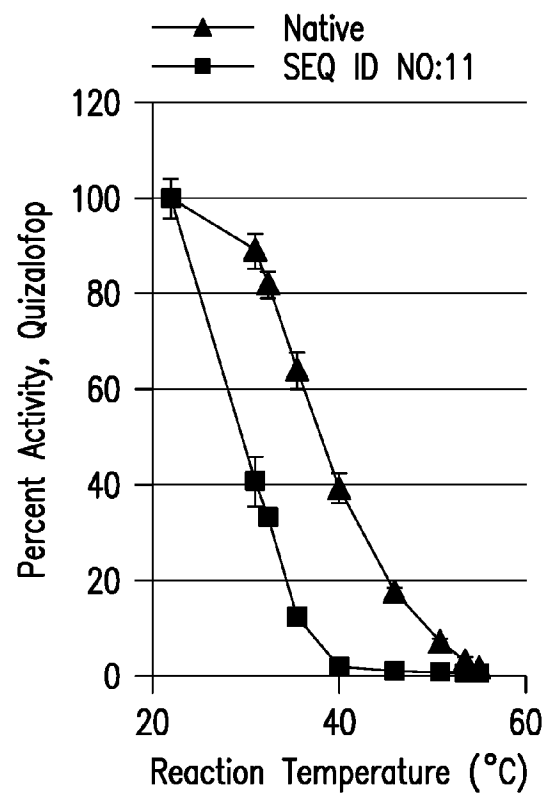


FIG.2A

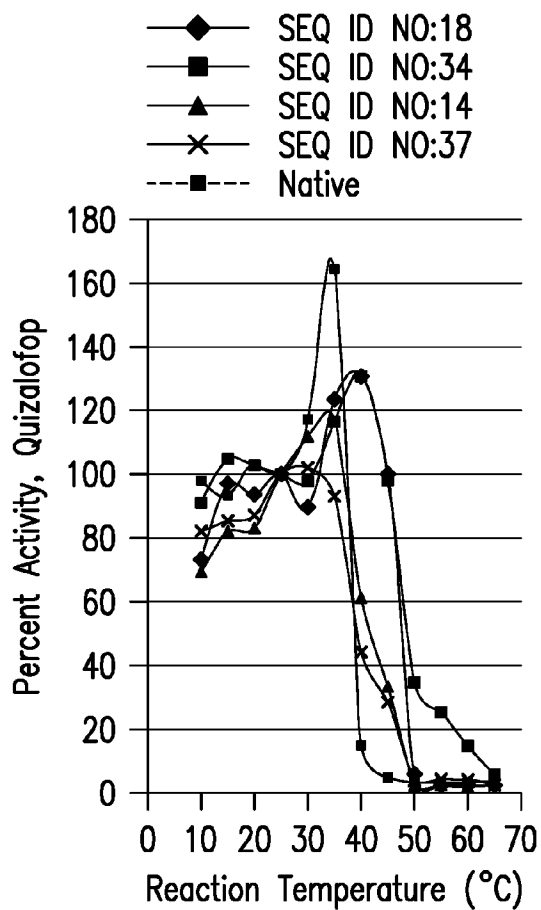


FIG.2B

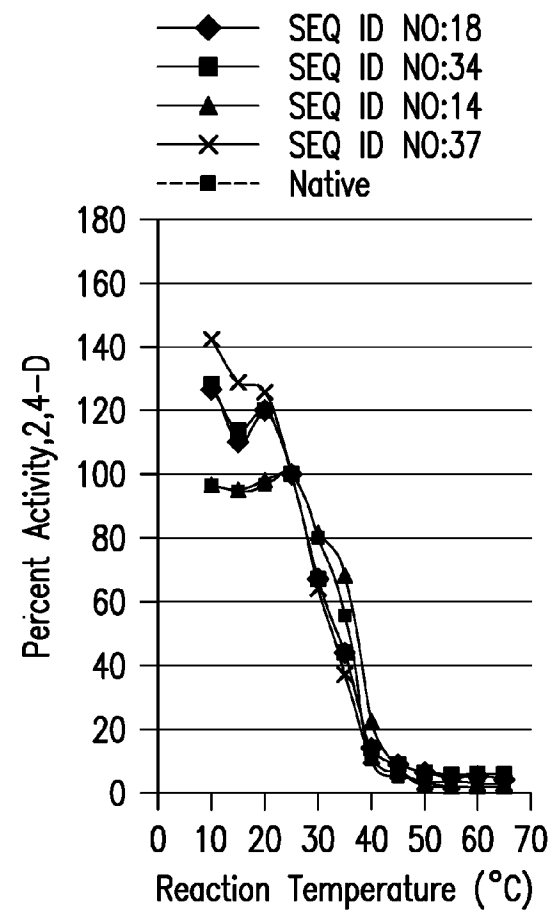


FIG.2C

MHAALSPLSQRFERIAVQPLTGVLGAEITGV¹DLREPLDD²STWNEIL
DAFHTYQVIYFPGQ³AITNEQH⁴AFSRRFGPVD⁵PV⁶PL⁷LKSIEGYPEV
QMIRREANES⁸GR⁹V¹⁰IGDDWH¹¹TD¹²ST¹³FLDAPPAAVVMRAIDVPEHGGDT
GF¹⁴L¹⁵SMY¹⁶T¹⁷AWETLSPTMQATIEGLNVVHSAT¹⁸RVFGSLYQA¹⁹Q²⁰NRRFSN
TSVKVMDVDAGDRETVHPLVVTHP²¹GS²²GRK²³GLYVNQVYCQRIEGMT²⁴D²⁵
AESK²⁶P²⁷LLQFLYEHA²⁸TR²⁹FDFTCRVRWKKDQVLWDNLCT³⁰M³¹HRA³²VPDY
AGKFRYLTRTTVGGVRP³³AR³⁴

FIG.3

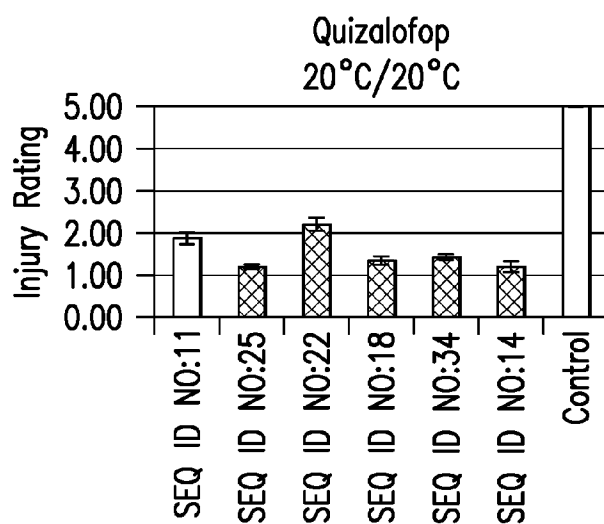


FIG.4A

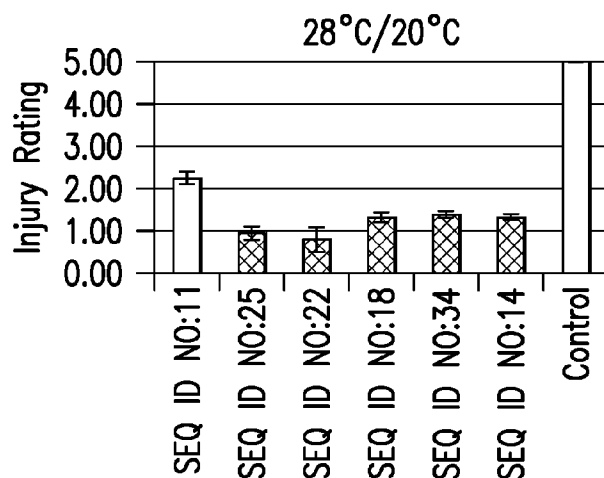


FIG.4B

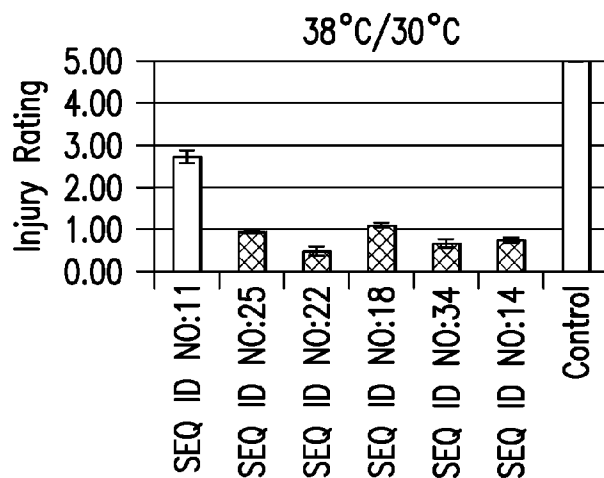


FIG.4C

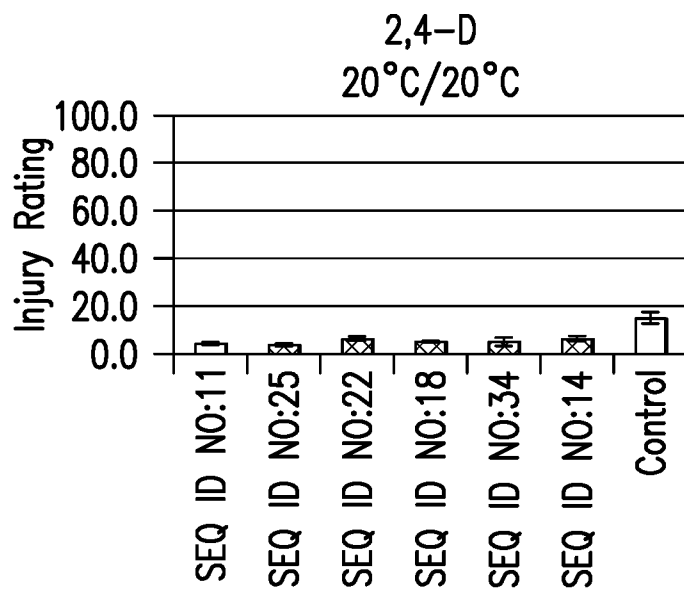


FIG.4D

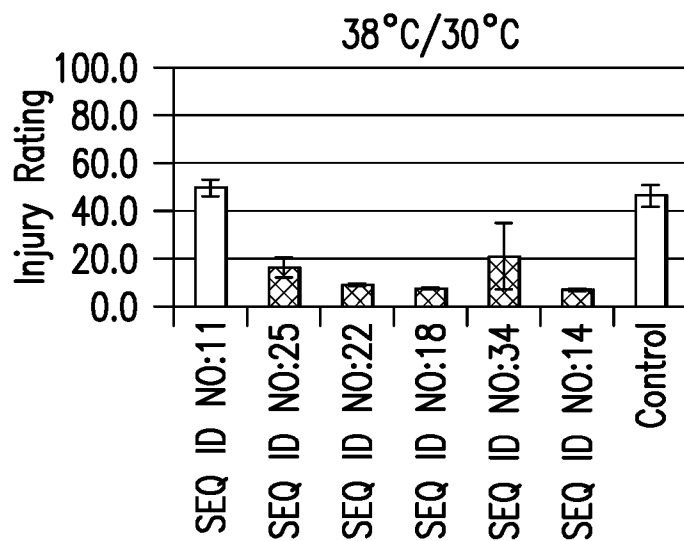


FIG.4E

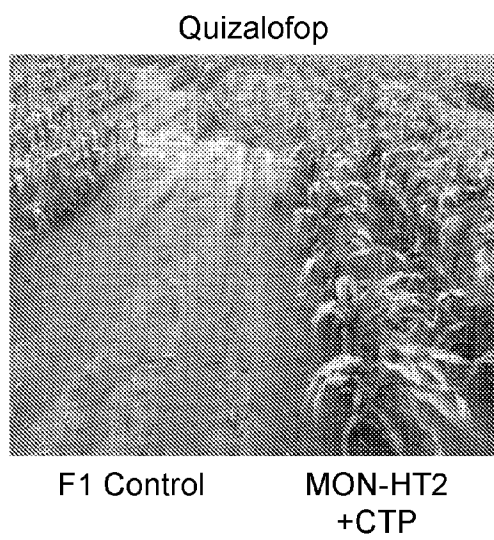


FIG.5A

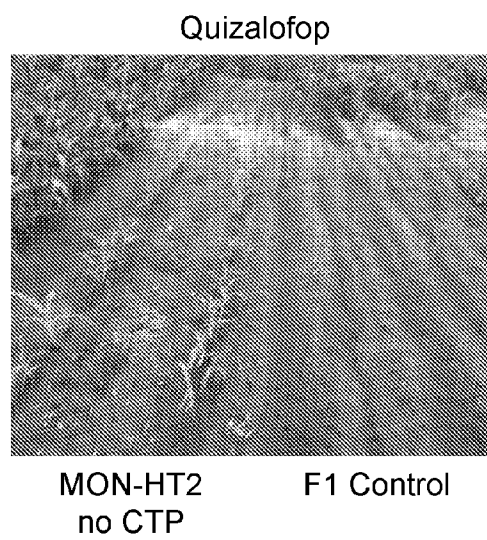


FIG.5B

SEQ ID NO:60	MHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:1	MHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLSDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:4	MHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQQAIF	70
SEQ ID NO:7	MHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLSDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:9	MHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLSDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:11	MHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:14	MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:18	MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:22	MHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:25	MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:28	MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:31	MHAALSPLSQKYRFIDVQPLTGVLGAEITGVTLREPLDDNTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:34	MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:37	MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:40	MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPAQQITNEQHISF	70
SEQ ID NO:43	MHAALTPLTNKYRFIDVQPLCGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:46	MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:47	MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:48	MHAALTPLTNKYRHIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:49	MHAALTPLTNKYRHIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:50	MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:51	MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
SEQ ID NO:52	MHAALTPLTNKYRHIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQAITNEQHIAF	70
Consensus	MHAAL•PL•.....I•VQPLtGVLGAEITGVdLREPLdDsTWNEILDAFHTYQVIYFgQaITNEQhIaF	70

FIG.6A

SEQ ID NO:60	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESGRVIGDDWHTDSTFLDAPPAAVVMRAIDVPEHGGDTGF	140
SEQ ID NO:1	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESGRVIGDDWHTDSSF	140
SEQ ID NO:4	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESGRVIGDDWHTDSTFLDAPPAAVVMRAIDVPEHGGDTGF	140
SEQ ID NO:7	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESGRVIGDDWHTDSTFLDAPPAAVVMRAIDVPEHGGDTGF	140
SEQ ID NO:9	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESGRVIGDDWHTDSTFLDAPPAAVVMRAIDVPEHGGDTGF	140
SEQ ID NO:11	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESGRVIGENWHTDSTFLDAPPAAVVMYAKEIPPYGGDTLF	140
SEQ ID NO:14	SRRFGPVDVPVILKSIEGYPEVQMIIRREANESSRFIGDDWHTDSTFLDAPPAAVVMRAIEVPEYGGDTGF	140
SEQ ID NO:18	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESSRFIGDDWHTDSTFLDAPPAAVVMRAIEVPEYGGDTGF	140
SEQ ID NO:22	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESGRVIGDDWHTDSTFLDAPPAAVVMYAKEVPPYGGDTLF	140
SEQ ID NO:25	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESGRVIGDDWHTDSTFLDAPPAAVVMYAREVPPYGGDTGF	140
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SEQ ID NO:31	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESGRVIGDDWHTDSTFLDAPPAAVVMYAREVPPYGGDTGF	140
SEQ ID NO:34	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESGRVIGDDWHTDSTFLDAPPAAVVMRAIEVPEYGGDTGF	140
SEQ ID NO:37	SRRFGPVDVPVILKSIEGYPEVQMIIRREANESGRVIGDDWHTDSTFLDAPPAAVVMRAIEVPEYGGDTGF	140
SEQ ID NO:40	SRRFGPVDVPVLLKSIEGYPEVQMIIRREANESGRVIGDDWHTDSTFLDAPPAAVVMRAIEVPEYGGDTGF	140
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SEQ ID NO:48	SRRFGPVDVPVILKSIEGYPEVQMIIRREANESSRYIGDDWHTDSTFLDAPPAAVVMRAIEVPEYGGDTGF	140
SEQ ID NO:49	SRRFGPVDVPVILKSIEGYPEVQMIIRREANESSRYIGDDWHTDSTFLDAPPAAVVMRAIEVPEYGGDTGF	140
SEQ ID NO:50	SRRFGPVDVPVILKSIEGYPEVQMIIRREANESSRYIGDDWHTDSTFLDAPPAAVVMRAIEVPEYGGDTGF	140
SEQ ID NO:51	SRRFGPVDVPVILKSIEGYPEVQMIIRREANESSRYIGDDWHTDSTFLDAPPAAVVMRAIEVPEYGGDTGF	140
SEQ ID NO:52	SRRFGPVDVPVILKSIEGYPEVQMIIRREANESSRYIGDDWHTDSTFLDAPPAAVVMRAIEVPEYGGDTGF	140
Consensus	SRRFGPVDVPV·LLKSIEGYPEVQMIIRREANES·R·iGddWH·DStFLDAPPAAVVMrAievPeyGGDTgf	140

FIG.6B

SEQ ID NO:60	LSMYTAWETLSPTMQATIEGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDAGDRETVHPLVWTHPGS	210
SEQ ID NO:1	LSMYTAYDALSDGLKKLISGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDAGDRETVHPLVWTHPGS	210
SEQ ID NO:4	LSMYTAYDALSDGLKKLISGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDAGDRETVHPLVWTHPGS	210
SEQ ID NO:7	LSMYTAYDALSDGLKKLISGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDAGDRETVHPLVWTHPGS	210
SEQ ID NO:9	LSMYTAYDALSDGLKKLISGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDAGDRETVHPLVWTHPGS	210
SEQ ID NO:11	TSMYTAWETLSPTMQATIEGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDAGDRETVHPLVWTHPET	210
SEQ ID NO:14	LSMYSAWETLSPTMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:18	LSMYSAWETLSPTMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:22	ASMYTAWETLSPTMQATIEGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDAGDRETVHPLVWTHPET	210
SEQ ID NO:25	LSMYTAWETLSPTMQATIEGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:28	LSMYSAWETLSPTMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:31	LSMYSAWDTLSDTMKATIEGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:34	LSMYSAWETLSPTMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:37	LSMYSAWETLSPTMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:40	LSMYSAWDTLSDTMKATIEGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:43	LSMYSAWDTLSDTMKATIEGLNVVHSATRVFGSLYQAQNRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:46	LSMYSAWETLSPTMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:47	LSMYSAWETLSPTMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:48	LSMYSAWETLSPTMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:49	LSMYSAWETLSPAMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:50	LSMYSAWETLSPTMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:51	LSMYSAWETLSPTMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210
SEQ ID NO:52	LSMYSAWETLSPTMQATIEGLNVVHSATKVFSGSLYQATNWRFSNTSVKVMVDAGDRETVHPLVWTHPVT	210

Consensus LSMYAw.tLS.tmatIeGLNVVHSATVFGSLYQA.N.RfSnTSVKVMVDaGDRETVHPLVWtHP.t 210

FIG.6C

SEQ ID NO:60	GRKGLYNQVYCCRIEGMTDAESKPLLQFLYEHAIRFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:1	GRKGLYNQVYCCRIEGMSEKESEPLLSEFLFAHATKPEFTCRVRWKKDQVLWMDNLCTMHYAINDYHGQT	280
SEQ ID NO:4	GRKGLYNQVYCCRIEGMSEKESEPLLSEFLFAHATKPEFTCRVRWKKDQVLWMDNLCTMHYAINDYHGQT	280
SEQ ID NO:7	GRKGLYNQVYCCRIEGMSEKESEPLLSEFLFAHATKPEFTCRVRWKKDQVLWMDNLCTMHYAINDYHGQT	280
SEQ ID NO:9	GRKGLYNQVYCCRIEGMSEKESEPLLSEFLFAHATKPEFTCRVRWKKDQVLWMDNLCTMHYAINDYHGQT	280
SEQ ID NO:11	GRKGLYNQVYCCRIEGMSEKESEPLLSEFLFAHATKPEFTCRVRWQEGDVLWMDNLCTQHAYVPDYAGKF	280
SEQ ID NO:14	GRRALYCNQVYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:18	GRRALYCNQVYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:22	GRKGLYNQVYCCRIEGMSEKESEPLLSEFLFAHATKPEFTCRVRWQEGQVLWMDNLCTQHFAVPDYAGKF	280
SEQ ID NO:25	GRRALYCNQVYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:28	GRRALYCNQVYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:31	GRRALYCNQVYCCRIEGMTDAESKPLLQFLYEHAIRFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:34	GRRALYCNQVYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHSVPDYAGKF	280
SEQ ID NO:37	GRRALYCNQVYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHSVPDYGNAF	280
SEQ ID NO:40	GRRALYCNQVYCCRIEGMTDAESKCLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:43	GRRALYCNQVYCCRIEGMTDAESKCLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYDGKF	280
SEQ ID NO:46	GRRALYCNQVYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:47	GRRALYCNQVYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:48	GRRALYCNQVYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:49	GRRALYCNQIYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:50	GRRALYCNQIYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:51	GRRALYCNQVYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280
SEQ ID NO:52	GRRALYCNQVYCQRIQGMTDAESKSLLOFLYEHAIRKFDFTCRVRWKKDQVLWMDNLCTMHRAVPDYAGKF	280

Consensus GR·LY·NQVYCQ·I·GMtdaESk·LLQFLYeHAT·fdTCRVRWkkdqVLWMDNLCTmH·avpDYagkf 280

FIG.6D

SEQ ID NO:60	RYLTRTTVGGVRPAR	295
SEQ ID NO:1	RILHRTTVGGVRPAR	295
SEQ ID NO:4	RILHRTTVGGVRPAR	295
SEQ ID NO:7	RILHRTTVGGVRPAR	295
SEQ ID NO:9	RILHRTTVGGVRPAR	295
SEQ ID NO:11	RYLTRTTVGGVRPAR	295
SEQ ID NO:14	RYLTRTTVAGDKPSR	295
SEQ ID NO:18	RYLTRTTVAGDKPSR	295
SEQ ID NO:22	RYLTRTTVGGVRPAR	295
SEQ ID NO:25	RYLTRTTVAGDKPSR	295
SEQ ID NO:28	RYLTRTTVAGDKPSR	295
SEQ ID NO:31	RYLTRTTVAGDKPSR	295
SEQ ID NO:34	RYLTRTTVAGDKPSR	295
SEQ ID NO:37	RYLTRTTVAGDKPSR	295
SEQ ID NO:40	RYLTRTTVAGDRPAR	295
SEQ ID NO:43	RYLTRTTVAGDKPSR	295
SEQ ID NO:46	RYLTRTTVAGDKPSR	295
SEQ ID NO:47	RYLTRTTVAGDKPSR	295
SEQ ID NO:48	RYLTRTTVAGDKPSR	295
SEQ ID NO:49	RYLTRTTVAGDKPSR	295
SEQ ID NO:50	RYLTRTTVAGDKPSR	295
SEQ ID NO:51	RYLTRTTVAGDKPSR	295
SEQ ID NO:52	RYLTRTTVAGDKPSR	295
Consensus	RYLTRTTV·G·P·R	295

FIG.6E

HERBICIDE TOLERANCE GENES AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/064,343, filed on Oct. 15, 2014, herein incorporated by reference in its entirety.

INCORPORATION OF SEQUENCE LISTING

[0002] The sequence listing that is contained in the file named "MONS378US_ST25", which is 118,584 bytes (measured in MS-WINDOWS) and created on Sep. 11, 2015, is filed herewith by electronic submission and incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0003] 1. Field of the Invention

[0004] The present invention generally relates to the field of biotechnology. More specifically, the invention relates to recombinant DNA molecules encoding enzymes that degrade herbicides. The invention also relates to transgenic plants, parts, seeds, cells, and plant parts containing the recombinant DNA molecules, as well as methods of using the same.

[0005] 2. Description of Related Art

[0006] Agricultural crop production often utilizes transgenic traits created using the methods of biotechnology. A heterologous gene, also known as a transgene, is introduced into a plant to produce a transgenic trait. Expression of the transgene in the plant confers a desirable trait, such as herbicide tolerance, on the plant. Examples of transgenic herbicide tolerance traits include glyphosate tolerance, glufosinate tolerance, and dicamba tolerance. With the increase of weed species resistant to the most commonly used herbicides, new herbicide tolerance traits are needed in the field. Herbicides of particular interest are the aryloxyphenoxypropionate (AOPP) herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides. AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides provide control of a spectrum of glyphosate-resistant weeds, thus making a trait conferring tolerance these herbicides particularly useful in a cropping system combined with other herbicide tolerance trait(s).

[0007] The *Sphingobium herbicidovorans* strain MH isolated from a dichloroprop-degrading soil sample was identified as being capable of cleaving the ether bond of various phenoxyalkanoic acid herbicides, utilizing this as its sole carbon and energy source for growth (HPE Kohler, Journal of Industrial Microbiology & Biotechnology (1999) 23:336-340). Catabolism of the herbicides is carried out by two different enantioselective alpha-ketoglutarate-dependent dioxygenases, RdpA (R-dichloroprop dioxygenase) and SdpA (S-dichloroprop dioxygenase). (A Westendorf, et al., Microbiological Research (2002) 157:317-322; Westendorf, et al., Acta Biotechnologica (2003) 23(1):3-17). RdpA has been isolated from *Sphingobium herbicidovorans* (GenBank Accessions AF516752 (DNA) and AAM90965 (protein)) and *Delftia acidovorans* (GenBank Accessions NG_036924 (DNA) and YP_009083283 (protein)) (TA Mueller, et al., Applied and Environmental Microbiology (2004) 70 (10): 6066-6075.) The RdpA and SdpA genes have been used for plant transformation to confer herbicide tolerance to crops (TR Wright, et al., Proceedings of the National Academy of Sciences USA, (2010) 107(47):20240-5). Improving the

activity of the RdpA enzyme using protein engineering techniques to create a protein for use in transgenic plants would permit higher rates of herbicide application, thus improving transgenic crop safety and weed control measures.

BRIEF SUMMARY OF THE INVENTION

[0008] The invention provides a polypeptide that has at least about 92% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52. In one embodiment, the polypeptide has oxygenase activity against at least one herbicide selected from the group consisting of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides.

[0009] The invention provides a recombinant DNA molecule comprising a nucleic acid sequence encoding a polypeptide that has at least about 92% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52. In one embodiment, the recombinant DNA molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:2, 3, 5, 6, 8, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, 41, 42, 44, 45, and 53-59. In another embodiment, the recombinant DNA molecule encodes a polypeptide with oxygenase activity against at least one herbicide selected from the group consisting of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides. In another embodiment, the recombinant DNA molecule is operably linked to a heterologous promoter functional in a plant cell. In another embodiment, the recombinant DNA molecule is operably linked to a DNA molecule encoding a chloroplast transit peptide that functions to localize an operably linked polypeptide within a cell.

[0010] The invention provides a DNA construct comprising a heterologous promoter functional in a plant cell operably linked to a recombinant DNA molecule comprising a nucleic acid sequence encoding a polypeptide that has at least about 92% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52. In one embodiment, the recombinant DNA molecule is operably linked to a DNA molecule encoding a chloroplast transit peptide that functions to localize an operably linked polypeptide within a cell. In another embodiment, the recombinant DNA molecule encodes a polypeptide that has an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52 and the expression of the polypeptide in a transgenic plant confers herbicide tolerance to the plant. In another embodiment, the DNA construct is present in the genome of a transgenic plant.

[0011] The invention provides a transgenic plant, seed, cell, or plant part comprising a recombinant DNA molecule comprising a nucleic acid sequence encoding a polypeptide that has at least about 92% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52. In one embodiment, the transgenic plant, seed, cell, or plant part comprises a transgenic trait for tolerance to at least one herbicide selected from the group consisting of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides. In another embodiment, the transgenic plant, seed, cell, or plant part comprises a DNA construct of the

invention. In another embodiment, the transgenic plant, seed, cell, or plant part comprises a polypeptide of the invention.

[0012] The invention provides a method for conferring herbicide tolerance to a plant, seed, cell, or plant part comprising expressing in the plant, seed, cell, or plant part a polypeptide of the invention. In one embodiment, the method for conferring herbicide tolerance is used with a transgenic plant, seed, cell, or plant part that comprises a transgenic trait comprising a recombinant DNA molecule of the invention. In one embodiment, the method for conferring herbicide tolerance is used with an herbicide selected from the group consisting of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides.

[0013] The invention provides a method of plant transformation, comprising introducing a DNA construct of the invention into a plant cell and regenerating a plant therefrom that comprises the DNA construct and is tolerant to at least one herbicide selected from the group consisting of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides. In one embodiment, the method of plant transformation includes crossing the regenerated plant with itself or with a second plant and collecting seed from the cross.

[0014] The invention provides a method for controlling weeds in a plant growth area by contacting a plant growth area comprising a transgenic plant or seed of the invention with at least one herbicide selected from the group consisting of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides, where the transgenic plant or seed is tolerant to the herbicide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIGS. 1A-1C. Control and MON-HT55 (SEQ ID NO:11) transgenic maize plants following treatment with quizalofop-P. FIG. 1A shows control and transgenic maize plants either untreated or treated with 1× quizalofop-P (0.08 lb ai/acre). FIG. 1B shows F1 hybrid control maize plants and FIG. 1C shows F1 hybrid MON-HT55 transgenic maize plants. Plants were grown at daytime/night-time temperatures of (1) 20° C./20° C., (2) 28° C./20° C., or (3) 38° C./30° C. prior to being sprayed with 2× quizalofop-P.

[0016] FIGS. 2A-2C. Graphs showing temperature-dependent activity of engineered proteins. FIG. 2A shows activity of MON-HT55 (SEQ ID NO:11) and the wild-type RdpA enzyme when tested with quizalofop-P as the substrate. FIG. 2B shows activity of MON-HT1 (SEQ ID NO:14), MON-HT2 (SEQ ID NO:18), MON-HT7 (SEQ ID NO:34), MON-HT8 (SEQ ID NO:37) and the wild-type RdpA when tested with quizalofop-P as the substrate. FIG. 2C shows activity of MON-HT1, MON-HT2, MON-HT7, MON-HT8 and the wild-type RdpA when tested with 2,4-D as the substrate. Data are normalized to the activity of each protein at 25° C.

[0017] FIG. 3. The protein sequence of wild-type RdpA (SEQ ID NO:60) with exemplary amino acid positions useful for protein engineering boxed.

[0018] FIGS. 4A-4E. The average injury rating after 2× quizalofop-P (0.16 lb ai/acre) (4A, 4B, and 4C) or 4× 2,4-D (4 lb ae/acre) (4D and 4E) application to F1 hybrid maize plants (homozygous R1 expressing MON-HT×MON89034 inbred) expressing MON-HT55 (SEQ ID NO:11), MON-HT1 (SEQ ID NO:14), MON-HT2 (SEQ ID NO:18), MON-HT3 (SEQ ID NO:22), MON-HT4 (SEQ ID NO:25), MON-HT7 (SEQ ID NO:34), or F1 hybrid control (NK603×MON89034). Data from plants acclimated at daytime and night time temperatures set at 20° C. (20° C./20° C.) prior to application of 2×

quizalofop-P (FIG. 4A) or 4× 2,4-D (FIG. 4D); FIG. 4B shows data from plants acclimated at daytime temperature of 28° C. and night time temperature of 20° C. (28° C./20° C.) prior to application of 2× quizalofop-P; FIG. 4C shows data from plants acclimated at daytime temperature of 38° C. and night time temperature of 30° C. (38° C./30° C.) prior to application of 2× quizalofop-P (FIG. 4C) or 4× 2,4-D (FIG. 4E).

[0019] FIGS. 5A-5B. FIG. 5A and FIG. 5B: Control and transgenic maize plants comprising MON-HT2 (SEQ ID NO:20, encoding SEQ ID NO:18) with a CTP or without a CTP where the plants received quizalofop-P at 16× rates (1.28 lb ai/acre) applied at V2 followed by V4 and the photos taken 10 to 14 days after quizalofop-P application.

[0020] FIGS. 6A-6E. Multi-sequence alignment of protein sequences for wild-type RdpA from *S. herbicidovorans* (SEQ ID NO:60) and SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52 with the consensus sequence (provided as SEQ ID NO:61) provided at the bottom of each of FIGS. 6A, 6B, 6C, 6D, 6E.

BRIEF DESCRIPTION OF THE SEQUENCES

[0021] SEQ ID NO:1-3 are the amino acid sequence, bacterial codon polynucleotide sequence, and monocot codon optimized polynucleotide sequence of MON-HT51.

[0022] SEQ ID NO:4-6 are the amino acid sequence, bacterial codon polynucleotide sequence, and monocot codon optimized polynucleotide sequence of MON-HT52.

[0023] SEQ ID NO:7-8 are the amino acid sequence and bacterial codon polynucleotide sequence of MON-HT53.

[0024] SEQ ID NO:9-10 are the amino acid sequence and bacterial codon polynucleotide sequence of MON-HT54.

[0025] SEQ ID NO:11-13 are the amino acid sequence, bacterial codon polynucleotide sequence, and monocot codon optimized polynucleotide sequence of MON-HT55.

[0026] SEQ ID NO:14-17 are the amino acid sequence, bacterial codon polynucleotide sequence, monocot codon optimized polynucleotide sequence, and dicot codon optimized polynucleotide sequence of MON-HT1.

[0027] SEQ ID NO:18-21 are the amino acid sequence, bacterial codon polynucleotide sequence, monocot codon optimized polynucleotide sequence, and dicot codon optimized polynucleotide sequence of MON-HT2.

[0028] SEQ ID NO:22-24 are the amino acid sequence, bacterial codon polynucleotide sequence, and monocot codon optimized polynucleotide sequence of MON-HT3.

[0029] SEQ ID NO:25-27 are the amino acid sequence, bacterial codon polynucleotide sequence, and monocot codon optimized polynucleotide sequence of MON-HT4.

[0030] SEQ ID NO:28-30 are the amino acid sequence, bacterial codon polynucleotide sequence, and monocot codon optimized polynucleotide sequence of MON-HT5.

[0031] SEQ ID NO:31-33 are the amino acid sequence, bacterial codon polynucleotide sequence, and monocot codon optimized polynucleotide sequence of MON-HT6.

[0032] SEQ ID NO:34-36 are the amino acid sequence, bacterial codon polynucleotide sequence, and monocot codon optimized polynucleotide sequence of MON-HT7.

[0033] SEQ ID NO:37-39 are the amino acid sequence, bacterial codon polynucleotide sequence, and monocot codon optimized polynucleotide sequence of MON-HT8.

[0034] SEQ ID NO:40-42 are the amino acid sequence, bacterial codon polynucleotide sequence, and monocot codon optimized polynucleotide sequence of MON-HT9.

[0035] SEQ ID NO:43-45 are the amino acid sequence, bacterial codon polynucleotide sequence, and monocot codon optimized polynucleotide sequence of MON-HT10.

[0036] SEQ ID NO:46-52 are the amino acid sequences of MON-HT11, MON-HT13, MON-HT14, MON-HT15, MON-HT16, MON-HT17, and MON-HT18.

[0037] SEQ ID NO:53-59 are the dicot codon optimized polynucleotide sequences of MON-HT11, MON-HT13, MON-HT14, MON-HT15, MON-HT16, MON-HT17, and MON-HT18.

[0038] SEQ ID NO:60 is the amino acid sequence for wild-type RdpA from *Sphingobium herbicidovorans*.

[0039] SEQ ID NO:61 is the consensus sequence of FIG. 6A-FIG. 6E.

DETAILED DESCRIPTION OF THE INVENTION

[0040] The following definitions and methods are provided to better define the invention and to guide those of ordinary skill in the art in the practice of the invention. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

[0041] The invention overcomes the limitations of the prior art by providing novel, engineered proteins, referred to herein as MON-HT proteins, and the recombinant DNA molecules that encode them as well as compositions and methods using these. The MON-HT proteins are oxygenases that can inactivate aryloxyphenoxypropionate (AOPP) herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides. As used herein, inactivating an herbicide means making the herbicide no longer have its herbicidal activity against a plant. The MON-HT proteins exhibit novel substrate selectivity, useful enzyme kinetics, and increased enzyme stability at elevated temperature. Transgenic plants expressing a MON-HT protein demonstrate improved tolerance to application of AOPP, phenoxy acid herbicides, and pyridinyloxy acid herbicides.

Engineered Proteins and Recombinant DNA Molecules

[0042] The invention provides novel, engineered proteins and the recombinant DNA molecules that encode them. As used herein, the term “engineered” refers to a non-natural DNA, protein, or organism that would not normally be found in nature and was created by human intervention. An “engineered protein” is a protein whose polypeptide sequence was conceived of and created in the laboratory using one or more of the techniques of protein engineering, such as protein design using site-directed mutagenesis and directed evolution using random mutagenesis and DNA shuffling. For example, an engineered protein may have one or more deletions, insertions, or substitutions relative to the coding sequence of the wild-type protein and each deletion, insertion, or substitution may consist of one or more amino acids. Examples of engineered proteins are provided herein as SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52.

[0043] Engineered proteins provided by the invention are enzymes that have oxygenase activity. As used herein, the term “oxygenase activity” means the ability to oxidize a substrate by transferring the oxygen from molecular oxygen to the substrate, co-product, or an intermediary. The oxygenase activity of the engineered proteins provided by the invention can inactivate one or more of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides.

[0044] As used herein, “wild-type” means naturally-occurring. As used herein, a “wild-type DNA molecule”, “wild-type polypeptide”, or a “wild-type protein” is a naturally-occurring DNA molecule, polypeptide, or protein, that is, a DNA molecule, polypeptide, or protein pre-existing in nature. A wild-type version of a polypeptide, protein, or DNA molecule may be useful for comparison with an engineered protein or gene. An example of a wild-type protein useful for comparison with the engineered proteins provided by the invention is the RdpA enzyme from *Sphingobium herbicidovorans* strain MH. An example of a wild-type DNA molecule useful for comparison with the recombinant DNA molecules provided by the invention is the RdpA gene from *Sphingobium herbicidovorans* strain MH. A wild-type version of a protein or DNA molecule may be useful as a control in an experiment.

[0045] As used herein, “control” means an experimental control designed for comparison purposes. For example, a control plant in a transgenic plant analysis is a plant of the same type as the experimental plant (that is, the plant to be tested) but does not contain the transgenic insert, recombinant DNA molecule, or DNA construct of the experimental plant. An example of a control plant useful for comparison with transgenic maize plants is non-transgenic LH244 maize (U.S. Pat. No. 6,252,148) and with transgenic soy plants is non-transgenic A3555 soybean (U.S. Pat. No. 7,700,846).

[0046] As used herein, the term “recombinant” refers to a non-natural DNA, polypeptide, or protein that is the result of genetic engineering and as such would not normally be found in nature and was created by human intervention. A “recombinant DNA molecule” is a DNA molecule comprising a DNA sequence that does not naturally occur and as such is the result of human intervention, for example, a DNA molecule that encodes an engineered protein. Another example is a DNA molecule comprised of a combination of at least two DNA molecules heterologous to each other, such as a protein-coding DNA molecule and an operably linked heterologous promoter. An example of a recombinant DNA molecule is a DNA molecule comprising at least one sequence selected from SEQ ID NO:2, 3, 5, 6, 8, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, 41, 42, 44, 45 and 53-59. A “recombinant polypeptide” or “recombinant protein” is a polypeptide or protein comprising an amino acid sequence that does not naturally occur and as such is the result of human intervention, for example, an engineered protein.

[0047] The term “transgene” refers to a DNA molecule artificially incorporated into the genome of an organism as a result of human intervention, such as by plant transformation methods. As used herein, the term “transgenic” means comprising a transgene, for example a “transgenic plant” refers to a plant comprising a transgene in its genome and a “transgenic trait” refers to a characteristic or phenotype conveyed or conferred by the presence of a transgene incorporated into the plant genome. As a result of such genomic alteration, the transgenic plant is something distinctly different from the related wild-type plant and the transgenic trait is a trait not naturally found in the wild-type plant. Transgenic plants of the invention comprise the recombinant DNA molecules and engineered proteins provided by the invention.

[0048] As used herein, the term “heterologous” refers to the relationship between two or more things derived from different sources and thus not normally associated in nature. For example, a protein-coding recombinant DNA molecule is heterologous with respect to an operably linked promoter if

such a combination is not normally found in nature. In addition, a particular recombinant DNA molecule may be heterologous with respect to a cell or organism into which it is inserted when it would not naturally occur in that particular cell or organism.

[0049] As used herein, the term “protein-coding DNA molecule” or “polypeptide-coding DNA molecule” refers to a DNA molecule comprising a nucleotide sequence that encodes a protein or polypeptide. A “protein-coding sequence” or “polypeptide-coding sequence” means a DNA sequence that encodes a protein or polypeptide. A “sequence” means a sequential arrangement of nucleotides or amino acids. The boundaries of a protein-coding sequence or polypeptide-coding sequence are usually determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A protein-coding molecule or polypeptide-coding molecule may comprise a DNA sequence encoding a protein or polypeptide sequence. As used herein, “transgene expression”, “expressing a transgene”, “protein expression”, “polypeptide expression”, “expressing a protein”, and “expressing a polypeptide” mean the production of a protein or polypeptide through the process of transcribing a DNA molecule into messenger RNA (mRNA) and translating the mRNA into polypeptide chains, which may be ultimately folded into proteins. A protein-coding DNA molecule or polypeptide-coding DNA molecule may be operably linked to a heterologous promoter in a DNA construct for use in expressing the protein or polypeptide in a cell transformed with the recombinant DNA molecule. As used herein, “operably linked” means two DNA molecules linked in manner so that one may affect the function of the other. Operably-linked DNA molecules may be part of a single contiguous molecule and may or may not be adjacent. For example, a promoter is operably linked with a protein-coding DNA molecule or polypeptide-coding DNA molecule in a DNA construct where the two DNA molecules are so arranged that the promoter may affect the expression of the transgene.

[0050] As used herein, a “DNA construct” is a recombinant DNA molecule comprising two or more heterologous DNA sequences. DNA constructs are useful for transgene expression and may be comprised in vectors and plasmids. DNA constructs may be used in vectors for the purpose of transformation, that is the introduction of heterologous DNA into a host cell, in order to produce transgenic plants and cells, and as such may also be contained in the plastid DNA or genomic DNA of a transgenic plant, seed, cell, or plant part. As used herein, a “vector” means any recombinant DNA molecule that may be used for the purpose of plant transformation. Recombinant DNA molecules as set forth in the sequence listing, can, for example, be inserted into a vector as part of a construct having the recombinant DNA molecule operably linked to a promoter that functions in a plant to drive expression of the engineered protein encoded by the recombinant DNA molecule. Methods for constructing DNA constructs and vectors are well known in the art. The components for a DNA construct, or a vector comprising a DNA construct, generally include, but are not limited to, one or more of the following: a suitable promoter for the expression of an operably linked DNA, an operably linked protein-coding DNA molecule, and a 3' untranslated region (3'-UTR). Promoters useful in practicing the present invention include those that function in a plant for expression of an operably linked polynucleotide. Such promoters are varied and well known in the art and include those that are inducible, viral, synthetic, con-

stitutive, temporally regulated, spatially regulated, and/or spatio-temporally regulated. Additional optional components include, but are not limited to, one or more of the following elements: 5'-UTR, enhancer, leader, cis-acting element, intron, chloroplast transit peptides (CTP), and one or more selectable marker transgenes.

[0051] The DNA constructs of the invention may include a CTP molecule operably linked to the protein-coding DNA molecules provided by the invention. A CTP useful in practicing the present invention includes those that function to facilitate localization of the engineered protein molecule within the cell. By facilitating protein localization within the cell, the CTP may increase the accumulation of engineered protein, protect it from proteolytic degradation, enhance the level of herbicide tolerance, and thereby reduce levels of injury after herbicide application. CTP molecules for use in the present invention are known in the art and include, but are not limited to the *Arabidopsis thaliana* EPSPS CTP (Klee et al., 1987), the *Petunia hybrida* EPSPS CTP (della-Cioppa et al., 1986), the maize cab-m7 signal sequence (Becker et al., 1992; PCT WO 97/41228) and the pea glutathione reductase signal sequence (Creissen et al., 1991; PCT WO 97/41228).

[0052] Recombinant DNA molecules of the present invention may be synthesized and modified by methods known in the art, either completely or in part, especially where it is desirable to provide sequences useful for DNA manipulation (such as restriction enzyme recognition sites or recombination-based cloning sites), plant-preferred sequences (such as plant-codon usage or Kozak consensus sequences), or sequences useful for DNA construct design (such as spacer or linker sequences). The present invention includes recombinant DNA molecules and engineered proteins having at least about 80% (percent) sequence identity, about 85% sequence identity, about 90% sequence identity, about 91% sequence identity, about 92% sequence identity, about 93% sequence identity, about 94% sequence identity, about 95% sequence identity, about 96% sequence identity, about 97% sequence identity, about 98% sequence identity, and about 99% sequence identity to any of the recombinant DNA molecule or engineered protein sequences provided herein, for instance, to a recombinant DNA molecule comprising a sequence selected from the group consisting of SEQ ID NO:2, 3, 5, 6, 8, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, 41, 42, 44, 45, and 53-59. As used herein, the term “percent sequence identity” or “% sequence identity” refers to the percentage of identical nucleotides or amino acids in a linear polynucleotide or polypeptide sequence of a reference (“query”) sequence (or its complementary strand) as compared to a test (“subject”) sequence (or its complementary strand) when the two sequences are optimally aligned (with appropriate nucleotide or amino acid insertions, deletions, or gaps totaling less than 20 percent of the reference sequence over the window of comparison). Optimal alignment of sequences for aligning a comparison window are well known to those skilled in the art and may be conducted by tools such as the local homology algorithm of Smith and Waterman, the homology alignment algorithm of Needleman and Wunsch, the search for similarity method of Pearson and Lipman, and by computerized implementations of these algorithms such as GAP, BESTFIT, FASTA, and TFASTA available as part of the Sequence Analysis software package of the GCG® Wisconsin Package® (Accelrys Inc., San Diego, Calif.), MEGA1ign (DNASar, Inc., 1228 S. Park St., Madison, Wis. 53715), and MUSCLE (version 3.6) (RC

Edgar, *Nucleic Acids Research* (2004) 32(5):1792-1797) with default parameters. An "identity fraction" for aligned segments of a test sequence and a reference sequence is the number of identical components which are shared by the two aligned sequences divided by the total number of components in the reference sequence segment, that is, the entire reference sequence or a smaller defined part of the reference sequence. Percent sequence identity is represented as the identity fraction multiplied by 100. The comparison of one or more sequences may be to a full-length sequence or a portion thereof, or to a longer sequence.

[0053] Engineered proteins may be produced by changing (that is, modifying) a wild-type protein to produce a new protein with a novel combination of useful protein characteristics, such as altered V_{max} , K_m , substrate specificity, substrate selectivity, and protein stability. Modifications may be made at specific amino acid positions in a protein and may be a substitution of the amino acid found at that position in nature (that is, in the wild-type protein) with a different amino acid. Exemplary amino acid positions relative to the protein sequence of wild-type protein RdpA (SEQ ID NO:60) useful for protein engineering are depicted in FIG. 3. FIGS. 6A, 6B, 6C, 6D, 6E provide a multi-sequence alignment of the wild-type RdpA protein sequence and engineered protein sequences SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52. An engineered protein can be designed that has at least about 92% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52 and comprises at least one of these amino acid mutations. Engineered proteins provided by the invention thus provide a new protein with one or more altered protein characteristics relative to the wild-type protein found in nature. In one embodiment of the invention, an engineered protein has altered protein characteristics such as improved or decreased activity against one or more herbicides or improved protein stability as compared to a similar wild-type protein, or any combination of such characteristics. In one embodiment, the invention provides an engineered protein, and the recombinant DNA molecule encoding it, having at least about 80% sequence identity, about 85% sequence identity, about 90% sequence identity, about 91% sequence identity, about 92% sequence identity, about 93% sequence identity, about 94% sequence identity, about 95% sequence identity, about 96% sequence identity, about 97% sequence identity, about 98% sequence identity, and about 99% sequence identity to an engineered protein sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52. Amino acid mutations may be made as a single amino acid substitution in the protein or in combination with one or more other mutation(s), such as one or more other amino acid substitution(s), deletions, or additions. Mutations may be made as described herein or by any other method known to those of skill in the art.

Transgenic Plants

[0054] An aspect of the invention includes transgenic plant cells, transgenic plant tissues, transgenic plants, and transgenic seeds that comprise the recombinant DNA molecules and engineered proteins provided by the invention. These cells, tissues, plants, and seeds comprising the recombinant DNA molecules and engineered proteins exhibit herbicide

tolerance to one or more of aryloxyphenoxypropionate (AOPP) herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides.

[0055] Suitable methods for transformation of host plant cells for use with the current invention include virtually any method by which DNA can be introduced into a cell (for example, where a recombinant DNA construct is stably integrated into a plant chromosome) and are well known in the art. An exemplary and widely utilized method for introducing a recombinant DNA construct into plants is the *Agrobacterium* transformation system, which is well known to those of skill in the art. Transgenic plants can be regenerated from a transformed plant cell by the methods of plant cell culture. A transgenic plant homozygous with respect to a transgene (that is, two allelic copies of the transgene) can be obtained by self-pollinating (selfing) a transgenic plant that contains a single transgene allele with itself, for example an R0 plant, to produce R1 seed. One fourth of the R1 seed produced will be homozygous with respect to the transgene. Plants grown from germinating R1 seed can be tested for zygosity, typically using a SNP assay, DNA sequencing, or a thermal amplification assay that allows for the distinction between heterozygotes and homozygotes, referred to as a zygosity assay.

[0056] Plants, seeds, plant parts, plant tissues, and cells provided by the invention exhibit herbicide tolerance to one or more of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides. AOPP herbicides target the plant's acetyl-coenzyme A carboxylase (ACCase), which is part of the fatty acid biosynthetic pathway. Grass plants are sensitive to these herbicides because they contain herbicide-sensitive ACCase in their plastids and cytosol. AOPP herbicides are well-known in the art and commercially available. Examples of AOPP herbicides include, but are not limited to, clodinafop, cyhalofop, diclofop, fenoxaprop, fenoxaprop-P, fenthiaprop, fluazifop, fluazifop-P, haloxyfop, isoxapyrifop, metamifop, propaquizafop, quizalofop, quizalofop-P, and trifop. The phenoxy acid and pyridinyloxy acid herbicides are synthetic auxins similar to the plant growth hormone indoleacetic acid (IAA). Broad-leaf plants are sensitive to these herbicides, which induce rapid, uncontrolled growth, eventually killing the plant. Examples of phenoxy acid herbicides include, but are not limited to, 2,4-D; 2,4-DB; clomeprop; dichlorprop; fenoprop; MCPA; MCPB, and mecoprop. Examples of pyridinyloxy acid herbicides include, but are not limited to, triclopyr; fluroxypyr; aminopyralid, clopyralid, and picloram.

[0057] Herbicides may be applied to a plant growth area comprising the plants and seeds provided by the invention as a method for controlling weeds. Plants and seeds provided by the invention comprise an herbicide tolerance trait and as such are tolerant to the application of one or more AOPP herbicides, or phenoxy acid herbicides, or pyridinyloxy acid herbicides. The herbicide application may be the recommended commercial rate (1x) or any fraction or multiple thereof, such as twice the recommended commercial rate (2x). Herbicide rates may be expressed as acid equivalent per pound per acre (lb ae/acre) or pounds active ingredient per acre (lb ai/acre). The herbicide application comprises at least one herbicide selected from the group consisting of AOPP herbicides, and phenoxy acid herbicides, and pyridinyloxy acid herbicides. The plant growth area may or may not comprise weed plants at the time of herbicide application. An herbicidally effective dose of AOPP herbicides for use in the area for controlling weeds should consist of a range from

about 0.01 lb ai/acre to about 16 lb ai/acre over a growing season. For example, a 1× rate of quizalofop-P would be a rate of 0.08 lb ai/acre. An herbicidally effective dose of phenoxy acid herbicides for use in the area for controlling weeds should consist of a range from about 0.01 lb ae/acre to about 16 lb ae/acre over a growing season. For example, a 1× rate of 2,4-D would be a rate of about 0.75 lb ae/acre to 1.0 lb ae/acre. An herbicidally effective dose of pyridinyloxy acid herbicides for use in the area for controlling weeds should consist of a range from about 0.01 lb ae/acre to about 16 lb ae/acre over a growing season. For example, a 1× rate of fluroxypyr would be a rate of about 0.13 to 0.48 lb ae/acre.

[0058] The herbicide application may be sequentially or tank mixed with one, two, or a combination of several AOPP herbicides, phenoxy acid herbicides, pyridinyloxy acid herbicides, or any other compatible herbicide. Multiple applications of one herbicide or of two or more herbicides, in combination or alone, may be used over a growing season to areas comprising transgenic plants of the invention for the control of a broad spectrum of dicot weeds, monocot weeds, or both, for example, two applications (such as a pre-planting application and a post-emergence application or a pre-emergence application and a post-emergence application) or three applications (such as a pre-planting application, a pre-emergence application, and a post-emergence application or a pre-emergence application and two post-emergence applications).

[0059] As used herein, “tolerance” or “herbicide tolerance” means a plant, seed, plant tissue, plant part, or cell’s ability to resist the toxic effects of one or more herbicide(s). The herbicide tolerance of a plant, seed, plant tissue, plant part, or cell may be measured by comparing the plant, seed, plant tissue, plant part, or cell to a suitable control. For example, the herbicide tolerance may be measured or assessed by applying an herbicide to a plant comprising a recombinant DNA molecule encoding a protein capable of conferring herbicide tolerance (the test plant) and a plant not comprising the recombinant DNA molecule encoding the protein capable of conferring herbicide tolerance (the control plant) and then comparing the plant injury of the two plants, where herbicide tolerance of the test plant is indicated by a decreased injury rate as compared to the injury rate of the control plant. An herbicide tolerant plant, seed, plant tissue, plant part, or cells exhibits a decreased response to the toxic effects of an herbicide when compared to a control plant, seed, plant tissue, plant part, or cell. As used herein, an “herbicide tolerance trait” is a transgenic trait imparting improved herbicide tolerance to a plant as compared to a wild-type plant or control plant.

[0060] The transgenic plants, progeny, seeds, plant cells, and plant parts of the invention may also contain one or more additional transgenic traits. Additional transgenic traits may be introduced by crossing a plant containing a transgene comprising the recombinant DNA molecules provided by the invention with another plant containing an additional transgenic trait(s). As used herein, “crossing” means breeding two individual plants to produce a progeny plant. Two transgenic plants may thus be crossed to produce progeny that contain the transgenic traits. As used herein “progeny” means the offspring of any generation of a parent plant, and transgenic progeny comprise a DNA construct provided by the invention and inherited from at least one parent plant. Alternatively, additional transgenic trait(s) may be introduced by co-transforming a DNA construct for that additional transgenic trait (s) with a DNA construct comprising the recombinant DNA

molecules provided by the invention (for example, with all the DNA constructs present as part of the same vector used for plant transformation) or by inserting the additional trait(s) into a transgenic plant comprising a DNA construct provided by the invention or vice versa (for example, by using any of the methods of plant transformation on a transgenic plant or plant cell). Such additional transgenic traits include, but are not limited to, increased insect resistance, increased water use efficiency, increased yield performance, increased drought resistance, increased seed quality, improved nutritional quality, hybrid seed production, and herbicide tolerance, in which the trait is measured with respect to a wild-type plant or control plant. Such additional transgenic traits are known to one of skill in the art; for example, a list of such traits is provided the United States Department of Agriculture’s (USDA) Animal and Plant Health Inspection Service (APHIS) and can be found on their website at www.aphis.usda.gov.

[0061] Transgenic plants and progeny that contain a transgenic trait provided by the invention may be used with any breeding methods that are commonly known in the art. In plant lines comprising two or more transgenic traits, the transgenic traits may be independently segregating, linked, or a combination of both in plant lines comprising three or more transgenic traits. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation. Descriptions of breeding methods that are commonly used for different traits and crops are well known to those of skill in the art. To confirm the presence of the transgene(s) in a particular plant or seed, a variety of assays may be performed. Such assays include, for example, molecular biology assays, such as Southern and northern blotting, PCR, and DNA sequencing; biochemical assays, such as detecting the presence of a protein product, for example, by immunological means (ELISAs and Western blots) or by enzymatic function; plant part assays, such as leaf or root assays; and also, by analyzing the phenotype of the whole plant.

[0062] Introgression of a transgenic trait into a plant genotype is achieved as the result of the process of backcross conversion. A plant genotype into which a transgenic trait has been introgressed may be referred to as a backcross converted genotype, line, inbred, or hybrid. Similarly a plant genotype lacking the desired transgenic trait may be referred to as an unconverted genotype, line, inbred, or hybrid.

[0063] As used herein, the term “comprising” means “including but not limited to”.

EXAMPLES

[0064] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the concept, spirit, and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein with the same or similar result achieved. All such similar substitutes and modi-

fications apparent to those skilled in the art are deemed to be within the spirit, scope, and concept of the invention as defined by the appended claims.

Example 1

Initial Protein Engineering and Enzyme Analysis

[0065] Novel, engineered proteins and recombinant DNA molecules encoding these proteins were conceived of and created in the laboratory using the techniques of protein engineering. The engineered proteins are enzymes that have oxygenase activity and were engineered to have altered abilities to inactivate AOPP herbicides, phenoxy acid herbicides, or both relative to the wild-type protein.

[0066] Sixteen known proteins with oxygenase activity were selected and used to create a consensus homology sequence alignment. This was used in combination with structure-guided analyses to inform rational design strategies. From these analyses, five regions each from 13 to 21 amino acids in length, referred to herein as “islands”, were selected for mutagenesis. Mutations in each of these regions were created using techniques known to those of skill in the art, such as Alanine-Scanning Mutations; Homology-Scanning Mutations; Pro/Gly Scanning Mutations; Region Swaps or Mutations; and combinations of these various techniques (see, M Lehmann and M Wyss, *Current Opinion in Biotechnology* (2001) 12(4):371-375; B Van den Burg and VGH Eijssink, *Current Opinion in Biotechnology* (2002) 13(4):333-337; and Weiss et al., *Proc Natl Acad Sci U S A* (2000) 97(16):8950-8954). Using these methods, more than 1,200 unique engineered proteins and the recombinant DNA molecules that encode them were generated for further analysis and characterization. Because of the large number of engineered proteins produced for testing, and the need to test and compare the enzymatic activity of each protein, a high-throughput bacterial protein expression and enzyme assay system was developed for rapid analysis using crude bacterial extracts.

[0067] The high-throughput bacterial protein expression was achieved by synthesizing a recombinant DNA molecule encoding each engineered protein and cloning this into a bacterial expression vector with a C-terminal histidine tag (His-tag) operably linked to the recombinant DNA molecule. The vectors were used to transform *Escherichia coli* (*E. coli*), and bacterial expression of the engineered proteins was induced. Overnight *E. coli* cultures were grown in 96-well plates and the cultures were centrifuged to pellet the bacteria. The bacterial pellets were lysed by adding 100 μ l lysis master mix (10 ml of Bacteria Protein Extraction Reagent (B-PER®) II (Pierce Biotechnology, Rockford, Ill.; cat. no. 78260); 10 μ l Lysozyme (10 μ g/ml final; Lysozyme American Bioanalytical, Natick, Mass.; cat. no. AB011780-00005); and 40 μ l Benzonase® Nuclease (100 Units/ml final, Novagen, Darmstadt, Germany; cat no 71206-3)) to each well. The plates were vortexed then incubated for 30 minutes at 4° C. 400 μ l of MOPS buffer (pH 6.57) was added to each well and the debris pelleted by centrifugation. The lysate supernatant was carefully removed and used as the crude bacterial extract for subsequent enzymatic analysis.

[0068] The high-throughput herbicide degradation enzyme assay was designed to assay the enzymatic activity of the engineered proteins towards various herbicides using crude bacterial extract. The engineered protein's oxygenase activity (that is, its enzymatic activity) was measured by an end point

colorimetric assay utilizing detection of phenol products through measurement of absorbance at 510 nm from 4-aminoantipyrine and potassium ferricyanide. This assay was based on the assay described in Fukumori and Hausinger, *Journal of Biological Chemistry* (1993) 268(32):24311-24317. Enzyme reactions were assayed in 96-well plates in a total volume of 150 μ l that contained: 20 mM MOPS pH 6.75, 50-200 μ M NH₄FeSO₄, 50-200 μ M sodium ascorbate, 1 mM alpha-ketoglutarate (aKG), 10 μ l of *E. coli* cell lysate containing expressed engineered protein, and substrate (either an AOPP herbicide or phenoxy acid herbicide). Upon initiation of the reaction with substrate, the plate was incubated at various temperatures for various times and quenched (terminated) by the addition of EDTA to a final concentration of 6.25 mM or by the addition of 15 μ l of pH 10 buffer (50 mM boric acid, 50 mM KCl) followed by 15 μ l of 0.2% 4-aminoantipyrine and 15 μ l of 0.8% potassium ferricyanide. Absorbance measurements were made on a standard laboratory spectrometer. Assays were scaled as needed to enhance throughput. Standard curves were generated using purified protein or product standards.

[0069] Using this high-throughput bacterial protein expression and enzyme assay system, activities of approximately 1200 engineered proteins were measured relative to the activity of a selected wild-type protein, RdpA. In a 96 well plate assay, there were 3 controls (crude bacterial extract without an engineered protein) and 3 positive controls (crude bacterial extract with wild-type protein). The absorbance of the wells was measured and the protein activity was calculated using the following formula:

$$Activity_i = \left(\frac{(Absorbance_i - Absorbance_{PET})}{(Absorbance_{WT} - Absorbance_{PET})} \right) \times 100$$

where, Activity, is the activity of the sample, Absorbance, is the absorbance of the sample, Absorbance_{WT} is the absorbance of the wells containing extract from *E. coli* expressing the wild-type enzyme, and Absorbance_{PET} is the absorbance of the wells containing extract from *E. coli* without an engineered protein. Activity for each unique engineered protein was measured in duplicate and was reported as an average of the two measurements.

[0070] Based on the results from the high-throughput enzyme assay system, approximately 545 unique engineered proteins were selected for further analysis using purified engineered protein. In the purified engineered protein prep assay, crude bacterial expression lysates were prepared using QUIAGEN® Ni-NTA Agarose (Qiagen, Valencia, Calif., cat. no. 30230) following manufacturer's protocol.

[0071] The purified engineered proteins were assayed using the herbicide degradation enzyme assay described in this Example 1 with the AOPP herbicide quizalofop as the substrate. The results of the purified engineered protein assays largely confirmed the results from the high-throughput enzyme assay. Assay results for seven of the approximately 545 engineered proteins are shown in Table 1, where the enzymatic activity is expressed as the activity of the sample relative to the activity of the wild-type RdpA enzyme (calculated as described in this Example 1). These data from these assays yielded the surprising result that combinations of specific mutations performed significantly better than others and demonstrated that the enzymatic activity of the engineered proteins could be altered significantly.

TABLE 1

Replicate Number	Island 1-2-3	Island 1-2-5	Island 1-3-4	Island 2-3-4	Island 2-3-5	Island 2-4-5	Island 3-4-5
1	-5.94	-4.14	-4.86	-2.84	138.99	48.12	4.06
2	-3.54	-2.64	-3.9	-0.05	117.67	48.84	5.56
3		-3.85			140.75		
4		-0.65			113.77		
5		-0.05			150.58		
6		2.14			141.27		

[0072] Using the information learned from the first assays, protein engineering was then done as described previously to produce additional engineered proteins, which were tested as described in this Example 1. The results of the high-throughput enzyme assay with quizalofop-P as the substrate for five of these additional engineered proteins are provided in Table 2.

TABLE 2

SEQ ID NO	MON-HT	Replicate 1	Replicate 2	Replicate 3	Average quizalofop-P activity
1	MON-HT51	117.02	124.87	110.17	117.35
4	MON-HT52	92.17	54.46	89.75	78.79
7	MON-HT53	105.34	105.31	106.13	105.60
9	MON-HT54	131.85	149.77	130.56	137.39
11	MON-HT55	118.48	99.79		109.13

[0073] Further protein characterization, such as K_m , V_{max} , and crystal structure analysis, was performed using the five engineered proteins from Table 2. For this detailed analysis, purified protein was prepared as follows: 2 ml overnight cultures of *E. coli* expressing a transgene encoding a given MON-HT protein were used to inoculate 500 ml of broth and grown at 37° C. for 4 hours followed by culture at 15° C. for approximately 36 hours. Then 250 ml of the 500 ml bacterial culture was pelleted by centrifugation and resuspended in 25 ml of extraction buffer (20 mM Tris, pH 7.8, 300 mM NaCl, 5 mM beta-mercaptoethanol (BME), 20 mM imidazole (Fluka/Sigma-Aldrich, St. Louis, Mo.), 125 units/ml of benzonase and 10K units/ml of lysozyme (Novagen,

buffer consisted of: 20 mM Tris pH 7.8, 300 mM NaCl, 20 mM imidazole and 5 mM BME. The composition of elution buffer was the same as the wash buffer except with 500 mM imidazole. The eluate from the nickel column was desalted on a Quick Spin Protein Sephadex G-25 fine column (Roche Applied Science, Indianapolis, Ind.) following the manufacturer's protocol. The eluted protein was in buffer consisting of: 20 mM Tris pH 7.8, 50 mM NaCl and 5 mM BME. Protein extract purity was assessed by SDS-PAGE analysis. Protein concentration was determined by Bradford assay using Bio-Rad Protein Assay dye reagent (Biorad, Hercules, Calif., cat no 500-0006).

[0074] Purified protein for the five engineered proteins was analyzed using the enzyme assay described in this Example 1, but with four different AOPP herbicides as substrates: quizalofop-P, haloxyfop, fenoxaprop, and fluazifop. Standard curves were generated using 2,4-dichlorophenol (2,4-DCP), which was used to produce a general phenol standard curve. The amount of phenol generated in the assay by the engineered proteins was calculated based on this standard curve. The controls were purified wild-type enzyme, no enzyme, and no substrate. Enzyme kinetic measurements of the five engineered proteins was done using 0, 20, 40, 80, 160, 320, 640, or 1280 μ M of quizalofop-P, haloxyfop, fenoxaprop, or fluazifop herbicides. Table 3 shows the K_m and V_{max} (expressed as relative values) measured for the five proteins with the four AOPP herbicide substrates. The protein characteristics of these five engineered proteins with each of the four AOPP herbicides as substrates demonstrated that the enzymatic activity, namely K_m and V_{max} , of the engineered proteins could be altered significantly through protein engineering.

TABLE 3

	Quizalofop-P		Haloxyfop		Fenoxaprop		Fluazifop	
	K_m (μ M)	V_{max}	K_m (μ M)	V_{max}	K_m (μ M)	V_{max}	K_m (μ M)	V_{max}
MON-HT51	570	120	250	48	870	180	4.0	12
MON-HT52	420	39	280	45	490	420	14	43
MON-HT53	690	95	290	64	1100	640	1.0	40
MON-HT54	560	32	380	40	2100	630	ND	ND
MON-HT55	250	320	450	190	200	450	50	320

Darmstadt, Germany) The cell slurry was passed through a cell disruptor once at 20000 psi and then this cell lysate was clarified by centrifugation at 35,000 $\times$ g for 20 min at 4° C. The supernatant containing the soluble His-tagged proteins was used for protein purification. For this purification, the supernatant was applied to a 1 ml HisTrapTM FF column (Nickel Sepharose) (GE Healthcare, Piscataway, N.J.) using an AKTexpressTM system (GE Healthcare, Piscataway, N.J.) following the standard manufacturer's protocol. The wash

Example 2

Expression of Engineered Proteins in Maize

[0075] Plant transformation vectors were constructed each comprising a recombinant DNA molecule encoding one of three engineered proteins with the protein-coding sequence optimized for monocot expression, MON-HT51 (SEQ ID NO:3), MON-HT52 (SEQ ID NO:6), and MON-HT55 (SEQ

ID NO:13). The vectors were created using different combinations of promoter, leader, intron, and 3'UTR and with and without a CTP operably linked to the protein-coding sequence. Also included in the vectors was a second DNA cassette comprising a cp4-EPSPS coding sequence to be used in transgenic plants for glyphosate tolerance. Immature maize (LH244) embryos were transformed with these vectors using *Agrobacterium tumefaciens* and standard methods known in the art. Regenerated R0 transgenic plantlets were grown in the green house and sprayed at approximately V2-V4 growth stage with 0.04 or 0.08 lb ai/acre quizalofop-P (Assure™ II, E.I. DuPont), representing 0.5× and 1× rates, respectively. Leaf samples were used to identify transgenic plants with a single copy of the transgenic DNA insert (that is, single event plants). R0 plants that contained only a single copy and passed either 0.5× or 1× quizalofop-P spray testing were selfed to produce R1 seed. No events were obtained with the constructs containing MON-HT52. Only one event was regenerated from the construct containing MON-HT51 with a CTP and from the construct containing MON-HT51 without a CTP. Two pairs of vectors containing MON-HT55 were transformed, with each pair differing only in containing a CTP or not containing a CTP.

[0076] R1 plants expressing MON-HT55 with and without an operably linked CTP were grown in the green house and quizalofop-P herbicide was applied at the V2 growth stage at a rate of 0.08 lb ae/acre (1×). Plants were evaluated for injury eleven days after treatment. The R1 plants were segregating for the trait in typical Mendelian ratio, and the expected numbers (~25%) of null segregants (progeny plants not containing the transgenic trait) were seen that did not survive the herbicide treatment. All R1 transgenic plants expressing MON-HT55 with an operably linked CTP, with the exception of those representing one event, showed only minor chlorotic speckling on the youngest exposed leaves following application of quizalofop-P. No injury scores over 5% were recorded for these plants after herbicide application. The unsprayed transgenic plants also did not differ phenotypically from the unsprayed control plants. FIG. 1A shows control LH244 plants and transgenic plants comprising MON-HT55 (SEQ ID NO:13) 18 days after application of quizalofop-P.

[0077] To assess the effect of using a CTP to target the engineered protein to the plant cell chloroplast, transgenic plants comprising a transgene insert with and without a CTP operably linked to the protein-coding sequence were compared. The plants comprising a CTP operably linked to the protein-coding sequence showed better tolerance to quizalofop-P compared to those without a CTP. In the R1 green house testing described in this Example 2, most of the transgenic plants comprising a CTP operably linked to the protein-coding sequence showed complete quizalofop-P tolerance. The plants not comprising a CTP operably linked to the protein-coding sequence showed quizalofop-P tolerance but with some moderate injury phenotypes. These results demonstrated that the use of a CTP to target the engineered protein to the plant cell chloroplast enhanced the transgenic plant's quizalofop-P tolerance. This unexpected finding was tested again in trait efficacy field trials with R1 plants comprising either MON-HT51 with or without a CTP operably linked to the protein-coding sequence or MON-HT55 with or without a CTP operably linked to the protein-coding sequence. These R1 plants were single-copy, but were still segregating. In this field trial, seed was planted in the field and treated as follows: 2× (0.16 lb ai/acre) quizalofop-P at pre-plant, 2× (0.14 lb

ai/acre) haloxyfop at V4 growth stage, then 2× quizalofop-P at V8 growth stage. A higher percentage of plants comprising a CTP operably linked to the protein-coding sequence survived quizalofop-P and haloxyfop applications and had lower injury scores compared to plants not comprising a CTP operably linked to the protein-coding sequence. Data are provided in Table 4. This confirmed the unexpected finding that a CTP operably linked to the protein-coding sequence confers higher plant tolerance to herbicide application for the engineered proteins.

TABLE 4

Protein	CTP	Unique events	Average % Herbicide Injury
MON-HT51	No	1	95%
MON-HT51	Yes	1	35%
MON-HT55	No	12	49%
MON-HT55	Yes	3	25%
MON-HT55	No	2	90%
MON-HT55	Yes	7	24%

[0078] Inbred trait efficacy field trials were conducted to assess tolerance to the AOPP herbicides and sensitivity to the cyclohexanediones (CHD) herbicides in an inbred background. R2 inbred plants were generated by selfing a homozygous transgenic R1 plant and collecting seed. R2 inbred plants containing MON-HT55 with or without a CTP or MON-HT51 with a CTP and were evaluated at two field locations. Herbicide treatment was 2× quizalofop-P at 0.16 lb ai/acre applied PRE (after planting but before emergence) followed by quizalofop-P at 0.16 lb ai/acre applied at V4 growth stage followed by quizalofop-P at 0.16 lb ai/acre applied at V8 growth stage. Plots were rated for crop injury 7-10 days after herbicide application on a scale of 0-100 with zero being no injury and 100 being complete crop death. All data were subjected to analysis of variance and means separated at LSD (0.05). Most of the inbred R1 plants showed no injury, confirming that both MON-HT55 and MON-HT51, with or without a CTP, confer quizalofop-P tolerance to maize. To test for sensitivity to CHD herbicides, which is desirable for use in volunteer control, plants were treated with a 1× rate of clethodim (0.25 lb ai/acre) at V8 growth stage. Volunteer control using a 1× rate of clethodim was 100% effective for all transgenic plants tested. Hybrid trait efficacy field trials were conducted to assess tolerance to the AOPP herbicides and sensitivity to the cyclohexanediones (CHD) herbicides in a hybrid background. F1 hybrid plants were produced by crossing an R1 inbred plant with a non-transgenic plant and collecting seed. The resulting F1 plants containing MON-HT55 (SEQ ID NO:13) with or without a CTP or MON-HT51 (SEQ ID NO:3) without a CTP were evaluated at six field locations. The hybrid trait efficacy field trials were conducted at six locations under a range of environmental conditions, including high heat and drought conditions during the field season. This permitted the engineered protein to be evaluated in maize under high temperature and water stress conditions. Data are provided in Table 5. Initial injury from 2× applications of quizalofop-P were higher than desired (>10% injury) at 7-10 days after application. Plants eventually grew out of most of the injury, with generally less injury from the V8 growth stage application as compared to the V4 growth stage application. Excessive injury was also noted when quizalofop-P was applied very early (for example, at VE-V2 growth stage).

TABLE 5

Protein	CTP	Unique events evaluated	% injury after V4 spray	% injury after V8 spray
MON-HT51	Yes	4	51.8	33.3
MON-HT55	No	9	46.8	78.8
MON-HT55	Yes	17	42.6-48.3	14.2-38.8

[0079] The finding that hybrid plants expressing the engineered proteins MON-HT55 or MON-HT51 were sensitive to quizalofop-P application when grown in field conditions of high temperature was confirmed using a plant-based assay. The plant-based assay was designed to test tolerance to quizalofop-P of F1 hybrids in the growth chamber prior to field testing. The assay was developed using F1 hybrids of plants containing maize events NK603 (U.S. Pat. No. 8,273,959)×MON89034 (U.S. Pat. No. 8,581,047) and F1 hybrids of plants containing MON-HT55×MON89034. F1 hybrid seed was germinated in a growth chamber for 1 week and then moved to one of three different growth chambers to acclimate for two days at day/night temperatures of 20° C./20° C., 28° C./20° C., and 38° C./30° C. prior to application of 2× (0.16 lb ai/acre) quizalofop-P. As expected, the plants not containing MON-HT55 at all temperature regimens were severely injured by 2× quizalofop-P application (FIG. 1B). The transgenic plants containing MON-HT55 showed good tolerance to the 2× quizalofop-P application when acclimated to day/night temperatures of 20° C./20° C. or 28° C./20° C., but showed significant sensitivity when acclimated to day/night temperatures of 38° C./30° C. (FIG. 1C). This confirmed that the plant-based assay could be used to screen proteins in plants in the growth chamber for temperature-sensitive activity.

[0080] The data demonstrated that the engineered proteins could be expressed in transgenic plants to confer herbicide tolerance and that unsprayed transgenic plants did not differ phenotypically from the unsprayed control plants. The data also confirmed that expression of the engineered proteins in plants permitted use of CHD herbicides for volunteer control. Unexpectedly, the data showed that use of a CTP for chloroplast targeting of the engineered protein enhanced the herbicide tolerance trait and that the herbicide tolerance provided by the engineered proteins was temperature-sensitive, decreasing under high temperature conditions.

Example 3

Optimizing Engineered Proteins

[0081] The finding that hybrid events expressing the engineered proteins MON-IIT55 or MON-IIT51 were sensitive to quizalofop-P application when grown in field conditions of high temperature was surprising and provided an additional protein characteristic capable of being altered through protein engineering. A new series of in vitro enzyme assays and a plant-based enzyme activity assay were developed to test proteins for sensitivity to high temperatures.

[0082] To create engineered proteins optimized for activity in higher temperatures, the protein motif analysis used in the first two rounds of protein engineering was combined with crystal structure data for several of the engineered proteins. This was used to inform additional rounds of mutagenesis, performed as described previously, and approximately 1400 additional engineered proteins were thus generated. These were combined with the approximately 1200 engineered pro-

teins described in Example 1 for a total of approximately 2600 engineered proteins for screening with the new temperature sensitivity assay to identify proteins optimized for activity in higher temperatures.

[0083] To analyze these engineered proteins for activity in higher temperatures, the in vitro enzyme assay in Example 1 was modified to specify pre-heating all of the assay components to the desired temperature for 5 minutes before combining the components and then maintaining the reaction at the desired temperature for the duration of the reaction. To normalize the assay measurements, quizalofop-P was used as the substrate for the reaction and enzyme activity was normalized based on the 25° C. readings. Using these parameters, the temperature at which the enzyme activity was half of maximum ($T_{1/2}$) was calculated. The $T_{1/2}$ for MON-HT55 was calculated to be 29° C., and the $T_{1/2}$ for wild-type RdpA was calculated to be 38° C. (FIG. 2A).

[0084] Because of the large number of variants to test, a five-level screening process was used. Table 6 shows the approximate number of variants tested in the different screenings.

TABLE 6

Engineered proteins tested	
First screen	~2600
Second screen	~1250
Third screen	94
Fourth screen	47
Fifth screen	11

[0085] The first screen was performed with approximately 2600 engineered proteins and used the high-throughput bacterial protein expression and enzyme assay system with crude bacterial lysates as described in Example 1 but modified to be conducted at the desired temperatures of 25° C. and 40° C. with the post-quenching color development done at 25° C. From this screen, approximately 1250 engineered proteins were selected and advanced. The second screen was similar, but included protein normalization across samples. From this screen, 94 engineered proteins were selected and advanced. The third screen used purified protein with the herbicide degradation enzyme assay as described in Example 1, but modified to be conducted at the desired temperatures of 25° C. and 40° C. with the post-quenching color development done at 25° C. From this screen, 47 engineered proteins were selected and advanced. The fourth screen was done using purified protein, the protein concentrations were normalized, and the screening included quizalofop-P and (for a subset of the proteins variants) 2,4-D as substrates with end-point assays done at 23° C. and 40° C. From this screen, thirteen engineered proteins were selected and advanced.

[0086] For the fifth screen, recombinant protein for each of the eleven engineered proteins was produced and purified for an in-depth biochemical analysis. This biochemical analysis included: (1) kinetic analysis (V_{max} and K_m), (2) activity assays over a range of temperatures, (3) protein melting analysis, (4) activity on additional AOPP herbicides substrates, and (5) mass spectrometry analysis on peptides to confirm identity. The biochemical analyses were also done with purified recombinant wild-type protein and MON-HT55 protein for comparison. For the kinetic analysis, a non-end-point assay was conducted at 23° C. with either quizalofop-P or 2,4-D as substrate. The recombinant proteins for these

assays were produced in bacteria and purified using a 6-His tag fused at the C-terminal end of the protein. Results of the kinetic analysis with either quizalofop-P or 2,4-D as substrate are presented for ten of the engineered proteins, wild-type protein, and MON-HT55 protein in Table 7 (standard error is shown in parenthesis). Vmax is expressed as specific activity, $\mu\text{mol herbicide product mg enzyme}^{-1} \text{ min}^{-1}$; Km expressed as mM herbicide substrate. NDB indicates that enzyme activity may be evident at higher concentrations of herbicide, but activity under the concentrations tested was not robust enough to provide proper kinetic characterization. For MON-HT55, low activity levels (Vmax) with 2,4-D as the substrate resulted in low confidence in the reported value. MON-HT7 had a Vmax with quizalofop-P that is approximately 40% greater than the wild-type enzyme and a Vmax for 2,4-D that is only about half of that of the wild-type enzyme. MON-HT1 had a Vmax for quizalofop-P that is about half that of the wild-type enzyme and a Vmax for 2,4-D that is 9.5-fold higher than that of the wild-type enzyme. The differentiation of the protein kinetics for MON-HT1 and MON-HT7 were surprising because there are only four amino acid differences between MON-HT1 and MON-HT7. Specifically, MON-HT1 has the following amino acids at the indicated position: I82; F105; T112; and V273, and MON-HT7 has the following amino acids at the indicated position: L82; V105; S112; and A273.

TABLE 7

Protein	Vmax Quizalofop	Km Quizalofop	Vmax 2,4-D	Km 2,4-D
MON-HT55 (SEQ ID NO: 11)	0.44 (−0.04)	0.32 (−0.07)	0.03 (−0.02)	0.69 (−0.66)
MON-HT1 (SEQ ID NO: 14)	0.76 (−0.04)	0.17 (−0.03)	0.95 (−0.03)	0.03 (0.00)
MON-HT2 (SEQ ID NO: 18)	1.41 (−0.11)	0.41 (−0.06)	0.23 (−0.01)	0.04 (−0.01)
MON-HT3 (SEQ ID NO: 22)	0.77 (−0.04)	0.38 (−0.05)	0.10 (−0.01)	0.27 (−0.06)
MON-HT4 (SEQ ID NO: 25)	0.30 (−0.02)	0.24 (−0.05)	NDB	NDB
MON-HT5 (SEQ ID NO: 28)	0.57 (−0.03)	0.17 (−0.03)	NDB	NDB
MON-HT6 (SEQ ID NO: 31)	0.39 (−0.03)	0.10 (−0.03)	NDB	NDB
MON-HT7 (SEQ ID NO: 34)	1.94 (−0.12)	0.33 (−0.05)	0.04 (−0.01)	0.06 (−0.04)
MON-HT8 (SEQ ID NO: 37)	0.46 (−0.02)	0.11 (−0.02)	0.11 (−0.01)	0.14 (−0.05)
MON-HT9 (SEQ ID NO: 40)	0.53 (−0.02)	0.10 (−0.01)	NDB	NDB
MON-HT10 (SEQ ID NO: 43)	0.78 (−0.05)	0.13 (−0.03)	NDB	NDB
Wild-type RdpA	1.38 (−0.06)	0.27 (−0.03)	0.10 (−0.01)	0.03 (−0.01)

[0087] Enzyme activity over a range of temperatures was analyzed in-depth for MON-HT1, MON-HT2, MON-HT7, and MON-HT8. These assays were conducted as described above. Quizalofop-P or 2,4-D was used as the substrate for these reactions and activity was normalized based on the activity of the wild-type enzyme at 25° C. The activity curves obtained are presented in FIG. 2B (with quizalofop-P as the substrate) and FIG. 2C (with 2,4-D as the substrate). Using quizalofop-P as the substrate, MON-HT55 was the most temperature sensitive, with a $T_{1/2}$ of 29° C. The wild-type enzyme had a $T_{1/2}$ of 38° C. MON-HT1 and MON-HT8 were less temperature sensitive than the wild-type enzyme with a $T_{1/2}$ of 42° C. and 41° C., respectively, at which temperatures the wild-type enzyme is 90% inactive. MON-HT2 and MON-HT7 were much less temperature sensitive with a $T_{1/2}$ of 46° C. and 47° C., respectively, at which temperatures the wild-type enzyme is completely inactive. When 2,4-D was used as the substrate, the wild-type enzyme had a $T_{1/2}$ of 36° C. MON-HT2, MON-HT7, and MON-HT8 were all slightly more temperature sensitive with a lower $T_{1/2}$ than the wild-type enzyme. MON-HT1 was slightly less temperature sensitive, with a $T_{1/2}$ about 1° C. higher than the wild-type enzyme.

[0088] A protein melting analysis was also conducted. For the protein melting determinations, purified enzyme was added to 96-well microtiter plates in standard storage buffer (30 mM Tris pH7.5, 150 mM NaCl) with or without 50 μM Fe2+ and 1.0 mM aKG. Protein unfolding was then detected with SYPRO® orange protein gel stain (Invitrogen™ catalog #S6651, Life Technologies, Grand Island, N.Y.) in a BioRad CFX96™ Real time PCR machine (BioRad, Hercules, Calif.) with readings taken between 10° C. to 95° C. in 0.5° C. steps. The $T_{1/2}$ (here, the temperature where 50% of the protein was unfolded) is shown in Table 8. The wild-type enzyme showed stabilization with 50 μM Fe2+ and 1.0 mM aKG. In contrast, 50 μM Fe2+ and 1.0 mM aKG had little effect on the stability of any of the engineered proteins. MON-HT55, MON-HT3, MON-HT4, MON-HT6, and MON-HT10 had melting temperatures in the range of 41° C. to 48° C., which is below the melting temperature of the wild-type enzyme. MON-HT1, MON-HT2, MON-HT5, MON-HT7, MON-HT8, and MON-HT9 had melting temperatures between 58° C. and 67° C., which is 8° C. to 17° C. higher than the wild-type enzyme. For MON-HT7 and MON-HT1, the difference in melting point was 11° C. in buffer without Fe2+ and aKG and 8° C. in buffer with Fe2+ and aKG. This was surprising since there are only four amino acid differences between the two enzymes. This data on the melting point of the enzymes confirms that the engineered proteins have been optimized for protein stability

at higher temperatures. This data also matches the enzyme activity assay results for the proteins conducted at different temperatures.

TABLE 8

Protein	Buffer	Buffer plus Fe2+ and aKG
MON-HT55 (SEQ ID NO: 11)	41° C.	41° C.
MON-HT1 (SEQ ID NO: 14)	56° C.	59° C.
MON-HT2 (SEQ ID NO: 18)	55° C.	58° C.
MON-HT3 (SEQ ID NO: 22)	46° C.	48° C.
MON-HT4 (SEQ ID NO: 25)	43° C.	44° C.
MON-HT5 (SEQ ID NO: 28)	60° C.	61° C.
MON-HT6 (SEQ ID NO: 31)	44° C.	44° C.
MON-HT7 (SEQ ID NO: 34)	67° C.	67° C.
MON-HT8 (SEQ ID NO: 37)	61° C.	61° C.
MON-HT9 (SEQ ID NO: 40)	61° C.	60° C.
MON-HT10 (SEQ ID NO: 43)	44° C.	44° C.
Wild-type RdpA	42° C.	50° C.

[0089] The enzyme activity of MON-HT protein variants with haloxyfop, fenoxaprop, fluazifop, and dichlorprop as

substrates was determined using the enzyme activity assay conducted at 23° C. with purified enzyme. The activity was recorded as the maximum activity as a percentage of the wild-type enzyme's activity, which was set at 100%. Data are provided in Table 9. MON-HT55, MON-HT3, MON-HT4, MON-HT5, and MON-HT9 had maximum activities for all four substrates lower than, or equal to, the maximum activity of the wild-type enzyme with the same substrate. With haloxyfop as the substrate MON-HT1, MON-HT2, MON-HT7, and MON-HT10 had a maximum activity that was greater than that of the wild-type enzyme. With fenoxaprop as the substrate MON-HT1, MON-HT2, MON-HT6, MON-HT7, MON-HT8, and MON-HT10 had a maximum activity that was greater than that of the wild-type enzyme. With fluazifop as the substrate MON-HT2 and MON-HT7 had a maximum activity that was greater than that of the wild-type enzyme. With dichlorprop as the substrate MON-HT1, MON-HT7, and MON-HT8 had a maximum activity that was greater than that of the wild-type enzyme.

TABLE 9

Protein	Haloxyfop	Fenoxaprop	Fluazifop	Dichlorprop
MON-HT55 (SEQ ID NO: 11)	58	34	38	31
MON-HT1 (SEQ ID NO: 14)	134	175	67	175
MON-HT2 (SEQ ID NO: 18)	142	121	124	92
MON-HT3 (SEQ ID NO: 22)	67	83	60	100
MON-HT4 (SEQ ID NO: 25)	49	53	52	39
MON-HT5 (SEQ ID NO: 28)	100	92	46	36
MON-HT6 (SEQ ID NO: 31)	75	333	47	37
MON-HT7 (SEQ ID NO: 34)	193	210	161	210
MON-HT8 (SEQ ID NO: 37)	99	106	50	106
MON-HT9 (SEQ ID NO: 40)	91	67	41	19
MON-HT10 (SEQ ID NO: 43)	124	233	48	29
Wild-type RdpA	100	100	100	100

[0090] The enzyme identities were confirmed using mass spectroscopy. For this analysis, purified protein was separated on a PAGE gel and stained. The stained bands were then cut out, destained, and trypsin digested using standard protocols. Trypsin digested protein preparations were separated on a Dionex UltiMate® 3000 RSLCnano LC System (Thermo Scientific, Sunnyvale, Calif.) using a Thermo Scientific™ AQUASIL™ C-18 Javelin™ Guard column under standard conditions and injected for MS-MS analysis using a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, Sunnyvale, Calif.).

[0091] To optimize proteins for increased activity in the presence of phenoxy acid herbicides, computational protein engineering was performed on the crystal structure for several of the engineered proteins. This was used to inform additional rounds of mutagenesis, performed as described previously but using the bacterial sequence, SEQ ID NO:15, encoding the protein sequence MON-HT1 (SEQ ID NO:14) as the starting sequence. Approximately 472 additional engineered

proteins were generated. These were combined with the approximately 2600 engineered proteins described in Example 1 and Table 6 for a total of approximately 3072 engineered proteins to identify proteins optimized for activity in the presence of 2,4-D. The first screen of the new variants was the high-throughput (HTP) bacterial protein expression and enzyme assay system with crude bacterial lysates as described in Example 1, but modified to be conducted at the desired temperatures of 25° C. and 40° C. with the post-quenching color development done at 25° C. Following this HTP screen, approximately 34 engineered proteins were selected and advanced into screening with protein normalization across all of the samples. From this screen, 12 engineered proteins were selected and advanced to screening with purified protein assayed with the herbicide degradation enzyme assay as described in Example 1. Enzyme heat stability was assayed with limited protein melting assays. From this screen, 7 engineered proteins were selected and advanced for in plant testing. Three enzyme variants were selected for

detailed characterization using purified protein, where the protein concentrations were normalized, and the screening included quizalofop-P and 2,4-D as substrates, as well as additional herbicides shown in Table 10 and Table 12 and protein melting characterization.

[0092] Kinetic analysis using a non-endpoint assay was conducted at 23° C. with either quizalofop-P or 2,4-D as substrate, as detailed above for purified protein of the wild-type enzyme, MON-HT1, MON-HT13, MON-HT15, and MON-HT17. The data demonstrate a significant and unexpected enhancement of enzymatic activity of MON-HT13, MON-HT15, and MON-HT17 relative to both the wild-type RdpA enzyme and the MON-HT1 enzyme when tested with 2,4-D as a substrate. Specifically, all three variants showed a roughly 2.5 to 3-fold increase in activity (V_{max}) relative to MON-HT1. Enzymatic activity of MON-HT13, MON-HT15, and MON-HT17 variants with quizalofop as the substrate was roughly similar to the activity of MON-HT1. See Table 10.

TABLE 10

Protein	Quizalofop		2,4-D	
	V _{max}	K _m	V _{max}	K _m
Wild-type RdpA	2.76 (0.11)	0.09 (0.013)	0.25 (0.01)	0.13 (0.017)
MON-HT1 (SEQ ID NO: 14)	1.62 (0.05)	0.12 (0.012)	1.17 (0.01)	0.03 (0.002)
MON-HT13 (SEQ ID NO: 47)	1.51 (0.05)	0.12 (0.013)	3.38 (0.05)	0.08 (0.004)

TABLE 10-continued

Protein	Quizalofop		2,4-D	
	Vmax	Km	Vmax	Km
MON-HT15 (SEQ ID NO: 49)	1.52 (0.06)	0.12 (0.017)	3.53 (0.04)	0.07 (0.004)
MON-HT17 (SEQ ID NO: 51)	1.53 (0.05)	0.14 (0.014)	2.91 (0.04)	0.07 (0.004)

[0093] A protein melting analysis was conducted as detailed above. The melting temperatures of MON-HT13, MON-HT15, and MON-HT17 was similar to the melting temperature of MON-HT1 with $T_{1/2}$ in buffer in the range of 55-58° C., and with $T_{1/2}$ in buffer plus Fe2+ and aKG in the range of 60-62° C. These data indicate that the MON-HT13, MON-HT15, and MON-HT17 variants have a similar enzyme heat stability compared to MON-HT 1. This data on the melting point of the enzyme variants confirms that the engineered proteins have been optimized for protein stability at higher temperatures. See Table 11.

TABLE 11

Protein	Melting temp ° C.	
	Buffer	Buffer plus Fe2+ and aKG
Wild-type RdpA	43	53
MON-HT1 (SEQ ID NO: 14)	58	62
MON-HT13 (SEQ ID NO: 47)	57	62
MON-HT15 (SEQ ID NO: 49)	55	60
MON-HT17 (SEQ ID NO: 51)	57	61

[0094] The enzyme activity of MON-HT protein variants with triclopyr, fluroxypyr, MCPA, MCPB, mecoprop, as substrates was determined using the enzyme activity assay conducted at 23° C. with purified enzyme. The activity was recorded as the maximum activity as a percentage of the wild-type RdpA enzyme's activity, which was set at 100%. Data are provided in Table 12. For each of the proteins assayed (MON-HT1, MON-HT13, MON-HT15, and MON-HT17) with the herbicides triclopyr and fluroxypyr as the substrate, there was detectable activity, especially in the engineered variants, but activity was not robust enough to quantify. There was no detectable activity for each of the proteins assayed (MON-HT1, MON-HT13, MON-HT15, and MON-HT17) with the herbicide MCPB as the substrate. The enzymatic activity with mecoprop as a substrate was reduced for each of the MON-HT1, MON-HT13, MON-HT15, and MON-HT17 variants compared to the wild-type RdpA enzyme. An unexpected result was that enzymatic activity with MCPA as a substrate was roughly 6-fold greater for MON-HT1, and approximately 10-fold greater for MON-HT13, MON-HT15, and MON-HT17 compared to the wild-type RdpA enzyme. See Table 12.

TABLE 12

Protein	Mecoprop		MCPA	
	Vmax	Km	Vmax	Km
Wild-type RdpA	100	100	100	100
MON-HT1 (SEQ ID NO: 14)	63	142	600	21
MON-HT13 (SEQ ID NO: 47)	37	92	1800	68

TABLE 12-continued

Protein	Mecoprop		MCPA	
	Vmax	Km	Vmax	Km
MON-HT15 (SEQ ID NO: 49)	25	57	1300	59
MON-HT17 (SEQ ID NO: 51)	27	65	1400	62

Example 4

Expression of Optimized Engineered Proteins in Maize

[0095] Ten unique engineered proteins optimized for activity at higher temperatures were selected for maize transformation and analysis in plants. DNA constructs were produced for expressing these engineered proteins with codon usage optimized for monocot expression using methods known to those skilled in the art. Enhancers, promoters, leaders, introns, CTPs, and 3'UTRs were tested in various combinations with the engineered proteins in these DNA constructs. The DNA constructs were used to transform immature maize (LH244) embryos with these vectors using *Agrobacterium tumefaciens* and standard methods known in the art. Regenerated R0 transgenic plantlets were grown in the green house.

[0096] Transgenic R0 maize plants were screened by the application of quizalofop-P (2×) plus 2,4-D (2×) at 7 to 10 days following transplant into plugs (generally corresponding to V3-V4 growth stage). All constructs tested produced plants containing unique events that passed the R0 screen. The R0 plants were selfed to generate R1 homozygous seed and the R0 was also used as the male to cross with inbred plants containing maize event MON89034 to generate segregating F1 hybrid seed for efficacy field trials.

[0097] An efficacy field trial was conducted with segregating F1 hybrid plants, with 50% hemizygous and 50% null for the transgene. Tolerance to quizalofop-P (2×) plus 2,4-D (2×) was assessed using two herbicide application regimens: (1) quizalofop-P (Assure II) at 0.16 lb ai/acre (2×) plus 0.25% v/v non-ionic surfactant (NIS) applied at VE-V2 growth stage followed by the same at V4 growth stage followed by the same at V8 growth stage and (2) 2,4-D amine at 2 lb ae/acre (2×) plus quizalofop-P at 0.04 lb ai/acre (0.5×) plus 0.25% v/v NIS applied at VE-V2 growth stage followed by 2,4-D amine at 2 lb ae/acre (2×) plus 0.25% v/v NIS applied at V4 growth stage followed by the same at V8 growth stage. The 50% of the plants that were null for transgene were removed by the first quizalofop-P application at VE-V2 growth stage. Plots were visually rated 10-14 days after application for crop injury on a scale of 0 to 100 with "0" being none and "100" being complete crop destruction. Table 13 shows the average injury rating at V4 and V8 growth stages for both spray regimens. Injury ratings of <10% were considered very good tolerance and injury ratings of <20% were considered good to fair tolerance. The percentage injury rating with 2× application of 2,4-D at V8 growth stage ranged from a high of 40% to

a low of 0. Similarly, the percentage injury rating with 2× application of quizalofop-P at V8 growth stage ranged from a high of 90% to a low of 0. Variation in injury rating between plants expressing the same protein is likely due to variations in construct design or transgene insertion location. This data confirmed that plants expressing the engineered proteins exhibited tolerance to 2,4-D and quizalofop herbicide application at the 2× rate.

TABLE 13

Protein	CTP	% Injury from V4 Quizalofop	% Injury from V8 Quizalofop	% Injury from V4 2,4-D	% Injury from V8 2,4-D
MON-HT1	A	8.33	8.33	7.5	5.83
MON-HT1	None	7.14	7.86	12.86	10.71
MON-HT1	B	7.5	8.75	10	10
MON-HT1	C	0.45	0.91	14.09	11.36
MON-HT2	A	0	0	20	34.17
MON-HT2	None	0	0	20	33.75
MON-HT2	B	11.67	11.67	10.56	13.33
MON-HT2	C	14.33	14.33	12	12
MON-HT3	A	1.6	0.5	21.7	9.2
MON-HT4	A	6.1	0	22.3	12.3
MON-HT5	A	6.8	1.7	27.2	17.4
MON-HT6	A	8	1.7	27	12.7
MON-HT7	A	31.2	5	21.6	16.6
MON-HT8	A	1.25	1.25	10	10
MON-HT8	None	85	90	0	0
MON-HT8	B	9.17	8.33	12.5	11.67
MON-HT8	C	30	32.5	20	21.25
MON-HT9	A	5	5	20	40
MON-HT10	A	0	0	28	38.33
MON-HT3 + MON-HT1	A + none	6.67	5.56	20.56	18.89
MON-HT4 + MON-HT8	A + none	11.25	11.88	9.38	4.38

[0098] A plant-based enzyme activity assay for heat sensitivity was used to determine the effect of elevated growth temperatures on the herbicide tolerance of transgenic plants containing the optimized engineered proteins. To test for quizalofop-P tolerance at elevated growth temperatures, F1 hybrid (produced by crossing an R1 homozygous plant expressing one of the MON-HT proteins with inbred maize event MON89034) maize seed was grown in a growth chamber for 10 days at a day temperature of 28° C. and a night temperature of 20° C. with 50% humidity. After 10 days, the plants were moved to acclimate for 3 days at one of three different day and night temperature regimens: (1) both day and night temperatures set at 20° C.; (2) day temperature at 28° C. and night temperature at 20° C.; or (3) day temperature at 38° C. and night temperature at 30° C. At the end of the acclimation period, the plants were generally at V4 growth stage and were sprayed with 2× quizalofop-P. Ten days post treatment the plants were scored for injury on a rating scale of 1 to 5 where '0' is no visible injury observed, '1' is chlorotic speckling, '2' is chlorotic streaking, '3' there are leaf gaps or tears, '4' are plants with stunted growth and/or twisted leaves, and '5' are dead plants or no growth observed. Results are presented in FIG. 4. F1 hybrid maize plants expressing MON-HT55 showed good tolerance (injury ratings of around 2) to the spray treatments when day/night temperatures were 20° C./20° C. (FIG. 4A) or 28° C./20° C. (FIG. 4B) relative to the F1 hybrid control plants (maize events NK603×MON89034) (injury rating of 5). When the day/night temperatures were 38° C./30° C. F1 hybrid control plants had an injury rating of 5, F1 hybrid plants expressing MON-HT55 had an average

injury rating of 3, and F1 hybrid plants expressing MON-HT1, MON-HT2, MON-HT3, MON-HT4, or MON-HT7 had injury ratings of ≤1 (FIG. 4C). The engineered proteins optimized for activity in higher temperatures provided AOPP herbicide tolerance when plants expressing these engineered proteins were exposed to high temperatures.

[0099] To test for 2,4-D tolerance at elevated growth temperatures, F1 hybrid plants were produced by crossing an R1 plant containing MON-HT1, MON-HT2, MON-HT3, MON-HT4, MON-HT7, or MON-HT55 with an inbred plant containing maize event MON89034. The F1 hybrid plants were grown in a greenhouse for one week at a minimum temperature of 20° C. and a maximum temperature of 28° C. with 50 to 80% humidity. After 1 week, the plants were moved to acclimate for three days at one of two different day and night temperature regimens: (1) both day and night temperatures set at 20° C. or (2) day temperature at 38° C. and night temperature at 30° C. At the end of the acclimation period, the plants were generally at V4 growth stage and were sprayed with 4× 2,4-D amine. Ten days after treatment the plants were scored for injury using an injury scale of 0 to 100 with "0" being no injury and "100" being a dead plant. When plants were acclimated at day/night temperatures of 20° C./20° C. prior to application of 4× 2,4-D amine, F1 plants containing MON-HT1, MON-HT2, MON-HT3, MON-HT4, MON-HT7, or MON-HT55 had injury rating averages of <10%, and the control plants had injury rating averages of <20% (FIG. 4D). When plants were acclimated at day/night temperatures of 38° C./30° C. prior to application of 4× 2,4-D amine, F1 plants containing MON-HT4 or MON-HT7 had injury rating averages of <20%, F1 plants containing MON-HT1, MON-HT2 or MON-HT3 had injury rating averages of <10% (FIG. 4E), and the control plants and plants containing MON-HT55 F1 plants had injury rating averages of 50% (FIG. 4E). These results demonstrated that the engineered proteins optimized for activity in higher temperatures provided 2,4-D herbicide tolerance when plants expressing these engineered proteins were exposed to high temperatures.

[0100] Separate trait efficacy field trials for quizalofop-P and 2,4-D were conducted at two locations each with F1 hybrid transgenic plants produced by crossing an inbred plant containing maize event MON89034 with an R1 plant containing MON-HT55 (with a CTP), MON-HT1 (with or without a CTP), MON-HT2 (with or without a CTP), MON-HT3 (with a CTP), MON-HT4 (with a CTP), MON-HT5 (with a CTP), MON-HT6 (with a CTP), or MON-HT7 (with a CTP). Transgenic F1 hybrid plants containing maize events NK603×MON89034 were used for comparison as a control.

[0101] In the efficacy field trial for quizalofop-P tolerance and clethodim sensitivity, one of four herbicide treatments was used: (1) quizalofop-P (Assure II) at 0.32 lb ai/acre (4×) plus 0.25% v/v non-ionic surfactant (NIS) applied at VE-V2 growth stage followed by the same at V4 growth stage followed by the same at V8 growth stage; (2) quizalofop-P at 0.64 lb ai/acre (8×) plus 0.25% v/v NIS applied at VE to V2 growth stage followed by the same at V4 growth stage followed by the same at V8 growth stage; (3) quizalofop-P at 1.28 lb ai/acre (16×) plus 0.25% v/v NIS applied at VE to V2 growth stage followed by the same at V4 growth stage followed by the same at V8 growth stage; or (4) clethodim at 0.25 lb ai/acre (1×) plus 0.25% v/v NIS applied at V8 growth stage. Plots were visually rated 10-14 days after application for crop injury on a scale of 0 to 100 with "0" being none and "100"

being complete crop destruction. Tables 14 and 15 show the average injury ratings after herbicide application at V4 or V8 growth stage, respectively.

[0102] Plants containing MON-HT1, MON-HT2, MON-HT3, MON-HT4, MON-HT5, MON-HT6, or MON-HT7 (all operably linked to a CTP) showed very good tolerance to quizalofop-P with injury ratings of less than 15% across all application rates and at both V4 and V8 growth stages. Plants containing MON-HT55 operably linked to a CTP showed moderate to poor tolerance with injury ratings from 0.8% to 78.8%. The injury ratings for the control plants after quizalofop-P application were 99.5%. These results indicate that plants containing MON-HT1, MON-HT2, MON-HT3, MON-HT4, MON-HT5, MON-HT6, or MON-HT7 operably linked to a CTP had very good tolerance to sequential applications of quizalofop-P.

[0103] Plants containing MON-HT1 or MON-HT2 with an operably linked CTP had better tolerance to quizalofop-P than plants containing MON-HT1 or MON-HT2 without an operably linked CTP. Plants containing MON-HT1 with an operably linked CTP had 0 to 5.5% injury rating across all quizalofop-P applications compared to the 3.3% to 18.8% injury ratings of plants containing MON-HT1 without an operably linked CTP. Plants containing MON-HT2 with an operably linked CTP had 1.5% to 10% injury rating across all quizalofop-P applications compared to the 16.3% to 82.5% injury ratings of plants containing MON-HT2 without an operably linked CTP. FIG. 5 shows plants containing MON-HT2 operably linked to a CTP (FIG. 5A) and plants containing MON-HT2 without a CTP (FIG. 5B) 10 to 14 days after quizalofop-P application (treatment 3) at 1.28 lb ai/acre (16x) plus 0.25% v/v NIS applied at VE to V2 growth stage followed by the same at V4 growth stage followed by the same at V8 growth stage. The control plants did not survive, the plants containing MON-HT2 without a CTP had moderate to poor tolerance, and the plants containing the MON-HT2 operably linked to a CTP had robust tolerance to the quizalofop-P application. These results confirmed that the use of an operably linked CTP greatly improves quizalofop-P tolerance.

[0104] All transgenic plants had injury ratings above 90% to an application of 1x clethodim (0.25 lb ai/acre) applied at the V8 growth stage, demonstrating the use of this herbicide for volunteer control in transgenic plants containing the engineered proteins.

[0105] In the efficacy field trial for 2,4-D tolerance, one of four herbicide treatments was used: (1) 2,4-D amine at 2 lb ae/acre (2x) plus 0.25% v/v non-ionic surfactant (NIS) applied to VE to V2 followed by V4 followed by V8; (2) 2,4-D amine at 4 lb ae/acre (4x) plus 0.25% v/v NIS applied to VE to V2 followed by V4 followed by V8; (3) 2,4-D amine at 8 lb ae/acre (8x) plus 0.25% v/v NIS applied to VE to V2 followed by V4 followed by V8 maize; or (4) 2,4-D amine at 16 lb ae/acre (16x) plus 0.25% v/v NIS applied to VE to V2 followed by V4 followed by V8. Plots were visually rated as above. Tables 14 and 15 show the average injury ratings after herbicide application at V4 or V8 growth stage, respectively.

[0106] Plants containing MON-HT1, MON-HT2, or MON-HT6 (all operably linked to a CTP) showed very good tolerance to 2,4-D with injury ratings of less than 10% and less than 17% across all application rates at V4 and V8 growth stages, respectively. Plants containing MON-HT3, MON-HT4, MON-HT5, or MON-HT7 (all operably linked to a CTP) showed good tolerance to 2,4-D with injury ratings of less than 20% and less than 22% across all application rates at V4 and V8 growth stages, respectively. Plants containing MON-HT55 operably linked to a CTP showed moderate to poor tolerance with injury ratings from 7.5% to 66%. The injury ratings for the control plants after 2,4-D application ranged from 40% to 82.2%. These results indicate that plants containing MON-HT1, MON-HT2, MON-HT3, MON-HT4, MON-HT5, MON-HT6, or MON-HT7 had good tolerance to sequential applications of 2,4-D.

[0107] Plants containing MON-HT1 or MON-HT2 with an operably linked CTP in general did not show the marked differences in tolerance to 2,4-D compared to plants containing MON-HT1 or MON-HT2 without an operably linked CTP as was seen with the quizalofop-P application. Plants containing MON-HT1 with an operably linked CTP had 0 to 16.3% injury rating across all 2,4-D applications compared to the 0 to 13.8% injury ratings of plants containing MON-HT1 without an operably linked CTP. Plants containing MON-HT2 with an operably linked CTP had 1.3% to 15% injury rating across all 2,4-D applications compared to the 1.3% to 21.3% injury ratings of plants containing MON-HT2 without an operably linked CTP. However, the difference was notable after 2,4-D application at V4 growth stage for plants containing MON-HT2 with an operably linked CTP (4.5% injury rating at 8x and 7.5% at 16x) compared to plants without the CTP (13.75% injury rating at 8x and 21.25% at 16x).

TABLE 14

V4 Growth Stage	2x 2,4-D	4x 2,4-D	8x 2,4-D	16x 2,4-D	4x Quizalofop	8x Quizalofop	16x Quizalofop
Control	52.5	64.4	73.8	74.7	99.5	99.5	99.5
MON-HT55	30.0	41.3	43.8	66.3	37.5	67.5	77.5
MON-HT55	20.0	23.1	50.0	55.0	12.5	43.8	62.5
MON-HT55	15.0	30.0	58.8	53.8	2.0	7.5	20.0
MON-HT55	12.5	30.0	48.8	52.5	8.0	18.8	48.8
MON-HT55	13.8	31.3	45.0	61.3	6.0	10.0	21.3
MON-HT55	n/a	n/a	n/a	n/a	0.8	13.8	16.3
MON-HT55	7.5	22.5	53.8	63.8	5.5	12.5	13.8
MON-HT1	0.0	0.0	5.8	12.5	5.8	11.3	18.8
(no CTP)							
MON-HT1	0.0	0.0	5.0	6.3	0.0	0.8	4.5
MON-HT2	1.3	2.5	13.8	21.3	42.5	76.3	82.5
(no CTP)							
MON-HT2	1.3	0.0	4.5	7.5	1.5	0.8	10.0
MON-HT3	3.8	6.3	8.8	11.3	0.8	0.0	4.3
MON-HT4	0.0	1.3	8.8	18.8	2.8	3.3	13.8
MON-HT5	2.5	2.5	7.5	17.5	1.3	4.5	4.5

TABLE 14-continued

V4 Growth Stage	2x 2,4-D	4x 2,4-D	8x 2,4-D	16x 2,4-D	4x Quizalofop	8x Quizalofop	16x Quizalofop
MON-HT6	0.0	2.0	5.8	8.8	0.0	1.3	2.8
MON-HT7	1.3	1.3	5.8	12.5	0.8	1.3	3.3

TABLE 15

V8 Growth Stage	1x Clethodim	2x 2,4-D	4x 2,4-D	8x 2,4-D	16x 2,4-D	4x Quizalofop	8x Quizalofop	16x Quizalofop
Control	93.6	40.0	52.8	64.1	82.2	99.5	99.5	99.5
MON-HT55	93.3	16.3	28.8	36.3	55.0	25.0	45.0	78.8
MON-HT55	93.3	14.4	20.0	39.4	56.9	10.0	31.3	41.3
MON-HT55	93.3	11.3	16.3	30.0	47.5	3.8	3.8	16.3
MON-HT55	93.3	11.3	20.0	35.0	47.5	3.8	20.0	38.8
MON-HT55	94.5	15.0	18.8	33.8	57.5	4.3	5.0	18.8
MON-HT55	93.3	n/a	n/a	n/a	n/a	2.5	6.3	18.8
MON-HT55	94.5	12.5	13.8	36.3	52.5	2.0	5.5	11.8
MON-HT1	94.5	2.5	4.0	8.8	13.8	3.3	7.5	16.3
(no CTP)								
MON-HT1	93.3	1.3	1.5	8.3	16.3	2.0	2.0	5.5
MON-HT2	92.0	5.0	8.8	13.8	25.0	16.3	52.5	77.5
(no CTP)								
MON-HT2	93.3	2.5	3.3	10.0	15.0	2.5	3.8	4.8
MON-HT3	94.5	5.5	5.5	11.3	13.8	2.5	2.0	6.0
MON-HT4	94.5	2.0	8.8	12.5	21.3	3.3	3.3	8.0
MON-HT5	92.0	3.3	4.0	10.0	21.3	2.5	0.0	8.0
MON-HT6	93.3	6.3	4.3	11.3	12.5	1.3	2.0	5.0
MON-HT7	93.3	2.0	2.8	9.3	20.0	1.5	0.8	5.5

Example 5

Evaluation of Chloroplast Targeting Peptides on Expression of Optimized Engineered Proteins in Maize

[0108] To evaluate different chloroplast targeting peptides (CTP), plant transformation vectors were constructed, each comprising a recombinant DNA molecule optimized for monocot expression and encoding MON-HT1 (SEQ ID NO:16), MON-HT2 (SEQ ID NO:20), and MON-HT8 (SEQ ID NO:39), MON-HT9 (SEQ ID NO:42), or MON-HT10 (SEQ ID NO:45). The vectors were created using the same combination of promoter, leader, intron, and 3'-UTR, but with one of three separate CTPs (A, B, or C) or without a CTP operably linked to the protein-coding sequence. See Table 16. The DNA constructs were used to transform immature maize (LH244) embryos using *Agrobacterium tumefaciens* and standard methods known in the art. Regenerated R0 transgenic plantlets were grown in the green house. The R0 plants were selfed to generate R1 homozygous seed. The R0 plants were also used as the male to cross with inbred plants containing maize event MON89034 to generate segregating F1 hybrid seed for trait efficacy field trials.

[0109] Separate trait efficacy field trials for quizalofop-P and 2,4-D were conducted at two locations each with the homozygous inbred transgenic plants (R2 or R4 generation). In these field trials, one of two herbicide treatments was used: (1) quizalofop-P (Assure II) at 0.16 lb ai/acre (2x) plus 0.25% v/v non-ionic surfactant (NIS) applied at V4 growth stage followed by the same at V8 growth stage; or (2) 2,4-D amine at 2 lb ae/acre (2x) plus 0.25% v/v non-ionic surfactant (NIS) applied at V4 followed by V8. Injury ratings (crop injury percentage at V4 (CIPV4) or V8 (CIPV8)) were taken 10 to 14 days after the V4 and V8 applications. Error was calculated

using LSD (0.05). The results demonstrated that these plants had tolerance to 2x sequential applications of either quizalofop-P or 2,4-D with injury ratings below 10% following V4 and V8 applications. See Table 16.

TABLE 16

Protein	CTP	2X Quizalofop-P		2X 2,4-D	
		CIPV4 LSD (0.05) =	CIPV8 LSD (0.05) =	CIPV4 LSD (0.05) =	CIPV8 LSD (0.05) =
		1.45	2.2	0.95	1.6
MON-HT1	A	0	2.125	0	2.5
MON-HT1	B	0	1.75	0	1.25
MON-HT1	C	0	1.75	1.25	1.25
MON-HT1	no CTP	0	1.25	0	1.25
MON-HT2	A	0	1.875	0.875	1.25
MON-HT2	B	0	0	0	2.5
MON-HT2	C	0	2	0	1.25
MON-HT2	no CTP	1.25	3.5	0	2.5
MON-HT8	A	0	1.25	1.75	1.25
MON-HT8	B	0.75	2.5	0	1.25
MON-HT8	C	0	1.25	1.25	0
MON-HT9	A	0	0	3.75	1.25
MON-HT10	A	0	0	0	1.25

[0110] Leaf samples were collected from the plants containing transgene cassettes encoding MON-HT1, MON-HT2, and MON-HT8 with and without CTP sequences to determine expression of the mRNA transcribed from the transgene cassette encoding the engineered proteins. Quantigene® analysis was done on the leaf sample extracts to determine mRNA expression of the transgene cassette. For these assays, the probe was to the common 3'-UTR sequence present in each expression cassette used to generate the transgenic plants. Relative expression was calculated by normal-

izing to maize housekeeping genes. A leaf sample was collected from each of eight plants for each construct configuration used to make the transgenic plants, and the reported relative mRNA expression data is an average of the eight samples with standard error.

[0111] Plants containing the transgene construct encoding either MON-HT1 (SEQ ID NO:14) or MON-HT2 (SEQ ID NO:18) had higher relative transgene mRNA expression for constructs containing either the 'A' or 'B' CTP than for constructs without a CTP or with the 'C' CTP. Plants containing the transgene construct encoding MON-HT8 (SEQ ID NO:37) had similar high relative transgene mRNA expression for constructs containing any of the three CTPs (A, B, or C). See Table17.

non-ionic surfactant (NIS) applied at VE-V2 growth stage followed by the same at V4 growth stage followed by the same at V8 growth stage; (2) quizalofop-P at 0.64 lb ai/acre (8x) plus 0.25% v/v NIS applied at VE-V2 growth stage followed by the same at V4 growth stage followed by the same at V8 growth stage; or (3) quizalofop-P at 1.28 lb ai/acre (16x) plus 0.25% v/v NIS applied at VE-V2 growth stage followed by the same at V4 growth stage followed by the same at V8 growth stage. Plots were visually rated as above. Table 18 shows the average injury ratings after herbicide application at V4 (CIPV4) or V8 (CIPV8) growth stage, respectively. The injury rating for the control plants after all quizalofop-P application was 100%. Error was calculated using LSD (0.05).

TABLE 18

MON-HT variant	CTP	4X Quizalofop-P		8X Quizalofop-P		16X Quizalofop-P	
		CIPV4 LSD (0.05) = 1.92	CIPV8 LSD (0.05) = 2.3	CIPV4 LSD (0.05) = 4.7	CIPV8 LSD (0.05) = 5.3	CIPV4 LSD (0.05) = 5.0	CIPV8 LSD (0.05) = 5.4
none	none	100	100	100	100	100	100
MON-HT1	A	0	7.5	2.5	10	5	15
MON-HT1	B	2.5	5	2.5	7.5	2.5	20
MON-HT1	C	0	5	2.5	10	5	20
MON-HT1	no CTP	2.5	10	10	22.5	22.5	37.5
MON-HT2	A	0	5	2.5	7.5	5	15
MON-HT2	B	0	5	0	10	0	20
MON-HT2	C	0	5	5	12.5	12.5	20
MON-HT2	no CTP	35	45	50	37.5	70	55
MON-HT8	A	15	15	37.5	35	60	50
MON-HT8	B	5	7.5	32.5	35	27.5	30
MON-HT8	C	55	45	75	55	85	72.5
MON-HT9	A	0	5	2.5	15	7.5	20
MON-HT10	A	0	5	2.5	10	5	21

TABLE 17

MON-HT variant	CTP	Relative Expression	Standard Error
MON-HT1	A	7.80	1.20
MON-HT1	B	5.95	1.63
MON-HT1	C	3.82	0.44
MON-HT1	no CTP	4.31	0.68
MON-HT2	A	6.96	0.66
MON-HT2	B	6.16	0.65
MON-HT2	C	4.11	0.29
MON-HT2	no CTP	4.66	0.45
MON-HT8	A	8.06	0.48
MON-HT8	B	7.62	0.46
MON-HT8	C	4.87	0.47
LH244 Control	none	0.00	0.00

[0112] Separate trait efficacy field trials for quizalofop-P and 2,4-D pressure screening were conducted at one location each with F1 hybrid transgenic plants produced by crossing an inbred plant containing maize event MON89034 with an R1 plant containing MON-HT1, MON-HT2, MON-HT8, MON-HT9, and MON-HT10 with and without operably linked CTP sequences. Transgenic F1 hybrid plants containing maize events NK603xMON89034 were used for comparison as a control.

[0113] In the trait efficacy field trial for quizalofop-P tolerance, one of three herbicide treatments was used: (1) quizalofop-P (Assure II) at 0.32 lb ai/acre (4x) plus 0.25% v/v

[0114] Plants containing MON-HT1 with any of the three operably linked CTPs (A, B, or C) had better tolerance to quizalofop-P than plants containing MON-HT1 without an operably linked CTP. Plants containing MON-HT1 with the operably linked 'A' CTP had 0 to 15% injury rating across all quizalofop-P applications. Plants containing MON-HT1 with the operably linked 'B' CTP had 2.5% to 20% injury rating across all quizalofop-P applications. Plants containing MON-HT1 with the operably linked 'C' CTP had 0 to 20% injury rating across all quizalofop-P applications compared to plants containing MON-HT1 without an operably linked CTP that had 2.5% to 37.5% injury ratings across all quizalofop-P applications.

[0115] Plants containing MON-HT2 with any of the three operably linked CTPs (A, B, or C) had better tolerance to quizalofop-P than plants containing MON-HT2 without an operably linked CTP. Plants containing MON-HT2 with the operably linked 'A' CTP had 0 to 15% injury rating across all quizalofop-P applications. Plants containing MON-HT2 with the operably linked 'B' CTP had 0 to 20% injury rating across all quizalofop-P applications. Plants containing MON-HT2 with the operably linked 'C' CTP had 0 to 20% injury rating across all quizalofop-P applications compared to plants containing MON-HT2 without an operably linked CTP that had 35% to 70% injury ratings across all quizalofop-P applications.

[0116] Plants containing MON-HT8 with either the operably linked 'A' or 'B' CTP had better tolerance to quizalofop-P than plants containing MON-HT8 with the operably linked 'C' CTP. Plants containing MON-HT8 with the oper-

ably linked 'A' CTP had 15% to 60% injury rating across all quizalofop-P applications. Plants containing MON-HT8 with the operably linked 'B' CTP had 5% to 35% injury rating across all quizalofop-P applications, compared to plants containing MON-HT8 with the operably linked 'C' CTP that had 45% to 85% injury rating across all quizalofop-P applications.

[0117] Plants containing MON-HT1 or MON-HT2 with any of the three operably linked CTPs and plants containing MON-HT9 or MON-HT10 with the operably linked 'A' CTP had better tolerance to quizalofop-P than plants containing

compared to plants containing either MON-HT9 or MON-HT10 with an operably linked 'A' CTP. Over the range of 2,4-D applications the relative ranking of tolerance was: plants containing MON-HT1 had better tolerance than plants containing MON-HT2 which were in turn better than plants containing MON-HT8. Consistent with the data from the quizalofop-P pressure testing trial, plants containing MON-HT1, MON-HT2, or MON-HT8 operably linked to the 'A' CTP showed a slight mathematical, but not statistically significant, advantage over the 'B' and 'C' transit peptide. Error was calculated using LSD (0.05).

TABLE 19

MON-HT variant	CTP	4X 2,4-D		8X 2,4-D		16X 2,4-D	
		CIPV4 LSD (0.05) = 3.4	CIPV8 LSD (0.05) = 3.33	CIPV4 LSD (0.05) = 2.1	CIPV8 LSD (0.05) = 3.5	CIPV4 LSD (0.05) = 3.4	CIPV8 LSD (0.05) = 3.5
None - Control	none	80	88.75	82.5	95	87.5	96.25
MON-HT1	A	5	5	5	10	7.5	15
MON-HT1	B	5	5	5	10	10	15
MON-HT1	C	5	5	5	10	10	17.5
MON-HT1	no CTP	5	5	7.5	10	10	22.5
MON-HT2	A	5	5	10	7.5	10	20
MON-HT2	B	5	5	5	10	10	25
MON-HT2	C	10	15	10	10	15	20
MON-HT2	no CTP	7.5	7.5	17.5	42.5	25	45
MON-HT8	A	7.5	5	10	12.5	15	30
MON-HT8	B	5	7.5	10	27.5	10	20
MON-HT8	C	10	7.5	10	17.5	17.5	25
MON-HT9	A	17.5	55	25	75	45	90
MON-HT10	A	32.5	70	55	87.5	45	90

MON-HT8 with any of the three operably linked CTPs. Plants containing MON-HT9 or MON-HT10 with the operably linked 'A' CTP had tolerance to quizalofop-P across all applications that was comparable to plants containing MON-HT1 or MON-HT2 with any of the three operably linked CTPs. At the highest rate (16x) of quizalofop application, plants containing MON-HT1 or MON-HT2 with an operably linked 'A' CTP had a slightly higher tolerance than plants containing MON-HT1 or MON-HT2 with an operably linked 'B' or 'C' CTP.

[0118] Three herbicide treatments were used in the trait efficacy field trial for 2,4-D tolerance: (1) 2,4-D amine at 4 lb ae/acre (4x) plus 0.25% v/v NIS applied to VE-V2 followed by V4 followed by V8; (2) 2,4-D amine at 8 lb ae/acre (8x) plus 0.25% v/v NIS applied to VE-V2 followed by V4 followed by V8 maize; or (3) 2,4-D amine at 16 lb ae/acre (16x) plus 0.25% v/v NIS applied to VE-V2 followed by V4 followed by V8. Plots were visually rated as above.

[0119] Table 19 shows the average injury ratings in maize after 2,4-D herbicide application at V4 (CIPV4) or V8 (CIPV8) growth stage, respectively. The injury rating for the control plants after all 2,4-D applications ranged from 80% to 96.25%. At the highest rate of 2,4-D (16x) applied through V8, the plants containing MON-HT1 or MON-HT2 operably linked to any of the three CTPs (A, B, or C) had better tolerance than plants containing MON-HT1 or MON-HT2 not operably linked to a CTP. Plants containing MON-HT1, MON-HT2, or MON-HT8 with or without an operably linked CTP had better tolerance to 2,4-D at all applications tested

Example 6

Expression of Optimized Engineered Proteins in Soy

[0120] Two engineered proteins were selected for analysis in transgenic soybean. DNA constructs were produced for expressing MON-HT1 (SEQ ID NO:14) and MON-HT2 (SEQ ID NO:18) with codon usage optimized for dicot expression using methods known to those skilled in the art. Enhancers, promoters, leaders, introns, CTPs, and 3'UTRs in various combinations were operably linked to the engineered proteins in these DNA constructs. The DNA constructs were used to transform soybean using *Agrobacterium tumefaciens* and standard methods known in the art. Regenerated R0 transgenic plantlets were grown in the green house. Approximately 9 weeks after transformation at the 1-2 trifoliate leaf stage, single copy R0 events were identified and sprayed with 2,4-D herbicide at a rate of 0.5x (0.375 lb ae/acre), 2x (1.5 lb ae/acre), or 4x (3.0 lb ae/acre). Approximately 2 weeks after herbicide application, the plants were rated for herbicide injury on a scale of 1 to 3, where 1=little to no injury (<20%), 2=moderate injury (20-50%) and 3=severe injury (>50%).

[0121] R0 soy plants containing each of the constructs showed tolerance to 2,4-D with little to no injury (<20% injury) or moderate injury (20-50%). Data are provided in Table 20. This indicated that the engineered proteins MON-HT1 and MON-HT2 can confer tolerance to 2,4-D in soybean plants.

TABLE 20

Protein	CTP	2,4-D rate	Single copy events	Events with <20% injury	Events with 20-50% injury	Events with injury >50%
MON-HT1	No	0.5X	130	120	10	0
MON-HT1	No	2X	36	6	30	0
MON-HT1	Yes	2X	136	101	35	0
MON-HT2	No	0.5X	22	3	19	0
MON-HT1	Yes	4X	11	5	6	0

[0122] An additional five engineered proteins optimized for activity for 2,4-D were then selected for analysis in transgenic soybean. DNA constructs were produced for expressing MON-HT13 (SEQ ID NO:47), MON-HT14 (SEQ ID NO:48), MON-HT15 (SEQ ID NO:49), MON-HT17 (SEQ ID NO:51), and MON-HT18 (SEQ ID NO:52) with codon usage optimized for dicot expression. The operably linked expression elements (promoter, leader, intron, CTP, and 3'UTR) were identical in all of the constructs. Leaf samples were taken from R0 plantlets and single copy plants were identified using a PCR-based assay. When the single-copy R0 plants had approximately two to three trifoliate leaves, they were treated with either 1.5 lb ae/acre (2×) or 3.0 lb ae/acre (4×) of 2,4-D. Seven days after herbicide application, the plants were scored for herbicide injury based on the percent area of the plant showing injury, as indicated above.

[0123] At the 2× application rate, soy plants containing any of six MON-HT variants (MON-HT1, MON-HT13, MON-HT14, MON-HT15, MON-HT17, and MON-HT18) showed

eleven single-copy plants containing MON-HT17, all eleven plants had an injury score of <20%. Of the twelve single-copy plants containing MON-HT18, nine plants had an injury score of <20% and three plants had an injury score of 20-50%. These results indicate that soy plants containing MON-HT1 (SEQ ID NO:14), MON-HT13 (SEQ ID NO:47), MON-HT14 (SEQ ID NO:48), MON-HT15 (SEQ ID NO:49), MON-HT17 (SEQ ID NO:51), or MON-HT18 (SEQ ID NO:52) had tolerance to 2,4-D at the 4× application rate. Furthermore, this demonstrated that soy plants containing MON-HT13 (SEQ ID NO:47), MON-HT14 (SEQ ID NO:48), MON-HT15 (SEQ ID NO:49), MON-HT17 (SEQ ID NO:51), or MON-HT18 (SEQ ID NO:52) had improved 2,4-D tolerance at the 4× application rate compared to MON-HT1 (SEQ ID NO:14). Based on the percentage of single-copy plants with an injury score of <20%, soy plants containing either MON-HT13 or MON-HT17 had better tolerance to 2,4-D applied at the 4× rate compared to soy plants containing MON-HT1, MON-HT14, MON-HT15, or MON-HT18. See Table 21.

TABLE 21

MON-HT	SEQ ID NO	2,4-D rate	Single copy events	Events with <20% injury	Events with 20-50% injury	Events with injury >50%
MON-HT1	14	2X	12	12	—	—
MON-HT13	47	2X	13	12	1	—
MON-HT14	48	2X	9	9	—	—
MON-HT15	49	2X	5	5	—	—
MON-HT17	51	2X	12	12	—	—
MON-HT18	52	2X	12	11	1	—
MON-HT1	14	4X	11	5	6	—
MON-HT13	47	4X	11	10	1	—
MON-HT14	48	4X	8	6	1	1
MON-HT15	49	4X	7	5	1	1
MON-HT17	51	4X	11	11	—	—
MON-HT18	52	4X	12	9	3	—

excellent tolerance to 2,4-D treatment, as evidenced by all but two of the single copy plants having injury rating of <20%; these two events (one for MON-HT13 and one for MON-HT18) had an injury rating of 20-30%. At the 4× application rate, of the eleven single-copy plants containing MON-HT1, five plants had an injury score of <20%, and six plants had an injury score of 20-50%. Of the eleven single-copy plants containing MON-HT13, ten plants had an injury score of <20% and one plant had an injury score of 20-50%. Of the eight single-copy plants containing MON-HT14, six plants had an injury score of <20%, one plant had an injury score of 20-50%, and one plant had an injury score of >50%. Of the seven single-copy plants containing MON-HT15, five plants had an injury score of <20%, one plant had an injury score of 20-50%, and one plant had an injury score of >50%. Of the

Example 7

Tolerance to Synthetic Auxins Fluroxypyr, Triclopyr, and MCPA

[0124] Tolerance of maize and soy plants containing MON-HT1 to applications of 2,4-D, fluroxypyr, triclopyr, and MCPA was determined F1 hybrid maize seed for three unique events containing MON-HT1 with the 'A' CTP and R2 soy seed were planted in pots. Hybrid maize seed containing NK603×MON89034 and the same soybean germplasm used for plant transformation were used as controls. Plants were grown in a green house and four plants were used for each treatment. The plants were sprayed with herbicide in a growth chamber when the plants were between 6-8 inches (soy) and 10-12 inches (corn) tall, then transferred to a greenhouse programmed to maintain optimum growth conditions.

[0125] For soy, a 2× herbicide application rate of each of the following was used: (1) 2,4-D Amine 4 (1680 g ae/ha) (2) triclopyr (840 g ae/ha, GARLON®); (3) fluroxypyr (840 g ae/ha, Starane®); or (4) MCPA (g ae/ha 1680). Following application of triclopyr, fluroxypyr, or MCPA the primary symptomology on soy was severe necrosis and epinasty. Visual plant injury ratings were made for all treatments on a rating scale from 0% to 100%, where 0% represented plants equivalent to untreated controls and 100% represented plants that were completely dead. All ratings were taken at seven days after treatment. Plants for all three soy MON-HT1 events showed good tolerance to 2,4-D Amine (2,4-D) averaging less than 7% crop injury compared to controls at 90-97% crop injury. No soybean events showed tolerance to triclopyr or fluroxypyr, with injury ratings across all three events averaged 81-97% crop injury compared to controls at 91% crop injury. One of the three soy events showed a low level of tolerance to MCPA with an average injury rating of 72% while the other two events had 90% crop injury compared to the controls at 90% injury. See Table 22.

TABLE 22

Average soy % crop injury for 4 reps						
TRT #	Herbicide	2X Rate (g ae/ha)	MON-HT1 Event 1	MON-HT1 Event 2	MON-HT1 Event 3	Control
1	2,4-D Amine	1680	4.5	6.5	5.3	90.0
2	triclopyr	840	81.3	90.0	90.0	91.3
3	fluroxypyr	840	96.3	93.8	97.5	91.3
4	MCPA	1680	90.0	90.0	72.5	90.0
5	Controls	0	0.0	0.0	0.0	0.0

[0126] For maize, a 4× herbicide application rate of each of the following was used: (1) 2,4-D Amine 4 (3360 g ae/ha); (2) triclopyr (1680 g ae/ha, GARLON®); (3) fluroxypyr (1680 g ae/ha, Starane®); or (4) MCPA (g ae/ha 3360). Following application of triclopyr, fluroxypyr, or MCPA to maize, the primary symptomology was lodging. Plants for all three maize MON-HT1 events were tolerant of 2,4-D averaging less than 15% injury compared to controls at 43% crop injury. The three MON-HT1 maize events appeared to show some low level tolerance to triclopyr with crop injury averaging 26%-37% compared to controls with 47% crop injury. The

three MON-HT1 maize events appeared to show some low level tolerance to fluroxypyr with crop injury averaging 20%-21% compared to controls with 55% crop injury. Two of the MON-HT1 maize events showed good tolerance to MCPA with an average crop injury of less than 6% compared to controls at 31% crop injury. The third maize MON-HT1 event had an average injury rating of 20%. See Table 23. These results of low tolerance to triclopyr and fluroxypyr and good tolerance to MCPA were consistent with the in vitro enzymatic data with purified MON-HT1 enzyme.

TABLE 23

Average maize % crop injury for 4 reps						
TRT #	Herbicide	4X Rate (g ae/ha)	MON-HT1 Event 1	MON-HT1 Event 2	MON-HT1 Event 3	LH244
1	2,4-D Amine	3360	5.8	15.0	5.3	43.8
2	triclopyr	1680	26.3	37.5	35.0	47.5
3	fluroxypyr	1680	20.0	20.0	21.3	55.0
4	MCPA	3360	3.8	5.0	20.0	31.3
5	Controls	0	0.0	0.0	0.0	0.0

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

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Leu Arg Glu Pro Leu Ser Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
35 40 45
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
50 55 60
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
65 70 75 80
Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
85 90 95
Arg Glu Ala Asn Glu Ser Gly Arg Val Ile Gly Asp Asp Trp His Thr
100 105 110
Asp Ser Ser Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
115 120 125
Ile Asp Val Pro Glu His Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
130 135 140
Thr Ala Tyr Asp Ala Leu Ser Asp Gly Leu Lys Lys Leu Ile Ser Gly
145 150 155 160
Leu Asn Val Val His Ser Ala Thr Arg Val Phe Gly Ser Leu Tyr Gln
165 170 175
Ala Gln Asn Arg Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
180 185 190
Ala Asp Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro
195 200 205
Gly Ser Gly Arg Lys Gly Leu Tyr Val Asn Gln Val Tyr Cys Gln Arg
210 215 220
Ile Glu Gly Met Ser Glu Lys Glu Ser Glu Pro Leu Leu Ser Phe Leu
225 230 235 240
Phe Ala His Ala Thr Lys Pro Glu Phe Thr Cys Arg Val Arg Trp Lys
245 250 255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Tyr Ala
260 265 270
Ile Asn Asp Tyr His Gly Gln Thr Arg Ile Leu His Arg Thr Thr Val
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Gly Gly Val Arg Pro Ala Arg
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<210> SEQ ID NO 2

<211> LENGTH: 906

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

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acctggaacg aaatcctcga cgcgttcac acttaccagg tcatctatct tcccgccag	180
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ccccctgctca agagcatcga aggggtatcca gaggtgcaga tgatccgccg cgaagccaac	300
gaaagcgggc gtgtgatcgg tgatgactgg cacaccgaca gcagcttcct ggacgcaccg	360
cgggccgcgc tgggtatgcg cgcgatcgac gtgcccgcgc atggcgccga caccggtttt	420
ctgagcatgt acaccgcgta tgatgcgctg tcggatggcc tgaagaaact gatcagcggg	480
ttgaacgtag tgcacagcgc caccgctgtg ttccgctcgc tctaccaggc ccagaaccgg	540
cgcttcagca acaccagcgt caagggtgatg gacgtcgagc atggcgaccg tgaaccgtg	600
cacccccctgg tggtagacca tccgggcgcgc ggcgcgaagg gcctgtacgt gaaccaggtc	660
tattgccagc gcacgcaggg catgagcgaa aaagaaagcg aaccgctgct gagcttctg	720
tttgcgcatg cgacaaaacc ggaattcacc tgcgcgctgc gctggaagaa ggaccaggtc	780
ctggtctggg acaacctgtg cagcatgcac tatgccatta acgactacca tggccagacc	840
cgcattctgc atcgaccac ggtcggtggc gtgcgcccg cgcccatca tcaccatcat	900
cactag	906

<210> SEQ ID NO 3
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 3

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acctggaacg agatcctcga cgccttcac acttaccag ttatctactt cccaggacag	180
gctatcacga acgaacagca catcgccctc tcgcgagggt tcgggccagt ggaccagtc	240
ccgctgctta agtctatcga aggtaccct gaggtgcaaa tgatccgccg cgaggcgaac	300
gaatccggga ggggtattgg cgacgattgg cacactgact ccagcttcct cgatgctcct	360
ccagcagccg tcgtgatgcg ggccatcgac gtgcccgcgc acggcgccga tacgggtttc	420
ctgtccatgt acactgctta cgacgctctt tctgatggcc tcaagaaact catcagcgga	480
ctcaatgtgg tccactctgc gaccgctgtc ttggctcgc tctatcaggc gcagaatagg	540
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tactgtcagc gaattgaggg tatgagtgag aaggagtccg agccgctgct cagtttctc	720
ttcgcgacg ccaccaagcc cgagttcacc tgcgcgctcc gctggaagaa ggatcaagtc	780
ctggtgtggg acaacctctg caccatgcac tacgccatca atgactatca tgggtcaaacc	840
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 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

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20     25     30
Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
35     40     45
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
50     55     60
Glu Gln Gln Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
65     70     75     80
Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
85     90     95
Arg Glu Ala Asn Glu Ser Gly Arg Val Ile Gly Asp Asp Trp His Thr
100    105    110
Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
115    120    125
Ile Asp Val Pro Glu His Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
130    135    140
Thr Ala Tyr Asp Ala Leu Ser Asp Gly Leu Lys Lys Leu Ile Ser Gly
145    150    155    160
Leu Asn Val Val His Ser Ala Thr Arg Val Phe Gly Ser Leu Tyr Gln
165    170    175
Ala Gln Asn Arg Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
180    185    190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro
195    200    205
Gly Ser Gly Arg Lys Gly Leu Tyr Val Asn Gln Val Tyr Cys Gln Arg
210    215    220
Ile Glu Gly Met Ser Glu Lys Glu Ser Glu Pro Leu Leu Ser Phe Leu
225    230    235    240
Phe Ala His Ala Thr Lys Pro Glu Phe Thr Cys Arg Val Arg Trp Lys
245    250    255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Tyr Ala
260    265    270
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<210> SEQ ID NO 5

<211> LENGTH: 906

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

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caccacctgg tggtagacca tccgggcgcg gcccgcaagg gcctgtacgt gaaccaggtc   660
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<210> SEQ ID NO 6
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 6

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gctatcacga acgaacagca gatcgcttc tcgcgagggt tcggggcagt ggaccagtc   240
ccgctgctta agtctatcga aggtaccct gaggtgcaaa tgatccgccg cgaggcgaaac   300
gaatccggga ggggtattgg cgacgattgg cacactgact ccaccttccct cgatgctcct   360
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cgcttcagca acacctccgt gaaggatcat gacgtggacg cgggagacag ggagactgtc   600
caccgcctcg tcgttactca cctgggtcc gcccgtaagg gtctgtacgt gaaccagggtg   660
tactgtcagc gaattgaggg tatgagttag aaggagtccg agccgctgct cagtttcttc   720
ttcgcgacg ccaccaagcc cgagttcacc tgccgcgtcc gctggaagaa ggatcaagtc   780
ctgggtgtggg acaacctctg caccatgcac tacgccatca atgactatca tgggtcaaacc   840
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<210> SEQ ID NO 7
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

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Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn	50	55	60
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val	65	70	75
Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg	85	90	95
Arg Glu Ala Asn Glu Ser Gly Arg Val Ile Gly Asp Asp Trp His Ser	100	105	110
Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala	115	120	125
Ile Asp Val Pro Glu His Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr	130	135	140
Thr Ala Tyr Asp Ala Leu Ser Asp Gly Leu Lys Lys Leu Ile Ser Gly	145	150	155
Leu Asn Val Val His Ser Ala Thr Arg Val Phe Gly Ser Leu Tyr Gln	165	170	175
Ala Gln Asn Arg Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val	180	185	190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro	195	200	205
Gly Ser Gly Arg Lys Gly Leu Tyr Val Asn Gln Val Tyr Cys Gln Arg	210	215	220
Ile Glu Gly Met Ser Glu Lys Glu Ser Glu Pro Leu Leu Ser Phe Leu	225	230	235
Phe Ala His Ala Thr Lys Pro Glu Phe Thr Cys Arg Val Arg Trp Lys	245	250	255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Tyr Ala	260	265	270
Ile Asn Asp Tyr His Gly Gln Thr Arg Ile Leu His Arg Thr Thr Val	275	280	285
Gly Gly Val Arg Pro Ala Arg	290	295	

<210> SEQ ID NO 8

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

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acctggaacg aaatcctega cgcgttcac acttaccagg tcatctattt tccggccag      180
gcgatcacca acgaacagca catgccttc agccggcgct tcggccccgt cgatcccgtg      240
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tattgccagc gcacgcaggg catgagcgaa aaagaaagcg aaccgctgct gagcttctctg 720
tttgcgcatg cgacaaaacc ggaattcacc tgccgcgtgc gctggaagaa ggaccaggtc 780
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<210> SEQ ID NO 9
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

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<400> SEQUENCE: 9

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20          25          30
Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
35          40          45
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
50          55          60
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
65          70          75          80
Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
85          90          95
Arg Glu Ala Asn Glu Ser Gly Arg Val Leu Gly Asp Asp Trp His Thr
100         105         110
Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
115         120         125
Ile Asp Val Pro Glu His Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
130         135         140
Thr Ala Tyr Asp Ala Leu Ser Asp Gly Leu Lys Lys Leu Ile Ser Gly
145         150         155         160
Leu Asn Val Val His Ser Ala Thr Arg Val Phe Gly Ser Leu Tyr Gln
165         170         175
Ala Gln Asn Arg Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
180         185         190
Ala Asp Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro
195         200         205
Gly Ser Gly Arg Lys Gly Leu Tyr Val Asn Gln Val Tyr Cys Gln Arg
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<400> SEQUENCE: 10

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<210> SEQ ID NO 11
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant
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<400> SEQUENCE: 11

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 100 105 110
 Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Tyr Ala
 115 120 125
 Lys Glu Ile Pro Pro Tyr Gly Gly Asp Thr Leu Phe Thr Ser Met Tyr
 130 135 140
 Thr Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly
 145 150 155 160
 Leu Asn Val Val His Ser Ala Thr Arg Val Phe Gly Ser Leu Tyr Gln
 165 170 175
 Ala Gln Asn Arg Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
 180 185 190
 Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro
 195 200 205
 Glu Thr Gly Arg Lys Gly Leu Tyr Val Asn Gln Val Tyr Cys Gln Arg
 210 215 220
 Ile Glu Gly Met Ser Glu Lys Glu Ser Glu Pro Leu Leu Ser Phe Leu
 225 230 235 240
 Phe Ala His Ala Thr Lys Pro Glu Phe Thr Cys Arg Val Arg Trp Gln
 245 250 255
 Glu Gly Asp Val Leu Val Trp Asp Asn Leu Cys Thr Gln His Tyr Ala
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<210> SEQ ID NO 12

<211> LENGTH: 906

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 12

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 cccctgctca agagcatcga agggatatca gaggtgcaga tgatccgccc cgaagccaac 300
 gaaagcgggc gtgtgatcgg tgaaaactgg cacaccgaca gcaccttctt ggacgcaccg 360
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 accagcatgt acaccgcgtg ggagacgctg tcgcccacca tgcaggccac catcgaaggg 480
 ttgaacgtag tgcacagcgc cacgcgtgtg ttcggctcgc tctaccaggc ccagaaccgg 540
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tttgcgcag	cgacaaaacc	ggaattcacc	tgccgcgtgc	gctggcagga	aggcgatgtc	780
ctgggtctggg	acaacctgtg	cacgcagcac	tatgccgtac	ccgactacgc	gggcaagtcc	840
cgctacctga	cgcgaccac	ggtcggtggc	gtgcgcccgg	cgcgccatca	tcaccatcat	900
cactag						906

<210> SEQ ID NO 13
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 13

atgcacgccg	ctctgagccc	gcttagccag	cgettcgagc	gcacgcctg	gcagccgctg	60
accggcgctg	taggcgtga	gatcacggc	gttgacctga	gggagccgct	tgacgactcc	120
acctggaacg	agatcctcga	cgcttcacc	acttaccag	ttatctactt	cccaggacag	180
gctatcacga	acgaacagca	catgccttc	tcgcgagggt	tcgggcccag	ggaccagtc	240
ccgctgctta	agttctatga	aggctaccct	gaggtgcaaa	tgatccgccg	cgaggcgaac	300
gaatccggga	gggttattgg	cgagaactgg	cacactgact	ccaccttcct	cgatgctcct	360
ccagcagccg	tcgtgatgta	cgccaaggag	atcccgccct	acggcgccga	tacgtctctc	420
acctccatgt	acactgcttg	ggagaccctt	tctccgacca	tgcaagccac	catcgaggga	480
ctcaatgtgg	tccactctgc	gacctgtg	tttggtcgc	tctatcaggc	gcagaatagg	540
cgettcagca	acacctccgt	gaaggctcat	gacgtggacg	ccggagacag	ggagactgtc	600
cacccgctcg	tcgttactca	ccctgagacc	ggccgtaagg	gtctgtacgt	gaaccagggtg	660
tactgtcagc	gaattgaggg	tatgagttag	aaggagtcgg	agccgctgct	cagtttctct	720
ttcgcgcacg	ccaccaagcc	cgagttcacc	tgccgcgtcc	gctggcaaga	ggcgacgctc	780
ctgggtgtggg	acaacctctg	caccagcac	tacgccgtgc	cggactatgc	cggaagtcc	840
cgctacctta	ccgcacaaac	ggttggcggc	gtgagacctg	cccgggtga		888

<210> SEQ ID NO 14
 <211> LENGTH: 295
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 14

Met	His	Ala	Ala	Leu	Thr	Pro	Leu	Thr	Asn	Lys	Tyr	Arg	Phe	Ile	Asp
1				5					10					15	
Val	Gln	Pro	Leu	Thr	Gly	Val	Leu	Gly	Ala	Glu	Ile	Thr	Gly	Val	Asp
			20				25					30			
Leu	Arg	Glu	Pro	Leu	Asp	Asp	Ser	Thr	Trp	Asn	Glu	Ile	Leu	Asp	Ala
		35				40					45				
Phe	His	Thr	Tyr	Gln	Val	Ile	Tyr	Phe	Pro	Gly	Gln	Ala	Ile	Thr	Asn
	50				55					60					
Glu	Gln	His	Ile	Ala	Phe	Ser	Arg	Arg	Phe	Gly	Pro	Val	Asp	Pro	Val
65				70					75				80		

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Pro Ile Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
85 90 95

Arg Glu Ala Asn Glu Ser Ser Arg Phe Ile Gly Asp Asp Trp His Thr
100 105 110

Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
115 120 125

Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
130 135 140

Ser Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly
145 150 155 160

Leu Asn Val Val His Ser Ala Thr Lys Val Phe Gly Ser Leu Tyr Gln
165 170 175

Ala Thr Asn Trp Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
180 185 190

Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro
195 200 205

Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Val Tyr Cys Gln Lys
210 215 220

Ile Gln Gly Met Thr Asp Ala Glu Ser Lys Ser Leu Leu Gln Phe Leu
225 230 235 240

Tyr Glu His Ala Thr Lys Phe Asp Phe Thr Cys Arg Val Arg Trp Lys
245 250 255

Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ala
260 265 270

Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val
275 280 285

Ala Gly Asp Lys Pro Ser Arg
290 295

<210> SEQ ID NO 15

<211> LENGTH: 906

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 15

atgcacgcgg ctctgacccc gcttaccaac aaataccggt tcatcgacgt tcagccgctg	60
accggtgttt taggtgtgta aatcacccgt gttgacctgc gtgaaccgct ggacgactct	120
acctggaacg aaatcctgga cgcgttccac acctaccagg ttatctactt cccgggtcag	180
gcgatcacca acgaacagca catcgcggtt tctcgtcgct tcgggtccggt tgacccggtt	240
ccgattctca aatctatcga aggttaccgc gaagtccaga tgatccgtcg cgaagcgaac	300
gaatctagcc gttttattgg tgacgattgg cacaccgact ccaccttctt ggacgcgccg	360
ccagctgcag ttgtgatgcg tgctattgaa gttccggaat acggtggcga caccggtttc	420
ctgtccatgt actctgcttg ggaaaccctg tccccgacca tgcaggctac cattgaaggt	480
ctgaacgttg tgcactccgc aaccaaagtg ttcggcagcc tgtaccaggc aaccaactgg	540
cgcttcagca acactagtgt gaaagtgatg gatgtggatg caggcgatcg tgagactgtg	600
caccgcgtgg tggttaactca cccggttacc ggccgctcgt cgctgtactg caaccaggta	660
tattgccaga aaattcaggg catgactgat gcagagtcaa aatctctgct ccaatttctg	720

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tatgagcacg ccactaaatt tgattttact tgccgtgtcc gttggaaaaa ggatcaagta	780
ctggtatggg ataactctgtg tacgatgcac cgcgccgtac ctgattatgc cggcaaattt	840
cgctatttga cgcgcacgac agtcgcgggg gacaaacctt ctgccatca ccatcatcat	900
cattag	906

<210> SEQ ID NO 16
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 16

atgcacgcgg cgctgactcc tctcaccaac aagtatcgct ttatcgacgt gcagccgctg	60
acaggcgctcc tcggtgcaga gattacaggc gtggatctgc gggagcctct cgatgacagc	120
acttggaatg agatcctgga cgcctttcac acctaccaag tgatctactt tccgggtcaa	180
gctatcacta acgagcagca catcgcgttc tcccgcgggt tcggccctgt ggaccgggtg	240
ccgatcttaa agagtatcga gggctatcca gaggtgcaga tgatacggcg cgaggcgaac	300
gagagcagcc ggttcacgag agatgactgg cacaccgatt ccaccttctt ggacgctccg	360
cctgccgcgg tcggtgatgag agctatcgaa gtgccggagt atggaggta cacaggcttc	420
ctctccatgt acagtgcctg ggagacactc tcgcctacga tgcaagctac catcgaaggc	480
ttaaacgtgg tccactcggc gacgaaggtc ttcgggtcat tgtaccaggc gactaattgg	540
cgtttctcga acaccagcgt gaaagtgatg gacgtggacg ccggagatag agagactgtg	600
caccactcgc tcgtgacgca tctgtttacg ggaaggcgcg cactctactg caaccagggtg	660
tactgccaga agatccaggg aatgacggac gcggagtcga agtccctgtt gcaattcctt	720
tacgagcacg ccaccaagtt cgacttcacc tgccgggtcc ggtggaagaa ggaccaagtc	780
ctggtgtggg acaacctgtg taccatgcac cgcgccgtcc cggactacgc tgggaaattc	840
agatacctga cccgcaccac cgtggcggga gacaagccgt cgcgttga	888

<210> SEQ ID NO 17
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 17

atgcacgcgg ctttgacacc ttgaccaac aagtatcggt tcatcgacgt tcaaccactc	60
acaggcgctgc tcggcgcaga gattaccgga gtggacctga gggagccctt agacgactcc	120
acttggaacg agatcctcga cgcctttcac acctaccaag ttatctactt tcttggaag	180
gcgatcacca acgagcagca cattgccttc tcaaggaggt tcggaccggt agatccagtt	240
ccaattctca aatccattga gggttatccc gaggtgcaga tgattagacg agaagccaac	300
gagtcctcac ggttcattag cgacgattgg cacacagaca gcaccttctt tgacgctcct	360
cgggtgcgg tcggtatgag cgcaatagag gtgccggagt acggcgcgca taccggtttc	420
ctatcaatgt actctgcatg ggagacgctc tcaccaacga tgcaagccac cattgaaggt	480
ctaaacgtgg ttcactcagc tactaaggtc ttcggaagtc tttaccaggc gacgaattgg	540

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agggttcagta acaccagtgt gaaggtgatg gatgtggacg ctggagacag ggagacggtg      600
catccactcg tagttacaca ccctgtaact ggacgcagag ccctttactg caaccaggtt      660
tactgccaga agatccaggg aatgactgat gcggagtcta agtccctgct tcaattcctc      720
tacgaacacg ccaccaaatt cgacttcact tgctcgtgttc ggtggaagaa ggaccaagtg      780
ctcgtgtggg ataacctttg caccatgcac cgagcagtac cagactacgc cgggaaattc      840
cgctatctca cccgcactac agtggccgga gacaagccta gccgctga      888

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<210> SEQ ID NO 18
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

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<400> SEQUENCE: 18

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Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg Phe Ile Asp
 1             5             10            15
Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
      20            25            30
Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
      35            40            45
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
      50            55            60
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
      65            70            75            80
Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
      85            90            95
Arg Glu Ala Asn Glu Ser Ser Arg Val Ile Gly Asp Asp Trp His Ser
      100           105           110
Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
      115           120           125
Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
      130           135           140
Ser Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly
      145           150           155           160
Leu Asn Val Val His Ser Ala Thr Lys Val Phe Gly Ser Leu Tyr Gln
      165           170           175
Ala Thr Asn Trp Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
      180           185           190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro
      195           200           205
Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Val Tyr Cys Gln Lys
      210           215           220
Ile Gln Gly Met Thr Asp Ala Glu Ser Lys Ser Leu Leu Gln Phe Leu
      225           230           235           240
Tyr Glu His Ala Thr Lys Phe Asp Phe Thr Cys Arg Val Arg Trp Lys
      245           250           255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ala
      260           265           270
Ala Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val
      275           280           285

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Ala Gly Asp Lys Pro Ser Arg
290 295

<210> SEQ ID NO 19
<211> LENGTH: 906
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 19

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atgcacgcgg ctctgacccc gcttaccaac aaataccggt tcatcgacgt tcagccgctg    60
accggtgttt taggtgctga aatcacccgt gttgacctgc gtgaaccgct ggacgactct    120
acctggaacg aaatcctgga cgcgttcac acctaccagg ttatctactt cccgggtcag    180
gcgatcacca acgaacagca catcgcggtc tctcgtcgct tcggtccggt tgaccgggtt    240
ccgctgctca aatctatcga aggttaccg gaagttcaga tgatccgctc cgaagcgaac    300
gaatctagcc gtgttattgg tgacgattgg cacacgact ccaccttct ggacgcgccg    360
ccagctgcag ttgtgatgcg tgctattgaa gttccggaat acggtggcga caccggtttc    420
ctgtccatgt actctgcttg ggaaccctg tccccgacca tgcaggctac cattgaaggt    480
ctgaacgttg tgcactccgc aaccaaagtg ttcggcagcc tgtaccaggc aaccaactgg    540
cgcttcagca acactagtgt gaaagtgatg gatgtggatg caggcgatcg tgagactgtg    600
caccgcgtgg tggtaactca cccggttacc ggccgctcgt cgctgtactg caaccaggta    660
tattgccaga aaattcaggg catgactgat gcagagtcaa aatctctgct ccaatttctg    720
tatgagcacg ccactaaatt tgattttact tgccgtgtcc gttggaaaaa ggatcaagta    780
ctggtatggg ataatctgtg tacgatgcac cgcgcgcgcg ctgattatgc cggcaaatTT    840
cgctatttga cgcgcacgac agtcgcgggg gacaaacctt ctgccatca ccatcatcat    900
cattag                                           906
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<210> SEQ ID NO 20
<211> LENGTH: 888
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 20

```
atgcacgcgg cgctgactcc tctcaccaac aagtatcgct ttatcgacgt gcagccgctg    60
acaggcgctc tcggtgcaga gattacaggc gtggatctgc gggagcctct cgatgacagc    120
acttggaatg agatcctgga cgcctttcac acctaccaag tgatctactt tccgggtcaa    180
gctatcacta acgagcagca catcgcggtc tcccgcgggt tcggccctgt ggaccgggtg    240
ccgctgttaa agagtatcga gggctatcca gaggtgcaga tgatacggcg cgaggcgaac    300
gagagcagcc ggggtgatcg agatgactgg cactccgatt ccaccttct ggacgctccg    360
cctgccgcgg tggatgatgag agctatcgaa gtgccggagt atggaggatga cacaggcttc    420
ctctccatgt acagtgcctg ggagacactc tcgcctacga tgcaagctac catcgaaggc    480
ttaaacgtgg tccactcggc gacgaaggtc ttcgggtcat tgtaccaggc gactaattgg    540
cgcttctcga acaccagcgt gaaagtgatg gacgtggacg ccggagatag agagactgtg    600
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caccactcg tcgtgacgca tcctgttacg ggaaggcgcg cactctactg caaccaggtg	660
tactgccaga agatccaggg aatgacggac gcggagtcga agtccctggt gcaattcctt	720
tacgagcacg ccaccaagtt cgacttcacc tgccgggtcc ggtggaagaa ggaccaagtc	780
ctggtgtggg acaacctgtg taccatgcac cgcccgcccc cggactacgc tgggaaattc	840
agatacctga cccgcaccac cgtggcgggg gacaagccgt cgcgttga	888

<210> SEQ ID NO 21
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 21

atgcacgcag cccttactcc actgacgaac aagtatcgct tcatcgacgt gcagccactc	60
acgggtgtac tcggagccga gatcacggga gtggatctgc gcgagccgct cgatgactct	120
acatggaacg agatcctaga cgctttccac acttatcaag ttatctactt tccaggacaa	180
gccatcacta acgagcaaca catcgcggtc tcacgtcggg tcgggcctgt tgatcctgtg	240
ccgctctcca agtcaatcga gggttatcca gaagttcaga tgatccggcg cgaggcta	300
gagtcacccc gtgttatcgg tgatgactgg cactcggaca gtacattcct cgacgcacca	360
ccggccgcag ttgtgatgag ggctatcgaa gtgccggaat acggtggtga cactgggttc	420
ctgtcaatgt actctgcatg ggagaccctt agtcccacta tgcaagcaac catcgaaggg	480
ctcaacgttg tgcattcagc tactaaagta ttcggttccc tttatcaggc gacaaactgg	540
cggttcagca ataccagtgt taaagttatg gatgtggatg ctggagacag ggaaacggtc	600
caccctcttg tcgtcacgca ccagttaca gggcgtcgag cgctttactg caatcaggtg	660
tactgtcaga agattcaagg aatgaccgat gcggagtcca aatcactggt acaattcttg	720
tacgagcacg ccactaagtt cgatttcacg tgccgagtgc gctggaagaa ggatcaagtg	780
ctcgtttggg acaactgtg caccatgcac cgggcagctc ccgactacgc gggcaagttc	840
cgttatctca ctgcacaac tgcgctgga gacaagccct ccgatga	888

<210> SEQ ID NO 22
 <211> LENGTH: 295
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 22

Met His Ala Ala Leu Ser Pro Leu Ser Gln Arg Phe Glu Arg Ile Ala	
1 5 10 15	
Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp	
20 25 30	
Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala	
35 40 45	
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn	
50 55 60	
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val	
65 70 75 80	
Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg	

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85	90	95
Arg Glu Ala Asn Glu Ser Gly Arg Val Ile Gly Asp Asp Trp His Thr		
100	105	110
Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Tyr Ala		
115	120	125
Lys Glu Val Pro Pro Tyr Gly Gly Asp Thr Leu Phe Ala Ser Met Tyr		
130	135	140
Thr Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly		
145	150	155
Leu Asn Val Val His Ser Ala Thr Arg Val Phe Gly Ser Leu Tyr Gln		
165	170	175
Ala Gln Asn Arg Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val		
180	185	190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro		
195	200	205
Glu Thr Gly Arg Lys Gly Leu Tyr Val Asn Gln Val Tyr Cys Gln Arg		
210	215	220
Ile Glu Gly Met Ser Glu Lys Glu Ser Glu Pro Leu Leu Ser Phe Leu		
225	230	235
Phe Ala His Ala Thr Lys Pro Glu Phe Thr Cys Arg Val Arg Trp Gln		
245	250	255
Glu Gly Gln Val Leu Val Trp Asp Asn Leu Cys Thr Gln His Phe Ala		
260	265	270
Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val		
275	280	285
Gly Gly Val Arg Pro Ala Arg		
290	295	

<210> SEQ ID NO 23

<211> LENGTH: 906

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 23

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atgcacgcgg ctctgagccc gcttagccag cgtttcgaac gtatcgcggt tcagccgctg      60
accggtgttt taggtgctga aatcaccggt gttgacctgc gtgaaccgct ggacgactct      120
acctggaacg aaatcctgga cgcgttcac acctaccagg ttatctactt cccgggtcag      180
gcgatcacca acgaacagca catcgcggtt tctcgctgct tcggtcgggt tgaccgggtt      240
ccgctgctca aatctatcga aggttaccgg gaagttcaga tgatccgctg cgaagcgaac      300
gaatctggtc gtgttattgg tgacgactgg cacaccgact ctaccttctt ggacgcgccg      360
ccagctgcag ttgtgatgta cgctaaagaa gtgccgccat acggtggcga caccctgttc      420
gcgtccatgt acacgcgttg ggaacccctg tccccgacca tgcaggctac cattgaagg      480
ctgaacgttg tgcactccgc aactcgtgtt ttcggctccc tgtaccaggc acagaaccgt      540
cgcttcagca aactagcgt taaagtgatg gatgtggatg caggcgatcg tgaaactgtg      600
caccgcgtgg tggtaactca cccggaaact ggccgtaaag gcctgtacgt gaaccaggta      660
tattgccagc gtattgaagg catgagtgag aaagagtcgg agccgctgct ctcatctctg      720
tttgcacacg ccactaaacc agagtttact tgccgtgtcc gttggcaaga gggccaggta      780

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ctggtatggg ataatctgtg tacgcaacac ttccgctac ctgattatgc cggcaagttt      840
cgctatttga cgcgcacgac agtcggcggg gtccgccctg cccgccatca ccatcaccat      900
cattag                                           906

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<210> SEQ ID NO 24
<211> LENGTH: 888
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

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<400> SEQUENCE: 24

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atgcacgcgg cctgtctcc tctgtcccag cggttcgagc gcacgcgggt ccagccgcta      60
acgggtgtcc tgggcgcgga aatcacggga gttgatctga gagagccttt agacgacagc      120
acctggaacg agatcctcga tgcctttcac acataccaag ttatctactt tcccggccaa      180
gccatcacga acgagcagca catcgcggtt agccggaggt ttggcccggg tgatccgggt      240
cctctgctta agtcaattga gggttacca gaggtgcaga tgatccgccg cgaggccaac      300
gaatctgggc gtgtcatagg cgacgattgg catacggaca gcacctttct cgacgctcct      360
ccggccgcag tcgtgatgta cgcgaaggag gtgccgcctt acggcggcga taccctgttc      420
gcgtcgatgt acacggcctg ggagacgctc agcccgaaca tgcaagccac aatagagggt      480
ctaaatgtgg tccactccgc gacgcgggtg ttccggagcc tctaccaggc gcagaacaga      540
cggttctcga acacatcagt caaggtgatg gacgtggatg ccggagatcg tgaaactgtt      600
caccacttg tggtcaccca tccagagacg ggaaggaaag gactttacgt gaaccagggtg      660
tactgccagc ggatcgaggg catgtccgag aaggagagtg agccattgct gagctttcta      720
ttcgcgcacg caactaagcc cgagttcacg tgccgcgtcc gatggcaaga gggccaagtt      780
ctcgtctggg ataacttggt cacacagcac ttccgggttc ccgattacgc cggaaagtgc      840
cgctatctca cagcaccac tgtgggaggc gttcgtcccg cgcggtga      888

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<210> SEQ ID NO 25
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

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<400> SEQUENCE: 25

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Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg Phe Ile Asp
 1             5             10             15
Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
          20             25             30
Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
          35             40             45
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
          50             55             60
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
          65             70             75             80
Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
          85             90             95
Arg Glu Ala Asn Glu Ser Gly Arg Val Ile Gly Asp Asp Trp His Thr

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100						105					110				
Asp	Ser	Thr	Phe	Leu	Asp	Ala	Pro	Pro	Ala	Ala	Val	Val	Met	Tyr	Ala
		115					120					125			
Arg	Glu	Val	Pro	Pro	Tyr	Gly	Gly	Asp	Thr	Gly	Phe	Leu	Ser	Met	Tyr
	130					135					140				
Thr	Ala	Trp	Glu	Thr	Leu	Ser	Pro	Thr	Met	Gln	Ala	Thr	Ile	Glu	Gly
145					150					155					160
Leu	Asn	Val	Val	His	Ser	Ala	Thr	Arg	Val	Phe	Gly	Ser	Leu	Tyr	Gln
				165					170					175	
Ala	Gln	Asn	Arg	Arg	Tyr	Ser	Asn	Thr	Ser	Val	Lys	Val	Met	Asp	Val
			180					185					190		
Asp	Ala	Gly	Asp	Arg	Glu	Thr	Val	His	Pro	Leu	Val	Val	Ser	His	Pro
		195					200					205			
Val	Thr	Gly	Arg	Arg	Ala	Leu	Tyr	Cys	Asn	Gln	Val	Tyr	Cys	Gln	Arg
	210					215					220				
Ile	Glu	Gly	Met	Thr	Asp	Ala	Glu	Ser	Lys	Ser	Leu	Leu	Gln	Phe	Leu
225					230					235					240
Tyr	Glu	His	Ala	Thr	Lys	Phe	Asp	Phe	Thr	Cys	Arg	Val	Arg	Trp	Lys
				245					250					255	
Lys	Asp	Gln	Val	Leu	Val	Trp	Asp	Asn	Leu	Cys	Thr	Met	His	Arg	Ala
			260				265						270		
Val	Pro	Asp	Tyr	Ala	Gly	Lys	Phe	Arg	Tyr	Leu	Thr	Arg	Thr	Thr	Val
		275					280					285			
Ala	Gly	Asp	Lys	Pro	Ser	Arg									
	290					295									

<210> SEQ ID NO 26

<211> LENGTH: 906

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 26

```

atgcacgcgg ctctgacccc gcttaccaac aaataccggt tcatcgacgt tcagccgctg      60
accggtgttt taggtgctga aatcaccggt gttgacctgc gtgaaccgct ggacgactct      120
acctggaacg aaatcctgga cgcgttcac acctaccagg ttatctactt cccgggtcag      180
gcgatcacca acgaacagca catcgcggtt tctcgtcgct tcggtcgggt tgaccgggtt      240
ccgctgctca aatctatcga aggttaccgg gaagttcaga tgatccgtcg cgaagcgaac      300
gaatctggtc gtgttattgg tgacgattgg cacaccgact ccaccttctt ggacgcgccg      360
ccagctgcag ttgtgatgta cgtcgtgtaa gttccgccgt acggtggcga caccggtttc      420
ctgtccatgt acaccgcttg ggaaaccttg tccccgacca tgcaggctac cattgaaggt      480
ctgaacgttg tgcaactccg aaccgctgtg ttccgcagcc tgtaccaggc acagaaccgt      540
cgctacagca acactagtgt gaaagtgatg gatgtggatg caggcgatcg tgagactgtg      600
caccgcgtgg tggtatctca cccggttacc ggccgctcgt cgctgtactg caaccaggta      660
tattgccagc gtattgaggg catgactgat gcagagtcaa aatctctgct ccaatttctg      720
tatgagcacg ccactaaatt tgattttact tgcctgttcc gttggaaaaa ggatcaagta      780
ctggtatggg ataatctgtg tacgatgcac cgcgccgtac ctgattatgc cggcaaat      840

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cgctatttga cgcgcacgac agtcgcgggg gacaaacctt ctcgccatca ccatcatcat 900
cattag 906

<210> SEQ ID NO 27
<211> LENGTH: 888
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 27

atgcacgctg ctctgactcc actcacaac aagtaccggt tcatcgacgt gcaaccgctg 60
accggcgctct tgggtgcgga aatcaccggc gtggacttgc gggagccgct ggatgacagc 120
acatggaacg agatcctcga tgcgttcac acctaccaag tgatctatct cccaggccag 180
gccattacga acgagcagca catcgcggtc agtcgaaggt tcgggcctgt ggaccgggtt 240
ccgctgctta agagtatcga gggctaccgc gaagtacaga tgattcgccg cgaagcgaat 300
gagtcggggc gagtgatcgg cgatgactgg cacaccgaca gcacgttcct cgacgcgccg 360
cctgccgctg tcgtgatgta cgcacgggag gtgccaccct acggcggaga tacgggattc 420
ctttcaatgt acacggcatg ggagacactc tctccgacca tgcaagcaac gatagagggc 480
ttgaacgtgg tgcactccgc caccagggtc ttcggcagcc tctaccaagc ccagaaccgc 540
cgggtactcca aactagcgt gaaagtgatg gacgtggatg cgggcgaccg ggagaccgtg 600
catcctctag ttgtgagcca cccggtgact ggccgacggg cgctgtactg caaccaggtc 660
tattgccagc gcacgaggg catgaccgac gcggagtcca aatctctgct ccaattcctg 720
tacgagcagc ccacgaagtt cgacttcacc tgccgggttc gctggaagaa ggatcaagtg 780
ctagtgtggg acaacctctg cactatgcac agggctgtgc cggactatgc tggcaaatc 840
cgttacctta cccggaccac tgtggcgggc gacaagccaa gcagatga 888

<210> SEQ ID NO 28
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 28

Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg Phe Ile Asp
1 5 10 15
Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
20 25 30
Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
35 40 45
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
50 55 60
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
65 70 75 80
Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
85 90 95
Arg Glu Ala Asn Glu Ser Gly Arg Val Ile Gly Asp Asp Trp His Thr
100 105 110
Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala

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115	120	125
Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr		
130	135	140
Ser Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly		
145	150	155
Leu Asn Val Val His Ser Ala Thr Lys Val Phe Gly Ser Leu Tyr Gln		
165	170	175
Ala Thr Asn Trp Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val		
180	185	190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro		
195	200	205
Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Val Tyr Cys Gln Lys		
210	215	220
Ile Gln Gly Met Thr Asp Ala Glu Ser Lys Ser Leu Leu Gln Phe Leu		
225	230	235
Tyr Glu His Ala Thr Lys Phe Asp Phe Thr Cys Arg Val Arg Trp Lys		
245	250	255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ala		
260	265	270
Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val		
275	280	285
Ala Gly Asp Lys Pro Ser Arg		
290	295	

<210> SEQ ID NO 29

<211> LENGTH: 906

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 29

```

atgcacgcgg ctctgacccc gcttaccaac aaataccggt tcatcgacgt tcagccgctg      60
accggtgttt taggtgctga aatcaccggt gttgacctgc gtgaaccgct ggacgactct      120
acctggaacg aaatcctgga cgcgttcac acctaccagg ttatctactt cccgggtcag      180
gcatcacca acgaacagca catcgcggtt tctcgctgct tcgggtccggt tgaccgggtt      240
ccgctgctca aatctatcga aggttaccgg gaagttcaga tgatccgctg cgaagcgaac      300
gaatctggtc gtgttattgg tgacgattgg cacaccgact ccacettctt ggacgcgccg      360
ccagctgcag ttgtgatgcg tgctattgaa gttccggaat acggtggcga caccggtttc      420
ctgtccatgt actctgcttg ggaaaccctg tccccgacca tgcaggctac cattgaaggt      480
ctgaacgttg tgcactccgc aaccaaagtg ttcggcagcc tgtaccaggc aaccaactgg      540
cgcttcagca acactagtgt gaaagtgatg gatgtggatg caggcgatcg tgagactgtg      600
caccgcgtgg tgtaactca cccggttacc ggccgctcgt cgctgtactg caaccaggta      660
tattgccaga aaattcaggg catgactgat gcagagtcaa aatctctgct ccaatttctg      720
tatgagcacg ccactaaatt tgattttact tgccgtgtcc gttggaaaaa ggatcaagta      780
ctggtatggg ataatctgtg tacgatgcac cgcgcgctac ctgattatgc cgcaaatatt      840
cgctatttga cgcgcacgac agtcgcgggg gacaaacctt ctgccatca ccatcatcat      900
cattag                                           906

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-continued

<210> SEQ ID NO 30
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 30

```

atgcacgcgg cgctgactcc tctcaccaac aagtatcgct ttatcgacgt gcagccgctg    60
acaggcgctcc tcggtgcaga gattacaggc gtggatctgc gggagcctct cgatgacagc    120
acttgaatg agatcctgga cgcctttcac acctaccaag tgatctactt tccgggtcaa    180
gctatcacta acgagcagca catcgcgctt tcccgccggt tcggccctgt ggaccgggtg    240
ccgctgttaa agagtatcga gggctatcca gaggtgcaga tgatacggcg cgaggcgaac    300
gagagcggcc ggggtgatcgg agatgactgg cacaccgatt ccaccttctt ggacgctccg    360
cctgccgcgg tggtgatgag agctatcgaa gtgccggagt atggaggtga cacaggttct    420
ctctccatgt acagtgcctg ggagacactc tcgcctacga tgcaagctac catcgaaggc    480
ttaaacgtgg tccactcggc gacgaaggtc ttcgggtcat tgtaccaggc gactaattgg    540
cgcttctcga acaccagcgt gaaagtgatg gacgtggacg ccggagatag agagactgtg    600
caccactcgt tcgtgacgca tctgtttacg ggaaggcgcg cactctactg caaccaggtg    660
tactgccaga agatccaggg aatgacggac gcggagtcga agtccctggt gcaattcctt    720
tacgagcacg ccaccaagtt cgacttcacc tgccgggtcc ggtggaagaa ggaccaagtc    780
ctggtgtggg acaacctgtg taccatgcac cgcgccgtcc cggactacgc tgggaaattc    840
agatacctga cccgcaccac cgtggcgggg gacaagccgt cgcgttga    888
  
```

<210> SEQ ID NO 31
 <211> LENGTH: 295
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 31

```

Met His Ala Ala Leu Ser Pro Leu Ser Gln Lys Tyr Arg Phe Ile Asp
 1             5             10             15
Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Thr
      20             25             30
Leu Arg Glu Pro Leu Asp Asp Asn Thr Trp Asn Glu Ile Leu Asp Ala
      35             40             45
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
      50             55             60
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
      65             70             75             80
Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
      85             90             95
Arg Glu Ala Asn Glu Ser Gly Arg Val Ile Gly Asp Asp Trp His Thr
      100            105            110
Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Tyr Ala
      115            120            125
Arg Glu Val Pro Pro Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
  
```

-continued

130	135	140
Ser Ala Trp Asp Thr Leu Ser Asp Thr Met Lys Ala Thr Ile Glu Gly		
145	150	155 160
Leu Asn Val Val His Ser Ala Thr Arg Val Phe Gly Ser Leu Tyr Gln		
	165	170 175
Ala Gln Asn Arg Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val		
	180	185 190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Ser His Pro		
	195	200 205
Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Val Tyr Cys Gln Arg		
	210	215 220
Ile Glu Gly Met Thr Asp Ala Glu Ser Lys Pro Leu Leu Gln Phe Leu		
	225	230 235 240
Tyr Glu His Ala Thr Arg Phe Asp Phe Thr Cys Arg Val Arg Trp Lys		
	245	250 255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ala		
	260	265 270
Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val		
	275	280 285
Ala Gly Asp Lys Pro Ser Arg		
	290	295

<210> SEQ ID NO 32

<211> LENGTH: 906

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 32

```

atgcacgcgg ctctgagccc gcttagccag aaataccgtt tcatcgacgt tcagccgctg      60
accggtgttt taggtgctga aatcaccggt gttaccctgc gtgaaccgct ggacgacaac      120
acctggaacg aaatcctgga cgcgttcac acctaccagg ttatctactt cccgggtcag      180
gcatcacca acgaacagca catcgcttc tctcgctcgt tcgggtccggt tgaccgggtt      240
ccgctgctca aatctatcga aggttaccgg gaagttcaga tgatccgtcg cgaagcgaac      300
gaatctggtc gtgttattgg tgacgattgg cacaccgact ccaccttctt ggacgcgccg      360
ccagctgcag ttgtgatgta cgctcgtgaa gttccgccgt acggtggcga caccggtttc      420
ctgtccatgt actctgcttg ggacaccctg tccgacacca tgaaagctac cattgaaggt      480
ctgaacgttg tgactccgc aacccgtgtg ttcggcagcc tgtaccaggc acagaaccgt      540
cgcttcagca acactagtgt gaaagtgatg gatgtggatg caggcgatcg tgagactgtg      600
caccgcgtgg tggatatcga cccggttacc ggccgtcgtg cgctgtactg caaccaggta      660
tattgccagc gtattgaggg catgactgat gcagagtcaa aaccactgct ccaatttctg      720
tatgagcacg cactcgttt tgattttact tgccgtgtcc gttggaaaaa ggatcaagta      780
ctggtatggg ataactctgtg tacgatgcac cgcgccgtac ctgattatgc cggcaaattt      840
cgctatttga cgcgcacgac agtcgcgggg gacaaacctt ctgccatca ccatcatcat      900
cattag                                           906

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<210> SEQ ID NO 33

-continued

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<211> LENGTH: 888
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 33
atgcacgccg cctctctccc tcttagccag aagtaccgat tcatcgacgt ccagccgctc   60
actggcgtag tgggcgctga gataacgggt gtgacctga gggagccgct ggacgacaaac   120
acttggaacg agatccttga cgccttccat acttatcaag ttatctactt tcttggaaca   180
gcgattacga atgagcagca catcgcggtc tcccgagggt tcgggccagt cgatccggtg   240
ccgctactca agtccatcga aggataccca gaagtccaga tgatccgtcg tgaggcaaac   300
gagtcgggcc gggtcacatcg cgacgattgg cacaccgact ctaccttctc tgacgcgcct   360
ccggccgcag tggtcagtga cgcgcgcgag gtgcctccct acggcggcga tacgggcttc   420
ctcagtatgt actctgcatg ggacacccta agcgacacca tgaaggccac catcgaagggt   480
ctgaacgtcg tgcactcggc aacacgagtc ttcggatcac tctaccaagc acagaatcgc   540
cgcttctcga acacctcggt taagggtgatg gacgtggatg cgggtgacag ggaaaccgtt   600
catcctctgg tgggtctcga tccgggttacg ggacgcgcgc ctctctactg caaccagggtg   660
tactgtcaaa ggatcgaagg aatgacagat gctgagagca agccgttgct ccaattctc   720
tatgaacacg caacaagggt cgacttcacc tgccgggttc gatggaagaa ggatcaagtg   780
ctagtctggg acaacctctg caccatgcac cgggccgtgc cggactatgc tgggaaattc   840
cgttacctga cccgcacgac cgtcgcggga gacaagccgt cgagatga   888

```

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<210> SEQ ID NO 34
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 34

```

```

Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg Phe Ile Asp
 1             5             10             15

Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
          20             25             30

Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
          35             40             45

Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
          50             55             60

Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
          65             70             75             80

Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
          85             90             95

Arg Glu Ala Asn Glu Ser Gly Arg Val Ile Gly Asp Asp Trp His Ala
          100            105            110

Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
          115            120            125

Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
          130            135            140

Ser Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly

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145	150	155	160
Leu Asn Val Val His Ser Ala Thr Lys Val Phe Gly Ser Leu Tyr Gln			
	165	170	175
Ala Thr Asn Trp Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val			
	180	185	190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro			
	195	200	205
Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Val Tyr Cys Gln Lys			
	210	215	220
Ile Gln Gly Met Thr Asp Ala Glu Ser Lys Ser Leu Leu Gln Phe Leu			
	225	230	235
Tyr Glu His Ala Thr Lys Phe Asp Phe Thr Cys Arg Val Arg Trp Lys			
	245	250	255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ser			
	260	265	270
Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val			
	275	280	285
Ala Gly Asp Lys Pro Ser Arg			
	290	295	

<210> SEQ ID NO 35
 <211> LENGTH: 906
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 35

atgcacgcgg ctctgacccc gcttaccac aaataccgtt tcatcgacgt tcagccgctg	60
accggtgttt taggtgtctga aatcaccggt gttgacctgc gtgaaccgct ggacgactct	120
acctggaacg aaatcctgga cgcgttcac acctaccagg ttatctactt cccgggtcag	180
gcgatcacca acgaacagca catcgcggtc tctcgctcgt tcggtcgggt tgaccgggtt	240
ccgctgtctca aatctatcga aggttaccgg gaagttcaga tgatccgtcg cgaagcgaac	300
gaatctggtc gtgttattgg tgacgattgg cagcgggact ccaccttctt ggacgcgccc	360
ccagctgcag ttgtgatgcg tgctattgaa gttccggaat acggtggcga caccggtttc	420
ctgtccatgt actctgcttg ggaaccctg tccccgacca tgcaggctac cattgaaggt	480
ctgaacgttg tgcaactccg aaccaaagtg ttcggcagcc tgtaccaggc aaccaactgg	540
cgcttcagca acactagtgt gaaagtgatg gatgtggatg caggcgatcg tgagactgtg	600
caccgcgtgg tggtaaactca cccggttacc ggccgctcgt cgctgtactg caaccaggta	660
tattgccaga aaattcaggg catgactgat gcagagtcaa aatctctgct ccaatttctg	720
tatgagcacg ccactaaatt tgattttact tgccgtgtcc gttggaaaaa ggatcaagta	780
ctggtatggg ataatctgtg tacgatgcac cgcagcgtac ctgattatgc cggcaaat	840
cgctatttga cgcgcacgac agtcgcgggg gacaaacctt ctgcacatca ccatcatcat	900
cattag	906

<210> SEQ ID NO 36
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 36

```

atgcacgcgg cgctgactcc tctcaccaac aagtatcgct ttatcgacgt gcagccgctg      60
acaggcgctcc tcggtgcaga gattacaggc gtggatctgc gggagcctct cgatgacagc      120
acttggaatg agatcctgga cgcctttcac acctaccaag tgatctactt tccgggtcaa      180
gctatcacta acgagcagca catcgcgctt tcccgccggt tcggccctgt ggacccggtg      240
ccgctgttaa agagtatcga gggctatcca gaggtgcaga tgatacggcg cgaggcgaac      300
gagagcgggc ggggtgatcg agatgactgg cagcccgatt ccaccttctt ggacgctccg      360
cctgccgcgg tggtgatgag agctatcgaa gtgccggagt atggaggatga cacaggcttc      420
ctctccatgt acagtgcctg ggagacactc tcgcctacga tgcaagctac catcgaaggc      480
ttaaacgtgg tccactcggc gacgaaggtc ttcgggtcat tgtaccaggc gactaattgg      540
cgcttctcga acaccagcgt gaaagtgatg gacgtggacg ccggagatag agagactgtg      600
caccactcgt tcgtgacgca tctgtttacg ggaaggcgcg cactctactg caaccagggtg      660
tactgccaga agatccaggg aatgacggac gcggagtcga agtcctctgt gcaattcctt      720
tacgagcacg ccaccaagtt cgacttcacc tgccgggtcc ggtggaagaa ggaccaagtc      780
ctggtgtggg acaacctgtg taccatgcac cgctccgtcc cggactacgc tgggaaattc      840
agatacctga cccgcaccac cgtggcgggg gacaagccgt cgcgttga      888

```

<210> SEQ ID NO 37

<211> LENGTH: 295

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 37

```

Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg Phe Ile Asp
 1             5             10             15

Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
      20             25             30

Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
      35             40             45

Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
 50             55             60

Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
 65             70             75             80

Pro Ile Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
      85             90             95

Arg Glu Ala Asn Glu Ser Gly Arg Val Ile Gly Asp Asp Trp His Thr
      100            105            110

Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
      115            120            125

Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
      130            135            140

Ser Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly
      145            150            155            160

Leu Asn Val Val His Ser Ala Thr Lys Val Phe Gly Ser Leu Tyr Gln

```

-continued

165	170	175
Ala Thr Asn Trp Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val		
180	185	190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro		
195	200	205
Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Val Tyr Cys Gln Lys		
210	215	220
Ile Gln Gly Met Thr Asp Ala Glu Ser Lys Ser Leu Leu Gln Phe Leu		
225	230	235
Tyr Glu His Ala Thr Lys Phe Asp Phe Thr Cys Arg Val Arg Trp Lys		
245	250	255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ser		
260	265	270
Val Pro Asp Tyr Gly Asn Ala Phe Arg Tyr Leu Thr Arg Thr Thr Val		
275	280	285
Ala Gly Asp Lys Pro Ser Arg		
290	295	

<210> SEQ ID NO 38
 <211> LENGTH: 906
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 38

```

atgcacgcgg ctctgacccc gcttaccac aaataccgtt tcatcgacgt tcagccgctg    60
accggtgttt taggtgctga aatcacccgt gttgacctgc gtgaaccgct ggacgactct    120
acctggaacg aaatcctgga cgcgttcac acctaccagg ttatctactt cccgggtcag    180
gcatcacca acgaacagca catcgcttc tctcgctgct tcggtcgggt tgaccgggtt    240
ccgattctca aatctatcga aggttaccgg gaagttcaga tgatccgtcg cgaagcgaac    300
gaatctggtc gtgttattgg tgacgattgg cacaccgact ccaccttctt ggacgcgccg    360
ccagctgcag ttgtgatgcg tgctattgaa gttccggaat acggtggcga caccggtttc    420
ctgtccatgt actctgcttg ggaaccctg tccccgacca tgcaggctac cattgaaggt    480
ctgaacgttg tgcaactccg aaccaaagtg ttcggcagcc tgtaccaggc aaccaaactg    540
cgcttcagca acactagtgt gaaagtgatg gatgtggatg caggcgatcg tgagactgtg    600
caccgcgtgg tggtaactca cccggttacc ggccgtcgtg cgctgtactg caaccaggta    660
tattgccaga aaattcaggg catgactgat gcagagtcaa aatctctgct ccaatttctg    720
tatgagcacg ccactaaatt tgattttact tgccgtgtcc gttggaaaaa ggatcaagta    780
ctggtatggg ataatctgtg tacgatgcac cgcagcgtac ctgattatgg caatgcgttt    840
cgctatttga cgcgcacgac agtcgcgggg gacaaacctt ctcgccatca ccatcatcat    900
cattag                                           906

```

<210> SEQ ID NO 39
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

-continued

<400> SEQUENCE: 39

```

atgcacgcgg cgctgactcc tctcaccaac aagtatcgct ttatcgacgt gcagccgctg      60
acaggcgctcc tcggtgcaga gattacaggc gtggatctgc gggagcctct cgatgacagc      120
acttggaatg agatcctgga cgcctttcac acctaccaag tgatctactt tccgggtcaa      180
gctatcacta acgagcagca catcgcggtc tcccgccggt tcggccctgt ggaccgggtg      240
ccgatcttaa agagtatcga gggctatcca gaggtgcaga tgatacggcg cgaggcgaac      300
gagagcggcc ggggtgatcg agatgactgg cacaccgatt ccaccttctt ggacgctccg      360
cctgccgcgg tggtgatgag agctatcgaa gtgccggagt atggaggatga cacaggcttc      420
ctctccatgt acagtgcctg ggagacactc tcgcctacga tgcaagctac catcgaaggc      480
ttaaacgtgg tccactcggc gacgaaggtc ttcgggtcat tgtaccaggc gactaattgg      540
cgcttctcga acaccagcgt gaaagtgatg gacgtggacg ccggagatag agagactgtg      600
caccactcgc tcgtgacgca tctgtttacg ggaaggcgcg cactctactg caaccagggtg      660
tactgccaga agatccaggg aatgacggac gcggagtcca agtcctctgt gcaattcctt      720
tacgagcacg ccaccaagtt cgacttcacc tgccgggtcc ggtggaagaa ggaccaagtc      780
ctggtgtggg acaacctgtg taccatgcac cgtccgctcc cggaactacg caacgccttc      840
agatacctga cccgcaccac cgtggcgggg gacaagccgt cgcgttga      888

```

<210> SEQ ID NO 40

<211> LENGTH: 295

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 40

```

Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg Phe Ile Asp
 1             5             10             15

Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
          20             25             30

Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
          35             40             45

Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Ala Gln Gln Ile Thr Asn
          50             55             60

Glu Gln His Ile Ser Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
          65             70             75             80

Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
          85             90             95

Arg Glu Ala Asn Glu Ser Gly Arg Ile Leu Gly Asp Asp Trp His Thr
          100            105            110

Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
          115            120            125

Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
          130            135            140

Ser Ala Trp Asp Thr Leu Ser Asp Thr Met Lys Ala Thr Ile Glu Gly
          145            150            155            160

Leu Asn Val Val His Ser Ala Thr Arg Val Phe Gly Ser Leu Tyr Gln
          165            170            175

Ala Gln Asn Trp Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val

```

-continued

180	185	190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Ser His Pro		
195	200	205
Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Val Tyr Cys Gln Arg		
210	215	220
Ile Glu Gly Met Thr Asp Ala Glu Ser Lys Cys Leu Leu Gln Phe Leu		
225	230	235
Tyr Glu His Ala Thr Lys Phe Asp Phe Thr Cys Arg Val Arg Trp Lys		
245	250	255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ala		
260	265	270
Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val		
275	280	285
Ala Gly Asp Arg Pro Ala Arg		
290	295	

<210> SEQ ID NO 41
 <211> LENGTH: 906
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 41

```

atgcacgcgg ctctgacccc gcttaccac aaataccggt tcatcgacgt tcagccgctg      60
accggtgttt taggtgtgta aatcacgggt gttgacctgc gtgaaccgct ggacgactct      120
acctggaacg aaatcctgga cgcgttcac acctaccagg ttatctactt cccggcgagc      180
cagatcacca acgaacagca catctctttc tctcgctgct tcgggtccggt tgaccggtt      240
ccgctgtctca aatctatcga aggttaccgg gaagttcaga tgatccgtcg cgaagcgaac      300
gaatctggtc gtatcctggg tgacgattgg cacaccgact ccaccttctt ggacgcgccg      360
ccagctgcag ttgtgatgcg tgctattgaa gttccggaat acggtggcga caccggtttc      420
ctgtccatgt actctgcttg ggacaccttg tccgacacca tgaaagctac cattgaaggt      480
ctgaacgttg tgcactccgc aacctgtgtg ttccgcagcc tgtaccaggc acagaactgg      540
cgcttcagca acactagtgt gaaagtgatg gatgtggatg caggcgatcg tgagactgtg      600
caccgcgtgg tggatatcga cccggttacc ggccgtcgtg cgctgtactg caaccaggta      660
tattgccagc gtattgaggg catgactgat gcagagtcaa aatgcctgct ccaatttctg      720
tatgagcacg ccactaaatt tgattttact tgccgtgtcc gttggaaaaa ggatcaagta      780
ctggtatggg ataatctgtg tacgatgcac cgcgccgtac ctgattatgc cggcataatt      840
cgctatttga cgcgcacgac agtcgcgggg gaccgccctg cccgccatca ccatcatcat      900
cattag                                           906

```

<210> SEQ ID NO 42
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 42

```

atgcacgcgg ccttaacgcc actcacgaac aagtatcgct tcatcgacgt ccagccgctc      60

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-continued

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actggcgtgc taggtgctga gatcacccgc gttgatctcc gcgagcctct tgacgactcg    120
acctggaacg agatcctgga tgccttcac acttaccaag tgatctactt cccggcccaa    180
cagatcacia acgagcagca catctccttt agtaggcgat tcggtccagt cgatccggtg    240
ccgctcctca agtcgattga gggctacccg gaggtccaga tgattcgtag ggaagccaac    300
gagtcaggcc gtattctggg cgacgactgg catacggact ccactttcct agacgcacct    360
ccggctgccg tcgtcatgag ggctattgaa gtgccggagt acggcggcga taccggattc    420
ctctctatgt actccgctg ggacacgctc tcagacacca tgaaggccac gatagagggc    480
ctgaacgttg tgcactccgc aacgagagta ttccgatctc tgtaccaggc acagaactgg    540
cggttcagta acacgagcgt gaaagtcacg gacgtggacg ctggcgacag gaaaactgtt    600
cacccgcttg tcgtttccca cctgtcact ggtcgccggg cactttactg caaccagggtg    660
tactgtcagc gcattgaggg catgacggat gctgagtcga agtgccctct ccaattcttg    720
tacgagcacg cgacaaagtt cgatttcacg tgccgggtcc gatggaagaa ggaccaagtg    780
ctagtgtggg acaacctttg cacgatgcac cgcgtgtcc cggtattacg cggaagttc    840
cgttacctca ccagaacaac agttgccggt gacagaccgc cgagatga    888

```

<210> SEQ ID NO 43

<211> LENGTH: 295

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 43

```

Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg Phe Ile Asp
 1             5             10             15

Val Gln Pro Leu Cys Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
          20             25             30

Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
          35             40             45

Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
 50             55             60

Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
65             70             75             80

Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
          85             90             95

Arg Glu Ala Asn Glu Ser Gly Arg Ile Leu Gly Asp Asp Trp His Thr
          100            105            110

Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
          115            120            125

Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
          130            135            140

Ser Ala Trp Asp Thr Leu Ser Asp Thr Met Lys Ala Thr Ile Glu Gly
145            150            155            160

Leu Asn Val Val His Ser Ala Thr Arg Val Phe Gly Ser Leu Tyr Gln
          165            170            175

Ala Gln Asn Trp Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
          180            185            190

Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Ser His Pro

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-continued

195	200	205
Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Val Tyr Cys Gln Arg		
210	215	220
Ile Glu Gly Met Thr Asp Ala Glu Ser Lys Cys Leu Leu Gln Phe Leu		
225	230	235 240
Tyr Glu His Ala Thr Lys Phe Asp Phe Thr Cys Arg Val Arg Trp Lys		
	245	250 255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Leu Ala		
	260	265 270
Val Pro Asp Tyr Asp Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val		
	275	280 285
Ala Gly Asp Lys Pro Ser Arg		
290	295	
<210> SEQ ID NO 44		
<211> LENGTH: 906		
<212> TYPE: DNA		
<213> ORGANISM: Artificial Sequence		
<220> FEATURE:		
<223> OTHER INFORMATION: Recombinant		
<400> SEQUENCE: 44		
atgcacgcgg ctctgacccc gcttaccac aaataccgtt tcatcgacgt tcagccgctg	60	
tcgggtgttt taggtgtgta aatcacgggt gttgacctgc gtgaaccgct ggacgactct	120	
acctggaacg aaatcctgga cgcgttcac acctaccagg ttatctactt cccgggtcag	180	
gcgatcacca acgaacagca catcgcggtt tctcgctcgt tcgggtccggt tgacccggtt	240	
ccgctgtctca aatctatcga aggttaccgg gaagttcaga tgatccgtcg cgaagcgaac	300	
gaatctggtc gtatcctggg tgacgattgg cacaccgact ccaccttctt ggacgcgccc	360	
ccagctgcag ttgtgatgcg tgctattgaa gttccggaat acgggtggcga caccggtttc	420	
ctgtccatgt actctgcttg ggacaccttg tccgacacca tgaaagctac cattgaaggt	480	
ctgaacgttg tgcaactccg aacccgtgtg ttccgcagcc tgtaccaggc acagaactgg	540	
cgtcttcagca acactagtgt gaaagtgtg gatgtggatg caggcgatcg tgagactgtg	600	
cacccgctgg tggtatctca cccggttacc ggccgtcgtg cgctgtactg caaccaggta	660	
tattgccagc gtattgaggg catgactgat gcagagtcaa aatgcctgct ccaatttctg	720	
tatgagcagc ccactaaatt tgattttact tgccgtgtcc gttggaaaaa ggatcaagta	780	
ctggtatggg ataactctgtg tacgatgcac ctggccgtac ctgattatga cggcaaat	840	
cgctatttga cgcgcacgac agtcgcgggg gacaaacctt ctgccatca ccatcatcat	900	
cattag	906	

<210> SEQ ID NO 45
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 45	
atgcacgcgg ctctcacacc gctcacaac aagtaccgct tcattgacgt gcagccgctg	60
tgtggcgtcc tgggcgcaga aatcacgggc gtggatctcc gcgagcctct ggacgacgc	120

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acctggaacg aaatcctgga tgctttccac acttaccaag tgatctactt tcccgacaaa 180
gccatcacta acgagcagca catcgctttc tcacggcgct tcggcccggt agatccggtg 240
ccgctactca agtcaattga aggctatccg gaggtgcaaa tgattcgccg cgaagctaac 300
gagagcgggc gcatactggg cgacgactgg catactgact ccaccttcct cgatgctcca 360
ccagccgcag tggatgatgc tgccatcgaa gttcccgagt atggtggcga cacgggtttc 420
ctgtctatgt actccgcttg ggatacactg tctgacacga tgaaggctac catcgagggc 480
ctgaatgtcg tccacagtgc tacgcgcgtg ttcggctcac tgtaccaggc gcagaactgg 540
aggttcagca acaccagtgt caaggatgat gacgttgatg ctggagacag ggaaactgtg 600
catcctcttg tggctctcca tccagttacc gggaggagag cactctactg caaccaggtt 660
tactgccagc gcacgagggg catgaccgat gcggagtcga agtgtctggt acagttcttg 720
tacgagcacg cgacgaagtt cgacttcacg tgccgggtgc ggtggaagaa ggaccaagtt 780
ctcgtctggg acaatctctg cacgatgcac ctgcgcgttc ccgactacga cggcaaatc 840
agatacttga cccgaacaac agtagcggga gataagcctt cccgttga 888

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<210> SEQ ID NO 46

<211> LENGTH: 295

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 46

```

Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg Phe Ile Asp
 1             5             10             15

Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
          20             25             30

Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
          35             40             45

Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
          50             55             60

Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
          65             70             75             80

Pro Ile Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
          85             90             95

Arg Glu Ala Asn Glu Ser Ser Arg Tyr Ile Gly Asp Asp Trp His Ala
          100            105            110

Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
          115            120            125

Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
          130            135            140

Ser Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly
          145            150            155            160

Leu Asn Val Val His Ser Ala Thr Lys Val Phe Gly Ser Leu Tyr Gln
          165            170            175

Ala Thr Asn Trp Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
          180            185            190

Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro
          195            200            205

Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Val Tyr Cys Gln Lys

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210	215	220
Ile Gln Gly Met Thr Asp Ala Glu Ser Lys Ser Leu Leu Gln Phe Leu		
225	230	235 240
Tyr Glu His Ala Thr Lys Phe Asp Phe Thr Cys Arg Val Arg Trp Lys		
	245	250 255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ala		
	260	265 270
Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val		
	275	280 285
Ala Gly Asp Lys Pro Ser Arg		
	290	295

<210> SEQ ID NO 47
 <211> LENGTH: 295
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant
 <400> SEQUENCE: 47

Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg Phe Ile Asp		
1	5	10 15
Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp		
	20	25 30
Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala		
	35	40 45
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn		
	50	55 60
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val		
	65	70 75 80
Pro Ile Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg		
	85	90 95
Arg Glu Ala Asn Glu Ser Ser Arg Tyr Ile Gly Asp Asp Trp His Ala		
	100	105 110
Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala		
	115	120 125
Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr		
	130	135 140
Ser Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly		
	145	150 155 160
Leu Asn Val Val His Ser Ala Thr Lys Val Phe Gly Ser Leu Tyr Gln		
	165	170 175
Ala Thr Asn Trp Arg Phe Ser Gly Thr Ser Val Lys Val Met Asp Val		
	180	185 190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro		
	195	200 205
Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Val Tyr Cys Gln Lys		
	210	215 220
Ile Gln Gly Met Thr Asp Ala Glu Ser Lys Ser Leu Leu Gln Phe Leu		
225	230	235 240
Tyr Glu His Ala Thr Gln Phe Asp Phe Thr Cys Arg Val Arg Trp Lys		
	245	250 255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ala		

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260	265	270
Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val		
275	280	285
Ala Gly Asp Lys Pro Ser Arg		
290	295	

<210> SEQ ID NO 48
 <211> LENGTH: 295
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant
 <400> SEQUENCE: 48

Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg His Ile Asp		
1	5	10
Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp		
20	25	30
Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala		
35	40	45
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn		
50	55	60
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val		
65	70	75
Pro Ile Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg		
85	90	95
Arg Glu Ala Asn Glu Ser Ser Arg Tyr Ile Gly Asp Asp Trp His Ala		
100	105	110
Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala		
115	120	125
Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr		
130	135	140
Ser Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly		
145	150	155
Leu Asn Val Val His Ser Ala Thr Lys Val Phe Gly Ser Leu Tyr Gln		
165	170	175
Ala Thr Asn Trp Arg Phe Ser Gly Thr Ser Val Lys Val Met Asp Val		
180	185	190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro		
195	200	205
Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Val Tyr Cys Gln Lys		
210	215	220
Ile Gln Gly Met Thr Asp Ala Glu Ser Lys Ser Leu Leu Gln Phe Leu		
225	230	235
Tyr Glu His Ala Thr Gln Phe Asp Phe Thr Cys Arg Val Arg Trp Lys		
245	250	255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ala		
260	265	270
Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val		
275	280	285
Ala Gly Asp Lys Pro Ser Arg		
290	295	

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<210> SEQ ID NO 49
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 49

Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg His Ile Asp
1 5 10 15
Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
20 25 30
Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
35 40 45
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
50 55 60
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
65 70 75 80
Pro Ile Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
85 90 95
Arg Glu Ala Asn Glu Ser Ser Arg Tyr Ile Gly Asp Asp Trp His Ala
100 105 110
Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
115 120 125
Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
130 135 140
Ser Ala Trp Glu Thr Leu Ser Pro Ala Met Gln Ala Thr Ile Glu Gly
145 150 155 160
Leu Asn Val Val His Ser Ala Thr Lys Val Phe Gly Ser Leu Tyr Gln
165 170 175
Ala Thr Asn Trp Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
180 185 190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro
195 200 205
Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Ile Tyr Cys Gln Lys
210 215 220
Ile Gln Gly Met Thr Asp Ala Glu Ser Lys Ser Leu Leu Gln Phe Leu
225 230 235 240
Tyr Glu His Ala Thr Gln Phe Asp Phe Thr Cys Arg Val Arg Trp Lys
245 250 255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ala
260 265 270
Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val
275 280 285
Ala Gly Asp Lys Pro Ser Arg
290 295

<210> SEQ ID NO 50
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 50

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Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg Phe Ile Asp
1          5          10          15

Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
          20          25          30

Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
          35          40          45

Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
50          55          60

Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
65          70          75          80

Pro Ile Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
          85          90          95

Arg Glu Ala Asn Glu Ser Ser Arg Tyr Ile Gly Asp Asp Trp His Ala
          100          105          110

Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
          115          120          125

Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
130          135          140

Ser Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly
145          150          155          160

Leu Asn Val Val His Ser Ala Thr Lys Val Phe Gly Ser Leu Tyr Gln
          165          170          175

Ala Thr Asn Trp Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
          180          185          190

Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro
          195          200          205

Val Thr Gly Arg Arg Ala Leu Tyr Cys Asn Gln Ile Tyr Cys Gln Lys
210          215          220

Ile Gln Gly Met Thr Asp Ala Glu Ser Lys Ser Leu Leu Gln Phe Leu
225          230          235          240

Tyr Glu His Ala Thr Gln Phe Asp Phe Thr Cys Arg Val Arg Trp Lys
          245          250          255

Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ala
          260          265          270

Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val
          275          280          285

Ala Gly Asp Lys Pro Ser Arg
          290          295

```

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<210> SEQ ID NO 51
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

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<400> SEQUENCE: 51

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Met His Ala Ala Leu Thr Pro Leu Thr Asn Lys Tyr Arg Phe Ile Asp
1          5          10          15

Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
          20          25          30

Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
35          40          45

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Phe	His	Thr	Tyr	Gln	Val	Ile	Tyr	Phe	Pro	Gly	Gln	Ala	Ile	Thr	Asn
50					55					60					
Glu	Gln	His	Ile	Ala	Phe	Ser	Arg	Arg	Phe	Gly	Pro	Val	Asp	Pro	Val
65					70					75					80
Pro	Ile	Leu	Lys	Ser	Ile	Glu	Gly	Tyr	Pro	Glu	Val	Gln	Met	Ile	Arg
		85					90					95			
Arg	Glu	Ala	Asn	Glu	Ser	Ser	Arg	Tyr	Ile	Gly	Asp	Asp	Trp	His	Ala
	100						105				110				
Asp	Ser	Thr	Phe	Leu	Asp	Ala	Pro	Pro	Ala	Ala	Val	Val	Met	Arg	Ala
115					120					125					
Ile	Glu	Val	Pro	Glu	Tyr	Gly	Gly	Asp	Thr	Gly	Phe	Leu	Ser	Met	Tyr
130					135					140					
Ser	Ala	Trp	Glu	Thr	Leu	Ser	Pro	Thr	Met	Gln	Ala	Thr	Ile	Glu	Gly
145					150					155					160
Leu	Asn	Val	Val	His	Ser	Ala	Thr	Lys	Val	Phe	Gly	Ser	Leu	Tyr	Gln
		165					170					175			
Ala	Thr	Asn	Trp	Arg	Phe	Ser	Asn	Thr	Ser	Val	Lys	Val	Met	Asp	Val
	180						185				190				
Asp	Ala	Gly	Asp	Arg	Glu	Thr	Val	His	Pro	Leu	Val	Val	Thr	His	Pro
195					200					205					
Val	Thr	Gly	Arg	Arg	Ala	Leu	Tyr	Cys	Asn	Gln	Val	Tyr	Cys	Gln	Lys
210					215					220					
Ile	Gln	Gly	Met	Thr	Asp	Ala	Glu	Ser	Lys	Ser	Leu	Leu	Gln	Phe	Leu
225					230					235					240
Tyr	Glu	His	Ala	Thr	Gln	Phe	Asp	Phe	Thr	Cys	Arg	Val	Arg	Trp	Lys
		245					250					255			
Lys	Asp	Gln	Val	Leu	Val	Trp	Asp	Asn	Leu	Cys	Thr	Met	His	Arg	Ala
	260					265					270				
Val	Pro	Asp	Tyr	Ala	Gly	Lys	Phe	Arg	Tyr	Leu	Thr	Arg	Thr	Thr	Val
275					280					285					
Ala	Gly	Asp	Lys	Pro	Ser	Arg									
290					295										

<210> SEQ ID NO 52

<211> LENGTH: 295

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 52

Met	His	Ala	Ala	Leu	Thr	Pro	Leu	Thr	Asn	Lys	Tyr	Arg	His	Ile	Asp
1				5					10					15	
Val	Gln	Pro	Leu	Thr	Gly	Val	Leu	Gly	Ala	Glu	Ile	Thr	Gly	Val	Asp
		20					25					30			
Leu	Arg	Glu	Pro	Leu	Asp	Asp	Ser	Thr	Trp	Asn	Glu	Ile	Leu	Asp	Ala
	35						40				45				
Phe	His	Thr	Tyr	Gln	Val	Ile	Tyr	Phe	Pro	Gly	Gln	Ala	Ile	Thr	Asn
50					55					60					
Glu	Gln	His	Ile	Ala	Phe	Ser	Arg	Arg	Phe	Gly	Pro	Val	Asp	Pro	Val
65					70					75					80
Pro	Ile	Leu	Lys	Ser	Ile	Glu	Gly	Tyr	Pro	Glu	Val	Gln	Met	Ile	Arg
		85						90					95		

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Arg	Glu	Ala	Asn	Glu	Ser	Ser	Arg	Tyr	Ile	Gly	Asp	Asp	Trp	His	Ala
			100					105					110		
Asp	Ser	Thr	Phe	Leu	Asp	Ala	Pro	Pro	Ala	Ala	Val	Val	Met	Arg	Ala
		115					120					125			
Ile	Glu	Val	Pro	Glu	Tyr	Gly	Gly	Asp	Thr	Gly	Phe	Leu	Ser	Met	Tyr
	130					135					140				
Ser	Ala	Trp	Glu	Thr	Leu	Ser	Pro	Thr	Met	Gln	Ala	Thr	Ile	Glu	Gly
145					150					155					160
Leu	Asn	Val	Val	His	Ser	Ala	Thr	Lys	Val	Phe	Gly	Ser	Leu	Tyr	Gln
				165					170					175	
Ala	Thr	Asn	Trp	Arg	Phe	Ser	Asn	Thr	Ser	Val	Lys	Val	Met	Asp	Val
		180						185					190		
Asp	Ala	Gly	Asp	Arg	Glu	Thr	Val	His	Pro	Leu	Val	Val	Thr	His	Pro
		195					200					205			
Val	Thr	Gly	Arg	Arg	Ala	Leu	Tyr	Cys	Asn	Gln	Val	Tyr	Cys	Gln	Lys
	210					215					220				
Ile	Gln	Gly	Met	Thr	Asp	Ala	Glu	Ser	Lys	Ser	Leu	Leu	Gln	Phe	Leu
225					230					235					240
Tyr	Glu	His	Ala	Thr	Gln	Phe	Asp	Phe	Thr	Cys	Arg	Val	Arg	Trp	Lys
				245					250					255	
Lys	Asp	Gln	Val	Leu	Val	Trp	Asp	Asn	Leu	Cys	Thr	Met	His	Arg	Ala
		260						265					270		
Val	Pro	Asp	Tyr	Ala	Gly	Lys	Phe	Arg	Tyr	Leu	Thr	Arg	Thr	Thr	Val
		275					280					285			
Ala	Gly	Asp	Lys	Pro	Ser	Arg									
	290					295									

<210> SEQ ID NO 53

<211> LENGTH: 888

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 53

atgcacgcgg ctttgacacc tttagaccaac aagtatcggg tcatcgacgt tcaaccactc	60
acaggcgtgc tcggcgacga gattaccgga gtggacctga gggagccctt agacgactcc	120
acttggaaac agatcctcga cgcctttcac acctaccaag ttatctactt tcctggacag	180
gcatcaccac acgagcagca cattgccttc tcaaggaggt tcggaccggt agatccagtt	240
ccaatttcta aatccattga gggttatccc gaggtgcaga tgattagacg agaagccaac	300
gagtcctcac ggtacatagg cgacgattgg cagcgagaca gcaccttcct tgacgctcct	360
cgggctgccg tggttatgcg cgcaatagag gtgccggagt acggcggcga taccggtttc	420
ctatcaatgt actctgcatg ggagacgctc tcaccaacga tgcaagccac cattgaaggt	480
ctaaacgtgg ttactcagc tactaaggtc ttcggaagtc tttaccagge gacgaattgg	540
aggttcagta acaccagtgt gaaggatgat gatgtggacg ctggagacag ggagacggtg	600
catccactcg tagttacaca cctgttaact ggacgcagag ccctttactg caaccaggtt	660
tactgccaga agatccaggg aatgactgat gcggagtcta agtcctgct tcaattctc	720
tacgaacacg ccaccaaatt cgacttcact tgctgtgttc ggtggaagaa ggaccaagt	780
ctcgtgtggg ataacctttg caccatgcac cgagcagtag cagactacgc cgggaaattc	840

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cgctatctca cccgcactac agtggccgga gacaagccta gccgctga 888

<210> SEQ ID NO 54
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 54

atgcacgcgg ctttgacacc ttgaccaac aagtatcggg tcatcgacgt tcaaccactc	60
acaggcggtgc tcggcgcgaga gattaccgga gtggacctga gggagccctt agacgactcc	120
acttggaacg agatcctcga cgcctttcac acctaccaag ttatctactt tctgggacag	180
gcgatcacca acgagcagca cattgccttc tcaaggaggt tcggaccggt agatccagtt	240
ccaattctca aatccattga gggttatccc gaggtgcaga tgattagacg agaagccaac	300
gagtcctcac ggtacatagg cgacgattgg cacgcagaca gcaccttctt tgacgctcct	360
ccggtgcccg tggttatgcg cgcaatagag gtgccggagt acggcgcgga taccggtttc	420
ctatcaatgt actctgcatg ggagacgctc tcaccaacga tgcaagccac cattgaaggt	480
ctaaacgtgg ttcactcagc tactaaggtc ttcggaagtc ttaccaggc gacgaattgg	540
aggttcagtg gaaccagtgt gaaggatgat gatgtggacg ctggagacag ggagacggtg	600
catccactcg tagttacaca cctgttaact ggacgcagag ccctttactg caaccagggt	660
tactgccaga agatccaggg aatgactgat gcggagtcta agtccctgct tcaattcttc	720
tacgaacacg ccaccaatcg cgacttact gtgtgtgttc ggtggaagaa ggaccaagtg	780
ctcgtgtggg ataacctttg caccatgcac cgagcagtag cagactacgc cgggaaattc	840
cgctatctca cccgcactac agtggccgga gacaagccta gccgctga	888

<210> SEQ ID NO 55
 <211> LENGTH: 888
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 55

atgcacgcgg ctttgacacc ttgaccaac aagtatcggg atatcgacgt tcaaccactc	60
acaggcggtgc tcggcgcgaga gattaccgga gtggacctga gggagccctt agacgactcc	120
acttggaacg agatcctcga cgcctttcac acctaccaag ttatctactt tctgggacag	180
gcgatcacca acgagcagca cattgccttc tcaaggaggt tcggaccggt agatccagtt	240
ccaattctca aatccattga gggttatccc gaggtgcaga tgattagacg agaagccaac	300
gagtcctcac ggtacatagg cgacgattgg cacgcagaca gcaccttctt tgacgctcct	360
ccggtgcccg tggttatgcg cgcaatagag gtgccggagt acggcgcgga taccggtttc	420
ctatcaatgt actctgcatg ggagacgctc tcaccaacga tgcaagccac cattgaaggt	480
ctaaacgtgg ttcactcagc tactaaggtc ttcggaagtc ttaccaggc gacgaattgg	540
aggttcagtg gaaccagtgt gaaggatgat gatgtggacg ctggagacag ggagacggtg	600
catccactcg tagttacaca cctgttaact ggacgcagag ccctttactg caaccagggt	660
tactgccaga agatccaggg aatgactgat gcggagtcta agtccctgct tcaattcttc	720

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tacgaacacg ccacccaatt cgacttcact tgtcgtgttc ggtggaagaa ggaccaagtg	780
ctcgtgtggg ataacctttg caccatgcac cgagcagtac cagactacgc cgggaaattc	840
cgctatctca ccgcactac agtggccgga gacaagccta gccgctga	888

<210> SEQ ID NO 56
<211> LENGTH: 888
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 56

atgcacgcgg ctttgacacc ttgaccaac aagtatcggc atatcgacgt tcaaccactc	60
acaggcgtgc tcggcgacga gattaccgga gtggacctga gggagccctt agacgactcc	120
acttgaacg agatcctcga cgcctttcac acctaccaag ttatctactt tcctggacag	180
gcgatcacca acgagcagca cattgccttc tcaaggaggt tcggaccggt agatccagtt	240
ccaattctca aatccattga gggttatccc gaggtgcaga tgattagacg agaagccaac	300
gagtcctcac ggtacatagg cgacgattgg cagcgagaca gcaccttcct tgacgctcct	360
ccggtgccc tggttatgcg cgcaatagag gtgccggagt acggcggcga taccggtttc	420
ctatcaatgt actctgcatg ggagacgctc tcaccagcta tgcaagccac cattgaaggt	480
ctaaacgtgg ttcactcagc tactaaggtc ttcggaagtc tttaccaggc gacgaattgg	540
aggttcagta acaccagtgt gaaggatgat gatgtggacg ctggagacag ggagacggtg	600
catccactcg tagttacaca cctgttaact ggacgcagag ccctttactg caaccagatt	660
tactgccaga agatccaggg aatgactgat gcggagtcta agtcctgct tcaattcctc	720
tacgaacacg ccacccaatt cgacttcact tgtcgtgttc ggtggaagaa ggaccaagtg	780
ctcgtgtggg ataacctttg caccatgcac cgagcagtac cagactacgc cgggaaattc	840
cgctatctca ccgcactac agtggccgga gacaagccta gccgctga	888

<210> SEQ ID NO 57
<211> LENGTH: 888
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

<400> SEQUENCE: 57

atgcacgcgg ctttgacacc ttgaccaac aagtatcggg tcatcgacgt tcaaccactc	60
acaggcgtgc tcggcgacga gattaccgga gtggacctga gggagccctt agacgactcc	120
acttgaacg agatcctcga cgcctttcac acctaccaag ttatctactt tcctggacag	180
gcgatcacca acgagcagca cattgccttc tcaaggaggt tcggaccggt agatccagtt	240
ccaattctca aatccattga gggttatccc gaggtgcaga tgattagacg agaagccaac	300
gagtcctcac ggtacatagg cgacgattgg cagcgagaca gcaccttcct tgacgctcct	360
ccggtgccc tggttatgcg cgcaatagag gtgccggagt acggcggcga taccggtttc	420
ctatcaatgt actctgcatg ggagacgctc tcaccaacga tgcaagccac cattgaaggt	480
ctaaacgtgg ttcactcagc tactaaggtc ttcggaagtc tttaccaggc gacgaattgg	540
aggttcagta acaccagtgt gaaggatgat gatgtggacg ctggagacag ggagacggtg	600

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catccactcg tagttacaca cctgttaact ggacgcagag ccctttactg caaccagatt    660
tactgccaga agatccaggg aatgactgat gcggagtcta agtccctgct tcaattcctc    720
tacgaacacg ccacccaatt cgacttcact tgctgtgttc ggtggaagaa ggaccaagtg    780
ctcgtgtggg ataacctttg caccatgcac cgagcagtac cagactacgc cgggaaattc    840
cgctatctca ccgcactac agtggccgga gacaagccta gccgctga                    888

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<210> SEQ ID NO 58
<211> LENGTH: 888
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

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<400> SEQUENCE: 58

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atgcacgcgg ctttgacacc ttgaccaac aagtatcggc tcatcgacgt tcaaccactc    60
acaggcgtgc tcggcgcaga gattaccgga gtggacctga gggagccctt agacgactcc    120
acttggaacg agatcctega cgcctttcac acctaccaag ttatctactt tcttgacag    180
gcgatcacca acgagcagca cattgccttc tcaaggaggt tcggaccggt agatccagtt    240
ccaattctca aatccattga gggttatccc gaggtgcaga tgattagacg agaagccaac    300
gagtcctcac ggtacatagg cgacgattgg cagcagaca gcaccttctc tgacgctcct    360
cgggctgccg tggttatgcg cgcaatagag gtgccggagt acggcggcga taccggtttc    420
ctatcaatgt actctgcatg ggagacgctc tcaccaacga tgcaagccac cattgaaggt    480
ctaaacgtgg ttcactcagc tactaaggtc ttcggaagtc tttaccaggc gacgaattgg    540
agggttcagta acaccagtgt gaagggtgat gatgtggacg ctggagacag ggagacggtg    600
catccactcg tagttacaca cctgttaact ggacgcagag ccctttactg caaccaggtt    660
tactgccaga agatccaggg aatgactgat gcggagtcta agtccctgct tcaattcctc    720
tacgaacacg ccacccaatt cgacttcact tgctgtgttc ggtggaagaa ggaccaagtg    780
ctcgtgtggg ataacctttg caccatgcac cgagcagtac cagactacgc cgggaaattc    840
cgctatctca ccgcactac agtggccgga gacaagccta gccgctga                    888

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<210> SEQ ID NO 59
<211> LENGTH: 888
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Recombinant

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<400> SEQUENCE: 59

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atgcacgcgg ctttgacacc ttgaccaac aagtatcggc atatcgacgt tcaaccactc    60
acaggcgtgc tcggcgcaga gattaccgga gtggacctga gggagccctt agacgactcc    120
acttggaacg agatcctega cgcctttcac acctaccaag ttatctactt tcttgacag    180
gcgatcacca acgagcagca cattgccttc tcaaggaggt tcggaccggt agatccagtt    240
ccaattctca aatccattga gggttatccc gaggtgcaga tgattagacg agaagccaac    300
gagtcctcac ggtacatagg cgacgattgg cagcagaca gcaccttctc tgacgctcct    360
cgggctgccg tggttatgcg cgcaatagag gtgccggagt acggcggcga taccggtttc    420
ctatcaatgt actctgcatg ggagacgctc tcaccaacga tgcaagccac cattgaaggt    480

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ctaaacgtgg ttcactcagc tactaaggtc ttcggaagtc tttaccaggc gacgaattgg    540
aggttcagta acaccagtgt gaaggatgat gatgtggacg ctggagacag ggagacgggtg    600
catccactcg tagttacaca ccttgtaact ggacgcagag ccctttactg caaccaggtt    660
tactgccaga agatccaggg aatgactgat gcggagtcta agtccctgct tcaattcctc    720
tacgaacacg ccaccaatt cgacttcact tgctgtgttc ggtggaagaa ggaccaagtg    780
ctcgtgtggg ataacctttg caccatgcac cgagcagtac cagactacgc cgggaaattc    840
cgctatctca cccgcactac agtggccgga gacaagccta gccgctga                888

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<210> SEQ ID NO 60
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Sphingobium herbicidovorans

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<400> SEQUENCE: 60

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Met His Ala Ala Leu Ser Pro Leu Ser Gln Arg Phe Glu Arg Ile Ala
1          5          10          15
Val Gln Pro Leu Thr Gly Val Leu Gly Ala Glu Ile Thr Gly Val Asp
20         25         30
Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
35         40         45
Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
50         55         60
Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
65         70         75         80
Pro Leu Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
85         90         95
Arg Glu Ala Asn Glu Ser Gly Arg Val Ile Gly Asp Asp Trp His Thr
100        105        110
Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
115        120        125
Ile Asp Val Pro Glu His Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
130        135        140
Thr Ala Trp Glu Thr Leu Ser Pro Thr Met Gln Ala Thr Ile Glu Gly
145        150        155        160
Leu Asn Val Val His Ser Ala Thr Arg Val Phe Gly Ser Leu Tyr Gln
165        170        175
Ala Gln Asn Arg Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
180        185        190
Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro
195        200        205
Gly Ser Gly Arg Lys Gly Leu Tyr Val Asn Gln Val Tyr Cys Gln Arg
210        215        220
Ile Glu Gly Met Thr Asp Ala Glu Ser Lys Pro Leu Leu Gln Phe Leu
225        230        235        240
Tyr Glu His Ala Thr Arg Phe Asp Phe Thr Cys Arg Val Arg Trp Lys
245        250        255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Arg Ala
260        265        270
Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val
275        280        285

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Gly Gly Val Arg Pro Ala Arg
290 295

<210> SEQ ID NO 61
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: consensus sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (9)..(14)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Thr can be Cys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (32)..(32)
<223> OTHER INFORMATION: Asp can be Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (38)..(38)
<223> OTHER INFORMATION: Asp can be Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (40)..(40)
<223> OTHER INFORMATION: Ser can be Asn
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (59)..(59)
<223> OTHER INFORMATION: Gly can be Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (61)..(61)
<223> OTHER INFORMATION: Ala can be Gln
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (67)..(67)
<223> OTHER INFORMATION: His can be Gln
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (69)..(69)
<223> OTHER INFORMATION: Ala can be Ser
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (82)..(82)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (103)..(103)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (105)..(105)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (106)..(106)
<223> OTHER INFORMATION: Ile can be Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (108)..(108)
<223> OTHER INFORMATION: Asp can be Glu
<220> FEATURE:

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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (109)..(109)
<223> OTHER INFORMATION: Asp can be Asn
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (112)..(112)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (115)..(115)
<223> OTHER INFORMATION: Thr can be Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (127)..(127)
<223> OTHER INFORMATION: Arg can be Tyr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (129)..(129)
<223> OTHER INFORMATION: Ile can be Arg or Lys
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Leu Arg Glu Pro Leu Asp Asp Ser Thr Trp Asn Glu Ile Leu Asp Ala
35 40 45

Phe His Thr Tyr Gln Val Ile Tyr Phe Pro Gly Gln Ala Ile Thr Asn
50 55 60

Glu Gln His Ile Ala Phe Ser Arg Arg Phe Gly Pro Val Asp Pro Val
65 70 75 80

Pro Xaa Leu Lys Ser Ile Glu Gly Tyr Pro Glu Val Gln Met Ile Arg
85 90 95

Arg Glu Ala Asn Glu Ser Xaa Arg Xaa Ile Gly Asp Asp Trp His Xaa
100 105 110

Asp Ser Thr Phe Leu Asp Ala Pro Pro Ala Ala Val Val Met Arg Ala
115 120 125

Ile Glu Val Pro Glu Tyr Gly Gly Asp Thr Gly Phe Leu Ser Met Tyr
130 135 140

Xaa Ala Trp Xaa Thr Leu Ser Xaa Thr Met Xaa Ala Thr Ile Glu Gly
145 150 155 160

Leu Asn Val Val His Ser Ala Thr Xaa Val Phe Gly Ser Leu Tyr Gln
165 170 175

Ala Xaa Asn Xaa Arg Phe Ser Asn Thr Ser Val Lys Val Met Asp Val
180 185 190

Asp Ala Gly Asp Arg Glu Thr Val His Pro Leu Val Val Thr His Pro
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Ile Xaa Gly Met Thr Asp Ala Glu Ser Lys Xaa Leu Leu Gln Phe Leu	
225	230 235 240
Tyr Glu His Ala Thr Xaa Phe Asp Phe Thr Cys Arg Val Arg Trp Lys	
	245 250 255
Lys Asp Gln Val Leu Val Trp Asp Asn Leu Cys Thr Met His Xaa Ala	
	260 265 270
Val Pro Asp Tyr Ala Gly Lys Phe Arg Tyr Leu Thr Arg Thr Thr Val	
	275 280 285
Xaa Gly Xaa Xaa Pro Xaa Arg	
290	295

What is claimed is:

1. A recombinant DNA molecule comprising a nucleic acid sequence encoding a polypeptide having at least 92% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52.

2. The recombinant DNA molecule of claim 1, wherein the nucleic acid sequence is selected from the group consisting of SEQ ID NO:2, 3, 5, 6, 8, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, 41, 42, 44, 45 and 53-59.

3. The recombinant DNA molecule of claim 1, wherein the recombinant DNA molecule is operably linked to a heterologous promoter functional in a plant cell.

4. The recombinant DNA molecule of claim 3, wherein the recombinant DNA molecule is further operably linked to a DNA molecule encoding a chloroplast transit peptide.

5. A DNA construct comprising a heterologous promoter functional in a plant cell operably linked to the recombinant DNA molecule of claim 1.

6. The DNA construct of claim 5, further comprising a DNA molecule encoding a chloroplast transit peptide operably linked to the recombinant DNA molecule.

7. The DNA construct of claim 5, wherein the nucleic acid sequence encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43 and 46-52.

8. The DNA construct of claim 5, wherein the DNA construct is present in the genome of a transgenic plant.

9. A plant, seed, plant tissue, plant part, or cell comprising the recombinant DNA molecule of claim 1.

10. The plant, seed, plant tissue, plant part, or cell of claim 9, wherein the plant, seed, plant tissue, plant part, or cell comprises tolerance to at least one herbicide selected from the group consisting of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides.

11. A plant, seed, plant tissue, plant part, or cell comprising the DNA construct of claim 5.

12. A plant, seed, plant tissue, plant part, or cell comprising the polypeptide encoded by the recombinant DNA molecule of claim 1.

13. A polypeptide having at least 92% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52.

14. The polypeptide of claim 13, wherein the polypeptide has oxygenase activity against at least one herbicide selected from the group consisting of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides.

15. A method for conferring herbicide tolerance to a plant, seed, cell, or plant part comprising expressing in said plant, seed, cell, or plant part the polypeptide of claim 13.

16. The method of claim 15, wherein said plant, seed, cell, or plant part comprises a DNA construct comprising a heterologous promoter functional in a plant cell operably linked to a recombinant DNA molecule comprising a nucleic acid sequence encoding a polypeptide having at least 92% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52.

17. The method of claim 15, wherein the plant, seed, cell, or plant part comprises tolerance to at least one herbicide selected from the group consisting of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides.

18. A method for producing an herbicide tolerant transgenic plant comprising transforming a plant cell or tissue with a recombinant DNA molecule comprising a nucleic acid sequence selected from the group consisting of:

- a) a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52;
- b) a nucleic acid sequence selected from the group consisting of SEQ ID NO:2, 3, 5, 6, 8, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, 41, 42, 44, 45 and 53-59; and
- c) a nucleic acid sequence encoding a polypeptide having at least 92% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52, and capable of conferring herbicide tolerance to a transgenic plant;

and regenerating an herbicide tolerant transgenic plant from the transformed plant cell or tissue.

19. The method of claim 18, wherein said herbicide tolerant transgenic plant comprises tolerance to at least one her-

bicide selected from the group consisting of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides.

20. A method for controlling weeds in a plant growth area, comprising contacting a plant growth area comprising a plant or seed comprising a recombinant DNA molecule comprising a nucleic acid sequence encoding a protein having at least 92% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1, 4, 7, 9, 11, 14, 18, 22, 25, 28, 31, 34, 37, 40, 43, and 46-52 with at least one herbicide selected from the group consisting of AOPP herbicides, phenoxy acid herbicides, and pyridinyloxy acid herbicides, wherein the plant or seed is tolerant to the at least one herbicide.

* * * * *