

CLAIMS

What is claimed is:

1. A polynucleotide encoding a modified luciferase polypeptide having at least 60% amino acid sequence identity to a wild-type *Oplophorus* luciferase comprising at least one amino acid substitution at a position corresponding to an amino acid in a wild-type *Oplophorus* luciferase of SEQ ID NO:1, wherein the modified luciferase polypeptide has at least one of enhanced luminescence, enhanced signal stability, and enhanced protein stability relative to the wild-type *Oplophorus* luciferase.
2. The polynucleotide of claim 1, wherein the encoded modified luciferase polypeptide has enhanced luminescence relative to the wild-type *Oplophorus* luciferase and an amino acid substitution at at least one of positions 2, 4, 11, 20, 23, 28, 33, 34, 44, 45, 51, 54, 68, 72, 75, 76, 77, 89, 90, 92, 99, 104, 115, 124, 135, 138, 139, 143, 144, 166, 167, or 169 corresponding to SEQ ID NO:1.
3. The polynucleotide of claim 1 or 2, wherein the modified luciferase polypeptide has enhanced signal stability relative to wild-type *Oplophorus* luciferase, and an amino acid substitution at at least one of positions 4, 11, 20, 28, 33, 54, 68, 75, 115, 124, 138, 143, or 166, corresponding to SEQ ID NO:1.
4. The polynucleotide of any one of claims 1-3, wherein the encoded modified luciferase polypeptide has enhanced protein stability relative to wild-type *Oplophorus* luciferase, and an amino acid substitution at at least one of positions 11, 20, 28, 33, 34, 44, 45, 51, 54, 68, 72, 75, 77, 89, 90, 92, 99, 104, 115, 124, 135, 138, 139, 143, 144, 166, 167, or 169 corresponding to SEQ ID NO:1.
5. The polynucleotide of any one of claims 1-4, wherein the encoded modified luciferase polypeptide further comprises a deletion of luciferase residues corresponding to the N-terminus of the wild-type *Oplophorus* luciferase of SEQ ID NO:10.
6. The polynucleotide of claim 5, wherein the deletion is at most 27 residues of the luciferase sequence.
7. The polynucleotide of any one of claims 1-6, wherein the wild-type *Oplophorus* luciferase is from *Oplophorus gracilirostris*, *Oplophorus grimaldii*, *Oplophorus spinicauda*, *Oplophorus foliaceus*, *Oplophorus novaezelandiae*, *Oplophorus typus*, or *Oplophorus spinous*.
8. The polynucleotide of any one of claims 1-7, wherein the encoded modified luciferase polypeptide comprises a substitution corresponding to at least one of T2S, A4E/S/R/G/D/T/L, Q11R/V/I/L/K/T, Q20R, E23V, S28P, A33K, L34M, V44I/L, V45E, G51V, A54F/T/V/G/S/W/L/I, F68S/Y/V, L72Q, M75R/K/Q/G/T/A, I76V, F77W, V79I, K89E, I90T/V, L92S/A/G/Q, I99V, P104L, P115E/I/Q/L/V/G/H/R/S/C/A/T, Q124K, N135K, Y138V/I/N/T/L/C/R/M/K, D139E, I143L, N144K, N166R/K/A/L/P/Q/S, I167V, or 169L corresponding to SEQ ID NO:1.
9. The polynucleotide of any one of claims 1-8, wherein the encoded modified luciferase polypeptide comprises amino acid substitutions at positions 11, 33, 44, 54, 115, 124, 138, and 166 corresponding to SEQ ID NO:1.
10. The polynucleotide of claim 9, wherein the substitutions comprise Q11R, A33K, V44I, A54F, P115E, Q124K, Y138I, and N166R.

11. The polynucleotide of claim 9 or 10, wherein the encoded modified luciferase further comprises an amino acid substitution at position 4 corresponding to SEQ ID NO:1.
12. The polynucleotide of claim 11, wherein the amino acid at position 4 is glutamic acid.
13. The polynucleotide of any one of claims 9-12, wherein the encoded modified luciferase further comprises amino acid substitutions at positions 45, 135, 104, 139, and 167 corresponding to SEQ ID NO:1.
14. The polynucleotide of claim 13, wherein the amino acid at position 45 is glutamic acid, position 104 is leucine, position 135 is lysine, position 139 is glutamic acid, and position 167 is valine.
15. The polynucleotide of any one of claims 9-12, wherein the encoded modified luciferase further comprises amino acid substitutions at positions 28, 34, 51, 99 and 143 corresponding to SEQ ID NO:1.
16. The polynucleotide of claim 16, wherein the amino acid at position 28 is proline, position 34 is methionine, position 51 is valine, position 99 is valine, and position 143 is leucine.
17. The polynucleotide of claim 11 or 12, further comprising a substitution of at least one amino acid at position 20, 54, 68, 72, 77, 79, 89, 90, or 92 corresponding to SEQ ID NO:1.
18. The polynucleotide of claim 17, wherein the amino acid at position 20 is arginine, position 54 is isoleucine, position 68 is serine, position 72 is glutamine, position 75 is lysine, position 77 is tryptophan, position 79 is isoleucine, position 89 is glutamic acid, position 90 is threonine or valine, and position 92 is glycine, glutamine, serine or alanine.
19. The polynucleotide of any one of claims 1-8, wherein the encoded modified luciferase polypeptide comprises amino acid substitutions at positions 23, 28, 143, and 166 corresponding to SEQ ID NO:1.
20. The polynucleotide of claim 19, wherein the substitutions comprise E23V, S28P, I143V, and N166R.
21. The polynucleotide of any one of claims 1-8, wherein the encoded modified luciferase polypeptide comprises amino acid substitutions at positions 4, 34, 76, and 166 corresponding to SEQ ID NO:1.
22. The polynucleotide of claim 21, wherein the substitutions comprise A4S, L34M, I76V, and N166R.
23. The polynucleotide of any one of claims 1-8, wherein the encoded modified luciferase polypeptide comprises amino acid substitutions at positions 51, 99, and 166 corresponding to SEQ ID NO:1.
24. The polynucleotide of claim 23, wherein the substitutions comprise G51V, I99V, and N166R.
25. The polynucleotide of any one of claims 1-24, wherein the polynucleotide further encodes a polypeptide of interest linked to the modified luciferase polypeptide, the polypeptide of interest and the modified luciferase polypeptide capable of being expressed as a fusion protein.

26. A vector comprising the polynucleotide of any of claims 1-25.
27. The vector of claim 26, wherein the polynucleotide is operably linked to a promoter.
28. A cell comprising the polynucleotide of any one of claims 1-25 or the vector of claim 26 or 27.
29. A non-human transgenic animal comprising the cell of claim 28.
30. A non-human transgenic animal comprising the polynucleotide of any one of claims 1-25 or the vector of claim 26 or 27.
31. A modified luciferase encoded by the polynucleotide of any one claims 1-25.
32. A polypeptide encoded by the polynucleotide of any one of claims 1-25.
33. A fusion protein comprising a modified luciferase encoded by the polynucleotide of any one of claims 1-24.
34. A method of producing a modified luciferase comprising growing the cell of claim 28 under conditions that permit expression of the modified luciferase.
35. A method of producing a modified luciferase comprising introducing the vector of claim 26 or 27 into a cell under conditions which permit expression of the modified luciferase.
36. A kit comprising the polynucleotide of any one of claims 1-25 or the vector of claim 26 or 27.
37. A kit comprising the modified luciferase of claim 31.
38. The polynucleotide of claim 1, wherein the modified luciferase polypeptide has luciferase activity and utilizes a coelenterazine as a substrate to generate luminescence.
39. The polynucleotide of claim 1, having at least 80% amino acid sequence identity to SEQ ID NO:1.

CLAIMS

1. A polynucleotide encoding a modified luciferase polypeptide comprising an amino acid sequence that is at least 80% identical to the amino acid sequence set forth in SEQ ID NO:1 and comprising at least one amino acid substitution at a position corresponding to position 2, 4, 11, 20, 23, 28, 33, 34, 44, 45, 51, 54, 68, 72, 75, 76, 77, 89, 90, 92, 99, 104, 115, 124, 135, 138, 139, 143, 144, 166, 167, or 169 in a wild-type *Oplophorus* luciferase of SEQ ID NO:1, wherein the modified luciferase polypeptide has at least one of enhanced luminescence, enhanced signal stability, and enhanced protein stability relative to a wild-type *Oplophorus* luciferase, wherein the modified luciferase polypeptide has luciferase activity and utilizes a coelenterazine as a substrate to generate luminescence.
2. The polynucleotide of claim 1, wherein the modified luciferase polypeptide has enhanced signal stability relative to that of a wild-type *Oplophorus* luciferase, and has at least one amino acid substitution at a position corresponding to at least one of positions 4, 11, 20, 28, 33, 54, 68, 75, 115, 124, 138, 143, or 166 of SEQ ID NO:1.
3. The polynucleotide of claim 1, wherein the encoded modified luciferase polypeptide has enhanced protein stability relative to that of a wild-type *Oplophorus* luciferase, and at least one amino acid substitution at a position corresponding to at least one of positions 11, 20, 28, 33, 34, 44, 45, 51, 54, 68, 72, 75, 77, 89, 90, 92, 99, 104, 115, 124, 135, 138, 139, 143, 144, 166, 167, or 169 of SEQ ID NO:1.
4. The polynucleotide of claim 1, wherein the encoded modified luciferase polypeptide further comprises a deletion of as many as 27 amino acid residues corresponding to the N-terminus of the wild-type *Oplophorus* luciferase of SEQ ID NO:10.
5. The polynucleotide of claim 4, wherein the deletion constitutes a deletion of the 27 amino acid residues corresponding to the N-terminus of SEQ ID NO:10.

6. The polynucleotide of claim 1, wherein the wild-type *Oplophorus* luciferase is from *Oplophorus gracilirostris*, *Oplophorus grimaldii*, *Oplophorus spinicauda*, *Oplophorus fofiaceus*, *Oplophorus novaezelandiae*, *Oplophorus typus*, or *Oplophorus spinous*.
7. The polynucleotide of claim 1, wherein the encoded modified luciferase polypeptide comprises a substitution corresponding to at least one of T2S, A4E/S/R/G/D/T/L, Q11R/V/I/L/K/T, Q20R, E23V, S28P, A33K, V44I/L, V45E, G51V, A54F/T/V/G/S/W/L/I, F68S/Y/V, L72Q, M75R/K/Q/G/T/A, I76V, F77W, V79I, K89E, I90T/V, L92S/A/G/Q, I99V, P104L, P115E/I/Q/L/V/G/H/R/S/C/A/T, Q124K, N135K, Y138V/I/N/T/L/C/R/M/K, D139E, I143L, N144K, N166R/K/A/L/P/Q/S, I167V, or A169L where the positions are numbered by correspondence with positions in the amino acid sequence of SEQ ID NO:1.
8. The polynucleotide of claim 1, wherein the encoded modified luciferase polypeptide comprises amino acid substitutions at positions corresponding to positions 11, 33, 44, 54, 115, 124, 138, and 166 in the amino acid sequence of SEQ ID NO:1.
9. The polynucleotide of claim 8, wherein the amino acid substitutions comprise Q11R, A33K, V44I, A54F, P115E, Q124K, Y138I, and N166R.
10. The polynucleotide of claim 8, wherein the encoded modified luciferase further comprises an amino acid substitution at a position corresponding to position 4 in the amino acid sequence of SEQ ID NO:1.
11. The polynucleotide of claim 10, wherein the amino acid substitution made at position 4 is glutamic acid.
12. The polynucleotide of claim 8, wherein the encoded modified luciferase further comprises amino acid substitutions at positions corresponding to positions 45, 104, 135, 139, and 167 in the amino acid sequence of SEQ ID NO:1.

13. The polynucleotide of claim 12, wherein the amino acid substitution made at position 45 is glutamic acid, the amino acid substitution made position 104 is leucine, the amino acid substitution made at position 135 is lysine, the amino acid substitution made at position 139 is glutamic acid, and the amino acid substitution made at position 167 is valine.

14. The polynucleotide of claim 10, wherein the encoded modified luciferase further comprises amino acid substitutions at positions corresponding to positions 28, 34, 51, 99 and 143 in the amino acid sequence of SEQ ID NO:1.

15. The polynucleotide of claim 14, wherein the amino acid substitution made at position 28 is proline, the amino acid substitution made at position 34 is methionine, the amino acid substitution made at position 51 is valine, the amino acid substitution made at position 99 is valine, and the amino acid substitution made at position 143 is leucine.

16. The polynucleotide of claim 10, wherein the encoded modified luciferase further comprises an amino acid substitution at one or more positions corresponding to positions 20, 54, 68, 72, 75, 77, 79, 89, 90, 92, and 164 in the amino acid sequence of SEQ ID NO:1.

17. The polynucleotide of claim 16, wherein an amino acid substitution made at position 20 is arginine, an amino acid substitution made at position 54 is isoleucine, an amino acid substitution made at position 68 is serine, an amino acid substitution made at position 72 is glutamine, the amino acid substitution made at position 75 is lysine, an amino acid substitution made at position 77 is tryptophan, an amino acid substitution made at position 79 is isoleucine, an amino acid substitution made at position 89 is glutamic acid, an amino acid substitution made at position 90 is either threonine or valine, an amino acid substitution made at position 92 is selected from the group consisting of glycine, glutamine, serine and alanine, and an amino acid substitution made at position 164 is serine.

18. The polynucleotide of claim 10, wherein the encoded modified luciferase polypeptide further comprises amino acid substitutions at positions corresponding to positions 23, 28, 143, and 166 in the amino acid sequence of SEQ ID NO:1.

19. The polynucleotide of claim 18, wherein the amino acid substitutions comprise E23V, S28P, I143V, and N166R.

20. The polynucleotide of claim 1, wherein the encoded modified luciferase polypeptide further comprises amino acid substitutions at positions corresponding to positions 4, 34, 76, and 166 in the amino acid sequence of SEQ ID NO:1.

21. The polynucleotide of claim 20, wherein the amino acid substitutions comprise A4S, L34M, I76V, and N166R.

22. The polynucleotide of claim 1, wherein the encoded modified luciferase polypeptide further comprises amino acid substitutions at positions corresponding to positions 51, 99, and 166 in the amino acid sequence of SEQ ID NO:1.

23. The polynucleotide of claim 22, wherein the amino acid substitutions comprise G51V, I99V, and N166R.

24. The polynucleotide of claim 1, wherein the polynucleotide further encodes a polypeptide of interest linked to the modified luciferase polypeptide and the polypeptide of interest and the modified luciferase polypeptide are capable of being expressed as a fusion protein.

25. A vector comprising the polynucleotide of claim 1.

26. The vector of claim 25, wherein the polynucleotide is operably linked to a promoter.

27. An isolated host cell comprising the polynucleotide of claim 1 or the vector of claim 25.

28. A kit comprising the polynucleotide of claim 1 or the vector of claim 25.

29. The polynucleotide of claim 1, wherein the modified luciferase polypeptide has at least 85% amino acid sequence identity to SEQ ID NO:1.