

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 corresponding to T2S, A4 to any amino acid, Q11 to any amino acid, Q20R/K/R/D/E/N, E23D/N/Q/V/I/L, S28P/T, A33K/R/G, V44I/L, V45I/L/D/E/Q/N, G51A/V/I/L, A54 to any amino acid, F68Y/V/L/I, L72I/V/Q/D/E/N, M75R/K/G/A/T/S, I76V/A, F77W, K89R/D/E/N/Q, I90T/S/V/L, L92 to any amino acid, I99V/A, P104L/V/I, P115 to any amino acid, Q124R/K/D/E/N, N135Q/D/E/K/R, Y138 to any amino acid, D139E/Q/N, I143L/V, N144D/E/Q/K/R, N166 to any amino acid, I167V/A, or A169G/L/V in a wild-type *Oplophorus gracilirostris* 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 gracilirostris* luciferase, and has at least one amino acid substitution corresponding to A4 to any amino acid, Q11 to any amino acid, Q20R/K/R/D/E/N, S28P/T, A33K/R/G, A54 to any amino acid, F68Y/V/L/I, M75R/K/G/A/T/S, P115 to any amino acid, Q124R/K/D/E/N, Y138 to any amino acid, I143L/V, or N166 to any amino acid 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 gracilirostris* luciferase.
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 gracilirostris* 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 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, K89E, I90T/V, L92S/A/G/Q, I99V, P104L, P115E/I/Q/L/V/G/H/R/S/C/A, 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.
7. 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.
8. The polynucleotide of claim 7, wherein the amino acid substitutions comprise Q11R, A33K, V44I, A54F, P115E, Q124K, Y138I, and N166R.
9. The polynucleotide of claim 7, 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.
10. The polynucleotide of claim 9, wherein the amino acid substitution made at position 4 is glutamic acid.
11. The polynucleotide of claim 7, 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.
12. The polynucleotide of claim 11, 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.

13. The polynucleotide of claim 9, 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.

14. The polynucleotide of claim 13, 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.

15. The polynucleotide of claim 9, 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.

16. The polynucleotide of claim 15, 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.

17. The polynucleotide of claim 9, 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.

18. The polynucleotide of claim 17, wherein the amino acid substitutions comprise E23V, S28P, I143V, and N166R.
19. 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.
20. The polynucleotide of claim 19, wherein the amino acid substitutions comprise A4S, L34M, I76V, and N166R.
21. 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.
22. The polynucleotide of claim 21, wherein the amino acid substitutions comprise G51V, I99V, and N166R.
23. 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.
24. A vector comprising the polynucleotide of claim 1.
25. The vector of claim 24, wherein the polynucleotide is operably linked to a promoter.
26. An isolated host cell comprising the polynucleotide of claim 1 or the vector of claim 24.
27. A kit comprising the polynucleotide of claim 1 or the vector of claim 24.
28. The polynucleotide of claim 1, wherein the modified luciferase polypeptide has at least 85% amino acid sequence identity to SEQ ID NO:1.