

What is claimed is:

1. A recombinant muscle-specific regulatory nucleic acid sequence, comprising:
a mammalian desmin promoter, wherein the mammalian desmin promoter comprises a nucleic acid sequence having at least 80% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10;
a mammalian desmin enhancer; and
one or more mammalian muscle creatine kinase (MCK) enhancers,
wherein the mammalian desmin promoter, mammalian desmin enhancer, and mammalian MCK enhancer are operably linked.
- ~~2. The recombinant muscle specific regulatory nucleic acid sequence of claim 1,~~
~~wherein the mammalian desmin promoter is a human desmin promoter.~~
- ~~3. The recombinant muscle specific regulatory nucleic acid sequence of claim 1,~~
~~wherein the mammalian desmin promoter comprises a nucleic acid sequence having at least 80% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.~~
- ~~4.2.~~ The recombinant muscle-specific regulatory nucleic acid sequence of claim 1,
wherein the mammalian desmin promoter comprises a nucleic acid sequence having at least 80% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 8, ~~SEQ ID NO: 9,~~ and SEQ ID NO: 10.
- ~~5. The recombinant muscle specific regulatory nucleic acid sequence of claim 1,~~
~~wherein the mammalian desmin promoter is a murine desmin promoter.~~
- ~~6. The recombinant muscle specific regulatory nucleic acid sequence of claim 1,~~
~~wherein the mammalian desmin promoter comprises a nucleic acid sequence having at least 80% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 4 and SEQ ID NO: 5.~~
- ~~7. The recombinant muscle specific regulatory nucleic acid sequence of claim 1,~~
~~wherein the mammalian desmin promoter comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 4 and SEQ ID NO: 5.~~
- ~~8.3.~~ The recombinant muscle-specific regulatory nucleic acid sequence of ~~any of~~ claims 1-7, wherein the mammalian desmin enhancer is a human desmin enhancer.
- ~~9.4.~~ The recombinant muscle-specific regulatory nucleic acid sequence of ~~any of~~ claims 1-7, wherein the mammalian desmin enhancer comprises a nucleic acid sequence having at least 80% identity to the nucleic acid sequence of SEQ ID NO: 6.

~~10-5.~~_____ The recombinant muscle-specific regulatory nucleic acid sequence of ~~any of~~ claims ~~1-7~~, wherein the mammalian desmin enhancer comprises the nucleic acid sequence of SEQ ID NO: 6.

~~11. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-7, wherein the mammalian desmin enhancer is a murine desmin enhancer.~~

~~12. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-7, wherein the mammalian desmin enhancer comprises a nucleic acid sequence having at least 80% identity to the nucleic acid sequence of SEQ ID NO: 3.~~

~~13. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-7, wherein the mammalian desmin enhancer comprises the nucleic acid sequence of SEQ ID NO: 3.~~

~~14-6.~~_____ The recombinant muscle-specific regulatory nucleic acid sequence of ~~any of~~ claims ~~1-13~~, wherein the one or more mammalian MCK enhancers are each a MCK CK7 enhancer.

~~15. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-14, wherein the one or more mammalian MCK enhancers are each a human MCK enhancer.~~

~~16. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-14, wherein the one or more mammalian MCK enhancers are each a murine MCK enhancer.~~

~~17-7.~~_____ The recombinant muscle-specific regulatory nucleic acid sequence of ~~any of~~ claims ~~1-13~~, wherein the one or more mammalian MCK enhancers each ~~have a~~ nucleic acid sequence with at least 80% identity to comprise a nucleic acid sequence selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 12.

~~18. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-13, wherein the one or more mammalian MCK enhancers each comprise a nucleic acid sequence selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 12.~~

~~19-8.~~_____ The recombinant muscle-specific regulatory nucleic acid sequence of ~~any of~~ claims ~~1-14~~, wherein the one or more mammalian MCK enhancers each have a nucleic acid sequence with at least 80% identity to the nucleic acid sequence of SEQ ID NO: 1.

20. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-14, wherein the one or more mammalian MCK enhancers each comprise the nucleic acid sequence of SEQ ID NO: 1
21. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-20, wherein the only enhancers of the recombinant muscle-specific regulatory nucleic acid sequence are the mammalian desmin enhancer and the one or more mammalian MCK enhancers.
22. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-20, wherein the recombinant muscle-specific regulatory nucleic acid sequence does not include any additional enhancers other than the mammalian desmin enhancer and the one or more mammalian MCK enhancers.
23. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-20, wherein the recombinant muscle-specific nucleic acid sequence does not include a vertebrate troponin I IRE (FIRE) enhancer.
24. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-20, further comprising one or more additional enhancers.
25. The recombinant muscle-specific regulatory nucleic sequence of any of claims 1-20, further comprising one or more additional enhancers comprising a nucleic acid sequence having at least 80% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16.
26. The recombinant muscle-specific regulatory nucleic sequence of any of claims 1-20, further comprising one or more additional enhancers comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, and SEQ ID NO: 16.
27. The recombinant muscle-specific regulatory nucleic acid sequence of any of the preceding claims, wherein the muscle-specific regulatory nucleic acid sequence comprises two or more of the mammalian MCK enhancer.
28. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1-27, wherein the muscle-specific regulatory nucleic acid sequence comprises 1 to 3 of the mammalian MCK enhancer.
29. The recombinant muscle-specific regulatory nucleic acid sequence of any of the preceding claims, further comprising one or more copies of an intron.

30. The recombinant muscle-specific regulatory nucleic acid sequence of claim 29, wherein the one or more copies of the intron each comprise a nucleic acid sequence having at least 80% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 17 and SEQ ID NO: 18.
31. The recombinant muscle-specific regulatory nucleic acid sequence of claim 29, wherein the one or more copies of the intron each comprise a nucleic acid sequence selected from the group consisting of SEQ ID NO: 17 and SEQ ID NO: 18.
32. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 29–31, wherein the one or more copies of the intron are positioned 3' to the mammalian desmin promoter.
33. The recombinant muscle-specific regulatory nucleic acid sequence of any of the preceding claims, wherein the mammalian desmin enhancer and the mammalian MCK enhancer are positioned 5' to the mammalian desmin promoter.
34. The recombinant muscle-specific regulatory nucleic acid sequence of claim 33, wherein the mammalian MCK enhancer(s) is/are positioned 5' to the mammalian desmin enhancer.
35. The recombinant muscle-specific regulatory nucleic acid sequence of any of the preceding claims, further comprising a transgene.
36. The recombinant muscle-specific regulatory nucleic acid sequence of claim 35, wherein the transgene is under the control of the mammalian desmin promoter.
37. The recombinant muscle-specific regulatory nucleic acid sequence of claim 35, wherein the transgene is positioned 3' of the mammalian desmin promoter.
38. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1–37, wherein the muscle-specific regulatory nucleic acid sequence includes only one mammalian desmin enhancer.
39. The recombinant muscle-specific regulatory nucleic acid sequence of any of claims 1–37, wherein the muscle-specific regulatory nucleic acid sequence does not include more than one mammalian desmin enhancer.
40. A recombinant muscle-specific regulatory nucleic acid sequence comprising a nucleic acid sequence having at least 80% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, and SEQ ID NO: 58.

41. A recombinant muscle-specific regulatory nucleic acid sequence comprising a nucleic acid sequence having a nucleic acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, and SEQ ID NO: 58.
42. A vector comprising the muscle-specific regulatory nucleic acid sequence of any of the preceding claims.
43. The vector of claim 42, wherein the vector is selected from the group consisting of a plasmid, a minicircle, a Doggybone, a MIDGE, a Nanoplasmid, and a viral vector.
44. The vector of claim 42, wherein the vector is selected from the group consisting of an episomal non-replicative expression vector, an episomal replicative expression vector, an transposon integration vector, an viral vector integration vector, and an identity directed repair integration vector.
45. The vector of claim 42, wherein the vector is a viral vector, and wherein the viral vector is selected from the group consisting of an adenovirus, an adeno-associated virus, and a lentivirus and a retrovirus.
46. The vector of claim 42, wherein the vector is a Nanoplasmid, and wherein the Nanoplasmid comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30.
47. A host cell comprising the muscle-specific regulatory nucleic acid sequence of any of claims 1-41 or the vector of any of claims 42-46.
48. A method for expressing a transgene in a eukaryotic muscle cell or myocyte, comprising:
transfecting a eukaryotic muscle cell or myocyte with the vector of any of claims 42-46.
49. A method for replicating a vector comprising a transgene in a cell, comprising:
transforming a host cell with the vector of any of claims 42-46;
incubating the cell under conditions sufficient to replicate the vector.
- 50.9. A vector, comprising:
- i) a eukaryotic region sequence comprising thea muscle-specific regulatory nucleic acid sequence wherein the muscle-specific regulatory nucleic acid sequence comprises a mammalian desmin promoter, a mammalian desmin enhancer; and one or more mammalian muscle creatine kinase (MCK) enhancers, wherein the mammalian desmin promoter, mammalian desmin

- enhancer, and mammalian MCK enhancer are operably linked of any of claims 35-37, having 5' and 3' ends; and
- ii) a spacer region having a length of less than 500 basepairs that links the 5' and 3' ends of the eukaryotic region sequence and that comprises a bacterial replication origin and a RNA selectable marker.
51. The vector of claim 50, wherein the muscle-specific regulatory nucleic acid sequence includes only one mammalian desmin enhancer.
52. The vector of claim 50, wherein the muscle-specific regulatory nucleic acid sequence does not include more than one mammalian desmin enhancer.
53. The vector of any of claims 50-52, wherein the muscle-specific regulatory nucleic acid sequence includes three mammalian MCK enhancers.
54. 10. The vector of any of claims 9-50-53, wherein the bacterial replication origin is a R6K bacterial replication origin.
55. 11. The vector of claim- 1054, wherein the bacterial replication origin comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 19-23.
56. 12. The vector of any of claims- 950-55, wherein the RNA selectable marker comprises a sequence having at least 80% identity to SEQ ID NO: 24 or SEQ ID NO: 26.
57. A method for producing a muscle-specific vector, comprising:
providing a vector comprising a non-muscle-specific promoter or non-desmin promoter;
modifying the vector to replace the non-muscle-specific promoter or non-desmin promoter; modifying the vector to replace the non-muscle-specific promoter or non-desmin promoter with a muscle-specific regulatory nucleic acid sequence of any one of claims 1-41.
58. The method of claim 57, wherein the vector is a Nanoplasmid.
59. The method of claim 58, wherein the bacterial replication origin is a R6K bacterial replication origin.
60. The method of claim 59, wherein the bacterial replication origin comprises a sequence having at least 80% identity to any one of SEQ ID NOs: 19-23.
61. The method of any one of claims 57-60, wherein the RNA selectable marker comprises a sequence having at least 80% identity to SEQ ID NO: 24 or SEQ ID NO: 26.

13. A recombinant muscle-specific regulatory nucleic acid sequence, comprising: a mammalian desmin promoter; a mammalian desmin enhancer, wherein the mammalian desmin enhancer comprises a nucleic acid sequence having at least 80% identity to the nucleic acid sequence of SEQ ID NO: 6; and one or more mammalian muscle creatine kinase (MCK) enhancers, wherein the mammalian desmin promoter, mammalian desmin enhancer, and mammalian MCK enhancer are operably linked.
14. The recombinant muscle-specific regulatory nucleic acid sequence of claim 13, wherein the mammalian desmin promoter comprises a nucleic acid sequence having at least 80% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 8-and SEQ ID NO: 10.
15. The recombinant muscle-specific regulatory nucleic acid sequence of claim 13, wherein the one or more mammalian MCK enhancers each comprise a nucleic acid sequence of SEQ ID NO: 2.
16. The recombinant muscle-specific regulatory nucleic acid sequence of claim 13, wherein the one or more mammalian MCK enhancers each have a nucleic acid sequence with at least 80% identity to a nucleic acid sequence of SEQ ID NO: 1.
17. The recombinant muscle-specific regulatory nucleic acid sequence of claim 13, wherein the mammalian desmin promoter comprises a nucleic acid sequence having at least 80% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 8-and SEQ ID NO: 10, and wherein the one or more mammalian MCK enhancers each comprise a nucleic acid sequence of SEQ ID NO: 2.
18. The recombinant muscle-specific regulatory nucleic acid sequence of claim 13, wherein the mammalian desmin promoter comprises a nucleic acid sequence having at least 80% identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 8-and SEQ ID NO: 10, and wherein the one or more mammalian MCK enhancers each have a nucleic acid sequence with at least 80% identity to a nucleic acid sequence of SEQ ID NO: 1.
19. The recombinant muscle-specific regulatory nucleic acid sequence of claim 13, wherein the mammalian desmin promoter comprises a nucleic acid sequence having at least 80% identity to a nucleic acid sequence of SEQ ID NO: 8, and wherein the one or more mammalian MCK enhancers each comprise nucleic acid sequence of SEQ ID NO: 2.
20. The recombinant muscle-specific regulatory nucleic acid sequence of claim 13, wherein the mammalian desmin promoter comprises a nucleic acid sequence having

at least 80% identity to a nucleic acid sequence of SEQ ID NO: 10, and wherein the one or more mammalian MCK enhancers each comprise a nucleic acid sequence of SEQ ID NO: 2.