

CLAIMS

1. A liver-specific nucleic acid regulatory element comprising a nucleic acid sequence having at least 93% identity to a sequence selected from the group consisting of: SEQ ID NOs: 1-80, 138, and 139.

2. The liver-specific nucleic acid regulatory element of claim 1, wherein the nucleic acid sequence has at least 94% identity to a sequence selected from the group consisting of: SEQ ID NOs: 1-80, 138, and 139; or

wherein the nucleic acid sequence has at least 95% identity to a sequence selected from the group consisting of: SEQ ID NOs: 1-80, 138, and 139; or

wherein the nucleic acid sequence has at least 95% identity to a sequence selected from the group consisting of: SEQ ID NOs: 1-80, 138, and 139; or

wherein the nucleic acid sequence has at least 97% identity to a sequence selected from the group consisting of: SEQ ID NOs: 1-80, 138, and 139; or

wherein the nucleic acid sequence has at least 98% identity to a sequence selected from the group consisting of: SEQ ID NOs: 1-80, 138, and 139; or

wherein the nucleic acid sequence has at least 95% identity to a sequence selected from the group consisting of: SEQ ID NOs: 1-80, 138, and 139; or

wherein the nucleic acid sequence consists of a sequence selected from the group consisting of: SEQ ID NOs: 1-80, 138, and 139.

3. A liver-specific expression cassette comprising the liver-specific nucleic acid regulatory element of claim 1 or 2, and a liver-specific promoter linked to a transgene;

optionally wherein the liver-specific expression cassette comprises at least two repeats of the liver-specific nucleic acid regulatory elements; further optionally wherein two or more nucleotides separate each liver-specific nucleic acid regulatory element;

further optionally wherein the liver-specific expression cassette comprises one or more FOXA and HNF4 transcription factor consensus sites.

4. The liver-specific expression cassette of claim 3, comprising at least three repeats of the liver-specific nucleic acid regulatory element and a liver-specific promoter linked to a transgene, and

wherein two or more nucleotides separate each liver-specific nucleic acid regulatory element.

5. The liver-specific expression cassette of any one of claims 3 or 4, wherein between 2 and 30 nucleotides separate each liver-specific nucleic acid regulatory element;

5 nucleotides separate each liver-specific nucleic acid regulatory element;

11 nucleotides separate each liver-specific nucleic acid regulatory element; or

30 nucleotides separate each liver-specific nucleic acid regulatory element.

6. The liver-specific expression cassette of any one of claims 3-5, wherein the liver-specific expression cassette comprises two, three, four, or five repeats of the liver-specific nucleic acid regulatory element, or wherein the liver-specific expression cassette comprises six, seven, eight, nine or ten repeats of the liver-specific nucleic acid regulatory element.

7. The liver-specific expression cassette of any one of claims 3-6, wherein the liver-specific nucleic acid regulatory element comprises one or more sites of CpG minimization.

8. The liver-specific expression cassette of any one of claims 3-7, wherein the liver-specific promoter is selected from the group consisting of: a transthyretin (TTR) promoter, minimal TTR promoter (TTRm), an AAT promoter, an albumin (ALB) promoter or minimal promoter, an apolipoprotein A1 (APOA1) promoter or minimal promoter, a complement factor B (CFB) promoter, a ketohexokinase (KHK) promoter, a hemopexin (HPX) promoter or minimal promoter, a nicotinamide N-methyltransferase (NNMT) promoter or minimal promoter, a carboxylesterase 1 (CES1) promoter or minimal promoter, a protein C (PROC) promoter or minimal promoter, an apolipoprotein C3 (APOC3) promoter or minimal promoter, a mannan-binding lectin serine protease 2 (MASP2) promoter or minimal promoter, a hepcidin antimicrobial peptide (HAMP) promoter or minimal promoter, and a serpin peptidase inhibitor, clade C (antithrombin), member 1 (SERPINC1) promoter or minimal promoter; optionally wherein said liver-specific promoter is a TTR promoter or a TTRm promoter; further optionally wherein the promoter comprises a sequence selected from the group consisting of: SEQ ID NOs 210-216 and 217.

9. The liver-specific expression cassette of any one of claims 3-8, wherein the transgene encodes a liver-specific therapeutic protein; optionally wherein the liver-specific therapeutic protein is coagulation factor VIII (FVIII); further optionally wherein the coagulation FVIII comprises a nucleic acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO: 143, wherein the coagulation FVIII comprises SEQ ID NO: 143, or wherein the coagulation FVIII consists of SEQ ID NO: 143.

10. A vector comprising the liver-specific nucleic acid regulatory element of any one of claims 1-2 or the liver-specific expression cassette of any one of claims 3-9.

11. The vector of claim 10, wherein the vector is a viral vector or a non-viral vector; optionally wherein the vector is a plasmid.

12. The vector of claim 10, wherein the vector is a closed-ended DNA (ceDNA) vector.

13. A pharmaceutical composition comprising the liver-specific expression cassette of any one of claims 3-9 or the vector of any one of claims 10-12, and a pharmaceutically acceptable excipient.

14. Use of the vector of any one of claims 10-12, or the pharmaceutical composition of claim 13 in the manufacture of a medicament for the treatment of a liver-specific disease or disorder in a subject.

15. The use of claim 14, wherein the subject is a human subject suffering from a genetic disorder; optionally wherein the genetic disorder is selected from the group consisting of sickle-cell anemia, melanoma, hemophilia A (clotting factor VIII (FVIII) deficiency) and hemophilia B (clotting factor IX (FIX) deficiency), cystic fibrosis (CFTR), familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson disease, phenylketonuria (PKU), congenital hepatic porphyria, inherited disorders of hepatic metabolism, Lesch-Nyhan syndrome, thalassaemias, xeroderma pigmentosum, Fanconi anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom syndrome, retinoblastoma, mucopolysaccharide storage diseases (*e.g.*, Hurler syndrome (MPS Type I), Scheie syndrome (MPS Type I S), Hurler-Scheie syndrome (MPS Type I H-S), Hunter syndrome (MPS Type II), Sanfilippo Types A, B, C, and D (MPS Types III A, B, C, and D), Morquio Types A and B (MPS IVA and MPS IVB), Maroteaux-Lamy syndrome (MPS Type VI), Sly syndrome (MPS Type VII), hyaluronidase deficiency (MPS Type IX)), Niemann-Pick Disease Types A/B, C1 and C2, Fabry disease, Schindler disease, GM2-gangliosidosis Type II (Sandhoff Disease), Tay-Sachs disease, Metachromatic Leukodystrophy, Krabbe disease, Mucopolysaccharidosis Type I, II/III and IV, Sialidosis Types I and II, Glycogen Storage disease Types I and II (Pompe disease), Gaucher disease Types I, II and III, Fabry disease, cystinosis, Batten disease, Aspartylglucosaminuria, Salla disease, Danon disease (LAMP-2 deficiency), Lysosomal Acid Lipase (LAL) deficiency, neuronal ceroid lipofuscinoses (CLN1-8, INCL, and LINCL), sphingolipidoses, galactosialidosis, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, Huntington's disease, spinocerebellar ataxia, spinal muscular atrophy, Friedreich's ataxia, Duchenne muscular dystrophy (DMD), Becker muscular dystrophies (BMD), dystrophic epidermolysis bullosa (DEB), ectonucleotide pyrophosphatase 1 deficiency, generalized arterial calcification of infancy (GACI), Leber Congenital Amaurosis, Stargardt macular dystrophy (ABCA4), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency), type II (ABCB11), type III (ABCB4), or type IV (TJP2) and Cathepsin A deficiency;
further optionally wherein the subject has hemophilia A.