

encapsulate nucleic acids. FVIII is a large glycoprotein, and the rAAV sequences necessary to encode and express FVIII generally exceed the packaging capacity of the capsid.

[0005] Recombinant capsid-free AAV vectors can be obtained as an isolated linear nucleic acid molecule comprising an expressible transgene and promoter regions flanked by two wild-type AAV inverted terminal repeat sequences (ITRs) including the Rep binding and terminal resolution sites. These recombinant AAV vectors are devoid of AAV capsid protein encoding sequences, and can be single-stranded, double-stranded or duplex with one or both ends covalently linked through the two wild-type ITR palindrome sequences (*e.g.*, WO2012/123430, U.S. Patent 9,598,703). They avoid many of the problems of AAV-mediated gene therapy in that the transgene capacity is much higher, transgene expression onset is rapid, and the patient immune system recognizes the DNA molecules as a virus to be cleared.

[0006] Non-viral gene therapy is assumed to be less toxic for the host and safer in terms of gene delivery compared to a viral vector. One example of a non-viral gene therapy, closed-ended DNA (“ceDNA”) vectors, has many attractive features for gene-based therapy. For example, ceDNA vectors have no packaging constraints imposed by the limiting space within the viral capsid. ceDNA vectors represent a viable eukaryotically-produced alternative to prokaryote-produced plasmid DNA vectors, as opposed to encapsulated AAV genomes. This permits the insertion of control elements, *e.g.*, large transgenes, multiple transgenes, regulatory switched, and incorporation of the native genetic regulatory elements of the transgene, if desired.

[0007] In most living organisms, and especially in eukaryotes with large genome sizes, however, there does not appear to be a driving force to limit enhancer/promoter size, and therefore most endogenous enhancer/promoters span hundreds, and more often thousands, of base pairs (bp) of DNA. Due to their size, these endogenous natural gene enhancers/promoters are generally not amenable to inclusion in gene therapy products due to size limitations.

[0008] Regardless of viral or non-viral delivery, there remains a need for a technology that permits robust expression of a therapeutic protein, such as a liver-specific therapeutic protein, in a cell, tissue or subject, to improve the efficiency and safety of treatment of a genetic disease or disorder.

SUMMARY

[0008a] In one aspect, provided herein is 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.

[0008b] In another aspect, provided herein is a liver-specific expression cassette comprising the liver-specific nucleic acid regulatory element as described herein, 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.

[0008c] In another aspect, provided herein is a vector comprising the liver-specific nucleic acid regulatory element as described herein or the liver-specific expression cassette as described herein.

[0008d] In another aspect, provided herein is a pharmaceutical composition comprising the liver-specific expression cassette as described herein or the vector as described herein, and a pharmaceutically acceptable excipient.

[0008e] In another aspect, provided herein is use of the vector as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for the treatment of a liver-specific disease or disorder in a subject.

[0009] The present disclosure has applied a range of bioinformatic analyses to identify a novel and inventive set of non-natural modifications to a native liver-specific Serpin enhancer region that surprisingly resulted in acute expression level and improved sequence characteristics known to impact expression durability of gene product.

[0010] The disclosure also provides an evolutionary conservation analysis to selective removal of CpGs in the enhancer without disrupting function. Enhancers are often combined in series to drive higher levels of transcription initiation. However, the principals underlying optimal number and orientation of enhancer regions remain not well understood. Spacing between transcription factor