Comparison Table: Non-viral DNA vectors VTvaf17 and GDTT1.8NAS VS other viral and non-viral vectors and mRNA

Parameter / Vector type	Viral vectors	"Sleeping Beauty" non-viral system (transposon/ transposase)	Existing plasmid vector	mRNA	Non-viral Gene therapy VTvaf17 and GDTT1.8NAS series vectors
Plasmid part size (for plasmids)	-	>3 000 bp	>3 000 bp	>3 000 bp	2 591 – 3 265 bp
Antibiotic resistance gene (for plasmids)	-	Yes	Yes	-	No
Selective agent (for plasmids)	-	Antibiotic	Antibiotic	-	Sucrose
Type of selection marker (for plasmids)	-	Protein	Protein	-	RNA
Tissue-specific transgene expression	No	Yes/No	Yes/No	No	Configured as required
Preclinical / Clinical trials currently underway worldwide	Yes	Yes	Yes	Yes	Yes
Approved by regulators for use as a medicinal product	Yes	Yes	Yes	Yes	Clinical trials are in process
Integral efficiency	High	High (when using high-performance delivery systems)	High (when using high-performance delivery systems)	High (when using high-performance delivery systems)	High (when using high-performance delivery systems)
Integration of a transgene into the genome	Yes	Yes	No	No	No
Risk of alternative spontaneous oncological transformation	Moderate	Moderate	Moderate	Absent	Absent
Compliance with the stringent regulatory requirements of the FDA and EMA*	Partially compliant	Partially compliant	Partially compliant	Partially compliant	Fully compliant

Integral safety	Moderate	Moderate	Moderate	Moderate	Highest
Target product yield (for plasmids)	-	<10 mg/litre	10 - 20 mg/litre	<10 mg/litre	>200 mg/litre
Production scale-up	Yes	Limited	Limited	Yes	Yes
Safety for the environment	Potentially hazardous	Limited hazard level. The gene is integrated only in the presence of transposase	Limited hazard level. Capable of replication in a variety of pUC-compatible strains	Safe. Rapid degradation caused by exonucleases	Safe. Capable of replication only in a specific strain
Production costs	Very expensive	Moderate	Moderate	Very expensive	Cheap
Logistical aspects (low temperature shipping)	Expensive	Cheap	Cheap	Expensive	Cheap
Integral level of production and logistics costs	High	Low	Low	High	Minimal
Patient affordability	Limited	Moderate	n/a	Moderate	High

*- "Draft Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products" (EMA/CAT/80183/2014, Committee for Advanced Therapies), "Reflection paper on design modifications of gene therapy medicinal products during development" (14 December 2011, EMA/CAT/GTWP/44236/2009, Committee for Advanced Therapies), USP 42 and NF 37, Chapter 1047: Gene Therapy Products, FDA-2008-D-0205 "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) Guidance for Industry", FDA-2015-D-3399 "Recommendations for Microbial Vectors Used for Gene Therapy".

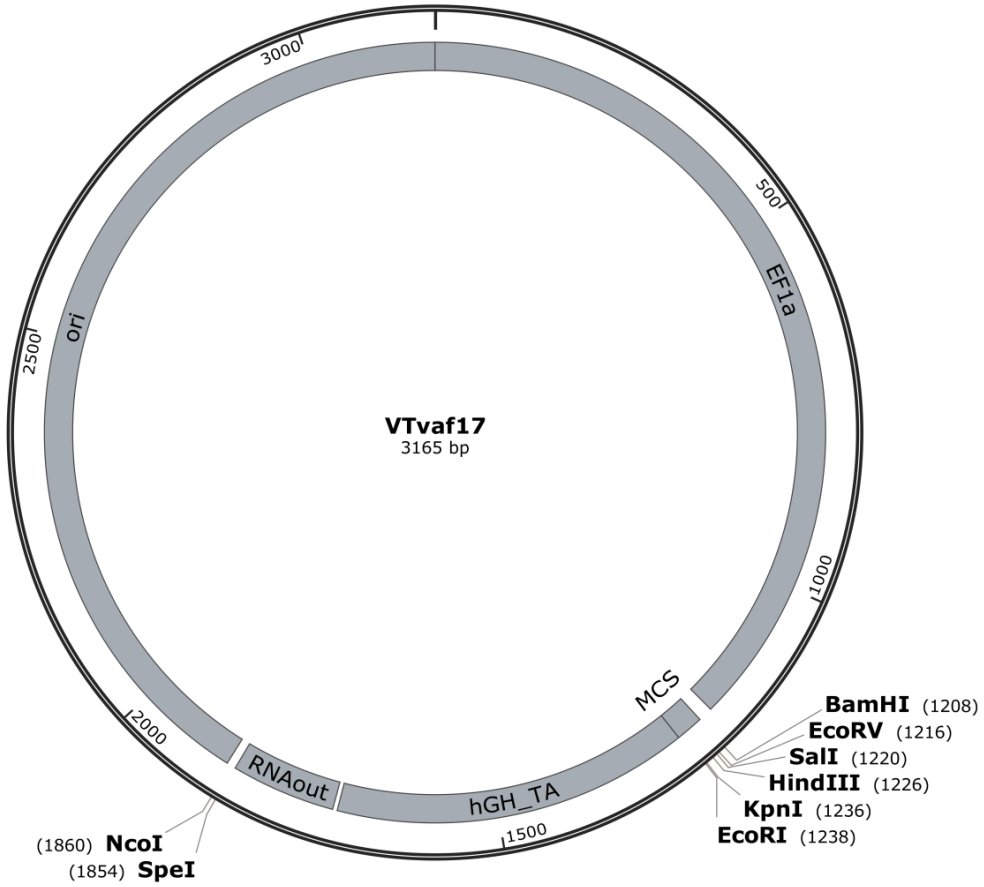
Key Advantages of the Non-Viral DNA Vectors of VTvaf17 Series

- **Absence of antibiotic-resistance genes.** Antisense RNA-out selection (Fig. 2) and dedicated production strains (E. coli SCS110-AF) eliminate antibiotics from the manufacturing process, thereby removing the risk of horizontal gene transfer generating pathogenic strains.
- **Exclusion of viral elements.** The vector architecture deliberately omits all sequences of viral origin (Fig. 1), in line with EMA and FDA recommendations (EMA/CAT/80183/2014; FDA-2008-D-0205), thereby lowering the probability of recombination and unintended gene expression.

- **Episomal persistence.** VTvaf17 remains episomal and does not integrate into the host genome; unlike viral vectors it virtually eliminates the risk of insertional mutagenesis and oncogenic rearrangements.

  Result. Compared with viral systems and existing DNA vectors, the VTvaf17 platform delivers an optimal efficacy-to-safety ratio, is non-immunogenic, and permits repeat dosing.

  Technical design details are described in patent JP6918231 (attached).



[Fig. 1] 図１は、大腸菌細胞において自律的複製が可能な３１６５ｂｐ環状二本鎖ＤＮＡ分子である、遺伝子治療ＤＮＡベクターＶＴｖａｆ１７の構造を示す。 図１は、ベクターの下記の構造要素を示している。（１）ＥＦ１ａ（１～１１８８ｂｐ）−遺伝子の第１イントロンに含まれる固有エンハンサーを伴うヒト伸張因子ＥＦ１Ａのプロモーター領域。それはほとんどのヒト組織で組換え遺伝子の効率的転写を保証する。（２）ＭＣＳ（１２０８～１２４３ｂ

ｐ）−制限酵素ＢａｍＨＩ、ＥｃｏＲＶ、ＳａｌＩ、ＨｉｎｄＩＩＩ、ＫｐｎＩ、およびＥｃｏＲＩの配列を含み、標的治療遺伝子のクローニングを可能とするポリリンカー（多重クローニング部位）。（３）ｈＧＨ−ＴＡ（１２４４〜１７１０ｂｐ）−ヒト増殖因子遺伝子の転写ターミネーターおよびポリアデニル化配列。（４）ＲＮＡ−ｏｕｔ（１７１７〜１８５３ｂｐ）−大腸菌株ＳＣＳ１１０を使用する場合に抗生物質を用いない正の選択を可能とするトランスポゾンＴｎ１０の調節要素ＲＮＡ−ＯＵＴ。（５）ｏｒｉ（１８６６〜３１６５ｂｐ）−ほとんどの大腸菌株の細胞でプラスミド生産を増加させるための一塩基置換を伴う自律的複製のための複製起点。

Figure 1 shows the structure of gene therapy DNA vector VTvaf17, which is a 3165-bp circular double-strand DNA molecule capable of autonomous replication in *Escherichia coli* cells.

Figure 1 illustrates the following structural elements of the vector:

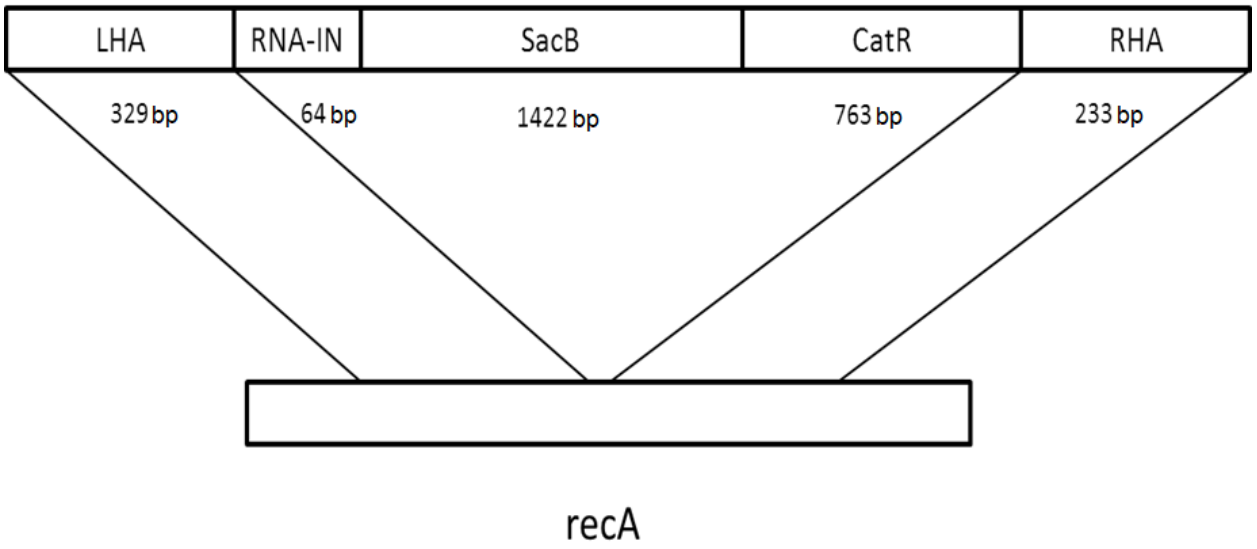
(1) EF1a (1 to 1188 bp) - the promoter region of human elongation factor EF1A with an intrinsic enhancer contained in the first intron of the gene. It ensures efficient transcription of the recombinant gene in most human tissues.

(2) MCS (1208 to 1243 bp) – the polylinker (multiple cloning site) which contains a sequence of restriction enzymes BamHI, EcoRV, SalI, HindIII, KpnI, and EcoRI and allows cloning the target therapeutic genes.

(3) hGH-TA (1244 to 1710 bp) – the transcription terminator and the polyadenylation sequence of the human growth factor gene.

(4) RNA-out (1717 to 1853 bp) – the regulatory element RNA-OUT of transposon Tn 10 allowing for antibiotic-free positive selection in case of the use of *Escherichia coli* strain SCS 110.

(5) ori (1866 to 3165 bp) – the origin of replication for autonomous replication with a single nucleotide substitution to increase plasmid production in the cells of most *Escherichia coli* strains.



[Fig. 2] 図２は、大腸菌株ＳＣＳ１１０を生産するための大腸菌の遺伝子ｒｅｃＡの領域における相同組換えのためのＤＮＡ断片の構造を示す。 直鎖断片は、抗生物質を用いない選択のためのトランスポゾンＴｎ１０の調節要素ＲＮＡ－ＩＮ（６４ｂｐ）、その産物がスクロース含有培地内での選択を保証するレバンスクラーゼ遺伝子ｓａｃＢ（１４２２ｂｐ）、相同組換えが生じるクローン株のピッキングに必要とされるクロラムフェニコール耐性遺伝子ｃａｔＲ（７６３ｂｐ）を有するカセットからなる。このカセットには、遺伝子の不活化を伴う遺伝子ｒｅｃＡの領域での組換えプロセスを保証する２つの相同アームによって挟み込まれている（左アームおよび右アーム、それぞれ３２９ｂｐおよび２３３ｂｐ）。

Figure 2 shows the structure of the DNA fragment for homologous recombination in the region of gene *recA* of *Escherichia coli* for producing *Escherichia coli* strain SCS 110.

The linear fragment consists of a cassette carrying the regulatory element RNA-IN of transposon Tn10 for antibiotic-free selection (64 bp), levansucrase gene *sacB* the product of which ensures selection within a sucrose-containing medium (1422 bp), and chloramphenicol resistance gene *catR* required for the picking of strain clones in which homologous recombination occurred (763 bp). The cassette is flanked by two homology arms that ensure the process of recombination in the region of gene *recA* with concurrent gene inactivation (329-bp and 233-bp for the left arm and for the right arm, respectively).