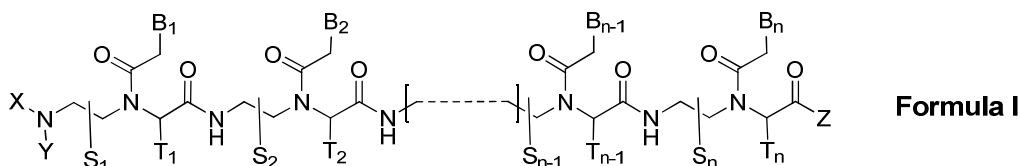


CLAIMS

1. A peptide nucleic acid derivative represented by **Formula I**, or a pharmaceutically acceptable salt thereof:



wherein,

n is an integer between 10 and 25;

the compound of **Formula I** possesses at least a 10-mer complementary overlap with a 14-mer target splice site sequence that consists of 7-mer from intron and 7-mer from exon within a target pre-mRNA;

the compound of **Formula I** is fully complementary to the target pre-mRNA sequence, or partially complementary to the target pre-mRNA sequence with one or two mismatches;

$S_1, S_2, \dots, S_{n-1}, S_n, T_1, T_2, \dots, T_{n-1},$  and  $T_n$  independently represent deuterido [D], hydrido [H], substituted or non-substituted alkyl, or substituted or non-substituted aryl radical;

X and Y independently represent hydrido, formyl [H-C(=O)-], aminocarbonyl [NH<sub>2</sub>-C(=O)-], aminothiocarbonyl [NH<sub>2</sub>-C(=S)-], substituted or non-substituted alkyl, substituted or non-substituted aryl, substituted or non-substituted alkylacyl, substituted or non-substituted arylacyl, substituted or non-substituted alkyloxycarbonyl, substituted or non-substituted aryloxycarbonyl, substituted or non-substituted alkylaminocarbonyl, substituted or non-substituted arylaminocarbonyl, substituted or non-substituted alkylaminothiocarbonyl, substituted or non-substituted arylaminothiocarbonyl, substituted or non-substituted alkyloxythiocarbonyl, substituted or non-substituted aryloxythiocarbonyl, substituted or non-substituted alkylsulfonyl, substituted or non-substituted arylsulfonyl, substituted or non-substituted alkylphosphonyl, or substituted or non-substituted arylphosphonyl radical;

Z represents hydrido, hydroxy, substituted or non-substituted alkyloxy, substituted or non-substituted aryloxy, non-substituted amino [-NH<sub>2</sub>], substituted or non-substituted alkylamino, substituted or non-substituted arylamino, substituted or non-substituted alkyl, or substituted or non-substituted aryl radical;

$B_1, B_2, \dots, B_{n-1}$ , and  $B_n$  are independently selected from natural nucleobases including adenine, thymine, guanine, cytosine and uracil, and unnatural nucleobases; and

at least four of  $B_1, B_2, \dots, B_{n-1}$ , and  $B_n$  are independently selected from unnatural nucleobases with a substituted or non-substituted amino radical covalently linked to the nucleobase moiety.

2. The peptide nucleic acid derivative according to claim 1, or a pharmaceutically acceptable salt thereof:

wherein,

$n$  is an integer between 10 and 25;

the compound of **Formula I** possesses at least a 10-mer complementary overlap with a 14-mer target splice site sequence that consists of 7-mer from intron and 7-mer from exon within a target pre-mRNA;

the compound of **Formula I** is fully complementary to the target pre-mRNA sequence, or partially complementary to the target pre-mRNA sequence with one or two mismatches;

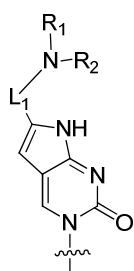
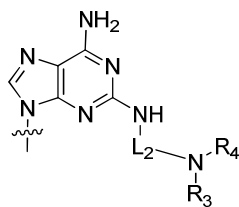
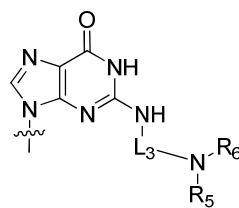
$S_1, S_2, \dots, S_{n-1}, S_n, T_1, T_2, \dots, T_{n-1}$ , and  $T_n$  independently represent deuterido, hydrido, substituted or non-substituted alkyl, or substituted or non-substituted aryl radical;

$X$  and  $Y$  independently represent hydrido, formyl, aminocarbonyl, aminothiocarbonyl, substituted or non-substituted alkyl, substituted or non-substituted aryl, substituted or non-substituted alkylacyl, substituted or non-substituted arylacyl, substituted or non-substituted alkyloxycarbonyl, substituted or non-substituted aryloxycarbonyl, substituted or non-substituted alkylaminocarbonyl, substituted or non-substituted arylaminocarbonyl, substituted or non-substituted alkylaminothiocarbonyl, substituted or non-substituted arylaminothiocarbonyl, substituted or non-substituted alkyloxythiocarbonyl, substituted or non-substituted aryloxythiocarbonyl, substituted or non-substituted alkylsulfonyl, substituted or non-substituted arylsulfonyl, substituted or non-substituted alkylphosphonyl radical, or substituted or non-substituted arylphosphonyl radical;

$Z$  represents hydrido, hydroxy, substituted or non-substituted alkyloxy, substituted or non-substituted aryloxy, non-substituted amino, substituted or non-substituted alkylamino, substituted or non-substituted arylamino, substituted or non-substituted alkyl, or substituted or non-substituted aryl radical;

$B_1, B_2, \dots, B_{n-1}$ , and  $B_n$  are independently selected from natural nucleobases including adenine, thymine, guanine, cytosine and uracil, and unnatural nucleobases;

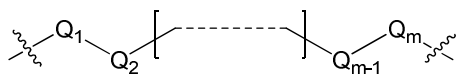
at least four of  $B_1$ ,  $B_2$ , ...,  $B_{n-1}$ , and  $B_n$  are independently selected from unnatural nucleobases represented by **Formula II**, **Formula III**, or **Formula IV**:

**Formula II****Formula III****Formula IV**

wherein,

$R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are independently selected from hydrido, and substituted or non-substituted alkyl radical;

$L_1$ ,  $L_2$  and  $L_3$  are a covalent linker represented by **Formula V** covalently linking the basic amino group to the nucleobase moiety:

**Formula V**

wherein,

$Q_1$  and  $Q_m$  are substituted or non-substituted methylene ( $-\text{CH}_2-$ ) radical, and  $Q_m$  is directly linked to the basic amino group;

$Q_2$ ,  $Q_3$ , ..., and  $Q_{m-1}$  are independently selected from substituted or non-substituted methylene, oxygen ( $-\text{O}-$ ), sulfur ( $-\text{S}-$ ), and substituted or non-substituted amino radical [ $-\text{N}(\text{H})-$ , or  $-\text{N}(\text{substituent})-$ ]; and

$m$  is an integer between 1 and 15.

3. The peptide nucleic acid derivative according to claim 1, or a pharmaceutically acceptable salt thereof:

wherein,

$n$  is an integer between 11 and 23;

the compound of **Formula I** possesses at least a 10-mer complementary overlap with a 14-mer target splice site sequence that consists of 7-mer from intron and 7-mer from exon within a target pre-mRNA;

the compound of **Formula I** is fully complementary to the target pre-mRNA sequence, or partially complementary to the target pre-mRNA sequence with one or two mismatches;

$S_1, S_2, \dots, S_{n-1}, S_n, T_1, T_2, \dots, T_{n-1},$  and  $T_n$  are hydrido radical;

X and Y independently represent hydrido, aminocarbonyl, substituted or non-substituted alkyl, substituted or non-substituted aryl, substituted or non-substituted alkylacyl, substituted or non-substituted arylacyl, substituted or non-substituted alkyloxycarbonyl, substituted or non-substituted alkylaminocarbonyl, or substituted or non-substituted arylsulfonyl radical;

Z represents non-substituted amino, or substituted or non-substituted alkylamino radical;

$B_1, B_2, \dots, B_{n-1},$  and  $B_n$  are independently selected from natural nucleobases including adenine, thymine, guanine, cytosine and uracil, and unnatural nucleobases;

at least four of  $B_1, B_2, \dots, B_{n-1},$  and  $B_n$  are independently selected from unnatural nucleobases represented by **Formula II**, **Formula III**, or **Formula IV**;

$R_1, R_2, R_3, R_4, R_5$  and  $R_6$  are independently selected from hydrido, and substituted or non-substituted alkyl radical;

$Q_1$  and  $Q_m$  are substituted or non-substituted methylene radical, and  $Q_m$  is directly linked to the basic amino group;

$Q_2, Q_3, \dots,$  and  $Q_{m-1}$  are independently selected from substituted or non-substituted methylene, oxygen, and amino radical; and

m is an integer between 1 and 11.

4. The peptide nucleic acid derivative according to claim 1, or a pharmaceutically acceptable salt thereof:

wherein,

n is an integer between 11 and 21;

the compound of **Formula I** possesses at least a 10-mer complementary overlap with a 14-mer target splice site sequence that consists of 7-mer from intron and 7-mer from exon within a target pre-mRNA;

the compound of **Formula I** is fully complementary to the target pre-mRNA sequence, or partially complementary to the target pre-mRNA sequence with one or two mismatches;

$S_1, S_2, \dots, S_{n-1}, S_n, T_1, T_2, \dots, T_{n-1},$  and  $T_n$  are hydrido radical;

X and Y independently represent hydrido, substituted or non-substituted alkyl, substituted or non-substituted aryl, substituted or non-substituted alkylacyl, substituted or non-substituted arylacyl, substituted or non-substituted alkyloxycarbonyl, substituted or non-substituted alkylaminocarbonyl, or substituted or non-substituted arylsulfonyl radical;

Z represents non-substituted amino, or substituted or non-substituted alkylamino radical;

$B_1$ ,  $B_2$ , ...,  $B_{n-1}$ , and  $B_n$  are independently selected from natural nucleobases including adenine, thymine, guanine, cytosine and uracil, and unnatural nucleobases;

at least four of  $B_1$ ,  $B_2$ , ...,  $B_{n-1}$ , and  $B_n$  are independently selected from unnatural nucleobases represented by **Formula II**, **Formula III**, or **Formula IV**;

$R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are independently selected from hydrido, and substituted or non-substituted alkyl radical;

$Q_1$  and  $Q_m$  are methylene radical, and  $Q_m$  is directly linked to the basic amino group;

$Q_2$ ,  $Q_3$ , ..., and  $Q_{m-1}$  are independently selected from methylene, oxygen, and amino radical; and

m is an integer between 1 and 11.

5. The peptide nucleic acid derivative according to claim 1, or a pharmaceutically acceptable salt thereof:

wherein,

n is an integer between 11 and 19;

the compound of **Formula I** possesses at least a 10-mer complementary overlap with a 14-mer target splice site sequence that consists of 7-mer from intron and 7-mer from exon within a target pre-mRNA;

the compound of **Formula I** is fully complementary to the target pre-mRNA sequence;

$S_1$ ,  $S_2$ , ...,  $S_{n-1}$ ,  $S_n$ ,  $T_1$ ,  $T_2$ , ...,  $T_{n-1}$ , and  $T_n$  are hydrido radical;

X and Y independently represent hydrido, substituted or non-substituted alkyl, substituted or non-substituted aryl, substituted or non-substituted alkylacyl, substituted or non-substituted arylacyl, substituted or non-substituted alkyloxycarbonyl, substituted or non-substituted alkylaminocarbonyl, or substituted or non-substituted arylsulfonyl radical;

Z represents non-substituted amino, or substituted or non-substituted alkylamino radical;

$B_1, B_2, \dots, B_{n-1}$ , and  $B_n$  are independently selected from natural nucleobases including adenine, thymine, guanine, cytosine and uracil, and unnatural nucleobases;

at least four of  $B_1, B_2, \dots, B_{n-1}$ , and  $B_n$  are independently selected from unnatural nucleobases represented by **Formula II**, **Formula III**, or **Formula IV**;

$R_1, R_3$ , and  $R_5$  are hydrido radical, and  $R_2, R_4$ , and  $R_6$  independently represent hydrido, or substituted or non-substituted alkyl radical;

$Q_1$  and  $Q_m$  are methylene radical, and  $Q_m$  is directly linked to the basic amino group;

$Q_2, Q_3, \dots$ , and  $Q_{m-1}$  are independently selected from methylene and oxygen radical;

and

$m$  is an integer between 1 and 9.

6. The peptide nucleic acid derivative according to claim 1, or a pharmaceutically acceptable salt thereof:

wherein,

$n$  is an integer between 12 and 19;

the compound of **Formula I** possesses at least a 10-mer complementary overlap with a 14-mer target splice site sequence that consists of 7-mer from intron and 7-mer from exon within a target pre-mRNA;

the compound of **Formula I** is fully complementary to the target pre-mRNA sequence;

$S_1, S_2, \dots, S_{n-1}, S_n, T_1, T_2, \dots, T_{n-1}$ , and  $T_n$  are hydrido radical;

$X$  and  $Y$  independently represent substituted or non-substituted alkylacyl, substituted or non-substituted arylacyl, or substituted or non-substituted alkyloxycarbonyl radical;

$Z$  represents non-substituted amino, or substituted or non-substituted alkylamino radical;

$B_1, B_2, \dots, B_{n-1}$ , and  $B_n$  are independently selected from adenine, thymine, guanine, cytosine, and unnatural nucleobases;

at least five of  $B_1, B_2, \dots, B_{n-1}$ , and  $B_n$  are independently selected from unnatural nucleobases represented by **Formula II**, **Formula III**, or **Formula IV**;

$R_1, R_2, R_3, R_4, R_5$ , and  $R_6$  are hydrido radical;

$Q_1$  and  $Q_m$  are methylene radical, and  $Q_m$  is directly linked to the basic amino group;

$Q_2, Q_3, \dots$ , and  $Q_{m-1}$  are independently selected from methylene and oxygen radical;

and

$m$  is an integer between 1 and 9.

7. The peptide nucleic acid derivative according to claim 1, or a pharmaceutical salt thereof:

wherein,

n is an integer between 12 and 18;

the compound of **Formula I** possesses at least a 10-mer complementary overlap with a 14-mer target splice site sequence that consists of 7-mer from intron and 7-mer from exon within a target pre-mRNA;

the compound of **Formula I** is fully complementary to the target pre-mRNA sequence;

$S_1, S_2, \dots, S_{n-1}, S_n, T_1, T_2, \dots, T_{n-1},$  and  $T_n$  are hydrido radical;

X is hydrido radical;

Y represents substituted or non-substituted alkylacyl, substituted or non-substituted arylacyl, or substituted or non-substituted alkyloxycarbonyl radical;

Z represents non-substituted amino, or substituted or non-substituted alkylamino radical;

$B_1, B_2, \dots, B_{n-1},$  and  $B_n$  are independently selected from adenine, thymine, guanine, cytosine, and unnatural nucleobases;

at least five of  $B_1, B_2, \dots, B_{n-1},$  and  $B_n$  are independently selected from unnatural nucleobases represented by **Formula II**, **Formula III**, or **Formula IV**;

$R_1, R_2, R_3, R_4, R_5,$  and  $R_6$  are hydrido radical;

$L_1$  represents  $-(CH_2)_2-O-(CH_2)_2-$ ,  $-CH_2-O-(CH_2)_2-$ ,  $-CH_2-O-(CH_2)_3-$ ,  $-CH_2-O-(CH_2)_4-$ ,  $-CH_2-O-(CH_2)_5-$ ,  $-CH_2-O-(CH_2)_6-$ , or  $-CH_2-O-(CH_2)_7-$  with the right end is directly linked to the basic amino group; and

$L_2$  and  $L_3$  are independently selected from  $-(CH_2)_2-$ ,  $-(CH_2)_3-$ ,  $-(CH_2)_4-$ ,  $-(CH_2)_5-$ ,  $-(CH_2)_6-$ ,  $-(CH_2)_7-$ ,  $-(CH_2)_8-$ ,  $-(CH_2)_2-O-(CH_2)_2-$ ,  $-(CH_2)_3-O-(CH_2)_2-$ , and  $-(CH_2)_2-O-(CH_2)_3-$  with the right end is directly linked to the basic amino group.

8. A method of using the peptide nucleic acid derivative of any one of claims 1-7 to induce in cells in vitro the skipping of the target exon within the target pre-mRNA.

9. A peptide nucleic acid derivative of any one of claims 1-7, for use in inducing in a subject the skipping of the target exon within the target pre-mRNA.

10. A peptide nucleic acid derivative of any one of claims 1-7, for use in the treatment of diseases or conditions involving the expression of the gene transcribing the target pre-mRNA.

11. A method of using the peptide nucleic acid derivative of any one of claims 1-7 to modulate in cells in vitro the functional activity of the gene transcribing the target pre-mRNA.

12. A peptide nucleic acid derivative of any one of claims 1-7, for use in modulating in a subject the functional activity of the gene transcribing the target pre-mRNA.

13. Use of a peptide nucleic acid derivative of any one of claims 1-7 in the manufacture of a medicament for the treatment of diseases or conditions involving the expression of the gene transcribing the target pre-mRNA.

14. Use of a peptide nucleic acid derivative of any one of claims 1-7 in the manufacture of a medicament for inducing in a subject the skipping of the target exon within the target pre-mRNA.

15. Use of a peptide nucleic acid derivative of any one of claims 1-7 in the manufacture of a medicament for modulating in a subject the functional activity of the gene transcribing the target pre-mRNA.

16. The peptide nucleic acid derivative of any one of claims 1-7, wherein the compound possesses at least a 10-mer complementary overlap with a 14-mer target splice site sequence that consists of 7-mer from intron and 7-mer from exon within a target pre-mRNA, wherein the target splice site sequence is not [(5' → 3') UUGCCUGGUAAGGA] within the human androgen receptor pre-mRNA, [(5' → 3') UUUUUGCGUAAGUA] within the human SCN9A pre-mRNA, [(5' → 3') UAAGUAGGAUAAGU] within the human HIF-1 $\alpha$  pre-mRNA, [(5' → 3') AUCCCAGGGUAACA] within the human SNAP25 pre-mRNA, [(5' → 3') UGUUUAGGUACACU] within the human SCN9A pre-mRNA, or [(5' → 3') UGUACAGAUUGUCU] within the human tyrosinase pre-mRNA.

17. The peptide nucleic acid derivative of any one of claims 1-7, wherein the compound possesses at least a 10-mer complementary overlap with a target splice site within

a target pre-mRNA, wherein the target splice site sequence does not comprise [(5' → 3') UUGCCUGGUAAGGA] within the human androgen receptor pre-mRNA, [(5' → 3') UUUUUGCGUAAGUA] within the human SCN9A pre-mRNA, [(5' → 3') UAAGUAGGAUAAGU] within the human HIF-1 $\alpha$  pre-mRNA, [(5' → 3') AUCCCAGGGUAACA] within the human SNAP25 pre-mRNA, [(5' → 3') UGUUUAGGUACACU] within the human SCN9A pre-mRNA, or [(5' → 3') UGUACAGAUUGUCU] within the human tyrosinase pre-mRNA.