In silico Design Bacterial Expression Vectors of Therapeutic Strategies of Lethal-Micro-RNA-7

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Abstract
Present study aims to design bacterial expression vectors produced microRNA let-7 types (a, b, c, d, e, f, g, i), bioinformatics software was used in present study. Nested PCR was used to add restriction site , supported sequences and poly u to primers of micro- RNA types, primers were tested for hairpin, self-complements and base running test, amplification of segments in first round of PCR were 100, 103, 104, 117, 99, 104, 117, 104 bp and the second round were114, 117, 118, 121, 113, 118, 121, 117 bp for types a, b, c, d, e, f, g and I respectively.

Target segments were digested by EcoRI and PstI, pET vector used to insert target sequences to create expression vectors (2929, 2932, 2933, 2933, 2928, 2933, 2936, 2933) bp for types a, b, c, d, e, f, g and I respectively with changes in some features without effect in main features of vectors elements. Poly u was added for isolation microRNA let-7 from other types of RNA in bacterial system.

Keywords: microRNA let-7, bacterial expression vectors, Poly u. pET vector.

Introduction

MicroRNAs (miRNAs) are most important discovery in last decade, it is short noncoding sequence of RNAs which have 20–25 nucleotides in length. Studies found that these sequences regulated gene expression by hybridization with the 3′ un-translated region (UTR) for mRNA of target genes, different mechanisms have been found for gene expression regulation; some microRNAs cause direct degradation of their target messenger RNAs or block the translation.[1]

miRNAs play major roles in some biological processes like metabolic control, immune modulation, neuronal development, muscle differentiation, cell cycle and stem cell differentiation. The first detection of Let-7 microRNA precursor in study of *C. elegans*, is shown that is a part of a big class of non-coding RNAs.[2,3]

Let-7 consists of some subtypes In human genome the cluster *(let-7a-1,let-7f-1, let-7d*) in the region B at 9q22.3, The cluster *(miR-99a, let-7c, miR-125b-2*)is in a 21p11.1 region of and The cluster *let-7g,miR-135-1* is in region 3 at 3p21.1-p21.2[4]. Some studies recorded deficiency in this type of micro-RNA let-7 especially in cancer cell types. As a results of therapeutic strategies development present study aims to design bio system for expressed let-7 microRNA in bacterial system for use in therapeutic strategies, there was poor information about using micro-RNA as therapy, but some reviewers focused on it as novel method in therapy of different disease as well as cancer, immune disease, metabolic disease etc.

Present study chose let-7 because several studies pointed its role in cancer cell regulation[5].

Material and Methods

The vectors created pET empty polycistronic destination vector from addgene as backbone for target sequence translated into *E coli DHα*. Isolation and purification using poly u was added at the end of the microRNA sequence. Vectors were as follows:

1. micro RNA sequence was downloaded from microbase, then it reversed transcriptase to obtain DNA sequence.

2. pET empty polycistronic destination vector sequences were downloaded from https://www.addgene.org/29775/ sequences/.

3. Primer design using primer3 software, linkers and supported sequences were added according to *biolabbs* (https://www.neb.com/tools-and-resources/usage-guidelines/cleavage-close-to-the-end-of-dna-fragments).[6]

4. Virtual amplification implemented using nested -PCR in to 2 steps F and R2.

5. PCR products and pET empty polycistronic destination vector digested by double digestion restriction enzymes (EcoRI g|aattc - PstI ctgca|g).

6. Digestion products were ligated in to suitable sites.

Results and Discussion

The present study was suggested to design bacterial expression vectors for micro-RNA expressions *in vitro* for therapeutic strategies, this suggestion was based on some points: first the expressions of micro-RNA let-7 types were varieties among disease,[5] second micro-RNA therapy is an important strategies of genetic engineering and therapy designs[1], third low side effect of these therapy because it is

expressed in normal cell and contributed biological processing , forth some of these micro-RNA were produced by synthetic method which needed high cost and advanced technology.

Table 2 showed micro RNA sequence and its size downloaded from micro base with accession number, different size of micro-RNA *precouser* while Mature microRNA let-7 sequences were shown in table 3 with its sites, consequence with *precouser*. All micro-RNA types were short sequence located in different sites of human genome.

Table 3 showed DNA sequence of micro-RNA types with accession number, these results were obtained from reverse transcriptase of RNA sequence after in alignment with genome data in NCBI. We used DNA data sequence for cloning these segments in bacterial expression vector as show in table 4.

The primer sequence and its features are shown in table 4. All primers were passed in SMS bioinformatics software \primer state like hairpin, self-annealing and nucleotide run. Annealing temperature was diverse among primers depending on the different factors like master-mix, concentration of salts and type of DNA polymerase. This was improved by Al-Terehi et al[6] when they found that there were differences between virtual and practical TM for some primers designed for nested PCR.

Nested PCR was used in present design to add restriction sites and poly U, nested PCR was used because the addition sequence was long, thus for more fidelity amplification it was added into two steps: supported sequence and EcoRI g|aattc in forward , two reversed primers were used for each types poly u (8) nucleotides and PstI ctgca|g restriction site with supported sequences for twice reversed (table 6). However, all primers in table 6 were passed in SMS bioinformatics software \primer state like hairpin, self-annealing and nucleotide run but some of them have high percentage of GC containing designers do not taking this in consideration when they designed primers for cloning because the addition of nucleotides did not affect in annealing TM. Present design deal with patents using same methods for primer.[8]

Amplification implanted into tow steps using same forward primer with two revers (R1 and R2) is shown in table 7.

Second round of PCR products was digested with double restriction enzyme EcoRI g|aattc - PstI ctgca|g, also map of pET empty polycistronic destination vector (2E) was digested with the same enzyme for assembly microRNA let-7 expressed vectors.

Table 9 shows restricted digest results of pET empty polycistronic destination vector (2E) about 53 bp from vector sequences (121 – 173) were removed from vector. It was located between restriction enzymes sites used in present study, each type of microRNA let-7 was ligated between segment 1 and 2 to create bacterial expression vectors (table 9).

Vectors were created for each types of microRNA let-7, vectors size were 2929, 2932, 2933, 2933, 2928, 2933, 2936, 2933 bp for types a, b, c, d, e, f, g and I respectively; maps and vectors features were shown in figure 2 and table 10 and 11. Some changes in features sites were observed in vectors because of divers of insertion sequences length, however all features did not affect these shifting because of insertion implemented in multiple cloning site, a study dealing with vector design for insertion regulatory sequences in non-viral vector design by Al-Terehi[9] showing shifting in vector feature site. Also, restriction enzyme sites were changed and a new restriction enzyme was created as in table 11.

Table 1
Bioinformatics software and data base of microRNA

Software	Links	Aims
Microbase	http://www.mirbase.org/cgi-bin/query.pl?terms=let-7&submit=Search	RNA sequence download
Transcription and Translation	http://www.attotron.com/cybertory/analysis/trans.htm	Reverse transcriptase
Sequence Manipulation Suite\primer states	http://www.bioinformatics.org/sms2/pcr_primer_stats.htm	Primer states
Sequence Manipulation Suite\ PCR Products	http://www.bioinformatics.org/sms2/pcr_products.html	Virtual amplification
Sequence Manipulation Suite\ Restriction Digest	http://www.bioinformatics.org/sms2/rest_digest.html	Sequence cutting by restriction enzyme
addgene\analyze sequence	https://www.addgene.org/analyze-sequence/	Vectors maps construction
The mfold Web Server	http://unafold.rna.albany.edu//?q=mfold/RNA-Folding-Form	RNA folding

Table 2
microRNA let-7 types sequence and accession number

microRNA let-7 types		Accession number	microRNA let-7 sequence
Homo sapiens (human) microRNA let-7a-1 precursor	100	MI0000060	UGGGAUGAGGUAGUAGGUUGUAUAGUUUUAGGGUCA CACCCACCACUGGGAGAUAACUAUACAAUCUACUGUC UUUCCUA
Homo sapiens (human) microRNA let-7b	103	MI0000063	CGGGGUGAGGUAGUAGGUUGUGUGGUUUCAGGGCAG UGAUGUUGCCCCUCGGAAGAUAACUAUACAACCUACU GCCUUCCCUG
Homo sapiens (human) microRNA let-7c precursor	104	MI0000064	GCAUCCGGGUUGAGGUAGUAGGUUGUAUGGUUUAGA GUUACACCCUGGGAGUUAACUGUACAACCUUCUAGCU UUCCUUGGAGC
Homo sapiens (human) microRNA let-7d precursor.	117	MI0000065	CCUAGGAAGAGGUAGUAGGUUGCAUAGUUUUAGGGC AGGGAUUUUGCCCACAAGGAGGUAACUAUACGACCUG CUGCCUUUCUUAGG
Homo sapiens (human) microRNA let-7e	99	MI0000066	CCCGGGCUGAGGUAGGAGGUUGUAUAGUUGAGGAGG ACACCCAAGGAGAUCACUAUACGGCCUCCUAGCUUUC CCCAGG
Homo sapiens (human) microRNA let-7g precursor	104	MI0000433	AGGCUGAGGUAGUAGUUUGUACAGUUUGAGGGUCUA UGAUACCACCCGGUACAGGAGAUAACUGUACAGGCCA CUGCCUUGCCA
Homo sapiens (human) microRNA let-7f-1 precursor.	117	MI0000067	UCAGAGUGAGGUAGUAGAUUGUAUAGUUGUGGGGUA GUGAUUUUACCCUGUUCAGGAGAUAACUAUACAAUCU AUUGCCUUCCCUGA
Homo sapiens (human) microRNA let-7i	104	MI0000434	CUGGCUGAGGUAGUAGUUUGUGCUGUUGGUCGGGUU GUGACAUUGCCCGCUGUGGAGAUAACUGCGCAAGCUA CUGCCUUGCUA

Table 3
Mature microRNA let-7 sequence

microRNA let-7 types	Accession number	Mature microRNA let-7 sequence
Homo sapiens (human) microRNA let-7a-1 precursor	MI0000060	57 - cuauacaaucuacugucuuuc - 77
Homo sapiens (human) microRNA let-7b	MI0000063	6 - ugagguaguagguuguguggguu- 27
Homo sapiens (human) microRNA let-7c precursor	MI0000064	11 - ugagguaguagguuguaugguu - 32
Homo sapiens (human) microRNA let-7d precursor.	MI0000065	8 - agagguaguagguugcauaguu- 29
Homo sapiens (human) microRNA let-7e	MI0000066	8 - ugagguaggagguuguauaguu - 29
Homo sapiens (human) microRNA let-7g precursor	MI0000433	5- ugagguaguaguuuguacau - 26
Homo sapiens (human) microRNA let-7f-1 precursor.	MI0000067	7 ugagguaguagauuguauauu- 28
Homo sapiens (human) microRNA let-7i	MI0000434	6 - ugagguaguaguuugugcuguu - 27

Table 4
Types of microRNA let-7 DNA sequences and accession number

microRNA let-7 types	Accession number	DNA sequence
Homo sapiens (human) microRNA let-7a-1 DNA	NR_029476.1	TGGGATGAGGTAGTAGGTTGTATAGTTTTAGGGTCACACCC ACCACTGGGAGATAACTATACAATCTACTGTCTTTCCTA
Homo sapiens (human) microRNA let-7b DNA	NC_000022.11	CGGGGTGAGGTAGTAGGTTGTGTGGTTTCAGGGCAGTGAT GTTGCCCCTCGGAAGATAACTATACAACCTACTGCCTTCCC TG
Homo sapiens (human) microRNA let-7c DNA	NR_029480.1	GCATCCGGGTTGAGGTAGTAGGTTGTATGGTTTAGAGTTAC ACCCTGGGAGTTAACTGTACAACCTTCTAGCTTTCCTTGGA GC
Homo sapiens (human) microRNA let-7d DNA	NR_029481.1	CCTAGGAAGAGGTAGTAGGTTGCATAGTTTTAGGGCAGGG ATTTTGCCCACAAGGAGGTAACTATACGACCTGCTGCCTTT CTTAGG
Homo sapiens (human) microRNA let-7e DNA	NC_000019.10	CCCGGGCTGAGGTAGGAGGTTGTATAGTTGAGGAGGACAC CCAAGGAGATCACTATACGGCCTCCTAGCTTTCCCCAGG
Homo sapiens (human) microRNA let-7g DNA	NR_029660.1	AGGCTGAGGTAGTAGTTTGTACAGTTTGAGGGTCTATGATA CCACCCGGTACAGGAGATAACTGTACAGGCCACTGCCTTG CCA
Homo sapiens (human) microRNA let-7f-1 DNA	NR_029483.1	TCAGAGTGAGGTAGTAGATTGTATAGTTGTGGGGTAGTGA TTTTACCCTGTTCAGGAGATAACTATACAATCTATTGCCTT CCCTGA
Homo sapiens (human) microRNA let-7f-1 DNA	NC_000012.12	CTGGCTGAGGTAGTAGTTTGTGCTGTTGGTCGGGTTGTGAC ATTGCCCGCTGTGGAGATAACTGCGCAAGCTACTGCCTTGC TA

Table 5
microRNA let-7 primers sequence states

Types	5´>3´	GC%	TM
microRNA let-7a-1	F- TGGGATGAGGTAGTAGGTT	47	59.51
	R- TAGGAAAGACAGTAGATTGTA	33	55.88
microRNA let-7b	F-CGGGGTGAGGTAGTAG	62.50	57.24
	R- CAGGGAAGGCAGTAGG	55.56	57.96
microRNA let-7c	F- GCATCCGGGTTGAGGTAG	61	62.36
	R- GCTCCAAGGAAAGCTAGAA	47	59.31
microRNA let-7d	F- CCTAGGAAGAGGTAGTAGG	52.63	57.42
	R- CCTAAGAAAGGCAGCAG	52.94	56.89
microRNA let-7e	F- CCCGGGCTGAGGTAGGA	75.00	63.46
	R- CCTGGGGAAAGCTAGGAG	61.11	75.00
microRNA let-7g	F- AGGCTGAGGTAGTAGTTTG	47.37	58.61
	R- TGGCAAGGCAGTGG	64.29	57.58
microRNA let-7f-1	F- TCAGAGTGAGGTAGTAGAT	42.11	56.20
	R- TCAGGGAAGGCAATAGA	47.06	56.53
microRNA let-7i	F- CTGGCTGAGGTAGTAGT	52.94	56.70
	R- TAGCAAGGCAGTAGCT	50.00	55.99

Table 6
microRNA let-7 primers sequence with sequence additive.

Types	5´>3´
microRNA let-7a-1	F- CAAATTgaattcTGGGATGAGGTAGTAGGTT
	R- aaaaaaaTAGGAAAGACAGTAGATTGTA
	R2-GCTACGctgcagaaaaaaaaaaTAGGAAAGACA
microRNA let-7b	F-CAAATTgaattcCGGGGTGAGGTAGTAG
	R- aaaaaaaCAGGGAAGGCAGTAGG
	R2-GCTACGctgcagaaaaaaaaaaCAGGGAAGGCAGTA
microRNA let-7c	F- CTGATA gaattc GCATCCGGGTTGAGGTAG
	R- aaaaaaaaGCTCCAAGGAAAGCTAGAA
	R2- GCTACGctgcagaaaaaaaaaaGCTCCAAGGAAAGCT
microRNA let-7d	F- TACCTGgaattcCCTAGGAAGAGGTAGTAGG
	R- aaaaaaaaCCTAAGAAAGGCAGCAG
	R2- GCTACGctgcagaaaaaaaaaaCCTAAGAAAGGCAG
microRNA let-7e	F- TACCTGgaattcCCCGGGCTGAGGTAGGA
	R- aaaaaaaaCCTGGGGAAAGCTAGGAG
	R2- GCTACGctgcagaaaaaaaaCCTGGGGAAAGCTAGG
microRNA let-7g	F- CGATAAgaattcAGGCTGAGGTAGTAGTTTG
	R- aaaaaaaaTGGCAAGGCAGTGG
	R2-GCTACGctgcag aaaaaaaaaaTGGCAAGGCAGTGG
microRNA let-7f-1	F- TCAGTCgaattcTCAGAGTGAGGTAGTAGAT
	R- aaaaaaaaTCAGGGAAGGCAATAGA
	R2- GCTACGctgcagaaaaaaaaaaTCAGGGAAGGCAAT
microRNA let-7i	F- TCAGTCgaattcCTGGCTGAGGTAGTAGT
	R- aaaaaaaaTAGCAAGGCAGTAGC
	R2- GCTACGctgcagaaaaaaaaaaTAGCAAGGCAGTA

Table 7
PCR products of microRNA let-7

Types	**First round**	**Second round**	**PCR products**
microRNA let-7a-1	100	114	CAAATTgaattcTGGGATGAGGTAGTAGGTTGTATAGTTTTAGGGTCACACCCACCACTGGGAGATAACTATACAATCTACTGTCTTTCCTAaaaaaaaaaactgcagGCATCG
	103	117	CAAATTgaattcCGGGGTGAGGTAGTAGGTTGTGTGGTTTCAGGGCAGTGATGTTGCCCCTCGGAAGATAACTATACAACCTACTGCCTTCCCTGaaaaaaaaaactgcagGCATCG
microRNA let-7c	104	118	CTGATAgaattcGCATCCGGGTTGAGGTAGTAGGTTGTATGGTTTAGAGTTACACCCTGGGAGTTAACTGTACAACCTTCTAGCTTTCCTTGGAGCaaaaaaaaaactgcagGCATCG
microRNA let-7d	117	121	TACCTGgaattcCCTAGGAAGAGGTAGTAGGTTGCATAGTTTTAGGGCAGGGATTTTGCCCACAAGGAGGTAACTATACGACCTGCTGCCTTTCTTAGGaaaaaaaaaactgcagGCATCG
microRNA let-7e	99	113	TACCTGgaattcCCCGGGCTGAGGTAGGAGGTTGTATAGTTGAGGAGGACACCCAAGGAGATCACTATACGGCCTCCTAGCTTTCCCCAGGaaaaaaaaaactgcagGCATCG
microRNA let-7g	104	118	CGATAAgaattcAGGCTGAGGTAGTAGTTTGTACAGTTTGAGGGTCTATGATACCACCCGGTACAGGAGATAACTGTACAGGCCACTGCCTTGCCAaaaaaaaaaactgcagGCATCG
microRNA let-7f-1	117	121	TCAGTCgaattcTCAGAGTGAGGTAGTAGATTGTATAGTTGTGGGGTAGTGATTTTACCCTGTTCAGGAGATAACTATACAATCTATTGCCTTCCCTGAaaaaaaaaaactgcagGCATCG
microRNA let-7i	104	117	TCAGTCgaattcCTGGCTGAGGTAGTAGTTTGTGCTGTTGGTCGGGTTGTGACATTGCCCGCTGTGGAGATAACTGCGCAAGCTACTGCCTTGCTAaaaaaaaaaactgcagGCATCG

Table 8
Digest results by (EcoRI g|aattc - PstI ctgca|g) of PCR products

Types	Sequence	Digest results (EcoRI g|aattc - PstI ctgca|g)
microRNA let-7a-1	aattcTGGGATGAGGTAGTAGGTTGTATAGTTTTAGGGTCACAC CCACCACTGGGAGATAACTATACAATCTACTGTCTTTCCTAaaa aaaaaaactgca	>100 bp linear fragment from linear parent Untitled, base 8 to base 107
microRNA let-7b	aattcCGGGGTGAGGTAGTAGGTTGTGTGGTTTCAGGGCAGTGA TGTTGCCCCTCGGAAGATAACTATACAACCTACTGCCTTCCCT Gaaaaaaaaaactgca	>103 bp linear fragment from linear parent Untitled, base 8 to base 110
microRNA let-7c	aattcGCATCCGGGTTGAGGTAGTAGGTTGTATGGTTTAGAGTTA CACCCTGGGAGTTAACTGTACAACCTTCTAGCTTTCCTTGGAG Caaaaaaaaaactgca	>104 bp linear fragment from linear parent Untitled, base 8 to base 111
microRNA let-7d	aattcCCTAGGAAGAGGTAGTAGGTTGCATAGTTTTAGGGCAGG GATTTTGCCCACAAGGAGGTAACTATACGACCTGCTGCCTTTC TTAGGaaaaaaaaaactgca	>107 bp linear fragment from linear parent Untitled, base 8 to base 114
microRNA let-7e	aattcCCCGGGCTGAGGTAGGAGGTTGTATAGTTGAGGAGGACA CCCAAGGAGATCACTA TACGGCCTCCTAGCTTTCCCCAGGaaaaaaaaaactgca	>99 bp linear fragment from linear parent Untitled, base 8 to base 106
microRNA let-7g	aattcAGGCTGAGGTAGTAGTTTGTACAGTTTGAGGGTCTATGAT ACCACCCGGTACAGGAGATAACTGTACAGGCCACTGCCTTGC CAaaaaaaaaaactgca	>104 bp linear fragment from linear parent Untitled, base 8 to base 111
microRNA let-7f-1	aattcTCAGAGTGAGGTAGTAGATTGTATAGTTGTGGGGTAGTG ATTTTACCCTGTTCAGGAGATAACTATACAATCTATTGCCTTC CCTGAaaaaaaaaaactgca	>107 bp linear fragment from linear parent Untitled, base 8 to base 114
microRNA let-7i	aattcCTGGCTGAGGTAGTAGTTTGTGCTGTTGGTCGGGTTGTGA CATTGCCCGCTGTGGAGATAACTGCGCAAGCTACTGCCTTGC TAaaaaaaaaaactgca	>104 bp linear fragment from linear parent Untitled, base 8 to base 111

Table 9
Restriction digest results of pET empty polycistronic destination vector (2E)

S.N.	Restriction digest of pET vector
1	>2709 bp linear fragment from linear parent Untitled, base 174 to base 2882 (PstI ctgca|g - sequence end).
2	>120 bp linear fragment from linear parent Untitled, base 1 to base 120 (sequence start - EcoRI g|aattc).
3	>53 bp linear fragment from linear parent Untitled, base 121 to base 173 (EcoRI g|aattc - PstI ctgca|g).

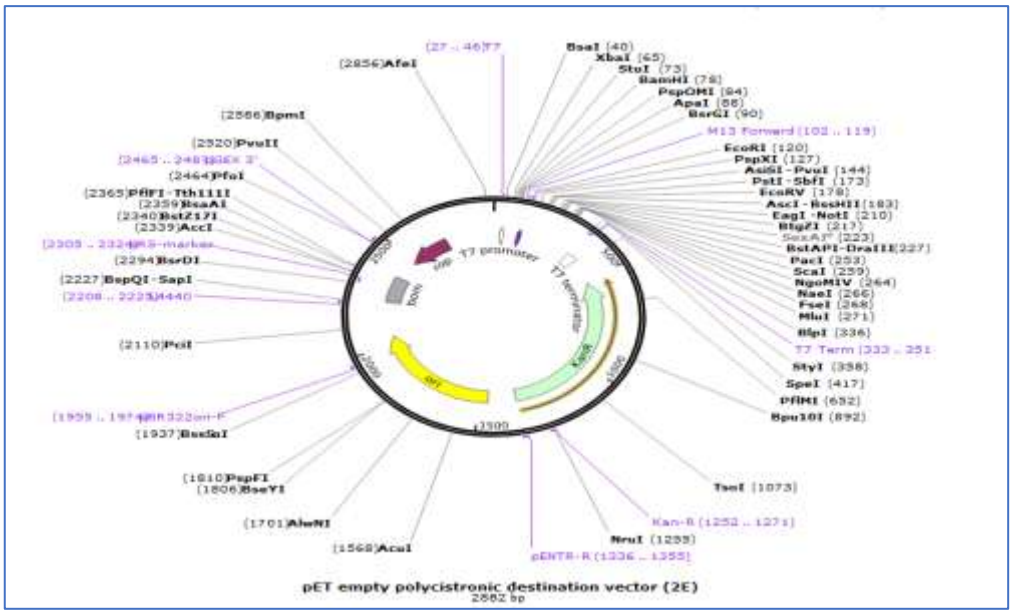

Figure 1: Map of pET empty polycistronic destination vector (2E) 2882 bp

Figure 2: Maps of pET empty polycistronic destination vector (2E) with micro- RNAlet-7 Sequence

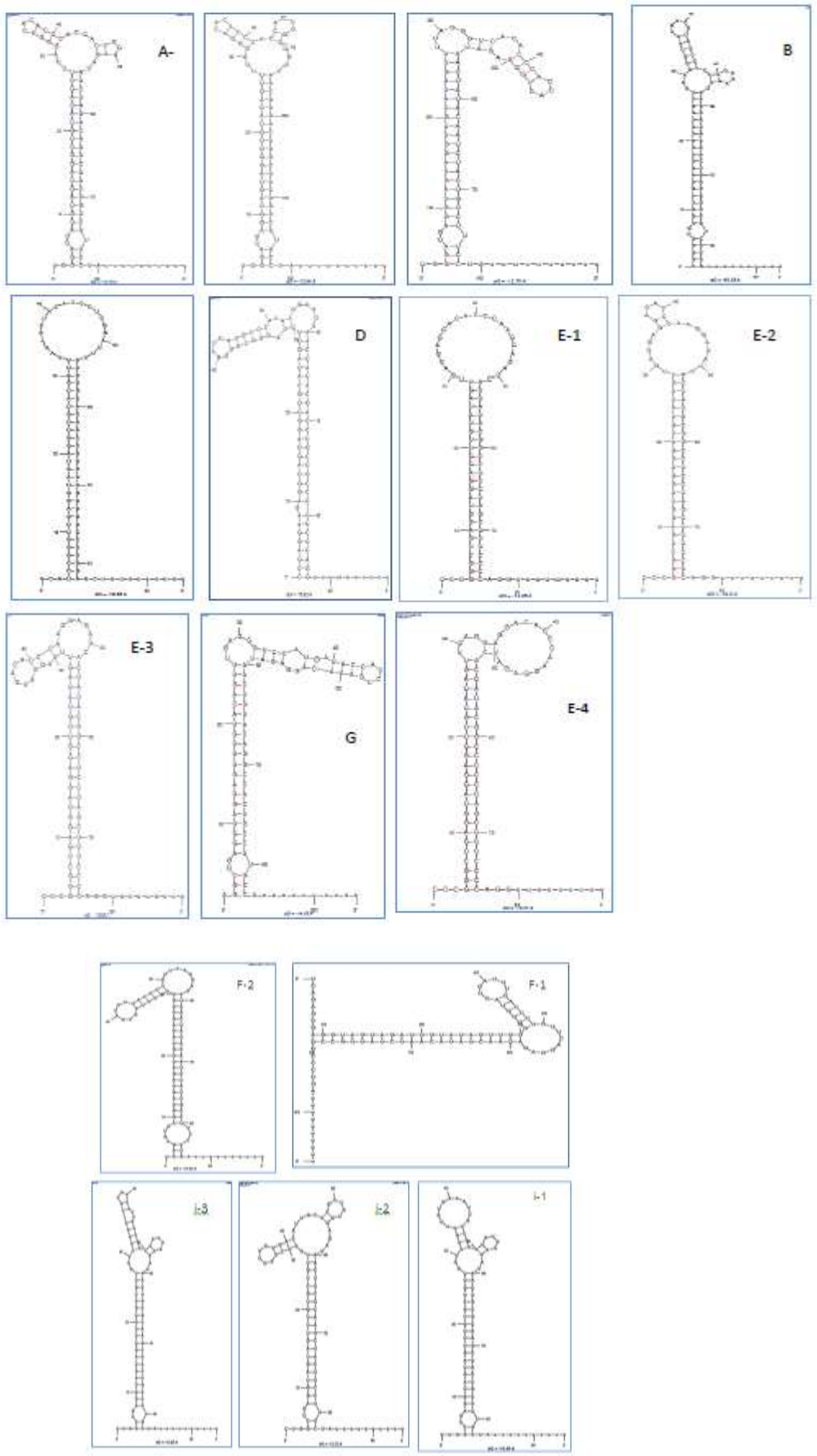

Figure 3: Secondary structure of microRNA let-7 types with possibility of each types

Table 10
Sites of vector elements for micro-RNA let-7 types

Features	C		A	B			C	D			E		F		G		I	
T7_promoter	27	45	27	45	27	45	27	45	27	45	27	45	27	45	27	45	27	45
M13_forward20_primer	103	119	103	119	103	119	103	119	103	119	103	119	103	119	103	119	103	119
T7_Terminal_primer	351	333	398	380	401	383	402	384	405	387	397	379	402	384	405	387	402	384
KanR2	1344	529	1391	576	1394	579	1395	580	1398	583	1390	575	1395	580	1398	583	1395	580
pBR322_origin	1450	2069	1497	2116	1500	2119	1501	2120	1504	2123	1496	2115	1501	2120	1504	2123	1501	2120
pGEX_3_primer	2465	2487	2512	2534	2515	2537	2516	2538	2519	2541	2511	2533	2516	2538	2519	2541	2516	2538
ROP	2672	2481	2719	2528	2722	2531	2723	2532	2726	2535	2718	2527	2723	2532	2726	2535	2723	2532

Table 11
Sites of restriction enzymes for micro-RNA let-7 types

C		A		B		C		D		E		F		G		I	
XbaI	65	XbaI	65	XbaI	65	XbaI	65	XbaI	65	XbaI	65	XbaI	65	XbaI	65	XbaI	65
StuI	73	StuI	73	StuI	73	StuI	73	StuI	73	StuI	73	StuI	73	StuI	73	StuI	73
BamHI	78	BamHI	78	BamHI	78	BamHI	78	BamHI	78	BamHI	78	BamHI	78	BamHI	78	BamHI	78
ApaI	88	ApaI	88	ApaI	88	ApaI	88	ApaI	88	ApaI	88	ApaI	88	ApaI	88	ApaI	88
EcoRI	120	EcoRI	120	EcoRI	120	EcoRI	120	EcoRI	120	EcoRI	120	EcoRI	120	EcoRI	120	EcoRI	120
PstI	173	PstI	220	PstI	223	HpaI	178	PstI	227	PstI	219	PstI	224	PstI	227	FspI	190
EcoRV	178	EcoRV	225	EcoRV	228	PstI	224	EcoRV	232	EcoRV	224	EcoRV	229	EcoRV	232	PstI	224
AscI	183	AscI	230	AscI	233	EcoRV	229	AscI	237	AscI	229	AscI	234	AscI	237	EcoRV	229
NotI	210	NotI	257	NotI	260	AscI	234	NotI	264	NotI	256	NotI	261	NotI	264	AscI	234
EagI	210	EagI	257	EagI	260	NotI	261	EagI	264	EagI	256	EagI	261	EagI	264	NotI	261
PacI	253	PacI	300	PacI	303	EagI	261	PacI	307	PacI	299	PacI	304	PacI	307	EagI	261
SpeI	417	XhoI	324	XhoI	327	PacI	304	XhoI	331	XhoI	323	XhoI	328	XhoI	331	PacI	304
NruI	1255	SpeI	464	SpeI	467	XhoI	328	SpeI	471	SpeI	463	SpeI	468	SpeI	471	XhoI	328
PvuII	2520	XmaI	1083	XmaI	1086	SpeI	468	XmaI	1090	NruI	1301	SmaI	1089	XmaI	1090	SpeI	468
		SmaI	1085	SmaI	1088	SmaI	1089	SmaI	1092	PvuII	2566	XmaI	1087	SmaI	1092	SmaI	1089
		NruI	1302	NruI	1305	XmaI	1087	NruI	1309			NruI	1306	NruI	1309	XmaI	1087
		PvuII	2567	PvuII	2570	NruI	1306	PvuII	2574			PvuII	2571	PvuII	2574	NruI	1306
						PvuII	2571									PvuII	2571

The present design aims to product microRNA let-7 in vitro using bacterial system for isolation. RNA poly u was added to the end of microRNA let-7 sequences, these features may be useful for using poly A for separation of this type as well as HIS tag in proteins sequences, these poly u did not effect in secondary structure of micro –RNA (figure 3), also separated vectors made investigators to controlling on RNA titers for therapy dose and for types choosing. For gene therapy polycistronic of microRNA let-7 type was created and inserted in mammalian expression vector[7,10,11]. Present study was needed for practical implementation for detection pharm kinetics and optimum features for its production.

Conclusion

The present results concluded that we can use bioinformatics for design of new vectors used in therapeutic strategies.

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