Designing L-Systems for making three and six open reading frames from the leading strand of a single DNA molecule

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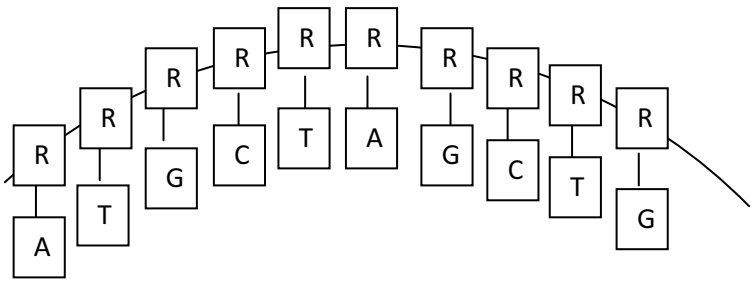
Current molecular biology dogma (except alternative splicing) suggests that leading strand of DNA produces mRNA and usually the leading strand gives one ORF (ORF) among the available six ORFs. We attempted to break this rule where three and six ORFs from the leading strand will be formed by designing L-Systems, originally proposed to study symmetry of biological world. We thought this would revolutionize the world of synthetic biology. While doing so, we found L-System containing A→T, T→G, C→A, and G→C, i.e., single nucleotide production rule will stop growing after one nucleotide. L-System containing either (a) A→TG, T→GC, C→GA, G→TC or (b) A→CTG, T→GAC, C→TAC, G→TGC will lead to generation of repetitive DNA sequence after few iteration. These repetitive sequences might become easy target for enzyme like restriction endonucleases and transposase or RNAse but may give repetitive DNA sequences which are normally found in the large non-coding region of the genome. Here we show that a number of L-Systems like with (Axiom: A) A→ATGC, C→CTCA, T→GCAG, G→CGAA and A→GTAC, C→TGCA, T→ CTGG, G→ATAT if used to generate either a short (~2000 bp) or long (~16000 bp) can give rise to three ORFs and six ORFs per DNA molecule when conceptually translated through Expasy tools respectively. To our surprise we find that L-System production rule with only four letter codes (not one, two, three, five, six) can produce this miraculous results which nothing but follows Young Fibonacci graph. We find that production rule with higher order of magnitude like 10 bp also give similar results but not 20 bp containing production rules.

Leading strand of DNA chain is constructed on the backbone of pentose sugar and nucleotide like A, T, G, C are arranged in following fashion where R boxes represent pentose sugar moieties and boxes with A, T, G, C represent nucleotides as shown in Fig. 1 below.

Fig. 1



DNA with 10 nucleotides per turn of a DNA helix would exactly look like Fig. 1. In this paper, we took following L-System production rule (Axiom: A)- A→ATGC, C→CTCA, T→GCAG, G→CGAA and made a 4096 bp long DNA sequence (Fig. 2) [1, 2, 3, 4].

Fig. 2

ATGCGCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCAATGCCTCACGAAATGCATGCCTCAGCAGCTCAATGCATGCGCAGCGAACT
CACTCACGAAATGCATGCCTCAGCAGCTCAATGCCTCACGAAATGCATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACTCAGCAGCTCAATGCCGAACTCAATGC
CGAACTCAGCAGCTCAATGCATGCGCAGCGAACTCACTCAGCAGCTCAATGCCTCACGAAATGCATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACTCAGCAGCT
CAATGCCGAACTCAATGCCGAACTCAGCAGCTCAATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAG
CTCAATGCCTCAGCAGCTCAATGCCTCACGAAATGCATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACTCAGCAGCTCAATGCCGAACTCAATGCCGAACTCAGC
AGCTCAATGCATGCGCAGCGAACTCACTCAGCAGCTCAATGCCTCACGAAATGCATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCAATGCGCAGCGAACTCACGAA
CTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCAATGCATGCGCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCAATGCCT
CAGCAGCTCAATGCCGAACTCAATGCCGAACTCAGCAGCTCAATGCATGCGCAGCGAACTCACTCACGAAATGCATGCCTCAGCAGCTCAATGCATGCGCAGCGAACTCA
CTCACGAAATGCATGCCTCAGCAGCTCAATGCCGAACTCAATGCCGAACTCAGCAGCTCAATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACGAACTCAATGCCG
AACTCACGAAATGCATGCCTCAGCAGCTCAATGCCTCAGCAGCTCAATGCCGAACTCAATGCCGAACTCAGCAGCTCAATGCATGCGCAGCGAACTCACTCAGCAGCTCA
ATGCCTCACGAAATGCATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCAATGCGCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCT
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CTCAATGCATGCGCAGCGAACTCACTCAGCAGCTCAATGCCGAACTCAATGCATGCGCAGCGAACTCACTCACGAAATGCATGCCTCAGCAGCTCAATGCCGAACTCAAT
CAATGCCGAACTCAGCAGCTCAATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCAATGCATGC
GCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCAATGCCTCACGAAATGCATGCCTCAGCAGCTCAATGCATGCGCAGCGAACTCACT
CACGAAATGCATGCCTCAGCAGCTCAATGCCTCACGAAATGCATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACTCAGCAGCTCAATGCCGAACTCAATGCCGAA
CTCAGCAGCTCAATGCATGCGCAGCGAACTCACTCAGCAGCTCAATGCCGAACTCAATGCCGAACTCAGCAGCTCAATGCATGCGCAGCGAACTCACTCAGCAGCTCAAT
GCCTCACGAAATGCATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCAATGCGCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCA
ATGCATGCGCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCAATGCCTCAGCAGCTCAATGCCGAACTCAATGCCGAACTCAGCAGCT
CAATGCATGCGCAGCGAACTCACTCACGAAATGCATGCCTCAGCAGCTCAATGCATGCGCAGCGAACTCACTCACGAAATGCATGCCTCAGCAGCTCAATGCCGAACTCA
ATGCCGAACTCAGCAGCTCAATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCAATGCCTCAGC
AGCTCAATGCCGAACTCAATGCCGAACTCAGCAGCTCAATGCATGCGCAGCGAACTCACTCAGCAGCTCAATGCCGAACTCAGCAGCTCAGCAGCTCAATGCATGCGCAG
GCAGCGAACTCAATGCGCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCAATGCCTCACGAAATGCATGCCTCAGCAGCTCAATGCCG
AACTCAATGCCGAACTCAGCAGCTCAATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCAATGC
CTCAGCAGCTCAATGCCTCACGAAATGCATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACTCAGCAGCTCAATGCCGAACTCAATGCCGAACTCAGCAGCTCAAT
GCATGCGCAGCGAACTCACTCACGAAATGCATGCCTCAGCAGCTCAATGCATGCGCAGCGAACTCACTCAGCAGCTCAATGCCGAACTCAATGCCGAACTCAGCAGCTCA
CGAACTCAGCAGCTCAATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCAATGCATGCGCAGCG
AACTCACGAACTCAATGCCGAACTCACGAAATGCATGCCTCAGCAGCTCAATGCCTCACGAAATGCATGCCTCAGCAGCTCAATGCATGCGCAGCGAACTCACTCACGAA
ATGCATGCCTCAGCAGCTCAATGCCTCACGAAATGCATGCATGCGCAGCGAACTCAATGCGCAGCGAACTCACTCAGCAGCTCAATGCCGAACTCAATGCCGAACTCAGC
AGCTCAATGCATGCGCAGCGAACTCA

Then we used Expasy tool (http://expasy.org/tools/dna.html) (freely available over internet) and translated the sequence into six ORFs (http://en.wikipedia.org/wiki/Open_reading_frame). We find that 5'→3' direction all three open ORFs but in the 3'→5' direction all three ORFs have stop codons. We then use another production rule (Axiom: A) A→GTAC, C→TGCA, T→ CTGG, G→ATAT, we made 4096 bp long stretch of DNA and translated into six ORFs.

Fig. 3

ATATCTGGGTACTGCATGCACTGGATATATATATATCTGGGTACTGCATGCACTGGATATATATCTGGATATTGCAGTACTGCACTGGATATATATGTACCTGGGTACCT
GGGTACCTGGGTACCTGGGTACCTGGGTACCTGGTGCACTGGATATATATATATCTGGGTACTGCACTGGATATTGCAGTACTGCACTGGATATATATGTACCTGGGTAC
CTGGCTGGATATTGCAGTACATATCTGGGTACTGCATGCACTGGATATATATGTACCTGGGTACCTGGCTGGATATTGCAGTACATATCTGGGTACTGCACTGGATATTG
CAGTACTGCACTGGATATATATGTACCTGGGTACCTGGGTACCTGGGTACCTGGATATCTGGGTACTGCATGCACTGGATATATATATCTGGGTACTGCATGCACTGG
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GGGTACTGCATGCACTGGATATATATCTGGATATTGCAGTACTGCACTGGATATATATGTACCTGGGTACCTGGGTACCTGGGTACCTGGGTACCTGGTGCA
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CTGGGTACCTGGTGCACTGGATATATATATATCTGGGTACTGCACTGGATATTGCAGTACCTGGATATTGCAGTACTGCACTGGATATATATGTACCTGGGTACCTGGGT
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GTACCTGGGTACCTGGGTACCTGGTGCACTGGATATATATATATCTGGGTACTGCACTGGATATTGCAGTACTGCACTGGATATATATGTACCTGGGTACCTGGCTGGAT
ATTGCAGTACATATCTGGGTACTGCA

To our surprise (Fig. 3) we find that all the six reading frames are open. This led to formation of a 16384 bp long stretch of DNA using the aforesaid L-System (Fig. 4).

Fig. 4

GTACCTGGGTACCTGGTGCACTGGATATATATATATCTGGGTACTGCACTGGATATTGCAGTACCTGGATATTGCAGTACTGCACTGGATATATATGTACCTGGGTACCT
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We find when conceptually translated via Expasy gave rise to six ORFs. Therefore, we can conclude that production rule starting with four letters can give rise to one to six ORFs (Data not shown). Now, we blasted all these aforesaid three sequences using Blastx and the results are summaries below (Table 1).

Table 1

L-System production rules (Axiom: A)	**Sequence length (bp)**	**Blastp results (name of the organism and percentage of alignment score in parenthesis)**
A→ATGC, C→CTCA, T→GCAG, G→CGAA	4096	*Streptococcus oralis* (80%-200%), *Streptococcus pneumoniae* (80%-200%)
A→GTAC, C→TGCA, T→ CTGG, G→ATAT	16384	*Vanderwaltozma polyspora* (200%)

Table 1 show that the above production rules identify organisms like *Streptococcus* bacteria and *Vanderwaltozyma* which is a fungus. Therefore, in conclusion the method of L-System which we have discovered can not only be used to generate recombinant proteins from a single DNA molecule using all its ORFs selectively. This would be a big step forward for synthetic biology researchers as well as industries in general. Apart from applied aspects this method can be used by evolutionary biologist to trace back genetic code evolution of virus, bacteria, and fungi to higher endangered animals like Giant Panda.

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