# Repurposing The Dark Genome. I - Antisense Proteins

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## Abstract

From the functional standpoint, genome may be considered as a collection of three types of sequences: protein encoding, RNA encoding and non-expressing. Based on the standard sequencing and annotation work, it is now well accepted that a small proportion of the genome has been allocated the job of encoding proteins, while most of the genome encodes RNA and some DNA sequences are not used for expression. The exact ratio among these three types of sequences vary based on the organism. We asked: Is it possible to artificially encode protein and peptide sequences from naturally non expressing (dark genome) sequences? This led to proof of the concept of making functional proteins from the intergenic sequences of E.coli (Dhar et al 2009). This study is an extension of the original concept and has been organized around antisense DNA sequences. The full length antisense gene equivalents in forward and reverse orientations were computationally studied for their structural, cellular location and functional properties, leading to a number of interesting observations. The current study points to a huge untapped genomic space that needs to be examined from cell physiology, evolutionary and application perspectives.

## Key Words

Antisense genes, synthetic biology, antisense peptides, proteins

## 1. Introduction

Genomes are the fundamental molecular chassis of organisms providing primary molecular codes for running the cell. Genomes come with various sizes and base pair proportions, hosting a unique arrangement of genes and non-genic regions providing the fundamental blueprint for activating the downstream molecular processes. Historically, DNA has been the main focus of several generations of scientists, as it provides an intellectual fuel to understand collective molecular events like replication, transcription, translation, pathways and so on. Following discovery of protein coding genes, the regulatory space defined by RNA coding genes have received an increased attention for the last several decades. It is now accepted that the so-called 'junk' is in fact a huge repository of RNA coding genes. Interestingly, the non-expressing part of the genome has only met a computational treatment leading to an unclear understanding of its role in the cell.

To fill up this gap, our lab provided preliminary experimental evidence of artificially encoding intergenic sequences into functional molecules (Dhar et al 2009). Extending the search further, the current study was organized to understand the potential of antisense strand in generating novel biomolecules.

## 2. Material and Methods

The *Escherichia coli* (Strain: K-12 MG1655) genome database and *Saccharomyces cerevisiae* (S288C) genome database and *Drosophila melanogaster* (fruit fly) genome database was used in this study (NCBI Resource Coordinators, 2013). Data was downloaded from the NCBI (National Center for Biotechnology Information) database. 4315 protein coding genes of *E. coli*, 6017 protein coding genes of the *S. cerevisiae* and 13,962 protein coding genes of the *Drosophila* were used to generate antisense DNA sequence equivalents.

### 2.1 Translation of Antisense and Reverse Antisense Sequences

Sequences on the minus (-) strand of *E. coli* and *S. cerevisiae* genomes were computationally translated into Protein Sequence with the help of UGENE tool (Okonechnikov et al., 2012). Only those translated sequences were used that were full length equivalents, not interrupted by stop codon (Figure 1). Sequences that showed stop codons within computationally translated antisense strand were discarded. Expasy-Translation Tool (Gasteiger, 2003) was used to quality check the result. In the antisense strand, both the directions were used to predict new genes as shown in Fig.1.

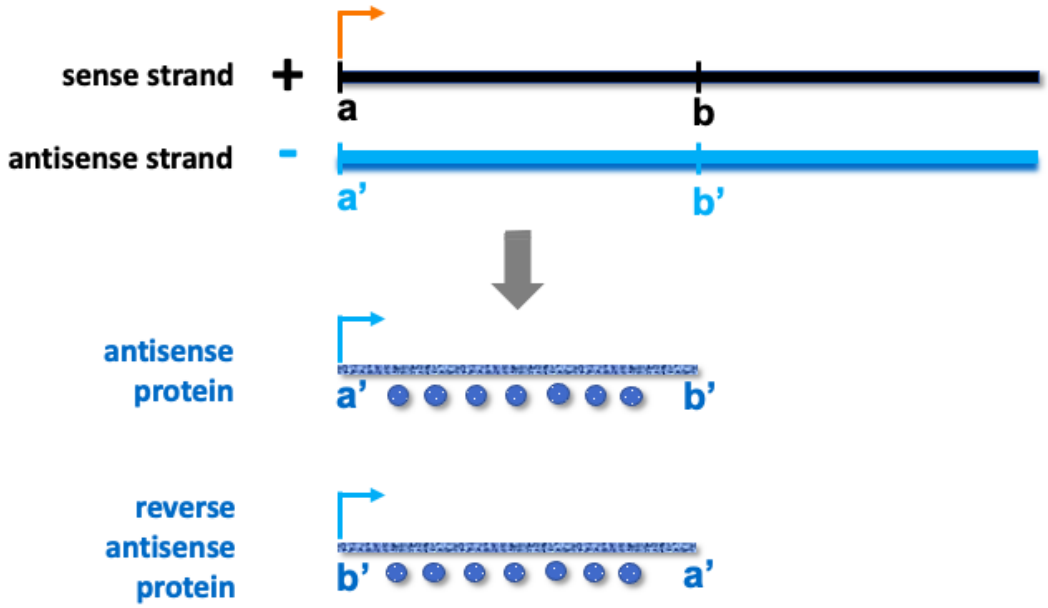



*Fig.1- Schematic diagram for making genes and proteins from antisense strand of DNA (indicated in blue color). Orange colored arrow indicates the direction of natural expression.*

## 2.2 Protein Sequence Similarity With Previously Available Database

In the next step, full length antisense translated sequences were matched against peptides and protein sequences available in the NCBI Non Redundant Protein Database (Altschul et al., 1990). Only those sequences were included further in the study that did not show any sequence similarity with the available protein sequences.

## 2.3 Physicochemical properties of protein Sequences

Physicochemical properties of Sequences checked by Expasy-ProtParam tool of SIB Swiss Institute of Bioinformatics (Roy et al., 2011). The ProtParam tool computes various physical and chemical properties of protein sequences such as molecular weight, theoretical pI, instability index and grand average of hydropathicity (GRAVY) index.

## 2.4 Structure and Function Prediction

Structure and Function was predicted by the I-TASSER Server (Iterative Threading ASSEmbly Refinement) tool (Zhang, 2008). I-TASSER predicts the secondary and tertiary structure of a protein along with probable functions. Subcellular localisation of protein was computed by WoLF PSORT tool (Horton et al., 2007).

### 2.5 Stereochemical properties of protein models

Stereochemical properties of the protein model were studied by using Procheck Server. The geometry of amino acids assessed by Ramachandran plot. Ramachandran Plot is a two-dimensional (2D) plot between the torsional angles of amino acids phi (φ) and psi (ψ) in protein sequences (Hollingsworth & Karplus, 2010).

## 3. Results

### 3.1 Translation of Antisense and Reverse Antisense Sequences

The computational translation of the antisense DNA sequences showed several full length gene sequence equivalents (i.e., without stop codon) in the forward and reverse directions (Table 1).

**Table 1 Full length gene sequence equivalents in *E. coli*, *S. cerevisiae* and *D. melanogaster***

| S.No. | Full length protein sequences | *E. coli* (4315 protein coding genes) | *S. cerevisiae* (6317 protein coding genes) | *D. melanogaster* (13,962 protein coding gene) |
|---|---|---|---|---|
| 1 | antisense proteins | 32 | 10 | 33 |
| 2 | reverse antisense proteins | 208 | 35 | 306 |

### 3.2 Sequence Similarity

The full length antisense and reverse antisense sequences from *E. coli*, S. cerevisiae and Drosophila were matched against the existing NCBI protein sequence database. Some sequences are not found to have any similarity with previously available protein databases (Novel protein) and remaining sequences show similarity in different percentage. (Table 2)

**Table 2 Sequence similarity of the full length antisense and reverse antisense sequences of *E. coli*, *S. cerevisiae* and *D. melanogaster* (with the existing protein sequence data)**

| S. No. | Similarity Range → / Full length sequences | Unique (No Similarity ) | Similarity < 30% | Similarity 30% - 80% | Similarity above 80% |
|---|---|---|---|---|---|
| **1.** | ***E. coli*** | | | | |
| a. | antisense protein Sequences | 9 | 0 | 18 | 5 |
| b. | reverse antisense protein sequences | 6 | 5 | 52 | 145 |
| **2.** | ***S. cerevisiae*** | | | | |
| a. | antisense protein sequences | 7 | 0 | 3 | 0 |
| b. | reverse antisense protein sequences | 18 | 1 | 6 | 10 |
| **3.** | ***D. melanogaster*** | | | | |
| a. | antisense protein sequences | 25 | 1 | 7 | 0 |
| b. | reverse antisense protein sequences | 209 | 32 | 56 | 9 |

**Table 3 Predicted identification and similarity of putative antisense and reverse antisense proteins of *E. coli*, *S. cerevisiae* and *D. melanogaster***

| S. No | Full Length Sequences | Name of Protein | Similarity (%) |
|---|---|---|---|
| **1.** | ***E. coli*** | | |
| a. | antisense protein Sequences | hypothetical protein E3J21_09715 [*Anaerolineales bacterium*] | ~90% |
| | | TonB-dependent receptor [*Thermonema lapsum*] | ~80% |
| b. | reverse antisense protein sequences | hypothetical protein DP20_3794 [*Shigella flexneri*] | ~100% |
| | | Hypothetical protein c4084 [*Escherichia coli* CFT073] | ~100% |
| **2.** | ***S. cerevisiae*** | | |
| a. | antisense protein sequences | SET domain [*Plasmodium cynomolgi* strain B] | ~55% |
| | | unnamed protein product [*Ostreobium quekettii*] | ~50% |
| b. | reverse antisense protein sequences | hypothetical protein SCEN_M04740 [*Saccharomyces cerevisiae*] | ~100% |
| | | hypothetical protein [*Virgibacillus massiliensis*] | ~100% |
| **3.** | ***D. melanogaster*** | | |
| a. | antisense protein sequences | hypothetical protein SLA_2901 [*Streptomyces laurentii*] | ~65% |
| | | hypothetical protein [*Vespula vulgaris*] | ~65% |
| b. | reverse antisense protein sequences | GM24575 [*Drosophila sechellia*] | ~100% |
| | | TPA: HDC08312 [*Drosophila melanogaster*] | ~100% |

**Table 4 Physicochemical properties of hypothetical antisense and reverse antisense proteins from *E. coli, S. cerevisiae and D. melanogaster***

| Type | amino acid residues Length (range) | Molecular weight (kDa) (range) | Theoretical pI (range) | Instability index (range) | Grand average of hydropathicity (GRAVY) (range) | G-C % (range) |
|---|---|---|---|---|---|---|
| ***E. coli*** | | | | | | |
| Antisense Protein | 31 - 78 | 3.8-9.1 | 6.69-12.55 | 10 - 118 | -0.496 - 0.672 | 40.9-50.8 |
| Reverse Antisense Protein | 44 - 70 | 5.1-8.3 | 6.68-11.84 | 30 - 71 | -1.792 - 0.468 | 39.5-59.7 |
| ***S. cerevisiae*** | | | | | | |
| Antisense Protein | 30 - 94 | 3.8-11.7 | 5.97-12.16 | 33 - 86 | -0.977 - 0.472 | 33.3-47.3 |
| Reverse Antisense Protein | 32 - 121 | 4.0-13.3 | 4.43-11.73 | 10 - 67 | -1.322 - 0.866 | 31.7-53.4 |
| ***D. melanogaster*** | | | | | | |
| Antisense Protein | 33 - 178 | 3.9-20.6 | 7.75-12.02 | 11 - 94 | -0.963 - 1.418 | 44.4-64.4 |
| Reverse Antisense Protein | 32 - 400 | 3.6-43.0 | 3.88-13.04 | 4.5-134 | -1.287 - 1.586 | 34.3-64.3 |

### 3.3 Physicochemical properties

Antisense and reverse antisense encoded proteins were studied for their physicochemical properties (Table 4). Molecular Weight of antisense proteins are found to range from 3.8 KDa to 43.0 KDa, with Isoelectric Point (pI) value ranging from 3.80 to 13.04 (pI <7 show acidic nature whereas pI >7 indicate basic nature), Instability index ranged from 4.5 - 134, hydropathicity value (GRAVY) ranged from -

0.496 to 0.672 showing interaction with water (low GRAVY score indicates better interaction with water), with G-C ratio ranging from 33.3 - 64.4.

### 3.4 Structure and Function Prediction

Structure and function prediction of dark proteins predicted by I-Tasser showed a favorable C-score i.e., within the range of -5 to +2.

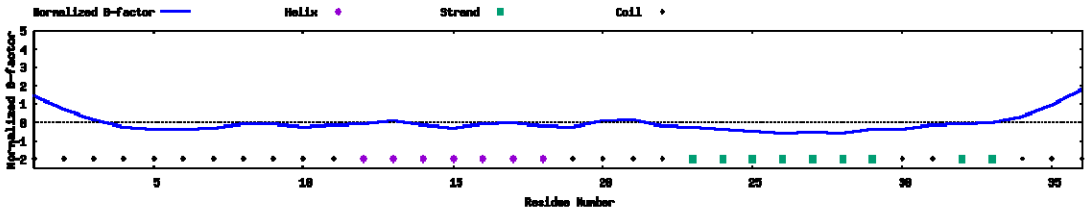
**Table 5. Predicted structure and function prediction of putative antisense and reverse antisense proteins of *E. coli*, *S. cerevisiae* and *D. melanogaster***

| Organisms | structure prediction (C-score range) | function prediction (types) | subcellular localizations |
|---|---|---|---|
| ***E. coli*** | *-1.76 to -3.29* | *transferase, electron transport, oxidoreductase, hydrolase* | *secreted, mitochondrial, cytoplasmic and nuclear* |
| ***S. cerevisiae*** | *-1.33 to -4.02* | *transferase, hydrolase, electron transport, protein transport, oxidoreductase* | *secreted, mitochondrial, cytoplasmic and nuclear* |
| ***D. melanogaster*** | *-0.67 to -4.11* | *hydrolase, lyase, ligase, oxidoreductase, isomerase, Transferase,* | *secreted, mitochondrial, peroxisomal, nuclear, and cytoplasmic* |

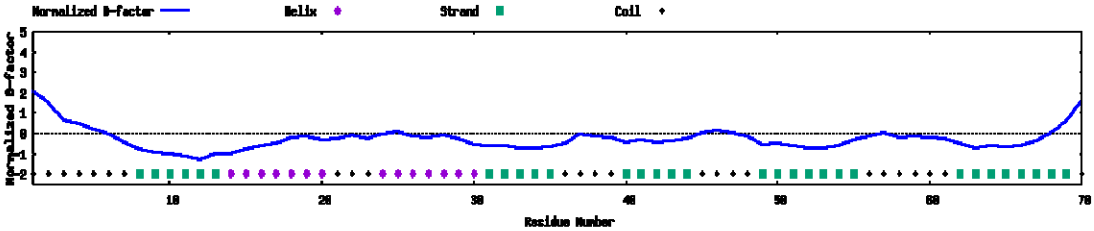
The secondary and tertiary prediction of protein structures (predicted by I-TASSER) alongwith normalized B factor (BFP) profile were found within a favorable range. BFP shows experimental structure stability of proteins. Subcellular localization (WoLF PSROT server) showed predominant localisation of proteins to mitochondria and several cytoplasmic destinations, while some were found to be secretory proteins (Table 5).

***E. coli***

## Antisense Protein

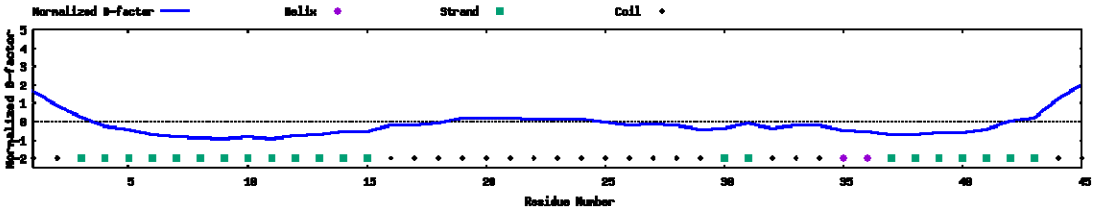



## Reverse Antisense Protein

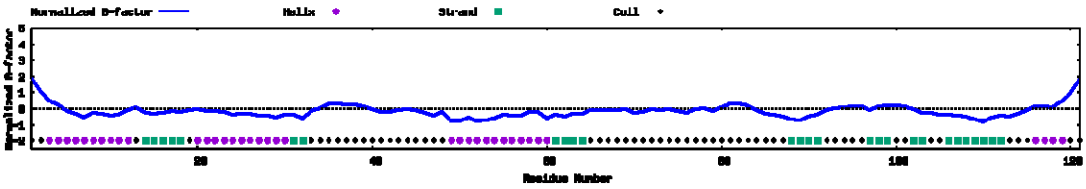



*S. cerevisiae*

## Antisense Protein

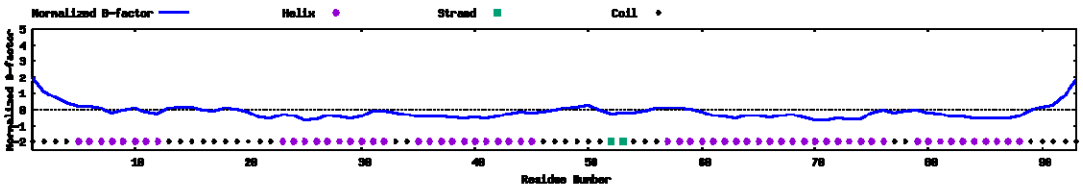



## Reverse Antisense Protein




*D. melanogaster*

## Antisense Protein




**Reverse Antisense Protein**

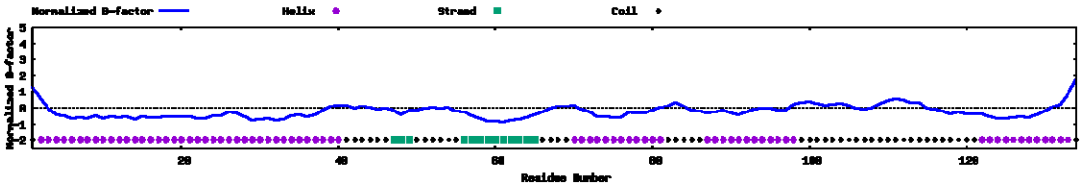



*Fig. 2 Normalized B-Factor Profile of antisense and reverse antisense proteins in E.coli, S.cerevisiae and D.melanogaster with most of the residues below 0 indicating the stability of proteins.*

| ***E. coli*** | |
|---|---|
| **Antisense Protein** | **Reverse Antisense Protein** |
| | |
| ***S. cerevisiae*** | |
| **Antisense Protein** | **Reverse Antisense Protein** |

***D. melanogaster***

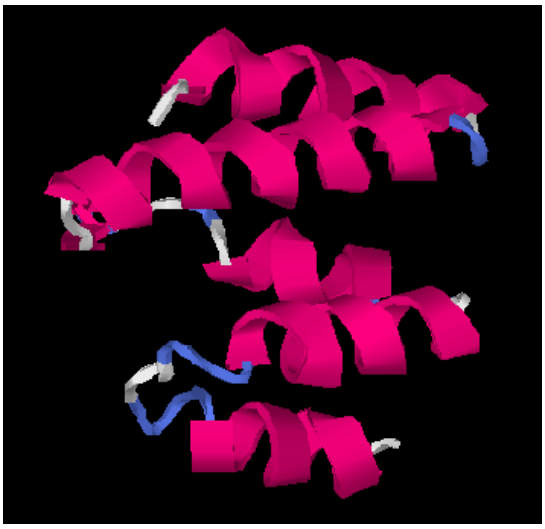
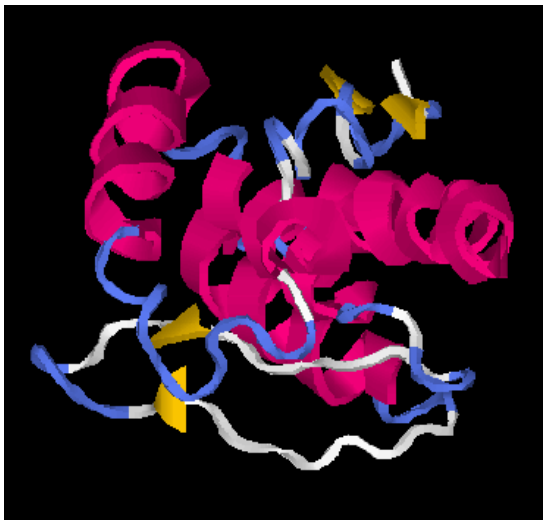
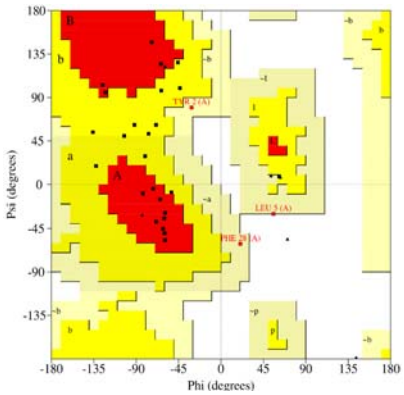
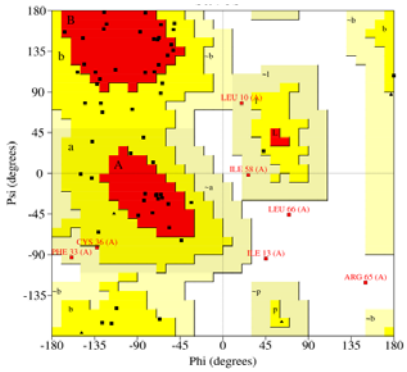
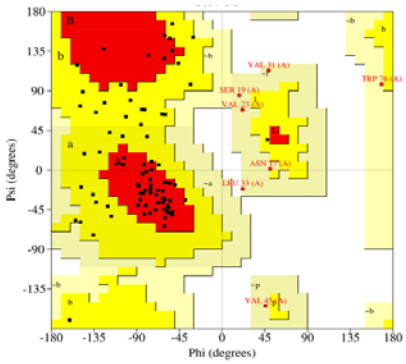
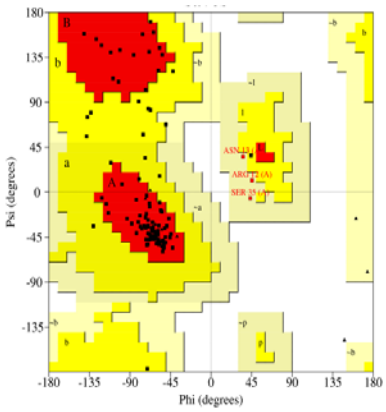
| Antisense Protein | Reverse Antisense Protein |
|---|---|
|  |  |

*Fig. 3 - Tertiary structure of antisense and reverse antisense protein sequences sampled from E.oli, S.cerevisiae and D.melanogaster resembling naturally expressing proteins (prediction tool: I-TASSER)*

### 3.5 Stereochemical properties

Ramachandran Plot was used to study the stereochemical properties of proteins (Fig 4). Most of the residues were found in the favourable regions indicating good protein folding profile.

| *E. coli* | |
|---|---|
| Antisense Protein | Reverse Antisense Protein |
|  |  |
| *S. cerevisiae* | |
| Antisense Protein | Reverse Antisense Protein |
|  |  |

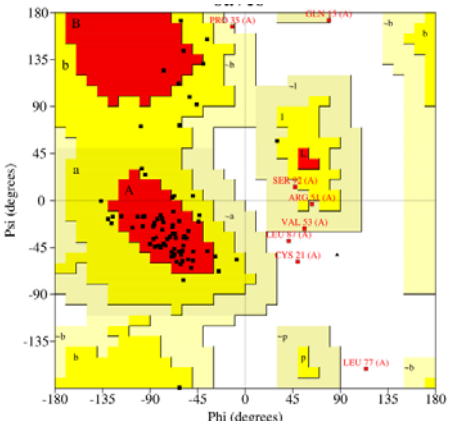
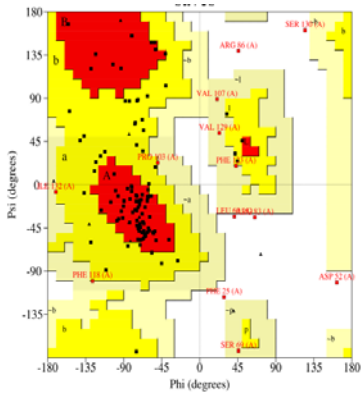
| *D. melanogaster* | |
|---|---|
| Antisense Protein | Reverse Antisense Protein |
|  |  |

*Fig. 4 - Ramachandran Plot of antisense and reverse antisense protein sequences of E.coli, S.cerevisiae and D.melanogaster. Most of the amino acid residues were found to be present in red and dark yellow regions.*

## 4 Discussion

The term ‘dark-genome‘ refers to fully dark (never expressing), partially dark (RNA expressing) and virtually dark (pseudogenes) sequences that can be artificially expressed into functional peptides and proteins. The fully dark sequences comprises antisense, reverse coding, highly repetitive sequences and intergenic sequences. The “partially dark” genome comprises sequences that didn’t go beyond transcription i.e., tRNA, long ncRNA, ribosomal RNA and intron sequences. The “virtually dark” genome comprises sequences like pseudogenes that were previously expressed but were retired during the course of evolution.

This study using *E.coli*, *S.cerevisiae* and *D.melanogaster* is an extension of our previous work (Dhar et al., 2009) where intergenic sequences of E.coli were artificially expressed into cell growth inhibiting proteins. Subsequently, it was observed that pseudogenes also have a potential to generate stable and functional proteins, if expressed (Shidhi et al., 2015)

Given that genes are found on both the strands, the term antisense does not indicate an “end-to-end” stretch of complementary DNA. Thus, sense and antisense only indicate a specific arrangement on a particular genomic address. In this study, we considered full length hypothetical genes on the antisense strands in the forward and

reverse directions. DNA sequences that showed stop codons on computational translation were discarded.

A strong evidence of unutilized genomic potential in the form of hypothetical full length antisense proteins and reverse antisense proteins were observed in *E.coli* (0.7% and 5.1%), *S.cerevisiae* (0.15%, 0.5%) and *D.melanogaster* (0.2%, 2.1%), respectively. The physicochemical properties of these molecules favored the possibility of a well defined structure and function.

Most of the proteins considered in this study were predicted to be located to various cellular locations with some proteins showing possibility of secretory nature. The functional prediction tools mapped many of these proteins to transporter and enzyme functions.

To our best knowledge, this is the first report that describes repurposing of antisense DNA sequences towards generating functional biomolecules. An additional parameter of “reverse antisense” proteins was considered in this study, to raise more evolutionary questions and increase the inventory of synthetic biomolecules. In future, more work is needed to release the raw antisense and reverse antisense data in the form of a publicly available database and experimentally validate some of the key predictions.

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