Getting Lucky: Using Proteomic Analysis and Creating CS-Beacon to Describe the Fortunato Genome

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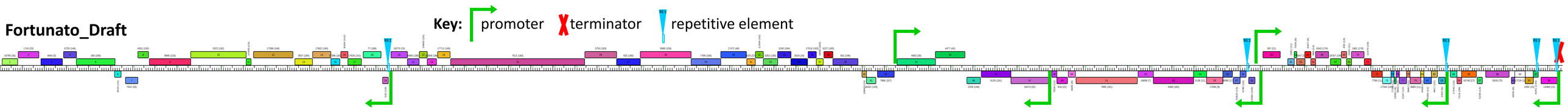
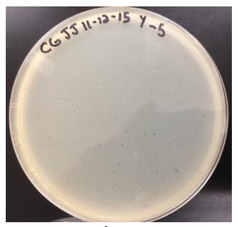
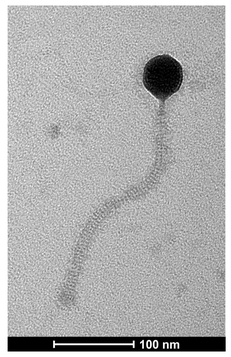


About Fortunato

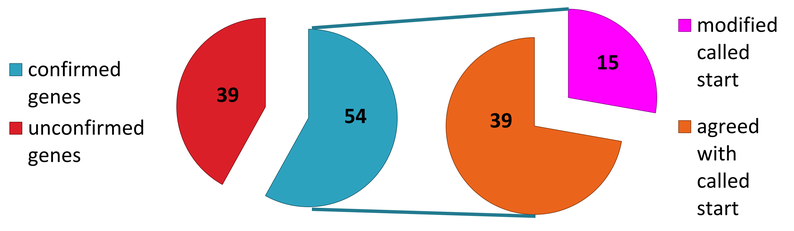
- B4 *Mycobacterium* phage
- Isolated by direct plating
- Plaques small (< 1mm) and hazy with sharp edges
  - B phage known not to have integrase or to produce lysogens
- Siphoviridae
- 70679 bp, circularly permuted genome
- 94 predicted genes
- 69.0% GC



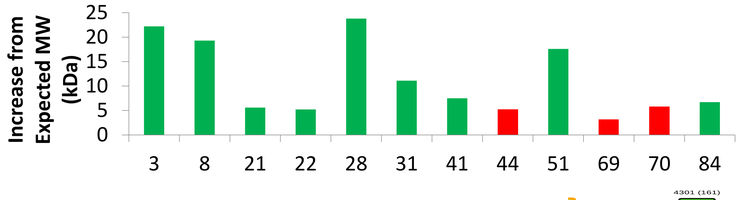


Proteomics

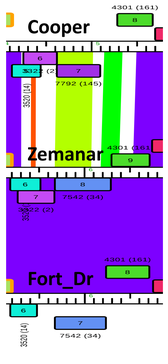
Fifty-four predicted genes were verified by mass spectrometry of Fortunato high-titer lysate. The called starts of **39 of the 54 verified genes were confirmed**.



The start sites of the remaining **15 genes were moved forward,** based on **observation of heavier-than-expected proteins**. The observed molecular masses of 12 of these proteins were at least 1 kDa greater than the expected mass.



Further, the **expression of ORF 6.5 was verified**. While ORF 6.5 has a 82bp overlap with ORF 6, proteins from both were detected by mass spectrometry. ORF 6.5 had previously been called in B4 phages Cooper and Zemanar.



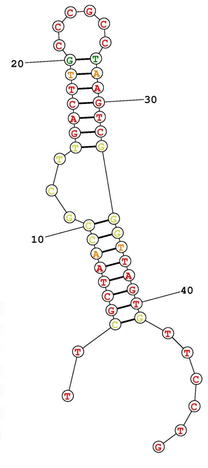
Promoters

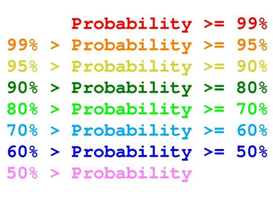
Two putative forward and five reverse promoters were identified using DNAMaster ( ) and PePPER ( ).

Location of putative promoter (bp of 5' end)	Direction of putative promoter	Found by DNAMaster?	Found by PePPER?
bp 17618	reverse		X
bp 40501	forward	X	X
bp 47540	reverse		X
bp 56854	forward		X
bp 56989	reverse		X
bp 65427	reverse	X	X
bp 70494	reverse	X	X

Terminators

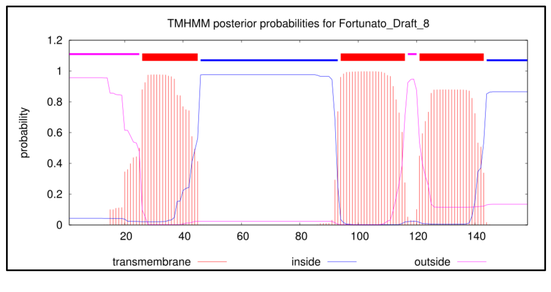
One putative rho-independent terminator was identified at bp 70493 using ARNold ( ). (FindTerm ( ) did not identify any terminators.)
The putative terminator, diagrammed using RNAStructure Web ( ) and shown at the right, had an estimated free energy of -7.00.





Transmembrane Proteins

A Fortunato protein fasta file downloaded from Phamerator was analyzed using TMHMM ( ) and SOSUI ( ), transmembrane helix predictors. **Eleven proteins with putative transmembrane domains** were identified in Fortunato.
The TMHMM output for gp 8 is shown at right; predicted transmembrane helices are indicated by red spikes.



Repetitive Regulatory Elements: CS-Beacon

Repetitive elements were identified using **CS-Beacon, ( ) a new, fully-customizable program that we wrote** to find sequences of specified similarity **in genomes of any length**.
CS-Beacon searches for putative repetitive elements **based on**

- **Percent similarity t**o another sequence in the genome
- **Sequence length**
- **Number of occurrences** in the genome

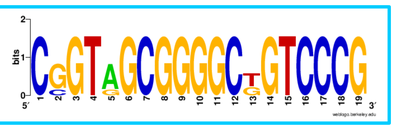
Remarkably, each of these parameters may be adjusted by the user through a command-line user interface.

Run with conservative settings of 80% sequence similarity and at least 4 occurrences, **CS-Beacon identified multiple putative repetitive elements** throughout the Fortunato genome. Of these, **one was consistently found in intergenic regions**, suggesting that it may have regulatory function.
In fact, this putative, repetitive regulatory element **frequently co-occurred with reverse promoters**. CS-Beacon sample outputs and the WebLogo representation are shown below.

```
From File:Fortunato.fasta
CGGTAGCGGGGCGGTCCCG
| |||||||||| ||||||
CCGTAGCGGGGCTGTCCCG
Direction:FWD
Starts at:17583
Found At:56387
Similarity:89.47%
Length of:19

From File:Fortunato.fasta
CGGTAGCGGGGCGGTCCCG
|||| ||||||| ||||||
CGGTGGCGGGGGCTGTCCCG
Direction:FWD
Starts at:17583
Found At:65392
Similarity:89.47%
Length of:19
```

Repetitive Element 1



Acknowledgements

The Fortunato electron micrograph was taken at the University of Arkansas for Medical Sciences (UAMS). Mass spectrometry of Fortunato high-titer lysate was performed at UAMS and was funded by Arkansas INBRE through a student voucher grant.

UAMS University of Arkansas for Medical Sciences

Arkansas INBRE IDeA Networks of Biomedical Research Excellence

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SEA PHAGES

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