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Identifying Genomic Islands with Deep Neural Networks

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ABSTRACT

Horizontal Gene Transfer(HGT) is the main source of adaptability for bacteria, allowing it to obtain genes from different sources like bacteria, archaea, viruses, and eukaryotes. This promotes the rapid spread of genetic information across lineages, typically in the form of clusters of genes referred to as genomic islands(GIs). There are different types of GIs, often classified by the content of their cargo genes or their means of integration and mobility. Different computational methods have been devised to detect different types of GIs, but there is no single method that is capable of detecting all GIs. The intrinsic value of machine learning methods lies in their ability to generalize. We propose a method(we call it Shutter Island) that uses deep learning, or more specifically, the Inception V3 model, to detect Genomic Islands in bacterial genomes. We show that using this approach, it is possible to generalize better than the existing tools, detecting more of their correct results than other tools, while making novel GI predictions.

INTRODUCTION

Interest in Genomic Islands resurfaced in the 1990s, when some Escherichia Coli strains were found to have exclusive virulence genes that were not found in other strains(4, 54). These genes were thought to have been acquired by these strains horizontally, and were referred to as pathogenicity islands (PAIs). Further investigations showed that other types of islands carrying other types of genes exist, giving rise to more names like secretion islands, resistance islands, and metabolic islands, considering the fact that the genes carried by these islands could promote not only virulence, but also symbiosis or catabolic pathways(5, 14, 28). Aside from functionality, different names are also assigned to islands on the basis of their mobility. Some GIs are mobile and can thus move themselves to new hosts, such as conjugative transposons, integrative and conjugative elements(ICEs), and prophages, whereas other GIs are not mobile anymore(42, 43). Prophages are viruses that infect bacteria and then remain inside the cell and replicate with the genome(44). They are also referred to as bacteriophages in some literature, constituting the majority of viruses, and outnumbering bacteria by a factor of 10 to 1(45, 46).

A Genomic Islands(GI) then is a cluster of genes that is typically between 10-200kb in length and has been transferred horizontally and (59).

HGT may contribute to anywhere between 1.6% to 32.6% of their genomes(15, 16, 17, 18, 38, 48, 49, 55, 57).This naturally implies that a major factor in the variability across bacterial species and clades can be attributed to GIs(19). Which also implies that they impose an additional challenge in our ability to reconstruct the evolutionary tree of life.

The identification of GIs is also important for the advancement of medicine, by helping develop new vaccines and antibiotics(20, 61), or even cancer therapies(2). For example, knowing that PAIs can carry many pathogenicity and virulence genes(11, 21, 22), potential vaccine candidates were found to reside within PAIs(23).

While early computational methods focused on manual inspection of disrupted genomic regions that may resemble GI attachment sites(56) or show unusual nucleotide content(34, 47), the most recent computational methods fall into two broad categories: methods that count on sequence composition, and methods that count on comparative genomics(54). They both focus on one or more of the features that make GIs distinct. A lot of research has been dedicated to identify these features such as compositional bias, mobility elements, and transfer RNA(tRNA) hotspots (6, 11, 12, 13, 14, 21, 43, 59). We discuss some of these features in more detail, listed by decreasing order of importance(7, 54):

- One of the most important features of GIs is that they are sporadically distributed, i.e only found in certain isolates from a given strain or species.
- Since GIs are transferred horizontally across lineages, and different bacterial lineages have different sequence compositions, measures such as GC content, or more generally oligonucleotides of various lengths(usually 2-9 nucleotides), are being used(3, 8, 24, 25, 26, 27, 51, 52). Codon usage is a well known metric, which is the special case of oligonucleotides of length three.
- Since the probability of having outlying measurements decreases as the size of the region increases, tools usually use cut-off values for the minimum size of a region(or gene cluster) to be identified as a GI.

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- Another type of evidence comes not from the attachment sites but whats in between, as some genes(e.g integrases, transposases, phage genes) are known to be associated with GIs(11).

- In addition to the size of the cluster, evidence from mycobacterial phages(32) suggests that the size of the genes themselves is shorter in GIs than in the rest of the bacterial genome. The reason may be unknown but different theories suggest that this may confer mobility or packaging or replication advantages(44).

- Some GIs integrate specifically into genomic sites such as tRNA genes, introducing flanking direct repeats. So the presence of such sites and repeats may be used as evidence for the presence of GIs(33, 58, 64).

"Other tools report the directionality of the transcriptional strand and the protein length to be among the most important features in GI prediction(44)." The available tools focus on one or more of the mentioned features. *Islander* works by first identifying tRNA and transfer-messenger RNA genes and their fragments as endpoints to candidate regions, then disqualifying candidates through a set of filters such as sequence length and the absence of an integrase gene(5).

IslandPick identifies GIs by comparing the query genome to a set of related genomes selected by an evolutionary distance function(31). It uses Blast and Mauve for the genome alignment. The outcome heavily depends on the choice of reference genomes selected. *Phaster* uses BLAST against a phage-specific sequence database(the NCBI phage database and the database developed by Srividhya et al in (39)), followed by DBSCAN(40) to cluster the hits into prophage-regions.

IslandPath-DIMOB considers a genomic fragment to be an island if it contains at least one mobility gene, in addition to 8 or more consecutive open reading frames with di-nucleotide bias(41). *SIGI-HMM* uses the Viterbi algorithm to analyze each genes most probable codon usage states, comparing it against codon tables representing microbial donors or highly expressed genes, and classifying it as native or non-native accordingly (60). *PAI-IDA* uses the sequence composition features, namely GC content, codon usage, and dinucleotide frequency to detect GIs(9).

Alien Hunter (or IVOM) uses k-mers of variable length to perform its analysis, assigning more weight to longer kmers (52).

Phispy uses random forests to classify windows based on features that include transcription strand directionality, customized AT and GC skew, protein length, and abundance of phage words (44).

Phage Finder classifies 10kb windows with more than 3 bacteriophage-related proteins as GIs (35).

MSGIP—Mean Shift Genomic Island Predictor is a non-parametric clustering algorithm that executes a gradient ascend on local estimated density until the convergence of each data point (8).

IslandViewer is an ensemble method. It combines the results of three other tools into one web resource. Specifically, it merges the results of SIGI-HMM, IslandPath-DIMOB, and IslandPick (36).

No single tool is able to detect all GIs in all bacterial genomes(47). Naturally, methods that narrow their search to GIs that integrate under certain conditions, such as into tRNAs, miss out on the other GIs. Similarly, not all GI regions exhibit atypical nucleotide content(20, 37). Evolutions such as gene loss and genomic rearrangement (14) present more challenges. Also, highly expressed genes(e.g genes in ribosomal protein operons), or having an island host and donor that belong to the same or closely related species, or the fact that amelioration would pressure even genes from distantly related genomes to adapt to the host over time, would lead to the host and the island to exhibit similar nucleotide composition(63). and subsequently to false negatives(61).

For tools that use windows, one challenge is the difficulty in adjusting their sizes, with small sizes leading to large statistical fluctuation and bigger sizes leading to a low resolution(62)."

When it comes to comparative genomics methods, the outcomes strongly depends on the choice of genomes used in the alignment process. Where very distant genomes may lead to false positives and very close genomes may lead to false negatives.

In general, the number of reported GIs may differ across tools, because one large GI is often reported as a few smaller ones or vice versa, also making it harder to detect end-points and boundaries accurately, even with the use of HMM by some tools like AlienHunter and SIGI-HMM.

"Last but certainly not least, there is no reliable GI dataset to validate all these computational methods predictions(42). " Although several databases exist, they usually cover only specific types of GIs [Islander, PAIDB, ICEberg], which would flag any extra predictions made by those tools as false positives. Moreover, "the reliability of the databases has not been verified by any convincing biological evidence(42)."

MATERIALS AND METHODS

PATRIC(The Pathosystems Resource Integration Center) is a bacterial Bioinformatics resource center(https://www.patricbrc.org)(10). It provides researchers with the tools necessary to analyze their private data, and the means to compare it to public data. It recently surpassed the 200,000 publicly sequenced genomes mark, further diminishing the challenge of having enough genomes in comparative genomics methods. One of the services PATRIC provides is the compare region viewer service, where a query genome is aligned against a set of other related genomes anchored at a specific focus gene/peg. The service starts with finding other pegs that are of the same family as the focus peg, and then aligning their flanking regions accordingly. Such graphical representations are appealing as they help users visualize the genomic areas of interest. Looking at the resulting plots, genomic islands should appear as gaps in alignment as opposed to conserved regions.

To get an idea about how the data looks like, figure 1 shows some sample visualizations of different genomic fragments belonging to the two classes. Each row represents a genome, with the query genome being the first row. Each arrow represents a single gene, capturing its size and strand directionality. The red arrow is reserved to represent the focus gene in the query genome, at which the alignment with the rest

of the genomes is anchored. The rest of the genes share the same color if they belong to the same family, or are colored black if they are not found in the query genome. Some colors are reserved to key genes, green for mobility genes, yellow for tRNA genes, and blue for phage related genes.

You could see from figure 1,a-b how lonely the query genome looks. The figures show that the focus peg lacks alignments in general, or is being aligned with genes from other genomes with different neighborhoods, containing genes with different functionalities than those in the query genome(functionality is color coded). On the contrary, figure 1,c-d show more conserved regions, which are what we expect to see in the absence of GIs.

This kind of representation also makes it easier to leverage the powerful machine learning(ML) technologies that have become the state of art in solving computer vision problems. Algorithms based on Deep Neural Networks have proven to be superior in competitions such as ImageNet Large Scale Visual Recognition Challenge(ILSVRS)(50). Deep learning is the process of training neural networks with many hidden layers. The depth of these networks allows them to learn more complex patterns and higher order relationships, while being more computationally expensive and requiring more data to work effectively. Improvements in such algorithms have been translated to improvements in a variety of domains reliant on computer visions tasks(29).

In particular, deep learning has been demonstrated to be very effective in solving a related task. Researchers trained the Inception V2 model(the predecessor of the model we are deploying) to call SNPs and small indel variants using graphical representations of alignment data. Their model performed better than other statistical methods and won the ”highest performance” award for SNPs in a FDA-administered variant calling challenge(53).

The images generated by PATRIC capture many of the most important features mentioned earlier. Namely the sporadic distribution of islands, the protein length, functionality, and strand directionality, using color coded arrows of various sizes.

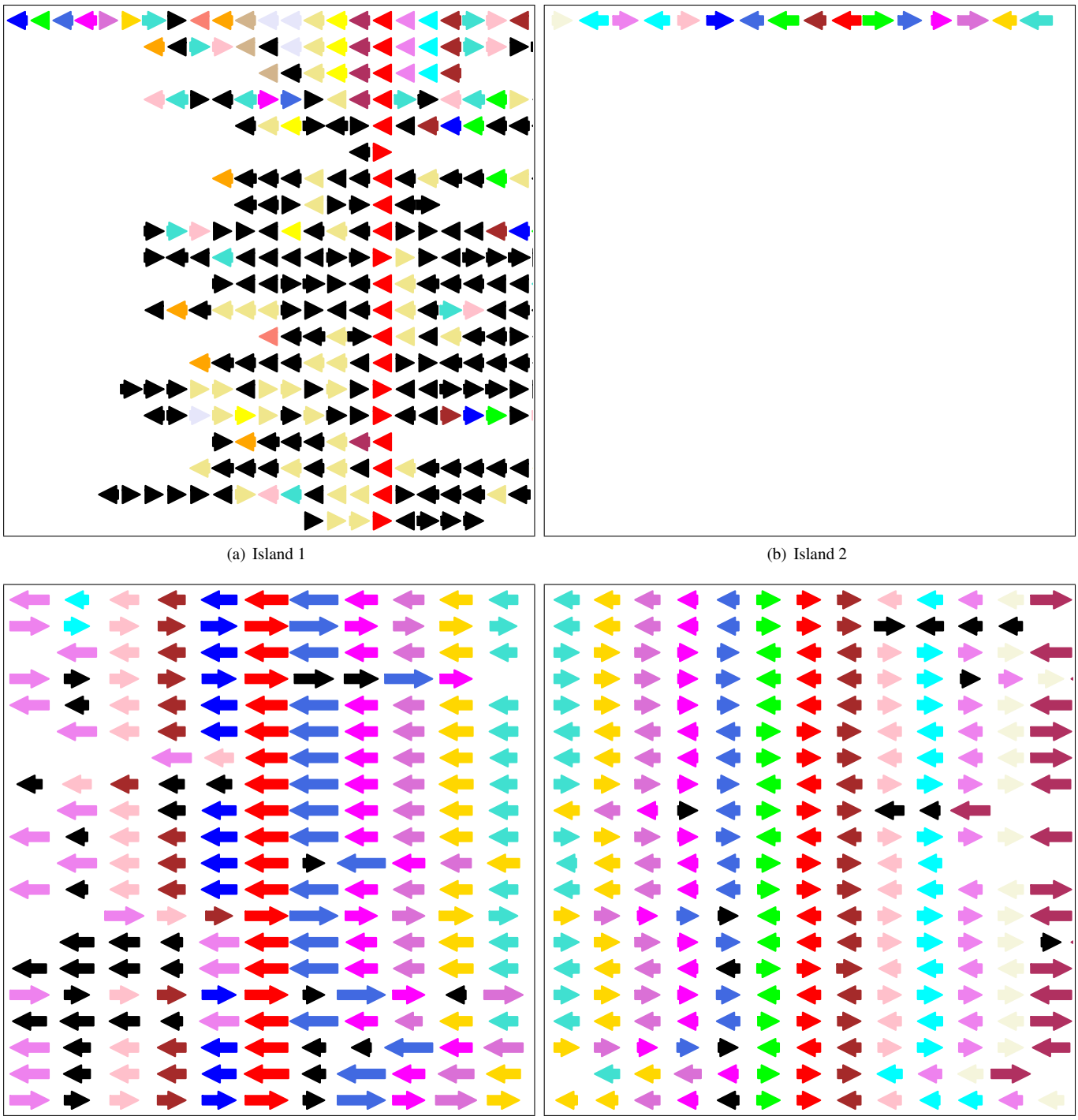
So while PATRIC provides a lot of genomic data, the challenge comes down to building a meaningful training dataset. The databases available are still very limited in size and specific in content, which in turn limits the ability even for advanced and deep models to learn and generalize well. Training deep models over a limited dataset puts the model at the risk of over-fitting. One way around this problem is using a technique referred to as transfer learning(30). In transfer learning, a model does not have to be trained from scratch. Instead, the idea is to retrain a model that has been previously trained on a related task. The newly retrained model should then be able to transfer its existing knowledge and apply it to the new task. This approach gives us the ability to reuse models that have been trained on huge amounts of data, while adding the necessary adjustments to make them available to work with more limited datasets, adding a further advantage to our approach of representing the data visually.

In our approach(we call it Shutter Island), we used Google’s Inception V3 architecture that has been previously trained on ImageNet. The Inception V3 architecture is a 48 layer deep convolutional neural network(29). Training such a deep network on a limited dataset like the one available for GIs wouldn’t promise great results. ImageNet is a database that contains more than a million images belonging to more than a thousands categories(50). The ImageNet project runs the aforementioned ILSVRC annually. The Inception V3 model reaches a 3.5% top-5 error rate on the 2012 ILSVRC dataset, where the winning model that year had a 15.3% error rate.

Thus, a model that was previously trained on ImageNet is already good at feature extraction and visual recognition. To make the model compatible with the new task, the top layer of the network is retrained on our GI dataset, while the rest of the network is left intact, which is more powerful than starting with a deep network with random weights.

For our training data, we used the set of reference+representative genomes found on PATRIC. For each genome, our program produces an image for every non-overlapping 10kb window. A balanced dataset was then curated from the total set of images created. Since this is a supervised learning approach, and our goal is to generalize over the tools predictions and beyond, we used Phispy and IslandViewers predictions to label the images that belong to candidate islands. IslandViewer captures the predictions of different methods that follow different approaches, while Phispy captures different GI features. While the primary goal is to predict the union of the predictions of other tools and to generalize, we labeled a genomic fragment as a GI only if it belonged to the intersection of the predictions made by these tools. This increases confidence that a certain candidate island is actually so. Overall, the intersection of these tools predictions spanned only almost half of the genome dataset. Our model reached a training accuracy of 92%, with a validation accuracy of 88%. Figure 2 shows the Receiver Operating Characteristic(roc) curve of our classifier. We considered a predicted gene to be a true positive if it overlaps with another tool’s prediction, or falls within a region that shows GI features, and a false positive otherwise. We considered a gene to be a true negative if it does not overlap with any tool’s predictions, or does not fall in an area that includes GI features, and a false negative otherwise. For the areas of interest, we used windows of 4 surrounding genes on each side.

Since there is no reliable benchmark out there, we resorted to using the set of genomes mentioned in (44). The set consists of 41 bacterial genomes that include 190 GIs. This set served as a good common ground for all the tools we mentioned. Some of those tools have not been updated for a while, but all thet tools had predictions made over the genomes in this set. The GIs in the set have also been reported to be manually verified by (44). We discarded the genomes that caused errors with any of the tools used in the comparison and any genomes that were part of the training set. All the presented results are aggregates over the mentioned genome dataset. When treating novel genomes, the same compare genome viewer service was used, aligning the query genome with the set of reference+representative genomes. The only difference is that an image is created for every gene in the genome, providing better resolution over having non-overlapping windows. Then, each window is classified as either part of a GI or not. That label belongs to the focus gene in that window. Eventually, every gene in the genome has a label, and these are clustered into GIs with a minimum cut off value of 8k base pairs(bp).



(a) Island 1

(b) Island 2

(c) Continent 1

(d) Continent 2

Figure 1. Examples of the images generated. Each arrow represents a gene(colors resemble functionality). The genomes are focused around the anchor peg(colored red).

In addition to making use of the powerful technologies and the extensive data, using this approach may add an extra advantage over whole genome alignment methods due to the the fact that performing the alignment over each gene may provide a higher local resolution, and aid in resisting evolutionary effects such as recombination and others that may have happened after the integration, and that usually affect GI detection efforts.

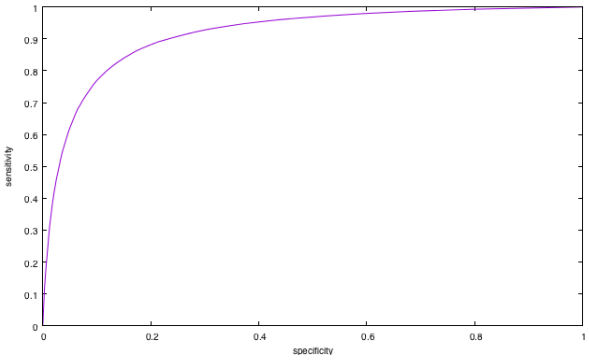


Figure 2. Figure 2: roc curve for our binary classifier. The roc curve plots the True Positive Rate as a function of the False Positive Rate. The greater the area under the curve is (the closer it is to the unrealistic ideal top left corner point), the better.

RESULTS

As mentioned earlier, we used the dataset[supplementary material] containing manually verified GIs as our testing set. Table 1 shows a summary of the tools predictions over the entire genome dataset, listing the number of islands and their total base pair value predicted by each tool. Different tools follow custom defined metrics to judge their results, typically by using a threshold representing the minimum values of features (like number of phage words) present in a region to be considered a GI. Admittedly, judging the results is not a trivial task, else the problem of detecting GIs would have been largely solved by the validation method itself. Since one of the main challenges is the absence of a reliable benchmark and a trivial way to verify the tools' predictions, we judge the results based on two factors: first, we consider the percentage of each tool's results that every tool predicts(table 2). This gives us a measure of how much a single tool can generalize, and how much of the union of all tools' predictions it can get. To give a better idea about each tool's quality of predictions, we show the percentage of those that include features we mentioned earlier that add confidence to our predictions, such as the presence of tRNAs or mobility or phage related genes, as is done by (19).(Tables 3). Second, we consider each tool's unique predictions and their quality based on content(table 4).

Since another challenge is getting precise endpoints for predicted islands, and different tools report a different number of islands owing to the nature of the features they use, where one island could be reported as many or vice versa, we considered a tool to predict anothers islands if any of its predictions overlap with that other tools predictions. Counting the percentage of bp coverage of that other tool as represented by its predicted endpoints. Since in reality, when investigating the islands getting some prediction or overlap is more important than getting the exact same prediction as the other tool.

For table 2, we included the tools that we were able to run. Some tools have not been updated for more than 10 years, we had a problem running those. Other tools(like MSGIP) did not return any GI predictions for our dataset. We used the default parameters for all tools.

Some tools simply make much more predictions than others. So, to get a better idea about the quality of the predictions made and missed by the other tools, we show a breakdown of the percentage of islands with known GI features(i.e tRNA or mobility or phage genes) in table 3. Naturally, tools who use these features to perform their classifications were omitted.

Table 4 shows each tools unique results, also highlighting which of those show GI features and which they dont.

Table 1. Number of islands and their total base pair value predicted by each tool over the testing genomes dataset.

Tool	Number of Islands	Number of Base pairs
Alien Hunter	1919	19,561,593
ShutterIsland	649	10,700,492
IslandViewer	701	10,571,974
IslandPath-Dimob	331	6,871,312
Phaster	109	4,334,225
Phispy	96	3,979,173
Phage Finder	85	3,656,950
IslandPick	356	3,020,733
SIGI-HMM	329	2,543,145
Islander	50	2,019,610

Table 4. Unique predictions made by each tool over the testing genomes dataset.

Tool	Unique(count)	Unique(bp)	with GI features(%)
Alien Hunter	1155	9,583,497	40%
ShutterIsland	280	3,647,377	65%
Phaster	2	30,814	0%
Phispy	1	26,890	100%

DISCUSSION

From table 1 we can see that AlienHunter calls the most GIs, with almost double the amount called by ShutterIsland and IslandViewer if measured by bp count, and even more if measured by island count. ShutterIsland's prediction count is close to IslandViewer's, which is remarkable considering the fact that IslandViewer is an ensemble method combining 4 other tools predictions.

Looking at table 2, it is clear that AlienHunter has the biggest coverage in general when it comes to predicting other tools' results, which is expected given that its predictions' bp coverage is almost 10 times as much as the tools with least coverage. ShutterIsland comes next and predicts the most out of 3 tools' predictions. What is clear is the models ability to generalize, considering that it was only trained on the intersection of the predictions made by Phispy and IslandViewer, but also got the most predictions for other tools

like PhageFinder and Phaster. Finally, you can see that specific tools such as Islander only detect a subset of the results, while the rest of the tools score somewhere in between.

Zooming in on this coverage in table 3, we can notice that on average, ShutterIsland is the tool with most predictions showing GI features being missed by other tools. It is also the tool that calls the most predictions showing GI features and misses the least such predictions made by other tools. So even though AlienHunter makes more predictions in general, more predictions made by ShutterIsland exhibit known GI features.

Both ShutterIsland and AlienHunter have a lot of unique predictions as is clear in table 4. AlienHunters unique predictions alone are almost more than every other tools total predictions. They average 8 kbp in length. ShutterIslands unique predictions are also more than most other tools predictions, with an average length of 14kbp. Applying the same length cutoff threshold(8kbp) on AlienHunter's unique predictions reduces them to 301 islands with a total of 3,880,000bp, which is on par with ShutterIsland's unique predictions. However, unlike AlienHunter, most of ShutterIslands unique predictions show GI features. These are genes that are known to be associated with GIs(e.g, mobile element proteins, phage genes, integrases). We present some snapshopts of typical unique predictions made by ShutterIsland, in addition to a breakdown of the most frequent gene annotations that are included in those predictions.

Example 1:

site-specific recombinase
Protein translocase subunit SecF
Protein translocase subunit SecD
hypothetical protein
Preprotein translocase subunit YajC (TC 3.A.5.1.1)
tRNA-guanine transglycosylase (EC 2.4.2.29)
S-adenosylmethionine:tRNA ribosyltransferase-isomerase
Transcriptional regulator, AsnC family
L-lysine 6-aminotransferase
hypothetical protein
FIG01209928: hypothetical protein
FIG01212260: hypothetical protein
FIG01213367: hypothetical protein
Mobile element protein
Mobile element protein
putative ISXo8 transposase
Mobile element protein
Mobile element protein

Example 2:

Mobile element protein
Phage tail fiber assembly protein
Phage tail fiber protein
Tail fiber assembly protein
Phage tail fiber protein
Putative phage tail protein
Putative phage tail protein
Phage minor tail protein
Putative phage tail protein
putative membrane protein
hypothetical protein

Example 3:

IncF plasmid conjugative transfer pilus assembly protein TraB
IncF plasmid conjugative transfer pilus assembly protein TraK
IncF plasmid conjugative transfer pilus assembly protein TraE
IncF plasmid conjugative transfer pilus assembly protein TraL
IncF plasmid conjugative transfer pilin protein TraA
hypothetical protein
Mobile element protein
IncF plasmid conjugative transfer protein TraD
hypothetical protein
IncF plasmid conjugative transfer DNA-nicking and unwinding protein TraI
IncF plasmid conjugative transfer pilin acetylase TraX
hypothetical protein
FinO, putative fertility inhibition protein
27kDa outer membrane protein
endonuclease
Replication regulatory protein repA2 (Protein copB)
DNA replication protein
hypothetical protein
Putative transposase
Mobile element protein

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Conflict of interest statement. None declared.

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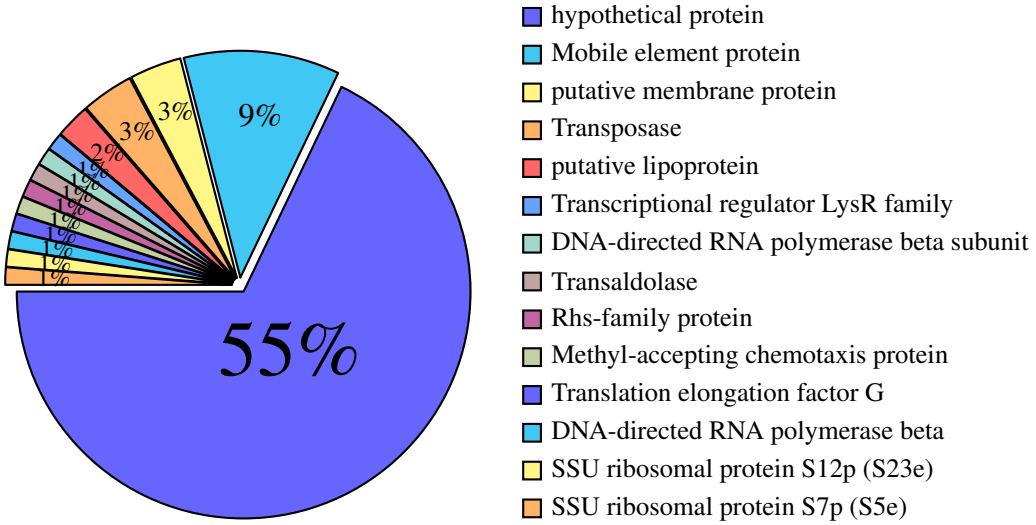

Figure 3. Top 10 genes with the percentage of unique AlienHunter predictions they reside in.

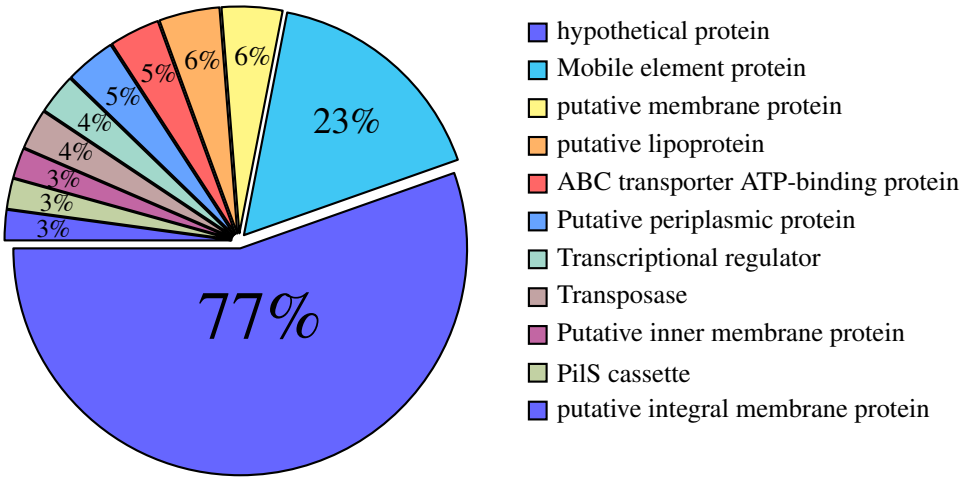

Figure 4. Top 10 genes with the percentage of unique ShutterIsland predictions they reside in.

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Table 2. Predictions of each tool predicted by other tools, over the testing genomes dataset.

Tool	ShutterIsland	IslandViewer	Phispy	PhageFinder	Islander	Phaster	Alien Hunter	IslandPick	Dimob	SIGI
ShutterIsland	N/A	45.7%	97.8%	99.1%	67.4%	92.9%	27%	20.3%	54%	28.8%
IslandViewer	42.8%	N/A	89.3%	89.2%	N/A	82.1%	39.4%	N/A	N/A	N/A
Phispy	29.1%	23.7%	N/A	98.3%	52.8%	79.3%	9%	10.8%	29.1%	11.5%
PhageFinder	28.1%	23.6%	92.8%	N/A	50.4%	79.8%	9%	10.1%	29/3%	12%
Islander	9.2%	21.2%	23.7%	25.7%	N/A	22.2%	8.3%	15.5%	22.9%	17%
Phaster	26.4%	22.5%	82.4%	86%	44.5%	N/A	10.4%	11.3%	27.5%	12.7%
AlienHunter	56.8%	78.9%	87.2%	86.5%	98%	87.2%	N/A	67.1%	82.8%	92.6%
IslandPick	10.6%	43.3%	25.4%	28.7%	51.7%	29%	13.8%	N/A	28.4%	31.2%
Dimob	34.9%	70.5%	86.1%	85.2%	87.8%	76.9%	25.7%	29.5%	N/A	50.3%
SIGI	17.2%	47.7%	34.4%	31.6%	63.2%	27.8%	22.6%	30.9%	44.8%	N/A

Table 3. Quality of tools predictions: % Predictions made with GI features — % Predictions missed with GI features, over the testing genomes dataset.

Tool	ShutterIsland	IslandViewer	AlienHunter	IslandPick	SIGI
ShutterIsland	N/A	91% — 64%	87% — 47%	89% — 31%	87% — 36%
IslandViewer	94% — 67%	N/A	89% — 45%	80% — n/a	87% — n/a
AlienHunter	74% — 70%	66% — 60%	N/A	73% — 21%	71% — 42%
IslandPick	69% — 76%	34% — 86%	49% — 53%	N/A	54% — 44%
SIGI	67% — 75%	45% — 77%	48% — 51%	50% — 35%	N/A
Dimob	n/a — 66%	n/a — 28%	n/a — 43%	n/a — 25%	n/a — 23%
Phispy	n/a — 68%	n/a — 70%	n/a — 50%	n/a — 33%	n/a — 39%
PhageFinder	n/a — 68%	n/a — 70%	n/a — 50%	n/a — 34%	n/a — 39%
Islander	n/a — 75%	n/a — 71%	n/a — 51%	n/a — 33%	n/a — 39%
Phaster	n/a — 75%	n/a — 71%	n/a — 51%	n/a — 33%	n/a — 39%