Taxonomic Assignment in Metagenomics with TANGO

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Abstract

One of the main computational challenges facing metagenomic analysis is the taxonomic identification of short DNA fragments. The combination of sequence alignment methods with taxonomic assignment based on consensus can provide an accurate estimate of the microbial diversity in a sample. In this note, we show how recent improvements to these consensus methods, as implemented in the latest release of the TANGO tool, can provide an improved estimate of diversity in simulated datasets.

Introduction

The diversity and richness of microbial populations can be characterised by several ecological indices, calculated by either grouping similar sequence reads into operational taxonomic units, or assigning them to the most similar taxa in a given taxonomy. While the former is useful for the study of unknown microbial communities, the latter is best suited when sequences and taxonomies of related species are already known.

The usual protocol for taxonomic assignment involves aligning the sequence reads to a set of reference sequences and, then, resolving any ambiguities (that is, a sequence being equally similar to more than one reference sequence) by assigning to a consensus sequence, such as the lowest common ancestor (LCA) of all the candidate sequences in a given taxonomy (Huson *et al.*, 2007; Kunin *et al.*, 2008; Liu *et al.*, 2008). Sequence composition-based methods have also been used in taxonomic assignment (Diaz *et al.*, 2009; McHardy *et al.*, 2007; Wang *et al.*, 2007).

Previous work on taxonomic assignment based on alignment has focused either on sequence reads of the 16S ribosomal RNA gene (Clemente *et al.*, 2010, 2011; Ribeca and Valiente, 2011), or on whole metagenomic shotgun sequence reads (Gerlach *et al.*, 2009; Krause et al., 2008). In this note, we show for the latter that recent improvements to consensus methods, as implemented in the latest release of the TANGO tool (Clemente *et al.*, 2011), bring about an accurate estimate of the actual taxonomic diversity in a metagenomic data-set.

In the improved consensus method, ambiguous sequence reads are assigned to consensus sequences at a lower taxonomic rank than the LCA of the candidate reference sequences (increased specificity), at the expense of discarding some candidate reference sequences (reduced sensitivity). This is done by optimising the combined precision and recall (F-measure) of the taxonomic assignment (Clemente *et al.*, 2010, 2011).

Metagenomic data-set

The complexity of the signal obtained when sequencing metagenomic data makes it necessary to take a standardised data-set as the basis for analysis (Ribeca and Valiente, 2011). We have chosen the metagenomic data-set of Mavromatis *et al.* (2007), which was designed with the goal of simulating microbial communities of varying complexity: low-complexity communities, with one dominant population (simLC), as seen in bioreactor communities (García Martín *et al.*, 2006; Strous *et al.*, 2006); medium-complexity communities, with more than one dominant population flanked by low-abundance populations (simMC), as seen in acid mine drainage biofilm (Tyson *et al.*, 2004)

Table 1. Phylogenetic distribution of the 113 microbial genomes.

Domain	Phylum	Class	Genomes
Bacteria	Actinobacteria	Actinobacteria	9
	Bacteroidetes	Cytophagia	1
	Chlorobi	Chlorobia	7
	Chloroflexi	Chloroflexi	1
	Cyanobacteria	Cyanobacteria	6
	Deinococcus-Thermus	Deinococci	1
	Firmicutes	Bacilli	13
		Clostridia	8
	Proteobacteria	Alphaproteobacteria	17
		Betaproteobacteria	13
		Gammaproteobacteria	25
		Deltaproteobacteria	6
		Epsilonproteobacteria	1
		unclassified Proteobacteria	1
Archaea	Euryarchaeota	Methanomicrobia	3
		Thermoplasmata	1

and symbiotic microbes from eukaryotes (Woyke *et al.*, 2006); and high-complexity communities, with no dominant population (simHC), as seen in agricultural soil (Tringe *et al.*, 2005).

The Mavromatis *et al.* data-set was built by combining Sanger sequence reads selected at random from 113 microbial genomes. The phylogenetic composition of the metagenomic data-set, summarised in Table 1, shows a high abundance of Proteobacteria, Actinobacteria, and Firmicutes, as usual in most metagenomic samples (Gabor *et al.*, 2004; Manichanh *et al.*, 2008).

Table 2. Distribution of sequence reads in the metagenomic data-set.

	simLC	simMC	simHC
Most abundant	28,861	22,956	2,384
2nd abundant	9,277	16,577	2,248
3rd abundant	5,168	10,484	2,191
4th abundant	1,149	6,107	2,127
5th abundant	1,109	4,868	2,083
6th abundant	1,074	1,146	2,051
Rest	50,857	52,319	103,687

The distribution of sequence reads in the metagenomic data-set, summarised in Table 2, shows a low-complexity microbial community, with one dominant population (28,861 sequence reads from *Rhodopseudomonas palustris HaA2*); a mediumcomplexity microbial community, with three dominant populations (22,956 sequence reads from *Bradyrhizobium sp. BTAi1*, 16,577 sequence reads from *Rhodopseudomonas palustris BisB5*, and 10,484 sequence reads from *Xylella fastidiosa Dixon*) flanked by low-abundance populations; and a high-complexity microbial community, with no dominant population.

Aligning sequence reads

The first step in the taxonomic analysis of a metagenomic data-set involves aligning the sequence reads to a database of known sequences from a large set of different organisms. Traditional alignment tools, such as BLAST (Altschul *et al.*, 1990) or BLAT (Kent, 2002), do not scale up to align millions or billions of sequence reads to a large reference genome (Horner *et al.*, 2010; Ribeca and Valiente, 2011; Trapnell and Salzberg, 2009). Microbial genomes are much shorter, though, making these tools appropriate for the alignment of sequence reads from envi-

Table 3: Ambiguous sequence reads in the metagenomic data-set.

Data-set	No hit	One hit	Ambiguous	Total
simLC	59	22,956	2,384	97,495
simMC	76	16,577	2,248	114,457
simHC	100	10,484	2,191	116,771

ronmental samples. Nevertheless, more efficient tools are available for the alignment of short and long sequence reads obtained using high-throughput sequencing technologies, including BWA (Li and Durbin, 2009), BWA/SW (Li and Durbin, 2010), and GEM (Ribeca, 2009).

We have used BLAST to align the 328,723 sequence reads to the 113 microbial genomes. Notice that a larger database is often used when the target sequences are not known beforehand. Ambiguities arise when a sequence read is aligned with more than one target sequence, and we have taken as candidate alignments all those sequences with the same E-value as the top BLAST hit. As shown in Table 3, ambiguous sequence reads represent about 20% of the metagenomic data-set. Sequence reads with no hit in the database of microbial genomes are the result of sequencing errors.

Assigning sequence reads

Once the sequence reads have been aligned to reference sequences, the second step in the taxonomic analysis of a metagenomic data-set involves resolving ambiguities by mapping those reads with more than one possible assignment to species at the closest possible taxonomic rank. We have chosen as taxonomic reference the NCBI taxonomy (Sayers *et al.*, 2009) for the 113 sampled microbial genomes. Again, notice that a larger taxonomy is often used when the target sequences are not known beforehand. Alternative taxonomies for microbial genomes include ARB-SILVA (Pruesse *et al.*, 2007), Greengenes (DeSantis *et al.*, 2006), RDP (Cole *et al.*, 2009), and TOBA (Garrity *et al.*, 2007).

We have used TANGO to assign the 328,723 sequence reads to the 113 microbial genomes at the closest possible taxonomic rank. As shown in Table 4, the optimal consensus method, F-measure-based assignment, resulted in assignments at a lower taxonomic rank than the classical consensus method, LCA-based assignment (Huson *et al.*, 2007).

Taxonomic diversity

Once the sequence reads have been assigned a taxonomy, the third and final step in the taxonomic analysis of a metagenomic data-set involves describing the diversity and richness of the sampled microbial population by means of ecological indices. Some widely accepted notions in ecology are those of α-diversity (species diversity within an ecosystem), β-diversity (change in species diversity within an ecosystem), and ω-diversity (phylogenetic difference between species in an ecosystem) (Faith, 1992; Whittaker, 1972). Among the latter, we have chosen the Clarke-Warwick taxonomic diversity index (Clarke and Warwick, 1998), which measures the

Table 4: Taxonomic distribution of the metagenomic data-set using consensus (LCA, top) and optimal (F-measure, bottom) taxonomic assignment.

Data-set	Taxonomic rank					
	Domain	Phylum	Class	Order	Family	Genus
simLC	126	104	134	56	2,785	5,295
simMC	194	176	174	101	2,784	5,219
simHC	272	219	230	111	822	11,164
simLC		1	65	46	1,236	3,241
simMC		10	90	104	1,179	3,191
simHC		12	145	77	414	6,847

Table 5: Taxonomic diversity (Clarke-Warwick index) of the metagenomic data-set for consensus (LCA) and optimal (F-measure) taxonomic assignment, together with the actual taxonomic diversity.

Data-set	Taxonomic diversity	
	LCA	F-measure
simLC	3.8193	4.5798
simMC	4.1485	4.7993
simHC	4.9433	5.7422

average distance in the taxonomic reference between the sampled species.

As shown in Table 5, the closer the measured taxonomic diversity in the metagenomic data-set is to the actual taxonomic diversity in the sampled population, the more accurate the assignment is: that is, when classical consensus (LCA) is replaced by the optimal consensus (F-measure) method.

Conclusion

The combination of sequence alignment methods with taxonomic assignment based on consensus provides an accurate estimate for the composition of a sample of sequence reads of the 16S ribosomal RNA gene. We have shown that for sequence reads of whole microbial genomes, recent improvements to consensus methods also bring about an accurate estimate of the microbial diversity in a metagenomic sample.

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