iTag amplicon sequencing for taxonomic identification at JGI

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The DOE Joint Genome institute sequences approximately 10,000 amplicon samples per year 3 using primer sets that target prokaryotic, eukaryotic or fungal organisms. This document describes the sequencing and bioinformatic processing of the samples. Information on the laboratory methods is available on the Protocols section of the JGI website.

Sequencing

JGI receives iTag samples in 96 well plates. Plates are quantified and individual libraries are amplified with single barcode primers (Figure 1) according the sequencing standard operating protocol. Samples are pooled at up to 184 samples per sequencing run and sequenced on an Illumina MiSeq sequencer in 2x300 run mode.

Analysis

All data from the sequencer is demultiplexed and stored in JGI's archiving and metadata organizer system (JAMO). Read data is then processed through JGI's centralized rolling quality control system. This verifies that there were no sequencing issues and removes known contaminant reads using the kmer filter in bbduk.

Quality controlled reads are then processed by **iTagger** (Tremblay *et al.*, 2015). The current version of iTagger (2.0) described in this document differs substantially from original version described in the cited paper. iTagger 2.0 processes sequencing amplicon data in three major steps: read clustering, the taxonomic assignment of operational taxonomic units (OTUs), and the analysis and summarization of ecological data. The iTagger program relies heavily on clustering and classification tools in the USEARCH software suite (Edgar, 2010) and ecological analysis scripts in QIIME (Caporaso *et al.*, 2010). Itagger also records

Amplicon primer sets at JGI

Archaeal and Bacterial 16S rRNA V4-V5
515F-Y GTGYCAGCMGCCGCGGTAA
926R CCGYCAATTYMTTTRAGTTT
(Parada *et al.*, 2015)

Eukaryotic 18S rRNA V4
565F CCAGCASCYGCGGTAATTCC
948R ACTTTCGTTCTTGATYRA
(Stoeck *et al.*, 2010)

Fungal ITS2
ITS9F GAACGCAGCRAAIIGYGA
ITS4R TCCTCCGCTTATTGATATGC
(Ihrmark *et al.*, 2012; White *et al.*, 1990)

Figure 1. The regions of the amplicon sequencing primers used for hybridization with target genes.

the OTU's from each project in a central database, creating clusters of centroids that can be queried to identify other samples with matching OTU's. This feature allows comparison across JGI's large amplicon sample collection. iTagger was designed to allow JGI to use the best amplicon analysis tools in a production environment. The itagger program is open source and licensed under the Perl license (individual components have their own licenses and Usearch is not open source) The source code for iTagger is available on Bitbucket: http://bitbucket.org/berkeleylab/jgi_itagger. A summary of the amplicon sequencing process is provided in Table 1. The exact methods and parameters used for each itags sequencing run are available in the method.txt and congig.ini files placed in every iTag project directory on the JGI genome portal.

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	Sequencing and QC	• Pool up to 184 samples • Sequence 2x300bp on an Illumina MiSeq (200K sequences per sample) • De-multiplex samples • Filter contaminants and trim adapters (Bbtools)
iTagger	Read preparation	• Merge read pairs (Usearch) • Match primers (Userach) • Remove reads with high expected errors • Dereplicate count and sort reads • Create seqobs file
	Clustering	• Remove samples with insufficient numbers of sequences • Combine seqobs files from all samples in a project • Sort by decreasing abundance • Cluster iteratively at 99%, 98%, 97% identity (Usearch cluster_otus and Usearch_global, chimera checking performed here too)

	Classification	• Classify centroids taxonomically using Usearch utax
		• One of three reference databases are used are used for annotation:
		  ○ 16S – Silva SSU, quality filtered, trimmed, V4-V5
		  ○ 18S – Silva LSU, quality filtered, trimmed, V4 (Quast *et al.*, 2013)
		  ○ ITS – Unite fungal database, ITS2 (Kõljalg *et al.*, 2013)
		• Generate Biom files (.json)

	Summary analysis	• Align centroids (Mafft) (Katoh *et al.*, 2002)
		• Build phylogenetic tree (FastTree 2) (Price *et al.*, 2010)
		• QIIME v1.91 core diversity analysis is run. This script creates:
		  ○ Alpha rarefaction plots
		  ○ Beta diversity and PCoA plots
		  ○ Graphical taxonomic summaries
		  ○ Lists of OTU's enriched in environmental conditions

	Linking to other projects	• OTU's from the project are entered into a database of OTU's from other projects • The centroids from all projects are clustered to identify centroids and samples from other projects that match

Table 1 The iTag amplicon sequencing and analysis process at JGI.

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