

Table A1: Performance Metrics for PARAFISH Workflow using Sample OF.216F.i

Main Step	Sub-Step / File	Metric	Value Obtained	Notes / Observations
1. Quality Control (FastQC)	OF.216F.i.raw.1.fastq.gz	Time (s)	4.52	Utilized multiple cores OF.216F.i.raw.1.fastqc.html
		CPU Peak (%)	199.60%	
		RAM Peak (MB)	278.98	
		Output HTML (MB)	1.00	
	OF.216F.i.raw.2.fastq.gz	Time (s)	4.83	Utilized multiple cores OF.216F.i.raw.2.fastqc.html
		CPU Peak (%)	189.80%	
		RAM Peak (MB)	254.86	
		Output HTML (MB)	0.94	
2. Preprocessing (Trimmomatic)	Paired-End (R1+R2)	Time (s)	4.82	Input: OF.216F.i.raw.R[1/2].fastq.gz Utilized multiple cores OF.216F.i.trimmed.R1.paired.fastq OF.216F.i.trimmed.R2.paired.fastq
		CPU Peak (%)	239.50%	
		RAM Peak (MB)	98.28	
		Output R1 Paired (MB)	41.46	
	Merge (Trimmed R1+R2)	Output R2 Paired (MB)	41.73	
		Time (s)	44.52	Input: Trimmed paired files Primarily single-threaded merged.assembled.fastq
2. Preprocessing (PEAR)	Merge (Trimmed R1+R2)	CPU Peak (%)	109.80%	
		RAM Peak (MB)	178.38	
		Output Merged (MB)	58.13	
		Time (s)	58.13	
3. Sequence Analysis (VSEARCH)	Input FASTA (input_from_pear.fasta)	Size (MB)	58.13	Converted from merged reads
	Dereplication	Time (s)	1.01	Input: input_from_pear.fasta VSEARCH derep subprocess VSEARCH derep subprocess 15,806 unique sequences
		CPU Peak (%)	30.00%	
		RAM Peak (MB)	12.00	
		Output Derep. (MB)	0.02	
	Clustering (OTUs @ 97%)	Time (s)	3.64	Input: derep.fasta Effective multi-core utilization VSEARCH cluster subprocess 63 OTUs (otus.fasta) clusters.uc
		CPU Peak (%)	734.60%	
		RAM Peak (MB)	30.33	
		Output OTUs (MB)	<0.01	
		Output UC (MB)	1.86	
	Total VSEARCH	Time (s)	4.66	Sum of derep + cluster
4. Ref. Databases (BOLD-igger)	Identification	Time (s)	14.45	Input: otus.fasta (63 OTUs) Multi-core usage boldigger.results.xlsx
		CPU Peak (%)	159.80%	
		RAM Peak (MB)	175.38	
		Output XLSX (MB)	0.01	
	MAFFT (Alignment)	Time (s)	2.85	Input: otus.fasta Primarily single-threaded (small input) Very low RAM usage aligned_safe_ids.fasta
5. Phylogenetic Analysis (63 OTUs)	FastTree2	CPU Peak (%)	49.90%	
		RAM Peak (MB)	4.12	
		Output Aligned (MB)	0.03	
		Time (s)	0.91	Input: aligned_safe_ids.fasta Efficient single-thread Very low RAM usage tree.nwk
	RAxML-NG (500 Bootstraps)	CPU Peak (%)	99.80%	
		RAM Peak (MB)	5.50	
		Output Tree (KB)	4.76	
		Time (s)	422.02	Input: aligned_safe_ids.fasta Effective multi-core utilization Moderate RAM usage raxml.raxml.bestTree
		CPU Peak (%)	209.80%	
		RAM Peak (MB)	56.61	
		Output BestTree (KB)	4.11	