# The Genome Sequence of the Marine Diatom *Pseudo-nitzschia australis*

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## *Pseudo-nitzschia australis*

- **Marine diatom**
- **Asexual and sexual reproduction**
- **Globally distributed**
- **Domoic acid producer**
- **Amnesic shellfish poisoning**

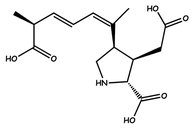




**Figure 1.** ***Pseudo-nitzschia australis***
(Image Credit: Northwest Fisheries Science Center)

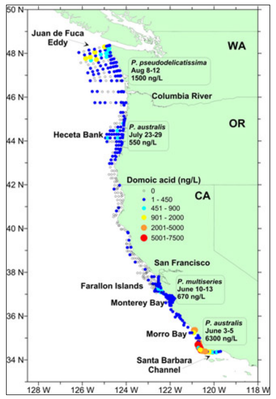



**Figure 2**. Domoic Acid concentrations along US West Coast during 1998.

## *De Novo* Genome Assembly

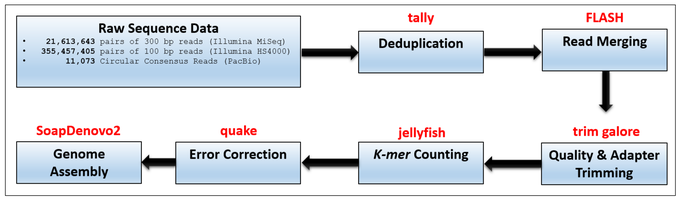



**Figure 3. Genome assembly pipeline.** Raw data was pre-processed to improve the quality of *de novo* genome assembly. We (1) collapsed duplicate paired-end reads, (2) merged overlapping pair-end reads, (3) quality and adapter trimmed reads, (4) quantified the occurrence of 17-mers for genome size estimation and error correction, and (5) corrected or discarded reads with low frequency 17-mers.

| Statistic | K61-81 |
|---|---|
| # Scaffolds | 1,990,571 |
| N50 (bp) | 4,085 |
| N90 (bp) | 120 |
| Longest Scaffold (bp) | 1,517,930 |
| Mean Scaffold Coverage | 27 |
| Cumulative Assembly Size (bp) | 767,504,633 |
| # Scaffolds ≥ 5,000 bp | 26,903 |
| Assembly Size Scaffolds ≥ 5,000 bp | 362,787,643 |

**Table 1.** ***De novo*** **Assembly Statistics.** We used **SOAPdenovo2** for genome assembly, using a *k-mer* range of 61-81. Overall, the assembly was highly fragmented, suggesting high rates of heterozygosity, and/or high levels of repetitive DNA.

## *Pseudo-nitzschia australis* Gene Prediction

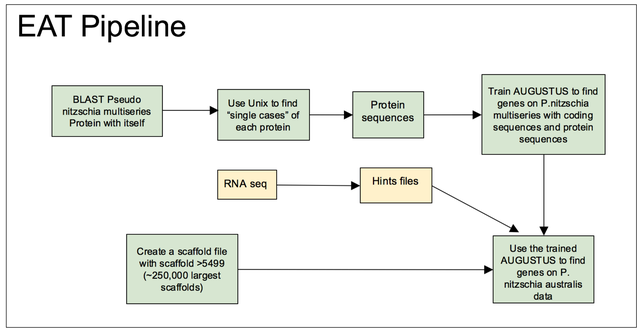



**Figure 4.** ***in silico*** **prediction of** ***Pseudo-nitzschia australis*** **genes.** We used AUGUSTUS to computationally predict genes. The *Pseudo-nitzschia multiseries* genome, proteome, and coding sequence files were used to train AUGUSTUS. Additionally, exonic “hints” were generated by mapping *Pseudo-nitzschia australis* RNA-seq data against the genome assembly. Together, this data was then used to predict protein-coding genes in the *Pseudo-nitzschia australis* genome.

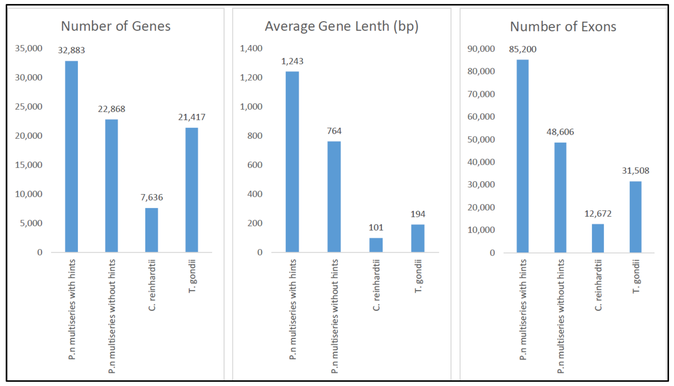



**Figure 5.** Results assessment of AUGUSTUS gene prediction on the largest 250,000 *Pseudo-nitzschia australis* scaffolds using different species-specific training sets (*Pseudo-nitzschia multiseries* (with and without hints), *Chlamydomonas reinhardtii* and *Toxoplasma gondii*).

## *Pseudo-nitzschia australis* Genome Size Estimation

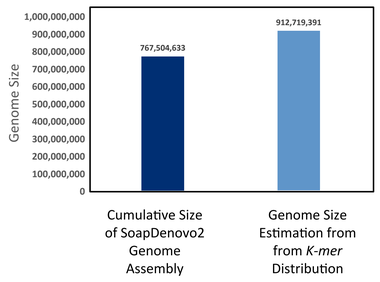



**Figure 6.** The *Pseudo-nitzschia australis* genome size was estimated from the cumulative size of the SoapDenovo2 assembly, and from the frequency distribution of 17-mers from the error-corrected Illumina MiSeq data. Our estimates suggest the *Pseudo-nitzschia australis* genome is between 700-900 Mb.

## Ecological Relevance of Bacterial Sequence Data

- Genes with outlier GC content may represent:
  (1) Contamination from ecologically relevant marine bacterial species
  (2) Genes horizontally transferred from bacteria to *Pseudo-nitzschia australis*

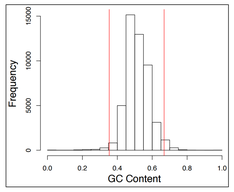



**Fig 7.** GC content distribution of the predicted *Pseudo-nitzschia australis* genes.

| %GC | Query cover | E value | Identity | Accession | Species | Class | Order | Family |
|---|---|---|---|---|---|---|---|---|
| 17.41% | 3% | 2.E-04 | 100% | CP008727.1 | *Burkholderia oklahomensis* | Betaproteobacteria | Burkholderiales | Burkholderiaceae |
| 33.62% | 19% | 8.E-09 | 77% | XM_014048693.1 | *Monorophalium neglectum* | Alphaproteobacteria | Rhizobiales | Methylobacteriaceae |
| 69.30% | 30% | 2.E-53 | 76% | CP000264.1 | *Jannaschia spp.* | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae |
| 69.35% | 36% | 2.E-64 | 81% | CP002279.1 | *Mesorhizobium opportunistum* | Alphaproteobacteria | Rhizobiales | Phyllobacteriaceae |
| 69.36% | 12% | 4.E-26 | 77% | CP000264.1 | *Jannaschia spp.* | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae |
| 69.73% | 17% | 4.E-21 | 81% | CP012908.1 | *Ketogulonicigenium vulgare* | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae |
| 70.11% | 57% | 4.E-61 | 75% | CP014028.1 | *Achromobacter xylosoxidans* | Betaproteobacteria | Burkholderiales | Alcaligenaceae |
| 70.72% | 67% | 4.E-62 | 75% | CP010855.1 | *Marinovum algicola* | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae |
| 71.28% | 63% | 1.E-106 | 74% | CP000031.2 | *Ruegeria pomeroyi* | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae |
| 71.49% | 93% | 3.E-83 | 81% | CP012960.1 | *Rhodobacter sphaeroides* | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae |
| 71.53% | 67% | 8.E-64 | 75% | CP010855.1 | *Marinovum algicola* | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae |
| 73.01% | 98% | 1.E-116 | 75% | CP000031.2 | *Ruegeria pomeroyi* | Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae |

**Table 2.** Best BLAST hit for the 10 highest and lowest GC content *Pseudo-nitzschia australis* genes against the NCBI non-redundant database. All top hits are of bacterial origin.

## Genome Assembly Improvement

- Long-read PacBio data is being integrated to improve genome assembly through 2 projects:
  (1) The development of a novel scaffolding algorithm
  (2) The evaluation of additional scaffolding software

**Fig 8. The Low Coverage Long Read Scaffolder (LCLRS).** The LCLRS algorithm incorporates a graph-based approach to best path reconstruction and aims to improve scaffolding through the use of low coverage PacBio data.

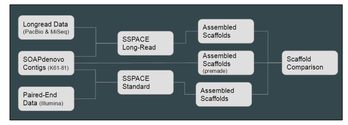
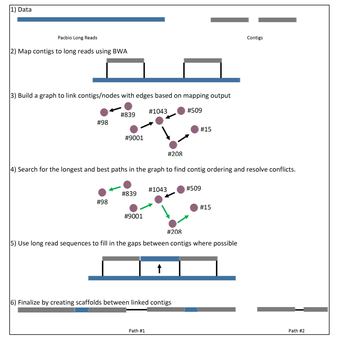
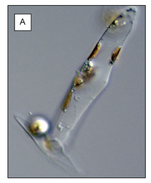



**Fig 9.** Evaluation of SSPACE and SSPACE-Long-Read scaffolding algorithms in comparison to SoapDenovo2.




## Future Directions

- Improvement of assembly quality through optimal scaffolding
- Functional annotation of predicted genes and proteins
- Identification of genomic elements contributing to genome size expansion
- Validation of putative HGT events through the analysis of flanking regions
- Identification of genes of genes involved in domoic acid production




*Trainer et al. 2012*

## Acknowledgments

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