



atlas

UNDERSTANDING DEEP ATLANTIC ECOSYSTEMS



Setting up RAD Seq protocols for selected species

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Objectives

- Test the performance of Restriction-Associated DNA (RAD) sequencing on a set of deep-sea invertebrates.
- 16 putative deep-sea species, 4 phyla
- 5 species of Cold-Water Corals (1 octocorallia, 4 hexacorallia)

Methods

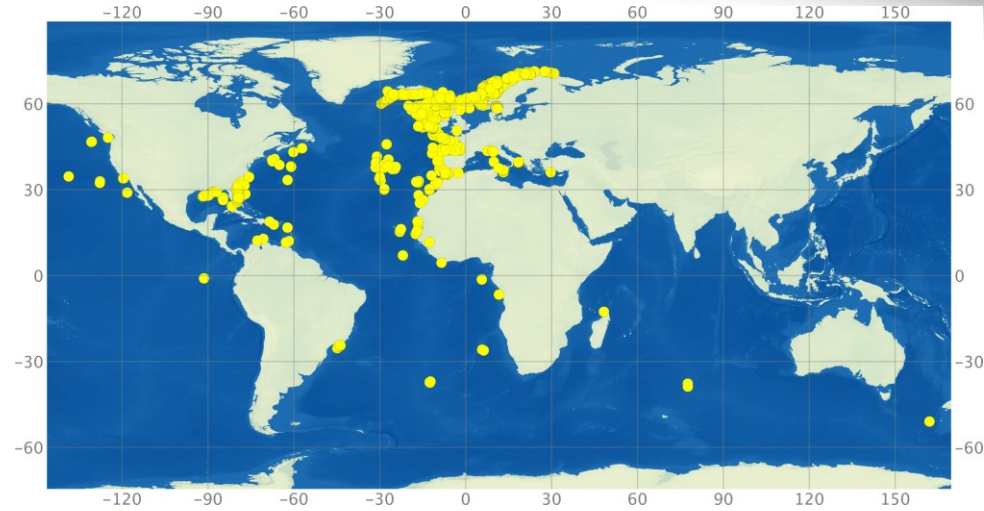
- Single-end RAD using two enzymes with different cut frequency: *SbfI* and 2 lanes of *PstI*, sequenced for 100 bp Illumina HiSeq
- Demultiplexed in Stacks v1.46
- Custom python script to optimize denovo assembly parameters followed by Stacks to build loci denovo and VCFtools for data filtering
- When samples were available for more than one location (*L. peetusa* and *M. oculata*), downstream analyses were performed (Admixture, PCA) to detect population groups.



Results

- Genomic DNA extraction of >10kb with no degradation from stored samples was challenging and had low rate of success.
- Extraction protocols were optimized, particularly for corals (<50% success).
- RAD-seq performed well for most species, and very well for all corals, 5-10k loci assembled with *SbfI* and 60-160k with *PstI*.
- The two enzymes give concordant nucleotide diversity estimates for all species tested.

Lophelia pertusa



Reef-building hard coral, scleractinian

RAD-seq compatible? YES

Restriction enzyme:

SbfI 1 lane and *PstI* 2 lanes, 100bp

Number of samples: 13

After filtering:

PstI 10

SbfI 10

Location: Alboran Sea, Bay of Cadiz, Azores archipelago, Iceland, Rockall Bank, Bay of Biscay

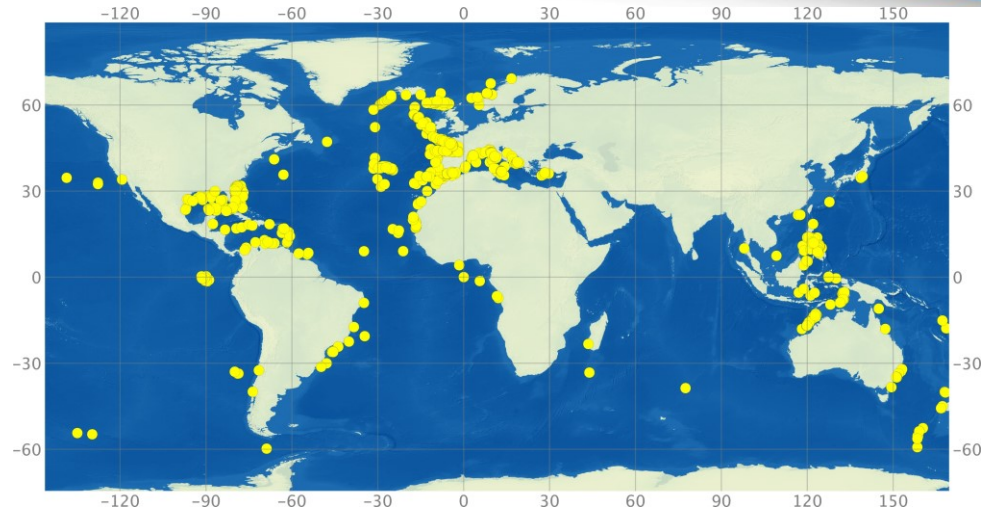
Polymorphic loci:

PstI 116 059

SbfI 9 776



Madrepora oculata



Reef-building hard coral, scleractinian

RAD-seq compatible? YES

Restriction enzyme:

SbfI 1 lane and *PstI* 2 lanes, 100bp

Number of samples: 16 (*PstI*) 13 (*SbfI*)

After filtering:

PstI 10

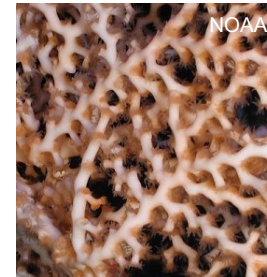
SbfI 8

Location: Alboran Sea, Azores archipelago, Rockall Bank, Bay of Biscay

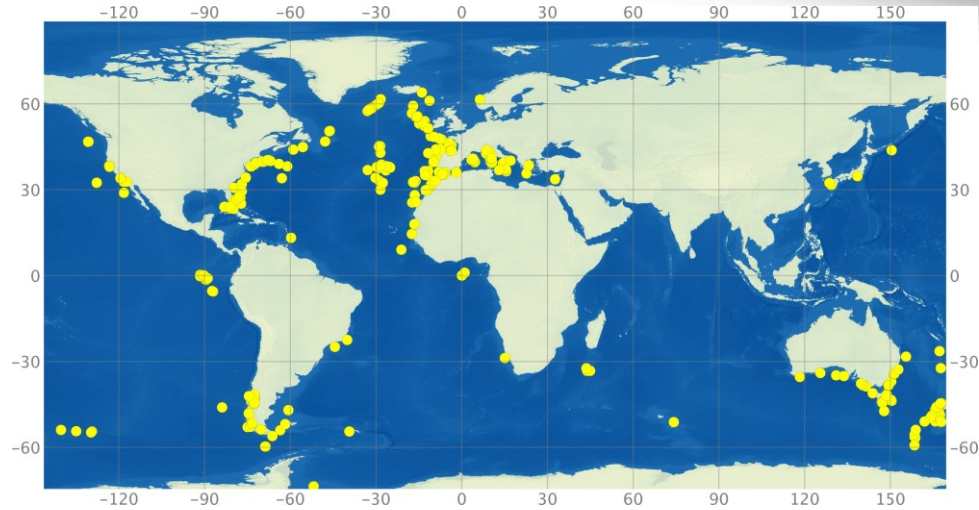
Polymorphic loci:

PstI 110 186

SbfI 5 315



Desmophyllum dianthus



Solitary hard coral, scleractinian

RAD-seq compatible? YES

Restriction enzyme:

Sbfl 1 lane and *PstI* 2 lanes, 100bp

Number of samples: 4

After filtering:

PstI 4

Sbfl 1

Location: Azores archipelago, Ionian Sea

Polymorphic loci:

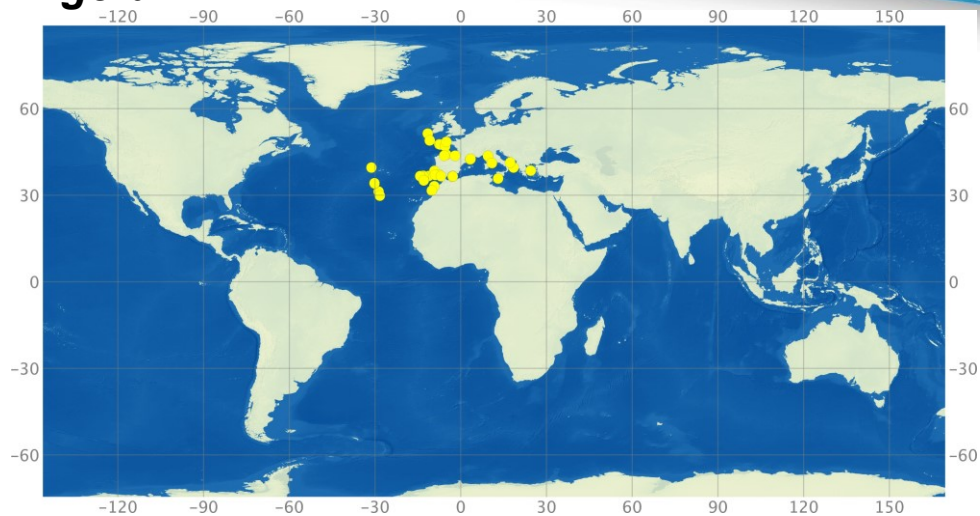
PstI 61 915

Sbfl 5 047



Gori et al
DOI_10.7717/peerj.1606

Dendrophyllia cornigera



Non reef-building hard coral, scleractinian

RAD-seq compatible? YES

Restriction enzyme:

SbfI 1 lane and *PstI* 2 lanes, 100bp

Number of samples: 5

After filtering:

PstI 5

SbfI 5

Location: Bay of Cadiz, Alboran Sea

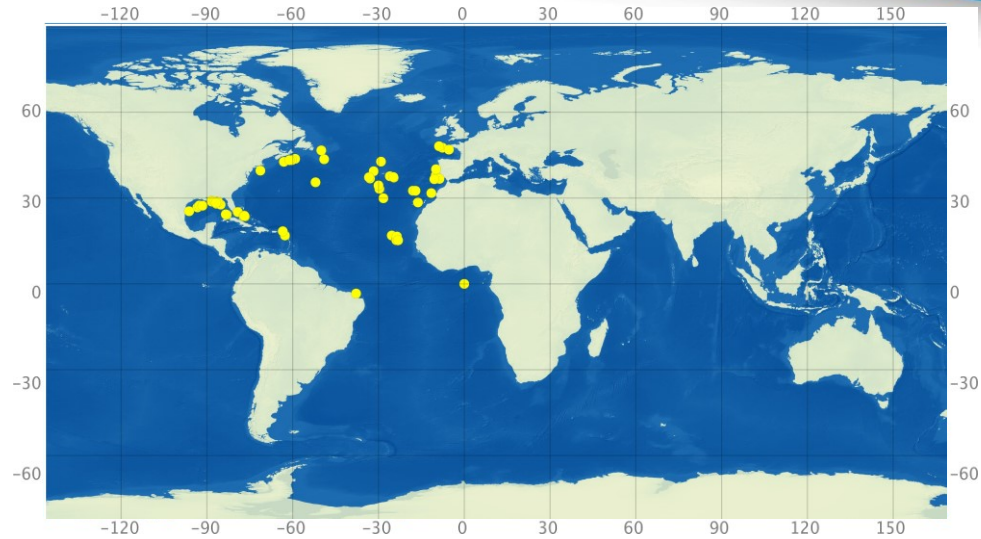
Polymorphic loci:

PstI 166 581

SbfI 9 660



Acanella arbuscula



Bamboo-coral, octocoral

RAD-seq compatible? YES

Restriction enzyme:

SbfI 1 lane and *PstI* 2 lanes, 100bp

Number of samples: 4

After filtering:

PstI 3

SbfI 4

Location: Azores archipelago

Polymorphic loci:

PstI 69 259

SbfI 6 151



Thank You!



Presenter details:

WP4: RADSeq protocols set up at Ifremer

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