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Development of a Molecular Assay for Caribbean Coral Identification

Elijah D. O'Cain

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DEVELOPMENT OF A MOLECULAR ASSAY FOR CARIBBEAN CORAL
IDENTIFICATION

by

Elijah D. O’Cain

(Under the direction of Daniel F. Gleason)

ABSTRACT

As coral cover has declined throughout the Caribbean, interest in the role that recruitment processes play in reef recovery has increased. Studies investigating these processes have been hampered by the inability to identify many species of coral larvae or recently settled recruits using morphology alone. In this study, the utility of targeting the non-coding internal transcribed spacer (ITS) regions with a multiplex PCR assay to identify common Caribbean coral species was explored. To design this assay, a database of ITS sequences was developed for 17 Caribbean scleractinian coral species that are important reef builders and/or are common in the Florida Keys. It was predicted that the ITS region would contain enough genetic variation to allow for separation of these corals to the species level, and that this variation could be targeted using a single-step nested multiplex PCR technique. Analyzing the ITS region, sufficient genetic variation was detected that would allow for nine of the seventeen Caribbean coral species targeted to be categorized to the genus level, and the remaining eight to the species level. Subsequently, three genus-specific primers (with a total of seven included species) and six species-specific primers were designed for use in a single-step nested multiplex PCR protocol that facilitates coral identification. While still under development, this genetic assay showed significant promise as an inexpensive and relatively straightforward method of identifying

planula larvae and recently settled coral recruits to the genus or species level. The increased accuracy and abbreviated timeframe offered by this technique for identifying coral larvae and recruits justifies its use as a tool for evaluating Caribbean reef recovery.

INDEX WORDS: Coral, Caribbean, Recruit, Larvae, Molecular assay, Single-step nested multiplex PCR, Internal transcribed spacer region

DEVELOPMENT OF A MOLECULAR ASSAY FOR CARIBBEAN CORAL
IDENTIFICATION

by

ELIJAH DYLAN O'CAIN

B.A., Georgia Southern University, 2013

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial
Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

STATESBORO, GA

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DEVELOPMENT OF A MOLECULAR ASSAY FOR CARIBBEAN CORAL
IDENTIFICATION

by

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July 2017

DEDICATION

I would like to dedicate this to my parents, Steve and Tisa O’Cain, as well as my brother, Luke O’Cain, who have supported and encouraged me in my pursuit of knowledge throughout my life.

I would also like to dedicate this to my closest confidante and future wife, Mallory Taylor, who has stood by my side ever since my final year of high school. Without her support, I would not have made it this far, and I look forward to our future together.

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS	3
LIST OF TABLES	5
LIST OF FIGURES	6
LITERATURE REVIEW	7
CHAPTER 1: EXPLORATION OF THE ITS REGION FOR SPECIES IDENTIFICATION OF CARIBBEAN CORALS	17
INTRODUCTION	17
METHODS	19
RESULTS	21
DISCUSSION	23
TABLES AND FIGURES	26
CHAPTER 2: A MOLECULAR ASSAY FOR CARIBBEAN CORAL IDENTIFICATION ...	33
INTRODUCTION	33
METHODS	35
RESULTS	37
DISCUSSION	38
TABLES AND FIGURES	44
REFERENCES	49
APPENDIX I: ALIGNMENT OF TARGET CORAL ITS REGIONS	57

LIST OF TABLES

TABLE 1.1: Target coral species and sample data	26
TABLE 1.2: Obtained sequence data	27
TABLE 1.3: Genetic distance matrix	28
TABLE 2.1: Developed primer data	44
TABLE 2.2: Sub-assay primer combinations	44
TABLE 2.3: Primer false positives	45
TABLE 2.4: Restriction enzyme digest results	46

LIST OF FIGURES

FIGURE 1.1: Schematic of target DNA region	29
FIGURE 1.2: Phylogenetic tree of target corals using ITS sequences	30
FIGURE 1.3: Phylogenetic tree of the <i>Diploria</i> genus and <i>Favia fragum</i> using ITS sequences..	31
FIGURE 1.4: Phylogenetic tree of the <i>Acropora</i> genus using ITS sequences	31
FIGURE 1.5: Phylogenetic tree of the <i>Orbicella</i> genus using ITS sequences.....	32
FIGURE 2.1: PCR products using SSNM-PCR assay	46
FIGURE 2.2: Restriction digest of similar-sized species-specific primer products	47
FIGURE 2.3: Flowchart explaining use of the SSNM-PCR assay	48

LITERATURE REVIEW

Coral reefs are important marine ecosystems providing vital economic and ecological goods and services. Although covering only 0.1-0.5% of the ocean floor, these ecosystems are home to almost a third of the world's marine fish species and provide about 10% of the fish consumed by humans (Moberg and Folke 1999, Adjeroud et al. 2016). Hermatypic corals provide the structure for these reefs, and create topographic complexity for the wide diversity of animals living there (Friedlander and Parrish 1998, Donahue et al. 2008, Adjeroud et al. 2016). Tens of millions of people living in countries near coral reefs depend on them and their associated biological communities for income or dietary needs (Salvat 1992, Moberg and Folke 1999, Adjeroud et al. 2016). Researchers have also identified several compounds found only on coral reefs that could be used to treat many different types of diseases (Moberg and Folke 1999). In addition to direct goods and services, these reefs protect coastal seagrass beds and mangroves from oceanic currents and tides, which in turn serve as tidal buffers for coastal areas and provide nursery habitat for many fish species (Ogden and Gladfelter 1983, Moberg and Folke 1999).

Corals are vulnerable to global stressors such as increased oceanic temperature (Hoegh-Guldberg 1999, Hughes et al. 2003) and declining pH from ocean acidification (Renegar and Riegl 2005, Anthony et al. 2008, Marubini et al. 2008). Bleaching, which is primarily caused by increased oceanic temperature and refers to the loss of color due to the expulsion of symbiotic algae, has caused massive declines in coral cover worldwide, including complete loss of all living corals in many countries (Goreau et al. 2000, Graham et al. 2015). In addition to these global stressors, corals can be harmed by more local impacts including enhanced sedimentation (Rogers 1990, Gilmour 1999), nutrient loading (Gilmour 1999, Renegar and Riegl 2005), and

overharvesting of herbivores (Williams and Polunin 2000, Rogers and Miller 2006, Doropoulos et al. 2014).

Historically, reefs in the Florida Keys National Marine Sanctuary (FKNMS) consisted primarily of large, long-lived, reef-building scleractinian corals such as those in the genera *Acropora*, *Montastraea*, and *Orbicella* (Gleason et al. 2001, Donahue et al. 2008, Huntington et al. 2011). Left undisturbed, these reef-builders grow large enough to become the dominant coral species in Caribbean reef ecosystems, assisted by their superior competitive ability to gain and maintain physical space (Connell et al. 2004). For example, corals in the genus *Acropora* are large branching species that grow over smaller, encrusting corals and prevent them from receiving adequate sunlight. In contrast, massive reef-building corals, such as those in the genera *Diploria*, are able to dominate space on the substrata, hindering larval settlement of other coral species (Connell et al. 2004). Areas dominated by large reef-building species are the preferred habitat for many fish and marine invertebrates, as these corals provide structural support against wave action and hiding spots from predators (Friedlander and Parrish 1998, Donahue et al. 2008, Ruzicka et al. 2013). However, these long-lived corals have low recruitment rates, and can take decades to reach maturity (Hughes and Tanner 2000, Green et al. 2008, Huntington et al. 2011). Thus, when these species face times of increased mortality, it becomes more difficult for their populations to be sustained through recruitment (Green et al. 2008, Huntington et al. 2011, Ruzicka et al. 2013).

Cover of reef-building corals in the Caribbean has steadily decreased since the 1970's (Hughes 1994, Hughes et al. 2003, Donahue et al. 2008). Widespread bleaching and disease of scleractinian corals beginning in the 1980's, exacerbated by global climate change, has led to massive decreases in richness and abundance in the Florida Keys (Donahue et al. 2008). Climatic

events like the one occurring in January 2010, where the Florida Keys experienced one of the coldest 12-day periods on record, have caused massive mortality (Kemp et al. 2011, Colella et al. 2012). Furthermore, the loss of *Diadema*, herbivorous fish, and other herbivores to issues including disease and overharvesting have contributed to reefs being dominated by macroalgae (Williams and Polunin 2000, Rogers and Miller 2006, Idjadi et al. 2010, Doropoulos et al. 2014).

As the abundance of primary reef-building corals has declined, there has been a marked increase in reefs dominated by smaller scleractinian corals (including those in the genera *Porites*, *Siderastrea*, and *Undaria*) that recruit in higher numbers, mature at a faster rate, and have shorter lifespans (Gleason et al. 2001, Green et al. 2008, Huntington et al. 2011). Reefs consisting primarily of these “weedy” species display a lower diversity of fish and corals than reefs populated with larger coral species (Donahue et al. 2008, Ruzicka et al. 2013). However, these weedy corals tend to be less vulnerable to threats including lowered pH and increased temperature, and can handle the changing global climate better than the major reef-building species (Green et al. 2008, Huntington et al. 2011). Furthermore, these weedy species can take over an area quickly after a disturbance event, making it more difficult for major reef-building species to repopulate (Rogers and Miller 2006, Ruzicka et al. 2013).

Another reef type being seen more frequently in the FKNMS is one dominated by gorgonians (Ruzicka et al. 2013, Ramsby et al. 2014). While scleractinian coral populations in the Florida Keys have decreased over the past few decades, gorgonian numbers have either stayed stable or increased (Donahue et al. 2008, Ruzicka et al. 2013). During the 1997-1998 El Niño event, corals, including gorgonians, in the Florida Keys were severely damaged, but gorgonians proved more resilient and were able to recover faster (Colella et al. 2012, Ruzicka et al. 2013). Gorgonians have proven to be more resistant to such factors as reduced pH, disease,

and large storms, and that has allowed them to become the dominant taxa on shallow fore-reefs of the Florida Keys (Gabay et al. 2014, Ramsby et al. 2014, Ruzicka et al. 2013).

Reefs lacking coral cover and dominated by fleshy macroalgae are also becoming more common globally, especially in areas with decreased herbivore abundances (Williams and Polunin 2000, Lirman 2001, Rogers and Miller 2006, Doropoulos et al. 2014, Toth et al. 2014). Without herbivores, fleshy macroalgae can overgrow corals, limit the amount of sunlight reaching the substrata, and inhibit new coral recruitment (Williams and Polunin 2000, Lirman 2001, Gleason et al. 2009, Gleason and Hoffman 2011, Doropoulos et al. 2014).

The primary management technique being implemented to protect and restore coral reefs to their original state after degradation is the creation of no-take marine reserves where fishing and other harvesting activities are strictly regulated (Williams and Polunin 2000, Donahue et al. 2008, Huntington et al. 2011, Toth et al. 2014). This method of reef management relies on a cascade of events whereby the recovery of herbivorous fish populations leads to increased grazing on macroalgae. In turn, the reduced macroalgal cover will result in enhanced coral larval settlement combined with improved survival and growth. A counter argument to this management strategy states that while there do seem to be fewer herbivorous fish on reefs dominated by macroalgae in the FKNMS, herbivorous fish were not historically targeted by fisheries in this region (Toth et al. 2014). This argument points to the alternative conclusion that rather than the departure of fish leading to increases in macroalgae and decreases in coral cover, corals may die off first causing the fish to leave and macroalgae to take over (Toth 2014). In either case, evidence suggest these marine reserves have been successful in reestablishing populations of herbivorous fish, but they have so far failed in returning reefs to their original

coral-dominated state (Williams and Polunin 2000, Donahue et al. 2008, Huntington et al. 2011, Toth et al. 2014.).

After periods of extensive coral mortality, reef recovery is only possible if there is suitable reproductive output, adequate larval supply, substantial settlement success, and high rates of post-settlement survivorship (Hughes and Tanner 2000, Gleason et al. 2001, Gleason et al. 2009, Gleason and Hoffman 2011, Kersting et al. 2014, Humanes and Bastidas 2015, Adjeroud et al. 2016). Therefore, it is necessary to understand what role recruitment, the process by which coral larvae establish themselves on a reef, may play in maintaining corals on reefs. Corals have two mechanisms of sexual reproduction: broadcasting (common for major reef-building corals) and brooding (common for weedy coral species and Caribbean gorgonians) (Brazeau and Lasker 1989, Baird et al. 2009, Gleason and Hoffman 2011). Broadcasting corals release eggs and sperm in synchronous mass spawning events coincident with the lunar cycle once or twice a year. These gametes combine in the water column to form embryos that develop into planula larvae that spend days to weeks in the water column (Babcock et al. 1986, Ritson-Williams et al. 2009). Brooding corals only release sperm, resulting in internal fertilization in the polyp of a conspecific neighbor and the eventual release of fully developed planula larvae (Ritson-Williams et al. 2009, Gleason and Hoffman 2011). Brooding corals can spawn several times per year, but generally release fewer propagules per cycle than broadcasting corals (Ritson-Williams et al. 2009). These larvae typically spend only hours to days in the plankton, but have the potential to spend weeks in the water column (Ritson-Williams et al. 2009). After dispersing in the plankton, planula larvae from both types of corals cement to the benthos and metamorphose (Ritson-Williams et al. 2009, Gleason and Hoffman 2011, Doropoulos et al. 2014). Once these individuals have metamorphosed and survived for long enough to establish

themselves on the reef and become visible to observers, they are considered recruits (Caley et al. 1996, Ritson-Williams et al. 2009).

Historically it was assumed that benthic marine invertebrate larvae recruit to areas far from their spawning sites (Caley et al. 1996, Pechenik 1999). However, verification that in some cases established adults are genetically similar to recruits provides evidence that propagules may settle close to their site of origin (Caley et al. 1996, Pechenik 1999, Cowen et al. 2000, Vermeij 2005). As larvae, corals may maintain proximity to natal reefs by responding to environmental cues including light, hydrostatic pressure, sound, and chemical signals. (Gleason and Hoffman 2011). Short dispersal distances may reduce coral mortality in the plankton (Pineda et al. 2009), and ensure that recruits are well-adapted for the area in which they settle (Strathmann et al. 2002). The benefits of short-range dispersal, however, must be balanced against the possibility of overcrowding and decreased genetic variability as the number of conspecific corals increase on a reef (Pechenik 1999, Ritson-Williams et al. 2009).

Differences in planktonic larval duration may impact community resilience, especially following major die-off events. Most long-lived, reef-building scleractinians are broadcast spawners so their larvae are more likely to be dispersed away from the natal reef (Ritson-Williams et al. 2009). In contrast, brooding Caribbean coral species exhibit a higher incidence of local recruitment (Brazeau et al. 2005, Ritson-Williams et al. 2009). Some brooding corals are known to self-fertilize, thus facilitating reproductive and recruitment success in conditions of low adult densities (Brazeau et al. 1998, Gleason et al. 2001). Therefore, it follows that if low abundances of brooding corals are left on a reef after a period of coral die-off, they could quickly recover and become the dominant reef taxa. However, to predict accurately how different species

of corals will repopulate degraded reefs, it is necessary to obtain additional information on coral recruitment (Adjeroud et al. 2016).

As coral cover has declined worldwide, there has been increased focus on the role that recruitment plays in reef recovery. Studies suggest lower recruitment rates in the Caribbean than in other regions such as the Indo-Pacific, with recruitment rates declining over the last few decades (Bak and Engel 1979, Rogers et al. 1984, Carlon 2001, Green and Edmunds 2011, van Woesik et al. 2014, Humanes and Bastidas 2015). Most recent investigations indicate no relationship between recruitment rates and reef recovery (van Woesik et al. 2014, Humanes and Bastidas 2015), although a few studies in Jamaica have documented a positive correlation between the recovery of large reef-building corals and recuperating populations of the long-spined urchin *Diadema* (Carpenter and Edmunds 2006, Idjadi et al. 2006, Idjadi et al. 2010). The commonly observed disconnect between coral recruitment rates and adult communities in the Caribbean could be due to several factors. First, even when there are high levels of recruitment, most of the recruits are from weedy species, such as *Undaria agaricites*, rather than primary reef-building corals (Bak and Engel 1979, Carlon 2001, Green and Edmunds 2011, Humanes and Bastidas 2015). Thus, high recruitment rates are not leading to recovery of the primary reef-building coral dominated condition (Carlon 2001, Green and Edmunds 2011, Humanes and Bastidas 2015). On the other hand, studies documenting high recruitment rates of primary reef-building corals, but low abundances of corresponding adults suggest that there is significant post-settlement mortality (van Woesik et al. 2014). A final explanation for the discrepancy between adult and recruit abundances could be the inability to identify young coral recruits to the species level using morphology alone. For example, van Woesik et al. (2014) noted that *Acropora* spp. recruits were common on Florida Keys reefs, although adults of these species were virtually

absent. However, a photo of an *Acropora* spp. recruit provided in van Woesik et al. (2014) is similar in appearance to an encrusting bryozoan and suggestive of a potential misidentification.

To predict accurately which reefs will recover to their original coral-dominated state, a reliable estimate of coral recruitment rates is necessary (Adjeroud et al. 2016). However, quantifying coral recruitment rates to the species level can be difficult as young coral recruits can be only millimeters in size making morphological characteristics among species difficult to distinguish (Baird and Babcock 2000, Babcock et al. 2003). While the relatively low coral diversity of the Caribbean can make coral identification simpler and easier than in other regions, still some species, such as *Siderastrea siderea* versus *Undaria agaricites*, and the genus *Diploria* versus *Favia fragum*, show striking similarities at smaller recruit sizes (Romano and Palumbi 1996, Edmunds 2010, Hoeksema et al. 2012). Compounding the problem of identification is the fact that some coral species, including *Montastraea cavernosa* and *Undaria agaricites*, that are commonly found in the FKNMS, can show high amounts of phenotypic plasticity during early development (Barnes 1973, Rylaarsdam 1983). These examples highlight the need for additional approaches to distinguish coral species, especially at early developmental stages. Molecular techniques targeting unique genetic signatures may provide the means to identify coral recruits and therefore be useful tools for assessing coral recruitment.

While DNA barcoding (the use of short, unique genetic sequences to identify an organism) using the 5' portion of the mitochondrial cytochrome oxidase I gene (COI) is the standard for identification of eukaryotes, especially larval or incomplete specimens, this method is not useful for corals because there is a limited database of COI sequences for corals, and what sequence data does exist indicates limited genetic diversity among species (Neigel et al. 2007, Hsu et al. 2014). This lack of diversity is due to slower evolutionary rates between different

species of the same genus compared to other metazoans (Romano and Palumbi 1997, Forsman et al. 2006, Neigel et al. 2007). Molecular markers commonly used for other organisms, such as the nuclear 18S rRNA and mitochondrial 16S rRNA genes, have also proven uninformative for corals due to the relatively slower evolutionary rates (Romano and Palumbi 1997, Forsman et al. 2006). In contrast, structural and genetic variation found in the internal transcribed spacer (ITS) regions between the 18S and 28S rRNA genes, which are commonly used to identify plant and fungal species, have shown promise for differentiating between some genera of scleractinian corals in the Caribbean (Chen et al. 2004, Forsman et al. 2006). While the 18S and 28S rRNA genes are both coding regions, the ITS 1 and 2 sequences found between them are non-coding and are thus less conserved (White et al. 1990, Forsman et al. 2006). The hypervariability of the ITS region allows for a more rapid pace of evolution and greater genetic differences between species (Forsman et al. 2006).

As a region of high variability flanked by two regions conserved among Cnidarian species, the ITS sequences are suitable targets for use in a single step nested multiplex polymerase chain reaction (SSNM-PCR) assay (Larsen et al. 2005). SSNM-PCR is a method that can be used to identify organisms quickly and inexpensively (Larsen et al. 2005). This method uses a PCR mix that contains one universal primer pair that amplifies a DNA region in all species of interest, and several species-specific primers that work with the universal primers to amplify a target region within the universal PCR product (Larsen et al. 2005). All primers are run at the same time and each reaction produces two separate PCR product bands when run on an agarose gel: one larger universal band that shows up for all target species, and one species-specific band that differs depending on which species is used as the PCR DNA template (Larsen et al. 2005). This technique is beneficial for the identification of early-stage corals, as the

amplification of the universal band creates more DNA template for amplification of the species-specific product, thus a smaller amount of DNA is needed for the identification of the sample to be completed successfully (Larsen et al. 2005). Furthermore, if no target-specific band is amplified, the universal band can then be sequenced for further analysis and identification of the unknown sample (Larsen et al. 2005). For the creation of an SSNM-PCR primer set, a region of high variability that can be targeted by the several species-specific primers must be flanked by two conserved regions that can be targeted by the universal primer pair (Larsen et al. 2005). The ITS region fits these criteria, and thus makes a suitable target for this type of assay.

In this study, it was predicted that the internal transcribed spacer sequences have enough interspecific variation to allow for differentiation of corals to the species level and that this region is suitable for the creation of a set of species-specific primers for use in a single-step nested multiplex PCR. To investigate this, a database of ITS sequences was developed (using DNA from known coral adults) for 17 different Caribbean scleractinian coral species that are either ecologically important to the Florida Keys region as primary reef builders, or are common as recruits in the area. After these sequences were generated and compared to ensure that there was enough genetic variation to allow for species differentiation, a set of primers was created for use in a SSNM-PCR assay. The ultimate goal was to develop an assay that facilitates identification of corals at life stages where morphological characteristics lack sufficient resolution to do so.

CHAPTER 1 EXPLORATION OF THE ITS REGION FOR SPECIES IDENTIFICATION OF CARIBBEAN CORALS

INTRODUCTION

A wide range of morphological characters are used to identify corals to the species level. For example, in the Caribbean the two common species in the genus *Acropora* are distinguished based on colony growth form: *Acropora palmata* grows large flat branches that resemble moose antlers, while *Acropora cervicornis* has cylindrical branches like the antlers of a deer (Walton Smith 1976, Veron 1993). Polyp shape and structure are also useful for categorizing adult corals. For example, *Siderastrea siderea* and *S. radians* can be distinguished from each other by the differing slopes of their septal margins (Walton Smith 1976, Veron 1993). Other features, such as polyp tissue color and number of corallite septa can be integrated in to the process of identifying adult scleractinian corals (Walton Smith 1976, Veron 1993, Budd and Stolarski 2009, Budd and Stolarski 2011).

In contrast to adults, coral recruits can be more difficult to identify to the species level due to their small sizes and overlapping physical characteristics (Hodgson 1985, Baird and Babcock 2000, Babcock et al. 2003, Jones et al. 2009). Coral recruit identification often centers around skeletal traits such as the number and shape of septa, presence or absence of a columella in the center of the corallite, or size of the corallite (Budd and Stolarski 2009, Budd and Stolarski 2011). However, species identifications are complicated at this point in development because single polyp recruits can display phenotypic plasticity sometimes in response to environmental factors such as sedimentation and light attenuation when it comes to features such as overall polyp shape, shape of coral septa, and thickness of the coral skeleton (Todd et al. 2000, Babcock et al. 2003). The small size of early coral recruits also necessitates that many features used for

identification must be observed using high-powered microscopes (Budd and Stolarski 2009).

These obstacles to identification often limit researchers studying coral recruitment to classifying recruits to the family level (Bak and Engel 1979, Carlon 2001, Miller and Barimo 2001, Green and Edmunds 2011, van Woesik et al. 2014, Humanes and Bastidas 2015).

Given the inherent difficulties of identifying young coral recruits to the species level, it is clear that alternative methods need to be investigated. One potential alternative is to incorporate molecular techniques. DNA barcoding using the 5' portion of the mitochondrial cytochrome oxidase I gene (COI) is the standard for identification of eukaryotes, especially those that are difficult to identify using morphological traits (Neigel et al. 2007, Hsu et al. 2014). However, the lack of an existing coral COI database and the slow evolutionary rate of Anthozoan mitochondrial genes prevent the use of this method for coral identification (Neigel et al. 2007, Fukami et al. 2008, Hsu et al. 2014). Other commonly used molecular markers, such as the nuclear 20S rRNA and mitochondrial 16S rRNA genes, have also proven uninformative for corals at the species level due to similarly slow evolutionary rates (Romano and Palumbi 1996, Forsman et al 2006). Therefore, genetic regions not commonly used for animals are needed to identify corals to the species level.

The internal transcribed spacer (ITS) 1 and 2 regions are located between the 18S, 5.8S, and 28S ribosomal RNA genes and have been used historically to identify to the species level and construct phylogenies for plants (Kim et al. 1996, LaJeunesse 2001), fungi (White et al. 1990, Gardes and Bruns 1993), nematodes (Powers et al. 1997), and some fish (Booton et al. 1999). By using this genetic region to build phylogenies for scleractinian corals, Chen et al. (2004) and Forsman et al. (2006) exhibited that it holds promise for differentiating between scleractinian coral species. The high genetic variability of the ITS regions can be attributed to the

fact that they are non-coding sequences and therefore not as highly conserved as coding regions (White 1990).

For this project, it was predicted that the ITS-1 and 2 regions exhibit sufficient interspecies variation to serve as viable targets for differentiating Caribbean corals to the species level. To test this prediction, ITS sequence data were generated in the lab for 15 different target coral species. To test the capacity of the ITS sequences for separating Caribbean coral species a phylogenetic tree was created by using the sequence data from the lab with information from GenBank for two other coral species. Finally, genetic distances were compared within and among species to investigate whether these sequences were sufficiently distinct from one another so as to be diagnostic.

METHODS

Coral species were selected based on their ecological role as reef builders and their prevalence in Caribbean reef communities. Seventeen coral species were selected as important targets for identification (Table 1.1). Twelve broadcasting corals, *Acropora cervicornis*, *Acropora palmata*, *Acropora prolifera*, *Dichocoenia stokesii*, *Diploria labyrinthiformis*, *Diploria strigosa*, *Montastraea cavernosa*, *Orbicella annularis*, *Orbicella faveolata*, *Orbicella franksi*, *Pseudodiploria clivosa*, and *Siderastrea siderea* were chosen based on their importance as reef builders (Donahue et al. 2008, Edmunds 2010, Ruzicka et al. 2013, Mercado-Molina et al. 2015). Five brooding species, *Favia fragum*, *Porites astreoides*, *Porites porites*, *Siderastrea radians*, and *Undaria agaricites*, were selected based on their abundance in reef communities (Chiappone and Sullivan 1996, Donahue et al. 2008, Edmunds 2010, Kemp et al. 2016). Samples identified as adults were obtained from various labs in the form of either preserved tissue or extracted DNA (Table 1.1). Two to five individuals were used to encompass genetic variability among

individuals during design of species-specific primers for the SSNM-PCR. The number of samples used for each species was a function of availability and sample quality.

DNA was extracted from coral tissue samples using DNeasy™ Blood & Tissue Kits (Qiagen, Valencia, CA, U.S.A.). PCR was run in duplicate on extracted DNA using two previously designed universal primers, Coral 18S 1648F (5'-gatygaayggttagtgagg) (Frischer unpublished) and ITS-4 (5'-tcctccgcttattgatatgc) (White et al. 1990), that target the region between 18S and 28S genes (Figure 1.1). The PCR protocol consisted of initial denaturing at 94°C for 10 minutes, followed by 30 cycles of denaturing at 94°C for 30 seconds, annealing for 30 seconds at 50°C, and extension at 72°C for 1 minute, followed by a final extension step for 7 minutes. Once PCR was completed, products were pooled by sample and run through an electrophoresis gel. Resulting bands on the gel were removed and the target region was extracted using Quantum Prep® Freeze 'N Squeeze™ tubes (Bio-Rad, Berkeley, CA, U.S.A.).

Once PCR products were extracted from the gel, a ligation reaction was completed using the TOPO TA Cloning® Kit for Sequencing (Invitrogen, Carlsbad, CA, U.S.A.). After ligation, transformation of bacterial *E. coli* cells was carried out using the ligation reactions, and 200 µL of competent cell solution was spread onto agar plates with a 50 µg/mL concentration of kanamycin and allowed to incubate overnight at 37°C. Three colonies from each cloning reaction were selected, then grown on a single patch plate (a singular agarose plate with several separated colonies from different cloning reactions growing on it at one time) overnight in order to obtain even larger concentrations of the target region.

Once a patch plate was incubated overnight, portions of each patch of *E. coli* cells were scraped off and grown in liquid LB media with a 50 µg/mL concentration of kanamycin. These colonies were incubated overnight. The next day, 0.5 mL of each liquid colony was saved in

50% glycerol stocks. Plasmids were extracted from the liquid colonies using a QIAprep® Spin Miniprep Kit (Qiagen, Valencia, CA, U.S.A.). Plasmids were sent to Functional Biosciences, INC. (Madison, WI), for sequencing in both directions to ensure quality reads for the whole target region for each plasmid sample.

Consensus sequences were created for each clone to enhance sequence accuracy. This was accomplished by aligning the two reads obtained from Functional Biosciences using ClustalW Multiple Alignment on the BioEdit software. Once an alignment was made, base pairs were checked by hand and excess sequence reads that went past the universal primer targets were trimmed, and a consensus sequence was formed with just the target area.

Sequences were checked by uploading them to GenBank's Basic Local Alignment Search Tool (BLAST), and then validated by determining if they were similar to any existing coral sequences already found in GenBank. Once confirmed as coral sequences, length and GC-contents of each individual sequence were compared using the BioEdit software. Sequences for *Montastraea cavernosa* (accession numbers KT254595-KT254598) and *Siderastrea radians* (accession numbers AY322604-AY322608) were found on GenBank and used for further comparisons. Finalized sequences were aligned using the MUSCLE alignment formula and a maximum likelihood tree was generated using SATé software (<http://phylo.bio.ku.edu/software/sate/sate.html>). Branches with bootstrap values of less than 50 were collapsed. Mean percent genetic distance within and among species were estimated based on pairwise differences using MEGA 7.0.

RESULTS

A total of 109 usable sequences were obtained from 15 target species (Appendix 1, Table 1.2). Sequence lengths ranged from 526 to 1121 base pairs (mean = 729.06 ± 150.13 S.D.).

Individuals in the genus *Acropora* had the shortest sequence lengths, while individuals of *Diploria labyrinthiformis* had the longest. G/C content ranged from 42.7% to 55.9% (mean = 53.44% \pm 3.93% S.D.), with *Pseudodiploria clivosa* having the lowest G/C content and *Favia fragum* the highest.

The phylogenetic tree generated using the SATé software (Figure 1.2) displayed consistent clustering of individuals of the same genus. The only genus that did not consistently group together was the genus *Diploria*, where *Diploria labyrinthiformis* grouped closer with *Favia fragum* than with *Diploria strigosa* (Figure 1.3), possibly caused by high amounts of intra-specific variation found among *Diploria labyrinthiformis* individuals. At the species level, however, intra-specific clustering was not found for the genera *Acropora* and *Orbicella* (Figure 1.4 and Figure 1.5), where there was no consistent clustering of individuals by species due to high amounts of intra-specific variation and low amounts of inter-specific variation respectively. Despite the inconsistent intra-specific groupings in the genera *Acropora* and *Orbicella*, all other individuals did group together by species. Nodes grouping individuals of the same genus and family exhibited high bootstrap support, while nodes at the species and within species levels were less conserved.

Mean genetic distance between individuals of the same species ranged from 0% to 2.3% and from 0.2% to 51% between individuals of different species (Table 1.3). Species within the genera *Acropora* and *Orbicella* had the greatest genetic distances within species, and relatively low genetic distances among species within the same genus. As expected, genetic distances are even greater at the generic level, with a high of 66.8% between individuals representing different genera.

DISCUSSION

Based on the phylogenetic analysis and the genetic distances between individuals of different species, I conclude that the ITS 1 and 2 sequences can be used to differentiate among all targeted Caribbean corals at the generic level, and among a subgroup of the targeted corals at the species level. The ITS sequences cannot, however, separate species in two genera containing major reef-building species: *Acropora* and *Orbicella*. These findings agree with others showing a lack of phylogenetic separation in Caribbean species of *Acropora* and *Orbicella* (Szmant et al. 1997, Medina et al. 1999, van Oppen et al. 2000, Vollmer and Palumbi 2006, Willis et al. 2006). The results of the current study also coincide with those of Forsman et al. (2006) and Chen et al. (2004), who found that the variation inherent in the ITS sequences can be used to create coral phylogenies that are similar to those constructed using coding regions or mitochondrial DNA, thus demonstrating that this region is separating coral species in a manner that is consistent with other genetic data.

The phylogenetic tree generated by the obtained sequences and those found on GenBank is similar to those developed previously using ITS sequences and other genetic regions (Medina et al. 1999, van Oppen et al. 2000, Vollmer and Polumbi et al. 2006, Forsman et al. 2006). This tree also closely matches current proposed coral taxonomy (Budd et al. 2012). While the ITS sequence was generally effective at grouping corals by species, genera, and families, in several instances unexpected results were found. For example, based on the phylogenetic tree generated using this sequence individuals from the genus *Diploria* were separated into *D. labyrinthiformis* and *D. strigosa*, but *D. labyrinthiformis* appeared to be more closely related to *Favia fragum* than its congener, *D. strigosa* (Figure 1.3). Additionally, *Pseudodiploria clivosa*, a member of the Faviinae sub-family (Budd et al. 2012), showed no relationship to other members of this sub-

family, which includes *D. strigosa*, *D. labyrinthiformis*, and *F. fragum*, despite normally grouping together with these species in phylogenies using other genetic regions. It should be noted that it is not uncommon for trees using only one genetic region to be different from those incorporating combinations of genetic markers. Also, despite their locations on the phylogenetic tree, the genetic distances between these species indicate that they can all be distinguished from one another using the ITS region.

While many individuals were separated by species on the phylogenetic tree, this was not the case in the genera *Acropora* and *Orbicella*, where small genetic distances between members of different species resulted in low bootstrap values. This result can be explained by two factors. First, the species targeted in these two genera have been shown to hybridize within their respective genus, implying close congeneric relationships (for *Orbicella* see Szmant et al. 1997 and Medina et al. 1999, for *Acropora* see van Oppen et al. 2000, Vollmer and Polunbi 2002, and Willis et al. 2006). Second, a common problem with using the ITS sequences for differentiating species comes from the rapid evolution of these non-coding regions (Álvarez and Wendel 2003, Forsman et al. 2006). This rapid evolution can allow for high ITS variation within species. This genetic divergence among individuals of the same species can create enough noise to obscure any useful differences between species (Álvarez and Wendel 2003, Forsman et al. 2006). This high amount of genetic variation can hinder species identification by making conspecific individuals just as genetically distant as congeneric individuals.

Another genus with low ITS variation among species was *Siderastrea*. Between *S. siderea* and *S. radians*, there was only a genetic distance of 0.3%. There was also no observed mean genetic distance among individuals of the same species. This corresponds with the findings of Forsman et al. (2006), where they also found a 0.3% genetic distance between the same two

species. However, in both this study and in Forsman et al. (2006), *S. siderea* and *S. radians* do separate by species on a phylogenetic tree, implying that there is enough variation to consistently distinguish them at the species level.

The genetic variation detected among Caribbean coral species in the ITS region represents a useful new characteristic that can be used to improve identification strategies for corals at early life cycle stages. Corals such as those generally grouped together as “Faviids” (i.e. *Diploria* spp., *Favia fragum*, and *Pseudodiploria clivosa*) in recruitment studies using physical characteristics (see Green and Edmunds 2011 and van Woesik et al. 2014) can be resolved to the genus or species levels using the ITS regions. Based on the variation of the ITS regions, Caribbean species of *Diploria*, *Siderastrea*, and *Porites* can be distinguished from one another, which is increasingly difficult at smaller sizes using morphological features. While there is not enough intrageneric variation to separate corals in the genera *Acropora* and *Orbicella* to the species level, the ITS region can be used to easily separate them from other species at the larval or early recruit stages.

For decades, researchers have struggled to find reliable methods of identifying coral larvae and young recruits to the species level (Forsman et al. 2006, Neigel et al. 2007, Hsu et al. 2014). While polyp morphology is adequate for species resolution in some genera, it is clear that molecular approaches are required in many cases. This study demonstrates that the ITS-1 and ITS-2 regions contain enough genetic variation to correctly classify 6 Caribbean coral species to the genus level and 11 to the species level. This is a marked improvement over identification using only morphological characteristics (Baird and Babcock 2000, Babcock et al. 2003). In order to take advantage of this genetic variation, in Chapter 2, methods were developed that allow researchers to identify coral samples quickly and efficiently using the ITS regions.

TABLES AND FIGURES

Table 1.1: Target coral species, their reproductive mode, the type of sample obtained, and the investigator who provided the samples. Species that were analyzed using sequences from GenBank have the accession numbers used in the Donor(s) column.

Species	Reproductive Mode	Type of Sample	Donor(s)
<i>Acropora cervicornis</i>	Broadcaster	Tissue and DNA	N. Fogarty and S. Schopmeyer
<i>Acropora palmata</i>	Broadcaster	Tissue	N. Fogarty
<i>Acropora prolifera</i>	Broadcaster	Tissue	N. Fogarty
<i>Dichocoenia stokesii</i>	Broadcaster	DNA	A. Baker
<i>Diploria labyrinthiformis</i>	Broadcaster	DNA	A. Baker
<i>Diploria strigosa</i>	Broadcaster	Tissue	N. Fogarty
<i>Favia fragum</i>	Brooder	DNA	A. Baker
<i>Montastraea cavernosa</i>	Broadcaster	Tissue and DNA	A. Baker, D. Brazeau, GenBank Accession Numbers KT254635-KT254638
<i>Orbicella annularis</i>	Broadcaster	DNA	A. Baker
<i>Orbicella faveolata</i>	Broadcaster	DNA	A. Baker
<i>Orbicella franksi</i>	Broadcaster	DNA	N. Fogarty
<i>Porites astreoides</i>	Brooder	DNA	N. Fogarty
<i>Porites porites</i>	Brooder	DNA	S. Schopmeyer
<i>Pseudodiploria clivosa</i>	Broadcaster	DNA	A. Baker
<i>Siderastrea radians</i>	Brooder	N/A	GenBank Accession Numbers AY322604-AY322608
<i>Siderastrea siderea</i>	Broadcaster	DNA	S. Schopmeyer
<i>Undaria agaricites</i>	Brooder	DNA	M.A. Coffroth

Table 1.2: Data for the ITS sequences used in this study. Sequence data includes the number of sequences obtained in the lab for each species, average sequence length, average G/C content, and GenBank accession numbers. Table includes *Montastraea cavernosa* and *Siderastrea radians* sequences obtained from GenBank.

Species	Number of Sequences	Average Length (bp) (\pm S.D.)	Average G/C content (%) (\pm S.D.)	GenBank Accession Numbers
<i>Acropora cervicornis</i>	14	537.2 (\pm 9.05)	56.0 (\pm 0.38)	KY867552- KY867565
<i>Acropora palmata</i>	8	544.6 (\pm 5.49)	55.0 (\pm 0.21)	KY867566- KY867573
<i>Acropora prolifera</i>	9	543.3 (\pm 7.11)	55.8 (\pm 0.30)	KY867574- KY867582
<i>Dichocoenia stokesii</i>	6	763 (\pm 0.00)	54.8 (\pm 0.05)	KY867583- KY867588
<i>Diploria labyrinthiformis</i>	5	1105.2 (\pm 12.68)	55.0 (\pm 0.40)	KY867589- KY867593
<i>Diploria strigosa</i>	5	1045 (\pm 8.00)	53.5 (\pm 0.26)	KY8675924- KY867598
<i>Favia fragum</i>	6	836.5 (\pm 0.40)	57.0 (\pm 0.10)	KY867599- KY867604
<i>Montastraea cavernosa</i>	4	891.5 (\pm 1.00)	53.9 (\pm 0.17)	KT254635- KT254638
<i>Orbicella annularis</i>	9	761.2 (0.44)	55.4 (0.16)	KY867605- KY867613
<i>Orbicella faveolata</i>	9	761 (\pm 0.00)	55.5 (\pm 0.00)	KY867614- KY867622
<i>Orbicella franksi</i>	9	761 (\pm 0.00)	55.5 (\pm 0.18)	KY867623- KY867632
<i>Porites astreoides</i>	6	771.2 (\pm 3.20)	45.3 (\pm 0.15)	KY867633- KY867637
<i>Porites porites</i>	6	766 (\pm 0.00)	45.1 (\pm 0.15)	KY867638- KY867643
<i>Pseudodiploria clivosa</i>	3	783 (\pm 0.00)	42.7 (\pm 0.06)	KY867644- KY867646
<i>Siderastrea radians</i>	4	605 (\pm 0.00)	48.8 (\pm 0.00)	AY322604- AY322608
<i>Siderastrea siderea</i>	6	732 (\pm 0.00)	48.7 (\pm 0.10)	KY867647- KY867652
<i>Undaria agaricites</i>	8	729 (\pm 0.00)	53.0 (\pm 0.10)	KY867653- KY867660

Table 1.3: Mean percent genetic distance matrix for individuals within species and between species generated using MEGA 7 software. Species with lower inter-specific distance than intra-specific distances are highlighted.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	113	14	15	16
0.015	1. <i>A. cervicornis</i>																
0.011	2. <i>A. palmata</i>	0.018															
0.024	3. <i>A. prolifera</i>	0.022	0.018														
0.003	4. <i>D. stokesii</i>	0.524	0.513	0.515													
0.013	5. <i>D. labyrinthiformis</i>	0.577	0.573	0.574	0.168												
0.008	6. <i>D. strigosa</i>	0.591	0.589	0.590	0.167	0.056											
0.001	7. <i>F. fragum</i>	0.579	0.574	0.576	0.141	0.065	0.060										
0.004	8. <i>M. cavernosa</i>	0.537	0.521	0.525	0.064	0.140	0.163	0.130									
0.004	9. <i>O. annularis</i>	0.550	0.537	0.543	0.070	0.169	0.193	0.147	0.043								
0.001	10. <i>O. faveolata</i>	0.545	0.532	0.538	0.069	0.168	0.193	0.147	0.042	0.003							
0.001	11. <i>O. franksi</i>	0.546	0.533	0.540	0.069	0.168	0.193	0.146	0.042	0.002	0.001						
0.004	12. <i>P. clivosa</i>	0.682	0.668	0.392	0.392	0.458	0.443	0.445	0.410	0.396	0.394	0.394					
0.003	13. <i>P. astreoides</i>	0.532	0.516	0.526	0.153	0.213	0.226	0.248	0.157	0.181	0.178	0.179	0.405				
0.000	14. <i>P. porites</i>	0.536	0.523	0.533	0.147	0.219	0.235	0.243	0.169	0.181	0.179	0.179	0.405	0.042			
0.000	15. <i>S. radians</i>	0.509	0.493	0.503	0.089	0.190	0.181	0.183	0.107	0.117	0.116	0.116	0.351	0.140	0.141		
0.000	16. <i>S. siderea</i>	0.507	0.492	0.502	0.086	0.190	0.181	0.183	0.103	0.113	0.113	0.112	0.356	0.140	0.141	0.003	
0.001	17. <i>U. agaricites</i>	0.511	0.507	0.514	0.101	0.186	0.195	0.179	0.116	0.123	0.121	0.121	0.401	0.158	0.098	0.098	0.098
Mean Within Species Distance		Mean Between Species Distances															

Figure 1.1: Schematic of the target DNA region. Primers targeting the highly conserved 3' end of the 18S rRNA coding gene (18S 1648F) and the highly conserved 5' end of the 28S rRNA coding gene (ITS-4) allow for the amplification of the hypervariable ITS 1 and ITS 2 regions, as well as the highly conserved 5.8S rRNA coding gene.

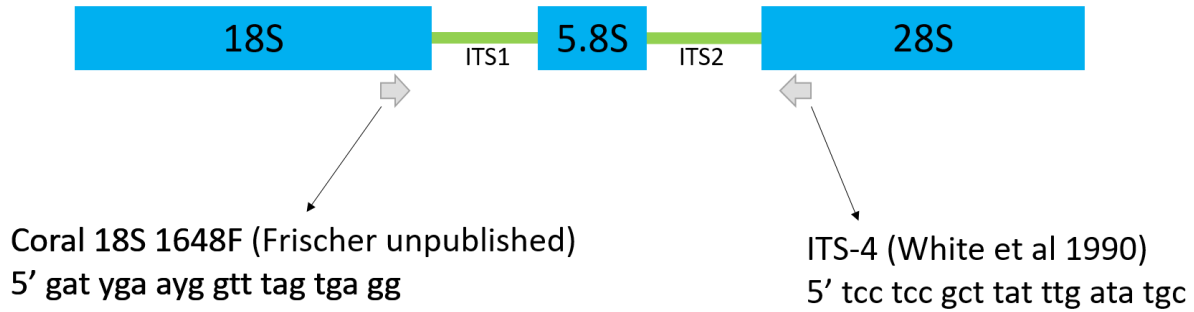


Figure 1.3: A closer look at the *Diploria* genus and *Favia fragum* branches of the generated phylogenetic tree (Figure 1.2). While *D. strigosa* and *D. labyrinthiformis* are in the same genus, *D. labyrinthiformis* displays closer relation to *F. fragum* using the ITS-1 and ITS-2 regions.

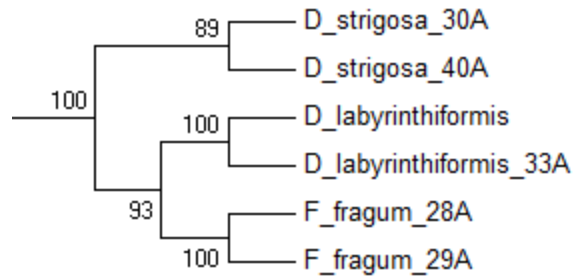


Figure 1.4: A closer look at the *Acropora* genus branches of the generated phylogenetic tree (Figure 1.2), displaying a lack of consistent grouping by species within this genus.

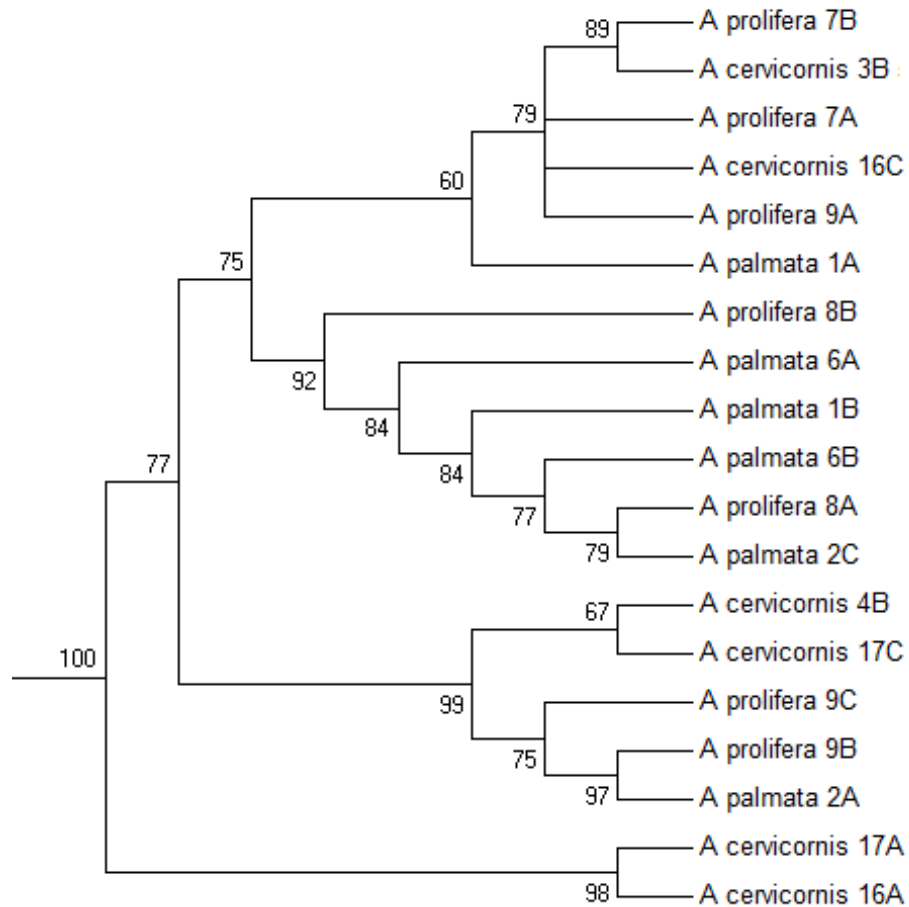
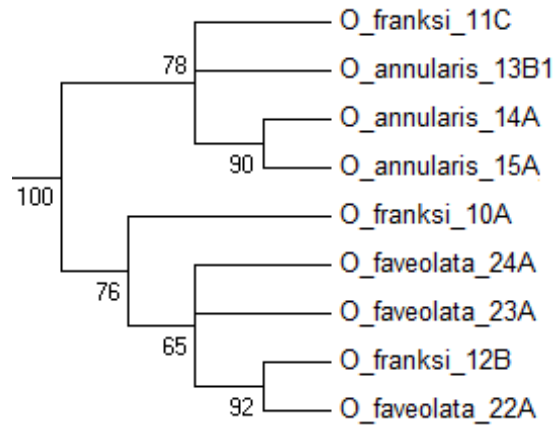


Figure 1.5: A closer look at the *Orbicella* genus branches of the generated phylogenetic tree (Figure 1.2). While *O. annularis* and *O. faveolata* separated from each other, *O. franksi* is found among individuals of both species.



CHAPTER 2 A MOLECULAR ASSAY FOR CARIBBEAN CORAL IDENTIFICATION

INTRODUCTION

Coral reefs in the Caribbean have faced rapid decline for several decades (Hughes 1994, Hughes et al. 2003, Donahue et al. 2008). As these reefs continue to lose ecologically important hermatypic corals, questions about the role that coral recruitment plays in reef recovery and sustainability have become increasingly important to address. Unfortunately, distinguishing between species of young coral recruits that are one to two polyps in size can be difficult due to their small size and similar morphologies. Thus, most recruitment studies only identify newly settled individuals to the familial or generic levels (Bak and Engel 1979, Carlon 2001, Miller and Barimo 2001, Green and Edmunds 2011, van Woesik et al. 2014, Humanes and Bastidas 2015).

The inability, in many cases, to reliably identify early-stage coral recruits to the species level poses problems for managing reefs, as species within the same family can have different ecological roles. For example, in the sub-family Faviinae, species in the genus *Diploria* are large, long-lived, reef-building corals that provide sufficient structure to support a diversity of fish and marine invertebrates (Edmunds 2010). In the same sub-family, *Favia fragum* is a small, short-lived “weedy” coral that can quickly recruit to a reef, but does not grow large enough to provide substantial structural habitat (Gleason et al. 2001, Hoeksema et al. 2012). These corals are difficult to separate at early juvenile stages and are often grouped together as “Faviids,” thereby reducing the ability of recruitment surveys to make conclusions about the potential for recovery of reef function (Green and Edmunds 2011, Humanes and Bastidas 2015).

Genetic markers provide a potential tool for species identification of corals when morphological resolution is insufficient. However, commonly used molecular techniques, such as barcoding the mitochondrial cytochrome oxidase I (COI) and the PaxC genes, have failed to

provide consistent taxonomic resolution past the family or genus level in corals due to the generally slow evolutionary rates for these genetic regions in anthozoans (Neigel et al. 2007, Hsu et al. 2014). Other molecular markers, such as the nuclear 20S rRNA and mitochondrial 16S rRNA genes, have also displayed little promise for determining coral species (Romano and Polumbi 1999, Forsman et al. 2006). Therefore, molecular approaches investigating different genetic regions are warranted.

In Chapter 1, it was confirmed that the rapid evolution of the internal transcribed spacer (ITS) regions 1 and 2 provides enough variation to separate Caribbean corals to the generic level, and many to the species level. In this chapter methods were developed that facilitate use of this variable genetic region for efficient identification of corals. Specifically, a single-step nested multiplex polymerase chain reaction (SSNM-PCR) assay was designed. The ITS-1 and ITS-2 regions are suitable targets for this type of assay because they have enough variation to differentiate corals to the species or genus level and are flanked by two coding regions conserved among Cnidarians (the 18S and 28S rRNA genes) (Larsen et al. 2005, Forsman et al. 2006). The SSNM-PCR assay utilizes a PCR mix that contains one pair of universal primers that amplify a region present in all individuals of the animal group of interest, and several individual primers that work within the universal region to amplify a second smaller product that is species-specific (Larsen et al. 2005). All primers are run in one step, and the size of the target-specific PCR product is used to identify the organism of interest (Larsen et al. 2005). Such a method has been used in the past to identify bivalve larvae to the species level, and may be useful for coral larvae and recruits as well (Larsen et al. 2005, 2007).

For this project, it was predicted that the ITS regions are viable targets for the creation of primers for use in a single-step nested multiplex PCR assay that can be used to identify target

coral species. To test this prediction, the ITS sequences obtained in Chapter 1 were used to design primers targeting certain Caribbean coral species or genera. The designed primers were then combined into three separate sub-assays in order to prevent unwanted interference between primers and tested in conjunction with the universal primer pair using plasmids containing the target species DNA to verify that the designed primers work for their targets.

METHODS

To create primers for Caribbean coral identification, consensus sequences of the ITS regions were made for 15 coral species that are either prevalent and/or ecologically important in the Caribbean using the sequence data gathered in the lab in Chapter 1 (Table 1.1). Potential species-specific target regions within the ITS sequences were identified by aligning sequences obtained for the target coral species and looking for regions that were conserved within species, but variable among species. Optimal primers were designed for these regions using Primer3Plus (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>). Potential primers were chosen based on their ability to only amplify a region for the intended species/genus, and whether or not they work without disrupting the universal primers 181648F and ITS-4. Primers generated for each species were compared against the sequences for non-target species. A primer was rejected if it had fewer than three base pair differences from a non-target species and an alternative primer was available. Primers were synthesized by Integrated DNA Technologies® (Coralville, Iowa) and ordered in 25 nmol concentrations with standard desalting purification.

Primers were first tested for specificity *in silico* by comparing their sequences to those available in the GenBank database using the BLAST tool (Basic Local Alignment Tool; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Primers were considered to show a false positive if they had a 100% match for another Cnidarian species in GenBank. Extracted plasmids containing known coral DNA, which were obtained through the cloning procedures in Chapter 1, were used

to test the primers experimentally. First, plasmids of target species were used as the DNA template for a PCR using the universal primer pair and the species-specific primer. PCR products were then run through electrophoresis on an agarose gel for visualization. Primers that successfully amplified both a universal PCR product and a species-specific product were subsequently used in PCR incorporating plasmids from non-target species as the template. If only a universal PCR product was generated in this reaction, then the primer was considered validated for the target coral species in question. If primers generated both a universal product and a “species-specific” product for species in the same genus, then the primer was either discarded or used as a genus-specific primer.

To ensure that the primers did not interfere with each other, verified primers were grouped into three sub-assays. Each sub-assay contained the universal primer pair and two to three species/genus-specific primers. The primers were grouped together based on *in silico* tests of whether or not they would interfere with each other in the assay using PriDimerCheck (biocomputer.bmi.ac.cn/MPprimer/primer_dimer.html). An attempt was also made to separate primers that amplified species/genus-specific products that were within 30 base pairs of each other. These sub-assays were tested as a whole using both extracted plasmids containing known coral DNA (obtained in Chapter 1), as well as extracted DNA from known corals when available. If primers did not work in one sub-assay, then they were shifted until all three sub-assays consisted of primers that worked for their targets without interference from the other primers. Interference included cross-hybridization between primers, the production of excessive primer-dimers (small oligonucleotides that form when primers hybridize with each other instead of with the DNA template), or the prevention of amplification in a PCR. For example, primers included in sub-assays 1 and 2 prevented the primers targeting *Favia fragum* and *Diploria* spp.

from amplifying the species/genus-specific product associated with their targeted corals.

Therefore, it was necessary to create a separate sub-assay containing just those two primers, where they would not be inhibited from amplifying their target PCR products.

To differentiate between species/genus-specific primers with similar sizes (within 30 base pairs of each other), restriction enzyme cut sites were investigated using Sequencher[®] software. Restriction enzymes ApoI and HaeII were selected based on their ability to make different-sized digestion products that were easier to differentiate than the species-specific products. These enzymes were tested using the species/genus-specific products from primers targeting *Undaria agaricites*, *Dichocoenia stokesii*, *Pseudodiploria clivosa*, *Porites astreoides*, *Porites* spp., and *Siderastrea siderea*, which all produced similarly-sized products. Restriction digest products were visualized using a 2% agarose gel instead of a 1.5% gel to allow for a wider spread of unequal-sized products.

RESULTS

The goal of this project was to design primers for use in a SSNM-PCR assay that will assist scientists and natural resource managers in identifying coral larvae and young recruits. Nine primers were developed during this project and grouped into 3 sub-assays (each sub-assay includes the universal cnidarian ITS primers, 18S1648F and ITS-4) (Table 2.1, Table 2.2, Figure 2.1). Sub-assay one consists of species-specific primers PCF3 (*Pseudodiploria clivosa*) and SSR5 (*Siderastrea siderea*), and the genus-specific primer, PPR1 (*Porites* spp.). Sub-assay two consists of two species-specific primers, DSR7 (*Dichocoenia stokesii*) and UAR1 (*Undaria agaricites*), and one genus-specific primer, O2R1 (*Orbicella* spp.). Sub-assay three consists of one species-specific primer, FFR3 (*Favia fragum*), and one genus-specific primer, DS2R2 (*Diploria* spp.). The final primer, PAF1 (*Porites astreoides*) is for use after a positive reaction

with the PPR1 primer in sub-assay 1. While the PPR1 primer works for both *P. astreoides* and *P. porites*, PAF1 only works for *P. astreoides*, and can thus be used to distinguish between the two *Porites* species found in the Caribbean.

One third of the designed primers produced false positives based on in silico searches on GenBank (Table 2.3). PAF1 was compatible with four *Porites* species native to the Red Sea and the Indo-Pacific, SSR5 showed compatibility with two other *Siderastrea* species (one native to the Caribbean and one from Brazil), and PCF3 displayed false positives for *Millepora exaesa*, a fire coral native to the Indo-Pacific, and two *Zanclaea* species, which are hydroids found in the Caribbean. DNA samples for the false positives were not available, so these were not tested in situ.

While UAR1, DSR7, PCF3, PAF1, PPR1, and SSR5 all generated similar-sized (within 30 base pairs) products, there was some success in differentiating between them by performing a restriction digest on the species/genus-specific product and running it on a 2% agarose gel (Table 2.4, Figure 2.2). Enzyme digests for *Siderastrea siderea* and *Pseudodiploria clivosa* using HaeII and ApoI respectively are easily distinguished from one another and can be used to differentiate between similarly-sized species-specific products generated by the designed assay. However, restriction digest products for DSR7 and UAR1 (both in sub-assay 2) are still close in size and may require a different method to tell them apart. Products generated by *Diploria*-specific primer DS2R2 were not tested for restriction digests as it is in a separate sub-assay with a primer that produces a distinctly-sized PCR product.

DISCUSSION

Currently, the only method of species identification commonly employed in coral recruitment studies is the use of morphological characters (Bak and Engel 1979, Carlon 2001,

Miller and Barimo 2001, van Woesik et al. 2014, Humanes and Bastidas 2015). Using this technique, researchers are only able to confidently identify most new coral recruits to the family or genus level (Bak and Engel 1979, Carlon 2001, Miller and Barimo 2001 van Woesik et al. 2014, Humanes and Bastidas 2015). Previous attempts to identify corals to the species level using molecular techniques such as the coral COI and PaxC regions have also met with limited success (Neigel et al. 2007, Hsu et al. 2014). For this project, the prediction that the ITS regions are viable targets for the creation of primers for use in a single-step nested multiplex PCR assay that can be used to identify targeted coral species and genera was tested. The goal was to develop a set of primers that can be used in a SSNM-PCR assay to identify corals more efficiently and with greater accuracy than is currently possible and at a reasonable cost. Six species-specific and three genus-specific primers were designed that allowed for identification of the species *Dichocoenia stokesii*, *Favia fragum*, *Porites astreoides*, *Porites porites*, *Pseudodiploria clivosa*, *Undaria agaricites*, and *Siderastrea siderea*, as well as corals in the genera *Diploria*, *Orbicella*, and *Porites* (Table 2.1, Figure 2.1). These primers work using a combination of PCR amplifications and restriction enzyme digests (Figure 2.3).

This SSNM-PCR technique has several advantages over commonly used molecular techniques. First, the amplification of a universal product in conjunction with the target-specific product allows researchers to sequence the ITS region of an unknown sample that does not exhibit a target-specific band on an electrophoresis gel. This gives investigators the opportunity to identify the sample based on its ITS sequence, and then potentially create a new primer or edit an existing primer based on the new genetic data. The universal band also serves as an internal positive control for the PCR reaction in that if the universal band does not amplify, then there is either a PCR error, or the DNA is not from a cnidarian. The universal band also enhances the

efficiency of the target-specific primers as it increases the amount of DNA template for the PCR reaction. Finally, using the developed primers within an assay is less expensive than using them in separate PCR amplifications, with the SSNM-PCR costing users approximately 40% of the price of a standard PCR using the same primers. It is even less expensive than sequencing samples, ranging from 58% to 89% less per sample. This lower cost will allow investigators on a limited budget to identify greater numbers of recruits and larvae.

The increased accuracy of coral identification provided by this assay represents a step forward for coral research. The ability to identify coral larvae using molecular techniques makes it possible for researchers to quantify larval supply to reefs, a task that has been hindered by the small sizes and lack of descriptive morphological features of coral planulae (Hodgson 1985, Jones et al. 2009). The ability to quantify larval supply will provide insight into whether or not larval supply is further limiting potential reef recovery. Additionally, this technique can be used as a tool for improving and refining coral recruit field guides that are based on morphology. Specifically, the molecular assay allows unambiguous identification of coral recruits if they are a member of one of the targeted genera or species. Once the identity is known, efforts to locate species of genus-specific, non-plastic skeletal characters can commence. Any such morphological features can then be used in the development of a field guide of coral recruits, thus increasing the speed at which scientists can identify these early-stage corals.

While the development of this SSNM-PCR assay targeting the ITS region is an improvement, further development is required to increase its usefulness. The rapid evolutionary rates of the ITS regions made it unsuitable for amplifying species- or genus-specific products for *Acropora palmata*, *Acropora cervicornis*, and *Acropora prolifera*. Furthermore, the small size of the ITS regions in *Acropora spp.* relative to the other coral species investigated (Table 1.1)

generated a second problem: all *Acropora* ITS primer sites suitable for targeting by this assay were close to the 5' or 3' ends leading to the genus-specific product being indistinguishable from the universal product when run on the gel. Finally, there is evidence that *Acropora prolifera* is an F1 hybrid of *A. palmata* and *A. cervicornis*, which would mean that the three species are closely related, creating further difficulties in distinguishing between them (van Oppen et al. 2000, Vollmer and Polunbi 2002, Willis et al. 2006). Therefore, a different genetic region will need to be explored for identification of recruits of *Acropora spp.*

Further limits of this assay include the inability to distinguish between species within the genera *Orbicella* and *Diploria*. However, the ability to differentiate between *Orbicella spp.*, *Diploria spp.* and *Favia fragum* is a step in the right direction as they are often grouped together as “faviids” when using morphological features (Green and Edmunds 2011). Furthermore, *Orbicella spp.* and *Diploria spp.* are ecologically important reef-building corals that have declined in number over the past few decades (Donahue et al. 2008, Edmunds 2010, Manzello et al. 2015).

In its current form, this coral identification method incorporates a combination of PCR sub-assays and restriction enzyme digests (Figure 2.3). To identify an unknown coral, an investigator will first run three separate PCR amplifications (one for each sub-assay). Each reaction consists of the extracted DNA of the unknown coral, the universal ITS primers 18S-1648F and ITS-4, a PCR mixture containing Taq polymerase, water, dNTPs, and buffer, and the appropriate primers for each sub-assay (sub-assay 1: PPR1, PCF3, and SSR5; sub-assay 2: DSR7, O2R1, and UAR1; sub-assay 3: DS2R2 and FFR3). The sub-assays will be run through a PCR amplification using the following parameters: initial denaturing at 94°C for 10 minutes, followed by 30 cycles of denaturing a 94°C for 30 seconds, annealing for 30 seconds at 50°C,

and extension at 72°C for 1 minute, followed by a final extension step for 7 minutes. Products can then be run on a 1.5% agarose gel. From there, the identity of the can either be determined from the size of the species/genus-specific product, or further steps involving either a restriction enzyme digest or a secondary PCR amplification will be needed.

The efficiency of this assay can be increased by making preliminary identifications from recruit morphology, thus saving PCR reagents and primers. For example, if morphological examination places the coral recruit in the genus *Porites*, it will be more efficient to run a single PCR amplification using only the PAF1 primer and determining whether the coral is *P. porites* or *P. astreoides*. Another way the efficiency of this assay can be improved is by separating the PCR products using a capillary gel electrophoresis sequencer instead of running them on a gel. The former allows for differentiation of PCR products with minimal size differences, thus rendering the secondary PCR reactions or restriction digests required for identification of some coral species unnecessary (Grossman and Colburn 2012). For example, the species-specific primers for *Dichocoenia stokesii* (DSR7) and *Siderastrea siderea* (SSR5) amplify 199 and 178 base pair products, respectively. While the 21 base pair difference is hard to recognize on a standard gel, capillary gel electrophoresis can register these differences and eliminate the need for a second round of PCR. A final way to streamline this assay is to use fluorescently labeled primers. With each primer having a different fluorescent label, the PCR products can be analyzed using a luminescence spectrometer, or a capillary gel sequencer with a fluorescence detector (Livak et al. 1995, Grossman and Colburn 2012). This technique allows for identification based on the fluorescent label of the species/genus-specific primer rather than the size of the PCR product itself.

Finally, while this assay can identify seven species and two genera of Caribbean corals that are either ecologically important or commonplace in the FKNMS, these species represent a subset of all species present. In order to be able to identify more species or genera of Caribbean corals, ITS sequence data and good-quality DNA extracts of additional target corals will be needed. The process of designing these primers is relatively straightforward once the DNA sequence is obtained, and in the future additional primers can be developed to allow for the identification of more coral species found in the Caribbean, or corals native to different regions altogether. Furthermore, for species such as those in the genus *Acropora*, for which primers for this assay could not be developed, or those in the genera *Diploria* and *Orbicella*, for which identification using this assay is only possible to the genus level, more genetic data will be needed to identify DNA regions that may be targeted in a similar assay that may allow for the separation of these species.

For this project, primers targeting six species and three genera of Caribbean corals were developed for use in a SSNM-PCR assay. This is a step forward for coral research, as this molecular technique will assist researchers in identifying coral larvae and early recruits with better accuracy than is currently possible. The increased accuracy will permit investigators to address questions about coral recruitment and larval supply that have thus far been intractable. The ability to identify coral recruits and larvae to the species level can enhance our understanding of how larval supply and recruitment are connected, and how they might contribute to reef recovery.

TABLES AND FIGURES

Table 2.1: Information pertaining to the developed primers. The primer orientation (forward or reverse) is indicated by the last letter in the primer name (F or R), with the exception of ITS-4, which is a previously developed primer with reverse orientation. The “Product Size” column contains the size of the band generated by the primer and the universal co-primer. If the species- or genus-specific product is a forward primer, its co-primer is ITS-4, if the species- or genus-specific primer is a reverse primer, it works with 18S1648F. The “Annealing Temperature” column displays the optimal annealing temperature for each primer working with its intended compliment. The product size of the universal primers varies depending on the species. The length of the universal band for each species can be found in Table 1.2 under “Average Sequence Length.”

Primer Name	Target Species	Sequence	Product Size (bp)	Annealing Temperature (°C)
18S1648F	Universal	5'-GATYGAAYGGTTTAGTGAGG-3'	567-1162	53.9
ITS-4	Universal	5'-TCCTCCGCTTATTTGATATGC-3'		
DSR7	<i>Dichocoenia stokesii</i>	5'-TCACACGGTAACAAAAACAA-3'	199	55.1
DS2R2	<i>Diploria</i> spp.	5'-CTTAAACAACCGGTTTCACAC-3'	219	55.7
FFR3	<i>Favia fragum</i>	5'-TGCAGGACAAAAATCGACG-3'	419	59.8
O2R1	<i>Orbicella</i> spp.	5'-ACCGTCAAAAGTTGTCTCTG-3'	393	53.6
PAF1	<i>Porites astreoides</i>	5'-TTGGACTCGCATTCTCTATT-3'	200	55.0
PPR1	<i>Porites</i> spp.	5'-ACCTGTGCGACCCCTAAAG-3'	217	59.1
PCF3	<i>Pseudodiploria clivosa</i>	5'-GAAGGCTCAACTAGCTTCTG-3'	166	54.6
UAR1	<i>Undaria agaricites</i>	5'-GTCTTTGAGACTCGTCTTGG-3'	178	55.0
SSR5	<i>Siderastrea siderea</i>	5'-CTTGGACACGTATCGGTAAT-3'	164	55.1

Table 2.2: The primer contents of each sub-assay developed. Each sub-assay is also run with the universal primers, 18S1648F and ITS-4. Primer PAF1 (targeting *Porites astreoides*) is used only if a reaction with sub-assay 1 identifies the sample as *Porites* spp.

Primer Name	Target Species	Sub-Assay
PPR1	<i>Porites</i> spp.	1
PCF3	<i>Pseudodiploria clivosa</i>	
SSR5	<i>Siderastrea siderea</i>	
DSR7	<i>Dichocoenia stokesii</i>	2
O2R1	<i>Orbicella</i> spp.	
UAR1	<i>Undaria agaricites</i>	3
DS2R2	<i>Diploria</i> spp.	
FFR3	<i>Favia fragum</i>	N/A
PAF1	<i>Porites astreoides</i>	

Table 2.3: Cross hybridization with non-targeted *Cnidarian* species identified by sequence comparison of all sequences available in GenBank using the BLAST search tool.

Primer	Target Species	“False Positives” Identified Through Blast Search	Accession No.
DSR7	<i>Dichocoenia stokesii</i>	None	N/A
DS2R2	<i>Diploria</i> spp.	None	N/A
FFF3	<i>Favia fragum</i>	None	N/A
O2R1	<i>Orbicella</i> spp.	None	N/A
PAF1	<i>Porites astreoides</i>	<i>Porites lutea</i> ^b	LT558237.1
		<i>Porites lobata</i> ^c	LT558234.1
		<i>Porites evermanni</i> ^c	LT558211.1
		<i>Porites compressa</i> ^c	LT558177.1
PPR1	<i>Porites porites</i>	None	
PCF3	<i>Pseudodiploria clivosa</i>	<i>Millepora exaesa</i> ^b	U65484.1
		<i>Zanclaea galli</i> ^a	LT606999.1
		<i>Zanclaea sango</i> ^a	LT607001.1
UAR1	<i>Undaria agaricites</i>	None	
SSR1	<i>Siderastrea siderea</i>	<i>Siderastrea stellata</i> ^d	KT750839.1
		<i>Siderastrea radians</i> ^a	KT750832.1

^aSpecies that are found in the Caribbean

^bSpecies that are found in the Red Sea

^cSpecies that are found in the Indo-Pacific

^dSpecies that are only confirmed in Brazil

Table 2.4: Sizes of products generated by restriction digests of similarly-sized PCR products. The change in product size after a restriction digestion reaction can be used to distinguish between two species that display similarly-sized products after being run through the designed assay.

Primer	Sub-Assay	Non-Digested Product Size	Restriction Enzyme	Digested Product Size (bp)
PCF3	1	166	<i>ApoI</i>	78
PPR1	1	200	<i>HaeII</i>	182
SSR5	1	164	<i>HaeII</i>	164
DSR7	2	199	<i>HaeII</i>	152
UAR1	2	178	<i>HaeII</i>	143

Figure 2.1: Examples of PCR products for each target species or genus. Species or genus name is followed by the size of the PCR product of the universal primer pair/the size of the PCR product of the species or genus-specific primer.

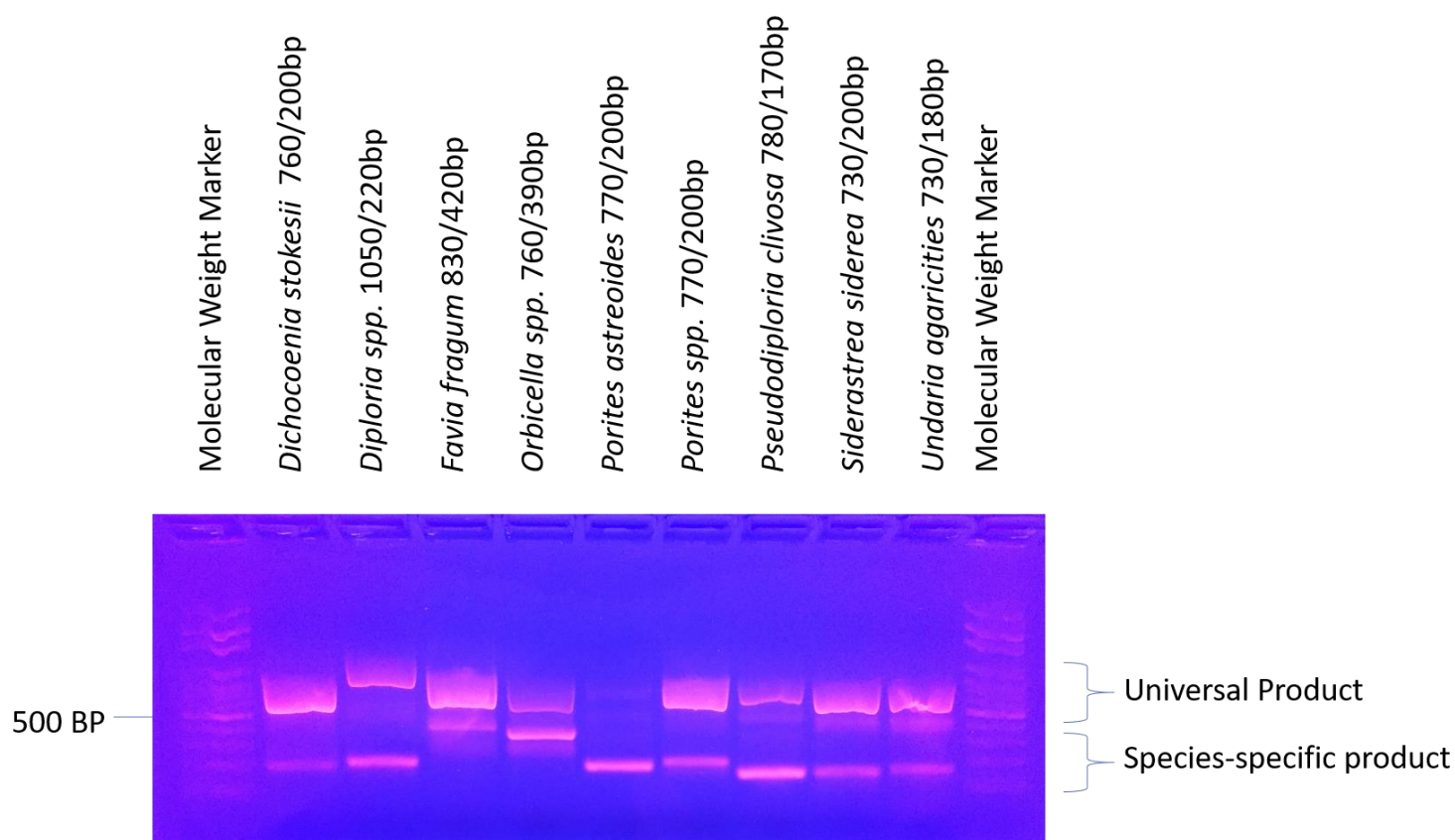


Figure 2.2: Separation of similarly-sized species or genus-specific PCR products using restriction digests. The top row displays the undigested species or genus-specific products, while the bottom row exhibits how these products look after being run through a restriction digest.

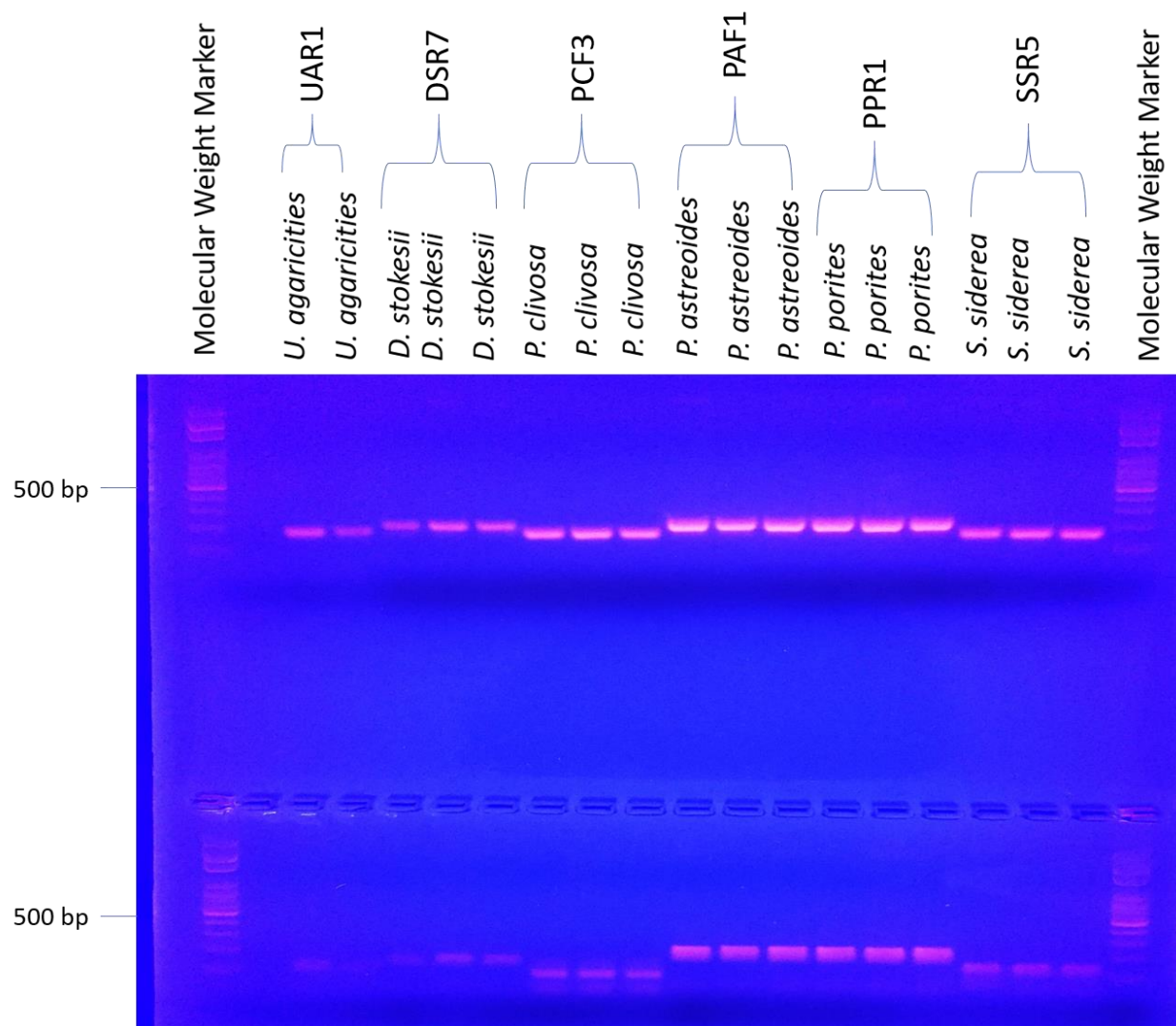
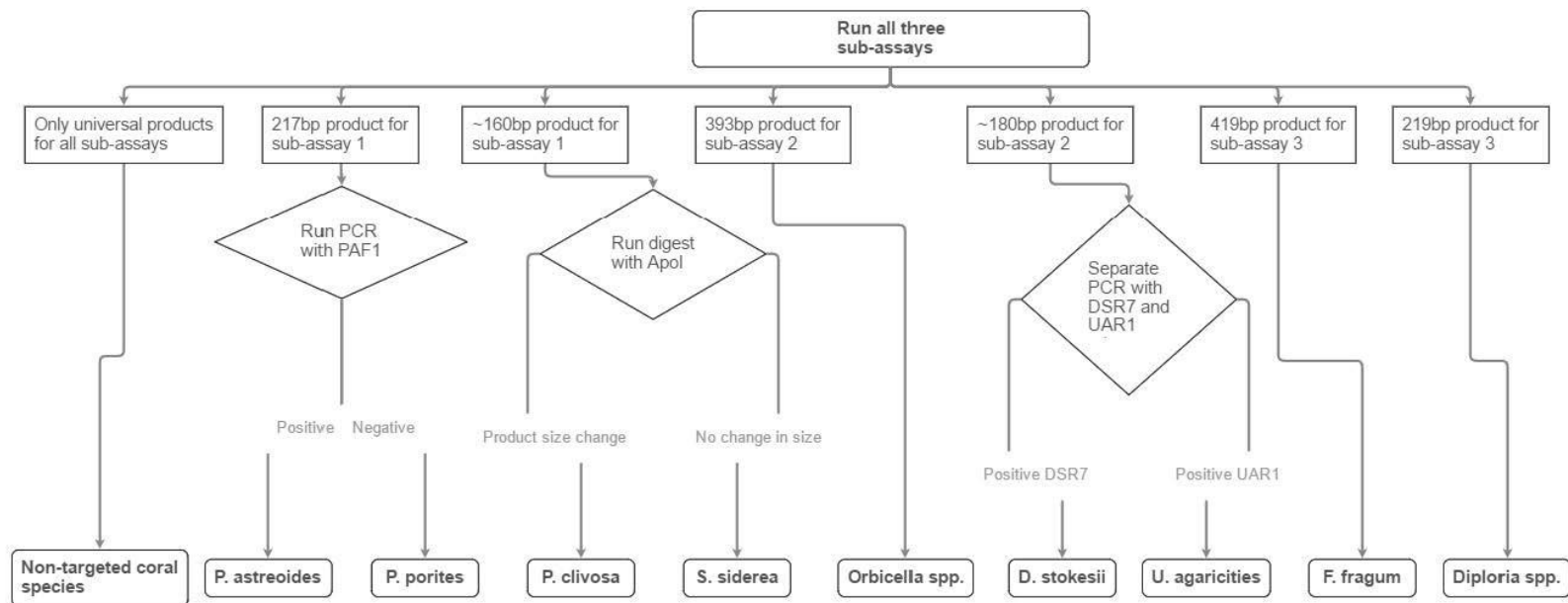


Figure 2.3: Procedure for identifying an unknown coral sample using the SSNM-PCR. To identify an unknown coral, first run three separate PCR amplifications (one for each sub-assay). Each reaction consists of the extracted DNA of the unknown coral, the universal ITS primers 18S-1648F and ITS-4, a PCR mixture containing Taq polymerase, water, dNTPs, and buffer, and the appropriate primers for each sub-assay (sub-assay 1: PPR1, PCF3, and SSR5; sub-assay 2: DSR7, O2R1, and UAR1; sub-assay 3: DS2R2 and FFR3). From there the coral can either be identified based on the size of the species/genus-specific product, or further steps involving either a restriction enzyme digest or a secondary PCR amplification will be needed based on the results of the different sub-assays.



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O_franksi_	GGAAGGATCA	TTACCGATGC	AATCC----	A	AAAACA	AACTC	CG-----	-----
O_franksi_	GGAAGGATCA	TTACCGATGC	AATCC----	A	AAAACA	AACTC	CG-----	-----
O_franksi_	GGAAGGATCA	TTACCGATGC	AATCC----	A	AAAACA	AACTC	CG-----	-----
P_clivosa_	GGAAGGATCA	TTACCGTTTG	TCAAT----	C	AACATGG	CAA	AAACCC----	-----
P_clivosa_	GGAAGGATCA	TTACCGTTTG	TCAAT----	C	AACATGG	CAA	AAACCC----	-----
P_clivosa_	GGAAGGATCA	TTACCGTTTG	TCAAT----	C	AACATGG	CAA	AAACCC----	-----
P_astreoid	GGAAGGATCA	TTACCGATAG	ATCTG----	A	AAACAAC	-AT	TAACCA----	-----
P_astreoid	GGAAGGATCA	TTACCGATAG	ATCTG----	A	AAACAAC	-AT	TAACCA----	-----
P_astreoid	GGAAGGATCA	TTACCGATAG	ATCTG----	A	AAACAAC	-AT	TAACCA----	-----
P_astreoid	GGAAGGATCA	TTACCGATAG	ATCTG----	A	AAACAAC	-AT	TAACCA----	-----
P_astreoid	GGAAGGATCA	TTACCGATAG	ATCTG----	A	AAACAAC	-AT	TAACCA----	-----
P_astreoid	GGAAGGATCA	TTACCGATAG	ATCTG----	A	AAACAAC	-AT	TAACCA----	-----
P_porites_	GGAAGGATCA	TTACCGATGA	ATGTG----	T	GATCATT	AAT	TGACCA----	-----
P_porites_	GGAAGGATCA	TTACCGATGA	ATGTG----	T	GATCATT	AAT	TGACCA----	-----
P_porites_	GGAAGGATCA	TTACCGATGA	ATGTG----	T	GATCATT	AAT	TGACCA----	-----
P_porites_	GGAAGGATCA	TTACCGATGA	ATGTG----	T	GATCATT	AAT	TGACCA----	-----
P_porites_	GGAAGGATCA	TTACCGATGA	ATGTG----	T	GATCATT	AAT	TGACCA----	-----
P_porites_	GGAAGGATCA	TTACCGATGA	ATGTG----	T	GATCATT	AAT	TGACCA----	-----
S_radians	-----	---CCGATAC	GTGTC----	C	AAGGT	TAACT	TTAATG----	-----
S_radians	-----	---CCGATAC	GTGTC----	C	AAGGT	TAACT	TTAATG----	-----
S_radians	-----	---CCGATAC	GTGTC----	C	AAGGT	TAACT	TTAATG----	-----
S_radians	-----	---CCGATAC	GTGTC----	C	AAGGT	TAACT	TTAATG----	-----
S_radians	-----	---CCGATAC	GTGTC----	C	AAGGT	TAACT	TTAATG----	-----
S_radians	-----	---CCGATAC	GTGTC----	C	AAGGT	TAACT	TTAATG----	-----
S_siderea_	GGAAGGATCA	TTACCGATAC	GTGTC----	C	AAGGT	TAACT	TTAACG----	-----
S_siderea_	GGAAGGATCA	TTACCGATAC	GTGTC----	C	AAGGT	TAACT	TTAACG----	-----
S_siderea_	GGAAGGATCA	TTACCGATAC	GTGTC----	C	AAGGT	TAACT	TTAACG----	-----
S_siderea_	GGAAGGATCA	TTACCGATAC	GTGTC----	C	AAGGT	TAACT	TTAACG----	-----
S_siderea_	GGAAGGATCA	TTACCGATAC	GTGTC----	C	AAGGT	TAACT	TTAACG----	-----
S_siderea_	GGAAGGATCA	TTACCGATAC	GTGTC----	C	AAGGT	TAACT	TTAACG----	-----
U_agaricit	GGAAGGATCA	TTACCGATGC	AATCC----	A	AGACGAG	TCT	CAA-----	-----
U_agaricit	GGAAGGATCA	TTACCGATGC	AATCC----	A	AGACGAG	TCT	CAA-----	-----
U_agaricit	GGAAGGATCA	TTACCGATGC	AATCC----	A	AGACGAG	TCT	CAA-----	-----
U_agaricit	GGAAGGATCA	TTACCGATGC	AATCC----	A	AGACGAG	TCT	CAA-----	-----
U_agaricit	GGAAGGATCA	TTACCGATGC	AATCC----	A	AGACGAG	TCT	CAA-----	-----
U_agaricit	GGAAGGATCA	TTACCGATGC	AATCC----	A	AGACGAG	TCT	CAA-----	-----
U_agaricit	GGAAGGATCA	TTACCGATGC	AATCC----	A	AGACGAG	TCT	CAA-----	-----
U_agaricit	GGAAGGATCA	TTACCGATGC	AATCC----	A	AGACGAG	TCT	CAA-----	-----
U_agaricit	GGAAGGATCA	TTACCGATGC	AATCC----	A	AGACGAG	TCT	CAA-----	-----
Alcyonium_	GGAAGGATCA	TCATCGCGTT	ACATC----	A	AAAAAAG	CAA	CAACGT----	-----

	245	255	265	275	285	295
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----GTCG	T-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGCA	T-----ATCG	T-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----GTCG	T-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----ATCG	T-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----ATCG	T-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----ATCG	C-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----ATCG	T-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----ATCG	T-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----ATCG	C-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----GTCG	T-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----ATCG	C-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----ATCG	C-----	-----
A_cervicor	-----	---CATCGTA	TATTGACGTA	T-----ATCG	C-----	-----
A_palmata_	-----	---CATCGTA	TATTGACGTA	T-----GTCG	T-----	-----
A_palmata_	-----	---CATCGTA	TATTGACGTA	T-----ATCG	T-----	-----
A_palmata_	-----	---CATCGTA	TATTGACGTA	T-----ATCG	T-----	-----
A_palmata_	-----	---CATCGTA	TATTGACGTA	T-----ATCG	T-----	-----
A_palmata_	-----	---CATCGTA	TATTGACGTA	T-----ATCG	T-----	-----
A_palmata_	-----	---CATCGTA	TATTGACGTA	T-----GTCG	T-----	-----
A_palmata_	-----	---CATCGTA	TATTGACGTA	T-----ATCG	T-----	-----
A_palmata_	-----	---CATCGTA	TATTGACGTA	T-----GTCG	T-----	-----
A_prolifer	-----	---CATCGTA	TATTGACGTA	T-----GTCG	T-----	-----
A_prolifer	-----	---CATCGTA	TATTGACGTA	T-----GTCG	T-----	-----
A_prolifer	-----	---CATCGTA	TATTGACGTA	T-----CTCG	T-----	-----
A_prolifer	-----	---CATCGTA	TATTGACGTA	T-----GTCG	T-----	-----
A_prolifer	-----	---CATCTTC	TATTGACCTA	TGAGAGAGAG	C-----	-----
A_prolifer	-----	---CATCTTC	TATTGACCTA	TGAGAGAGAG	C-----	-----
A_prolifer	-----	---CATCGTA	TATTGACGTA	T-----GTCG	T-----	-----
A_prolifer	-----	---CATCGTA	TATTGACGTA	T-----CTCG	T-----	-----
A_prolifer	-----	---CATCGTA	TATTGACGTA	T-----ATCG	T-----	-----
D_stokesii	CAGGT-----	-----CCGTT	AAATGGCGGC	CGGACGCGAG	C-----	-----
D_stokesii	CAGGT-----	-----CCGTT	AAATGGCGGC	CGGACGCGAG	C-----	-----
D_stokesii	CAGGT-----	-----CCGTT	AAATGGCGGT	CGGACGCGAG	C-----	-----
D_stokesii	CAGGT-----	-----CCGTT	AAATGGCGGT	CGGACGCGAG	C-----	-----
D_stokesii	CAGGT-----	-----CCGTT	AAATGGCGGT	CGGACGCGAG	C-----	-----
D_labyrint	CAGAAT----	---TGCCGTT	AAATGGCGAT	GG--CACTTG	CATTGGTGTT	CTTGCGCTGT
D_labyrint	CAGAAT----	---TGCCGTT	AAATGGCGAT	GG--CACTTG	CATTGGTGTT	CTTGCGCTGT

O_franksi_	CCCGG-----	----GCCGTT	AAATGGCGAT	CGGACGTGCA	A-----	-----
O_franksi_	CCCGG-----	----GCCGTT	AAATGGCGAT	CGGACGTGCA	A-----	-----
O_franksi_	CCCGG-----	----GCCGTT	AAATGGCGAT	CGGACGTGCA	A-----	-----
P_clivosa_	CGAGGTGC--	---ATTCCTT	TTATAATCGG	C-----CCC	AACGTGC---	-----
P_clivosa_	CGAGGTGC--	---ATTCCTT	TTATAATCGG	C-----CCC	AACGTGC---	-----
P_clivosa_	CGAGGTGC--	---ATTCCTT	TTATAATCGG	C-----CCC	AACGTGC---	-----
P_astreoid	CACAGGCGTG	---TGTCGTT	AAATGGCGGA	T---AGGTTC	C-----	-----
P_astreoid	CACAGGCGTG	---TGTCGTT	AAATGGCGGA	T---AGGTTC	C-----	-----
P_astreoid	CACAGGCGTG	---TGTCGTT	AAATGGCGGA	T---AGGTTC	C-----	-----
P_astreoid	CACAGGCGTG	---TGTTGTT	AAATGGCGGA	T---AGGTTC	C-----	-----
P_astreoid	CACAGGCGTG	---TGTCGTT	AAATGGCGGA	T---AGGTTC	C-----	-----
P_astreoid	CACAGGCGTG	---TGTTGTT	AAATGGCGGA	T---AGGTTC	C-----	-----
P_porites_	CACAGGTAGA	AGTGGTCGTT	AAATGGTGGG	T-----ATTC	C-----	-----
P_porites_	CACAGGTAGA	AGTGGTCGTT	AAATGGTGGG	T-----ATTC	C-----	-----
P_porites_	CACAGGTAGA	AGTGGTCGTT	AAATGGTGGG	T-----ATTC	C-----	-----
P_porites_	CACAGGTAGA	AGTGGTCGTT	AAATGGTGGG	T-----ATTC	C-----	-----
P_porites_	CACAGGTAGA	AGTGGTCGTT	AAATGGTGGG	T-----ATTC	C-----	-----
P_porites_	CACAGGTAGA	AGTGGTCGTT	AAATGGTGGG	T-----ATTC	C-----	-----
S_radians	CCTGATG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	T-----G---	-----
S_radians	CCTGATG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	T-----G---	-----
S_radians	CCTGATG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	T-----G---	-----
S_radians	CCTGATG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	T-----G---	-----
S_radians	CCTGATG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	T-----G---	-----
S_radians	CCTGATG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	T-----G---	-----
S_siderea_	CCTAAGG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	TGTGAAA---	-----
S_siderea_	CCTAAGG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	TGTGAAA---	-----
S_siderea_	CCTAAGG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	TGTGAAA---	-----
S_siderea_	CCTAAGG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	TGTGAAA---	-----
S_siderea_	CCTAAGG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	TGTGAAA---	-----
S_siderea_	CCTAAGG---	---TGCCGTT	AAATGGTCGT	C---CGTCGT	TGTGAAA---	-----
U_agaricit	TCTCCG----	---TGCCGTT	AAATGG---T	CGTTCGCTCG	C-----	-----
U_agaricit	TCTCCG----	---TGCCGTT	AAATGG---T	CGTTCGCTCG	C-----	-----
U_agaricit	TCTCCG----	---TGCCGTT	AAATGG---T	CGTTCGCTCG	C-----	-----
U_agaricit	TCTCCG----	---TGCCGTT	AAATGG---T	CGTTCGCTCG	C-----	-----
U_agaricit	TCTCCG----	---TGCCGTT	AAATGG---T	CGTTCGCTCG	C-----	-----
U_agaricit	TCTCCG----	---TGCCGTT	AAATGG---T	CGTTCGCTCG	C-----	-----
U_agaricit	TCTCCG----	---TGCCGTT	AAATGG---T	CGTTCGCTCG	C-----	-----
U_agaricit	TCTCCG----	---TGCCGTT	AAATGG---T	CGTTCGCTCG	C-----	-----
Alcyonium_	TGGATGGCGA	---CATTCTT	AAATGGGTGG	A---TGGACA	C-----	-----

	545	555	565	575	585	595
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATAGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATAGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATAGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATAGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATAGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_cervicor	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_palmata_	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_palmata_	-ATCACCTGA	C-----	-----	-----	-----	--CTCATCGT
A_palmata_	-ATCACCTGA	C-----	-----	-----	-----	--CTCATCGT
A_palmata_	-ATCACCTGA	C-----	-----	-----	-----	--CTCATCGT
A_palmata_	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_palmata_	-ATCACCTGA	C-----	-----	-----	-----	--CTCATCGT
A_palmata_	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_palmata_	-ATCACCTGA	C-----	-----	-----	-----	--CTCATCGT
A_palmata_	-ATCACCTGA	C-----	-----	-----	-----	--CTCATCGT
A_prolifer	-ATCACCGGA	C-----	-----	-----	-----	--CTCATAGT
A_prolifer	-ATCACCTGA	C-----	-----	-----	-----	--CTCATCGT
A_prolifer	-ATCACCTGA	C-----	-----	-----	-----	--CTCATCGT
A_prolifer	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_prolifer	-CTCACCTGA	C-----	-----	-----	-----	--CTCATCGT
A_prolifer	-CTCACCTGA	C-----	-----	-----	-----	--CTCATCGT
A_prolifer	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
A_prolifer	-ATCACCTGA	C-----	-----	-----	-----	--CTCATCGT
A_prolifer	-ATCACCGGA	C-----	-----	-----	-----	--CTCATCGT
D_stokesii	GCTCCGCCAG	C-----	-----	-----	-----	--CCCGCCTG
D_stokesii	GCTCCGCCAG	C-----	-----	-----	-----	--CCCGCCTG
D_stokesii	GCTCCGCCAG	C-----	-----	-----	-----	--CCCGCCTG
D_stokesii	GCTCCGCCAG	C-----	-----	-----	-----	--CCCGCCTG
D_stokesii	GCTCCGCCAG	C-----	-----	-----	-----	--CCCGCCTG
D_labyrinth	GCGCTGTCCC	CTTACAGGTG	GAGGTTC---	-----	-----TTTG	GTCCCCACAA
D_labyrinth	GCGCTGTCCC	CTTACAGGTG	GAGGTTC---	-----	-----TTTG	GTCCCCACAA

O_franksi_	GCCTCGCCTG	C-----	-----	-----	-----	--CCCGCCTG
O_franksi_	GCCTCGCCTG	C-----	-----	-----	-----	--CCCGCCTG
O_franksi_	GCCTCGCCTG	C-----	-----	-----	-----	--CCCGCCTG
P_clivosa_	ATCTAAAATA	A-----	-----	-----	-----	--TTTATTTT
P_clivosa_	ATCTAAAATA	A-----	-----	-----	-----	--TTTATTTT
P_clivosa_	ATCTAAAATA	A-----	-----	-----	-----	--TTTATTTT
P_astreoid	-TTCCGCCAG	C-----	-----	-----	-----	--CCCGCCAT
P_astreoid	-TTCCGCCAG	C-----	-----	-----	-----	--CCCGCCAT
P_astreoid	-TTCCGCCAG	C-----	-----	-----	-----	--CCCGCCAT
P_astreoid	-TTCCGCCAG	C-----	-----	-----	-----	--CCCGCCAT
P_astreoid	-TTCCGCCAG	C-----	-----	-----	-----	--CCCGCCAT
P_astreoid	-TTCCGCCAG	C-----	-----	-----	-----	--CCCGCCAT
P_porites_	-TTCCAGCAG	C-----	-----	-----	-----	--CCCGCCAT
P_porites_	-TTCCAGCAG	C-----	-----	-----	-----	--CCCGCCAT
P_porites_	-TTCCAGCAG	C-----	-----	-----	-----	--CCCGCCAT
P_porites_	-TTCCAGCAG	C-----	-----	-----	-----	--CCCGCCAT
P_porites_	-TTCCAGCAG	C-----	-----	-----	-----	--CCCGCCAT
P_porites_	-TTCCAGCAG	C-----	-----	-----	-----	--CCCGCCAT
S_radians	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGCCTT
S_radians	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGCCTT
S_radians	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGCCTT
S_radians	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGCCTT
S_radians	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGCCTT
S_siderea_	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGCCTT
S_siderea_	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGCCTT
S_siderea_	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGCCTT
S_siderea_	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGCCTT
S_siderea_	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGCCTT
S_siderea_	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGCCTT
U_agaricit	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGACCC
U_agaricit	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGACCC
U_agaricit	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGACCC
U_agaricit	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGACCC
U_agaricit	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGACCC
U_agaricit	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGACCC
U_agaricit	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGACCC
U_agaricit	GTTCGGTCAG	C-----	-----	-----	-----	--CCCGACCC
Alcyonium_	-ATCGAACAC	A-----	-----	-----	-----	--TGCGAGTT

O_franksi_	TG-TTTTTCC	T-----	-----	-----	-----	-----
O_franksi_	TG-TTTTTCC	T-----	-----	-----	-----	-----
O_franksi_	TG-TTTTTCC	T-----	-----	-----	-----	-----
P_clivosa_	AGCACACTTT	T-----	-----	-----	-----	-----
P_clivosa_	AGCACACTTT	T-----	-----	-----	-----	-----
P_clivosa_	AGCACACTTT	T-----	-----	-----	-----	-----
P_astreoid	TGTGTTTATT	A-----	-----	-----	-----	-----
P_astreoid	TGTGTTTATT	A-----	-----	-----	-----	-----
P_astreoid	TGTGTTTATT	A-----	-----	-----	-----	-----
P_astreoid	TGTGTTTATT	A-----	-----	-----	-----	-----
P_astreoid	TGTGTTTATT	A-----	-----	-----	-----	-----
P_astreoid	TGTGTTTATT	A-----	-----	-----	-----	-----
P_astreoid	TGTGTTTATT	A-----	-----	-----	-----	-----
P_porites_	TGTGTCTTAC	T-----	-----	-----	-----	-----
P_porites_	TGTGTCTTAC	T-----	-----	-----	-----	-----
P_porites_	TGTGTCTTAC	T-----	-----	-----	-----	-----
P_porites_	TGTGTCTTAC	T-----	-----	-----	-----	-----
P_porites_	TGTGTCTTAC	T-----	-----	-----	-----	-----
P_porites_	TGTGTCTTAC	T-----	-----	-----	-----	-----
S_radians	TGTATTCTTT	A-----	-----	-----	-----	-----
S_radians	TGTATTCTTT	A-----	-----	-----	-----	-----
S_radians	TGTATTCTTT	A-----	-----	-----	-----	-----
S_radians	TGTATTCTTT	A-----	-----	-----	-----	-----
S_radians	TGTATTCTTT	A-----	-----	-----	-----	-----
S_siderea_	TGTTTTATTT	A-----	-----	-----	-----	-----
S_siderea_	TGTTTTATTT	A-----	-----	-----	-----	-----
S_siderea_	TGTTTTATTT	A-----	-----	-----	-----	-----
S_siderea_	TGTTTTATTT	A-----	-----	-----	-----	-----
S_siderea_	TGTTTTATTT	A-----	-----	-----	-----	-----
S_siderea_	TGTTTTATTT	A-----	-----	-----	-----	-----
U_agaricit	GATGTTTTTA	C-----	-----	-----	-----	-----
U_agaricit	GATGTTTTTA	C-----	-----	-----	-----	-----
U_agaricit	GATGTTTTTA	C-----	-----	-----	-----	-----
U_agaricit	GATGTTTTTA	C-----	-----	-----	-----	-----
U_agaricit	GATGTTTTTA	C-----	-----	-----	-----	-----
U_agaricit	GATGTTTTTA	C-----	-----	-----	-----	-----
U_agaricit	GATGTTTTTA	C-----	-----	-----	-----	-----
U_agaricit	GATGTTTTTA	C-----	-----	-----	-----	-----
Alcyonium_	GGCAACGACT	T-----	-----	-----	-----	-----

O_franksi_	-----	-----	-----	-----	-GTGGCCAAT	GTACGTGTCT
O_franksi_	-----	-----	-----	-----	-GTGGCCAAT	GTACGTGTCT
O_franksi_	-----	-----	-----	-----	-GTGGCCAAT	GTACGTGTCT
P_clivosa_	-----	-----	-----	-----	---TACTGTT	TTCCAGTAAG
P_clivosa_	-----	-----	-----	-----	---TACTGTT	TTCCAGTAAG
P_clivosa_	-----	-----	-----	-----	---TACTGTT	TTCCAGTAAG
P_astreoid	-----	-----	-----	-----	---TATCACT	TTGAACGTTA
P_astreoid	-----	-----	-----	-----	---TATCACT	TTGAACGTTA
P_astreoid	-----	-----	-----	-----	---TATCACT	TTGAACGTTA
P_astreoid	-----	-----	-----	-----	---TATCACT	TTGAACGTTA
P_astreoid	-----	-----	-----	-----	---TATCACT	TTGAACGTTA
P_astreoid	-----	-----	-----	-----	---TATCACT	TTGAACGTTA
P_porites_	-----	-----	-----	-----	---ATGCATA	TTGAACGTAA
P_porites_	-----	-----	-----	-----	---ATGCATA	TTGAACGTAA
P_porites_	-----	-----	-----	-----	---ATGCATA	TTGAACGTAA
P_porites_	-----	-----	-----	-----	---ATGCATA	TTGAACGTAA
P_porites_	-----	-----	-----	-----	---ATGCATA	TTGAACGTAA
P_porites_	-----	-----	-----	-----	---ATGCATA	TTGAACGTAA
S_radians	-----	-----	-----	-----	---TATCAAA	CTTTGTCCAG
S_radians	-----	-----	-----	-----	---TATCAAA	CTTTGTCCAG
S_radians	-----	-----	-----	-----	---TATCAAA	CTTTGTCCAG
S_radians	-----	-----	-----	-----	---TATCAAA	CTTTGTCCAG
S_radians	-----	-----	-----	-----	---TATCAAA	CTTTGTCCAG
S_siderea_	-----	-----	-----	-----	---TATCAAA	CGTTGTGCAG
S_siderea_	-----	-----	-----	-----	---TATCAAA	CGTTGTGCAG
S_siderea_	-----	-----	-----	-----	---TGTCAAA	CTTTGTGCAG
S_siderea_	-----	-----	-----	-----	---TATCAAA	CGTTGTGCAG
S_siderea_	-----	-----	-----	-----	---TGTCAAA	CTTTGTGCAG
S_siderea_	-----	-----	-----	-----	---TATCAAA	CGTTGTGCAG
U_agaricit	-----	-----	-----	-----	-----	-----
U_agaricit	-----	-----	-----	-----	-----	-----
U_agaricit	-----	-----	-----	-----	-----	-----
U_agaricit	-----	-----	-----	-----	-----	-----
U_agaricit	-----	-----	-----	-----	-----	-----
U_agaricit	-----	-----	-----	-----	-----	-----
U_agaricit	-----	-----	-----	-----	-----	-----
U_agaricit	-----	-----	-----	-----	-----	-----
Alcyonium_	-----	-----	-----	-----	---CATCATT	T----CGCCA

	725	735	745	755	765	775
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_cervicor	-----	-----	-----	-----	-----	-----
A_palmata_	-----	-----	-----	-----	-----	-----
A_palmata_	-----	-----	-----	-----	-----	-----
A_palmata_	-----	-----	-----	-----	-----	-----
A_palmata_	-----	-----	-----	-----	-----	-----
A_palmata_	-----	-----	-----	-----	-----	-----
A_palmata_	-----	-----	-----	-----	-----	-----
A_palmata_	-----	-----	-----	-----	-----	-----
A_palmata_	-----	-----	-----	-----	-----	-----
A_palmata_	-----	-----	-----	-----	-----	-----
A_palmata_	-----	-----	-----	-----	-----	-----
A_prolifer	-----	-----	-----	-----	-----	-----
A_prolifer	-----	-----	-----	-----	-----	-----
A_prolifer	-----	-----	-----	-----	-----	-----
A_prolifer	-----	-----	-----	-----	-----	-----
A_prolifer	-----	-----	-----	-----	-----	-----
A_prolifer	-----	-----	-----	-----	-----	-----
A_prolifer	-----	-----	-----	-----	-----	-----
A_prolifer	-----	-----	-----	-----	-----	-----
A_prolifer	-----	-----	-----	-----	-----	-----
D_stokesii	TATGTCTGAC	CATGTTCG---	GTGCGACCGC	TTCGCGGAAG	TGCGTCGTCA	CCT-----GG
D_stokesii	TATGTCTGAC	CATGTTCG---	GTGCGGCCGC	TTCGCGGAAG	TGCGTCGTCA	CCT-----GG
D_stokesii	TATGTCTGAC	CATGTTCG---	GTGCGGCCGC	TTCGCGGAAG	TGCGTCGTCA	CCT-----GG
D_stokesii	TATGTCTGAC	CATGTTCG---	GTGCGGCCGC	TTCGCGGAAG	TGCGTCGTCA	CCT-----GG
D_stokesii	TATGTCTGAC	CATGTTCG---	GTGCGGCCGC	TTCGCGGAAG	TGCGTCGTCA	CCT-----GG
D_stokesii	TATGTCTGAC	CATGTTCG---	GTGCGGCCGC	TTCGCGGAGG	TGCGTCGTCA	CCT-----GG
D_labyrint	TAATCGTACC	TTAACCCCAA	GTCTGATT--	--TGCTGCAG	-CCCCAGGGC	TGT-----GC
D_labyrint	TAATCGTACC	TTAACCCCAA	GTCTGATT--	--TGCTGCAG	-CCCCAGGGC	TGT-----GC

O_franksi_	ATTGTCTGAT	GTGACTGTGA	CGGCGTCCGC	CGACCGGCGG	TTCGCCCGAA	AGT-----TG
O_franksi_	ATTGTCTGAT	GTGACTGTGA	CGGCGTCCGC	CGACCGGCGG	TTCGCCCGAA	AGT-----TG
O_franksi_	ATTGTCTGAT	GTGACTGTGA	CGGCGTCCGC	CGACCGGCGG	TTCGCCCGAA	AGT-----TG
P_clivosa_	TGATTTTCTT	GTGTTTCGTT	GTTTAAACGTA	TAAATGAACG	ACGACAGAAG	A-----
P_clivosa_	TGATTTTCTT	GTGTTTCGTT	GTTTAAACGTA	TAAATGAACG	ACGACAGAAG	A-----
P_clivosa_	TGATTTTCTT	GTGTTTCGTT	GTTTAAACGTA	TAAATGAACG	ACGACAGAAG	A-----
P_astreoid	AAG----ACT	GAGAACGTAA	GTAATTGATG	TGTGCGAACA	GCGTGCATTA	ATT----A--
P_astreoid	AAG----ACT	GAGAACGTAA	GTAATTGATG	TGTGCGAACA	GCGTGCATTA	ATT----A--
P_astreoid	AAG----ACT	GAGAACGTAA	GTAATTGATG	TGTGCGAACA	GCGTGCATTA	ATT----A--
P_astreoid	AAG----ACT	GAGAACGTAA	GTAATTGATG	TGTGCGAACA	GCGTGCATTA	ATT----A--
P_astreoid	AAG----ACT	GAGAACGTAA	GTAATTGATG	TGTGCGAACA	GCGTGCATTA	ATT----A--
P_astreoid	AAG----ACT	GAGAACGTAA	GTAATTGATG	TGTGCGAACA	GCGTGCATTA	ATT----A--
P_porites_	AAG----ACT	GAGAG-----	--AACGTATC	TTCATGATCA	CCATGAAAAGG	AAA----AAC
P_porites_	AAG----ACT	GAGAG-----	--AACGTATC	TTCATGATCA	CCATGAAAAGG	AAA----AAC
P_porites_	AAG----ACT	GAGAG-----	--AACGTATC	TTCATGATCA	CCATGAAAAGG	AAA----AAC
P_porites_	AAG----ACT	GAGAG-----	--AACGTATC	TTCATGATCA	CCATGAAAAGG	AAA----AAC
P_porites_	AAG----ACT	GAGAG-----	--AACGTATC	TTCATGATCA	CCATGAAAAGG	AAA----AAC
P_porites_	AAG----ACT	GAGAG-----	--AACGTATC	TTCATGATCA	CCATGAAAAGG	AAA----AAC
S_radians	-----TCT	GAAAACAATT	GTGCTACTCT	TAAAAAGGTA	GTAACAAATG	A-----
S_radians	-----TCT	GAAAACAATT	GTGCTACTCT	TAAAAAGGTA	GTAACAAATG	A-----
S_radians	-----TCT	GAAAACAATT	GTGCTACTCT	TAAAAAGGTA	GTAACAAATG	A-----
S_radians	-----TCT	GAAAACAATT	GTGCTACTCT	TAAAAAGGTA	GTAACAAATG	A-----
S_radians	-----TCT	GAAAACAATT	GTGCTACTCT	TAAAAAGGTA	GTAACAAATG	A-----
S_siderea_	-----CCT	GAAAAC----	CTACTACTTT	TGAGTAAGTA	GTAAGAAATG	A-----
S_siderea_	-----CCT	GAAAAC----	CTACTACTTT	TGAGTAAGTA	GTAAGAAATG	A-----
S_siderea_	-----CCT	GAAAAC----	GTACTACTTT	TGAGTAAGTA	GTAAGAAATG	A-----
S_siderea_	-----CCT	GAAAAC----	CTACTACTTT	TGAGTAAGTA	GTAAGAAATG	A-----
S_siderea_	-----CCT	GAAAAC----	GTACTACTTT	TGAGTAAGTA	GTAAGAAATG	A-----
S_siderea_	-----CCT	GAAAAC----	CTACTACTTT	TGAGTAAGTA	GTAAGAAATG	A-----
U_agaricit	-----G	AACGTTG---	TTCAGTCAGT	CTGAGAGCCA	ACAGTAAGTG	TTG-----GA
U_agaricit	-----G	AACGTTG---	TTCAGTCAGT	CTGAGAGCCA	ACAGTAAGTG	TTG-----GA
U_agaricit	-----G	AACGTTG---	TTCAGTCAGT	CTGAGAGCCA	ACAGTAAGTG	TTG-----GA
U_agaricit	-----G	AACGTTG---	TTCAGTCAGT	CTGAGAGCCA	ACAGTAAGTG	TTG-----GA
U_agaricit	-----G	AACGTTG---	TTCAGTCAGT	CTGAGAGCCA	ACAGTAAGTG	TTG-----GA
U_agaricit	-----G	AACGTTG---	TTCAGTCAGT	CTGAGAGCCA	ACAGTAAGTG	TTG-----GA
U_agaricit	-----G	AACGTTG---	TTCAGTCAGT	CTGAGAGCCA	ACAGTAAGTG	TTG-----GA
U_agaricit	-----G	AACGTTG---	TTCAGTCAGT	CTGAGAGCCA	ACAGTAAGTG	TTG-----GA
Alcyonium_	TTG----CCC	GGCCTCTCGA	TTCATTTATA	CAAACAAACT	ATTGACGTTT	AAC----TTA

O_franksi_	TCATCCATCG	C----	AGGC-	-----	AA	A---	AACTTG	CCGGCGGCCT	TGAGGCGTCA
O_franksi_	TCATCCATCG	C----	AGGC-	-----	AA	A---	AACTTG	CCGGCGGCCT	TGAGGCGTCA
O_franksi_	TCATCCATCG	C----	AGGC-	-----	AA	A---	AACTTG	CCGGCGGCCT	TGAGGCGTCA
P_clivosa_	AAACCTA---	C----	ACAC-	-----		--	TTCTGGTT	TTTGTGGCTA	GGGAGCTTCA
P_clivosa_	AAACCTA---	C----	ACAC-	-----		--	TTCTGGTT	TTTGTGGCTA	GGGAGCTTCA
P_clivosa_	AAACCTA---	C----	ACAC-	-----		--	TTCTGGTT	TTTGTGGCTA	GGGAGCTTCA
P_astreoid	TTATCGAACG	C----	GCAT-	-----		----	GAATG	TGTGCGGTAT	TGAGGTGTCA
P_astreoid	TTATCGAACG	C----	GCAT-	-----		----	GAATG	TGTGCGGTAT	TGAGGTGTCA
P_astreoid	TTATCGAACG	C----	GCAT-	-----		----	GAATG	TGTGCGGTAT	TGAGGTGTCA
P_astreoid	TTATCGAACG	C----	GCAT-	-----		----	GAATG	TGTGCGGTAT	TGAGGTGTCA
P_astreoid	TTATCGAACG	C----	GCAT-	-----		----	GAATG	TGTGCGGTAT	TGAGGTGTCA
P_astreoid	TTATCGAACG	C----	GCAT-	-----		----	GAATG	TGTGCGGTAT	TGAGGTGTCA
P_porites_	TTATCGAACG	C----	ACAC-	-----	T	CACTCGTGAG	TGTGCGGTAT	TGAGGTGTCA	
P_porites_	TTATCGAACG	C----	ACAC-	-----	T	CACTCGTGAG	TGTGCGGTAT	TGAGGTGTCA	
P_porites_	TTATCGAACG	C----	ACAC-	-----	T	CACTCGTGAG	TGTGCGGTAT	TGAGGTGTCA	
P_porites_	TTATCGAACG	C----	ACAC-	-----	T	CACTCGTGAG	TGTGCGGTAT	TGAGGTGTCA	
P_porites_	TTATCGAACG	C----	ACAC-	-----	T	CACTCGTGAG	TGTGCGGTAT	TGAGGTGTCA	
P_porites_	TTATCGAACG	C----	ACAC-	-----	T	CACTCGTGAG	TGTGCGGTAT	TGAGGTGTCA	
S_radians	CCATCTAACG	C----	ACAC-	-----		-----	GTG	TGTGCGGCCT	TGAGGCGTCA
S_radians	CCATCTAACG	C----	ACAC-	-----		-----	GTG	TGTGCGGCCT	TGAGGCGTCA
S_radians	CCATCTAACG	C----	ACAC-	-----		-----	GTG	TGTGCGGCCT	TGAGGCGTCA
S_radians	CCATCTAACG	C----	ACAC-	-----		-----	GTG	TGTGCGGCCT	TGAGGCGTCA
S_radians	CCATCTAACG	C----	ACAC-	-----		-----	GTG	TGTGCGGCCT	TGAGGCGTCA
S_radians	CCATCTAACG	C----	ACAC-	-----		-----	GTG	TGTGCGGCCT	TGAGGCGTCA
S_siderea_	CCATCTAACG	T----	ACAC-	-----		-----	GATG	TGTGCGGCCT	TGAGGCGTCA
S_siderea_	CCATCTAACG	T----	ACAC-	-----		-----	GATG	TGTGCGGCCT	TGAGGCGTCA
S_siderea_	CCATCTAACG	T----	ACAC-	-----		-----	GATG	TGTGCGGCCT	TGAGGCGTCA
S_siderea_	CCATCTAACG	T----	ACAC-	-----		-----	GATG	TGTGCGGCCT	TGAGGCGTCA
S_siderea_	CCATCTAACG	T----	ACAC-	-----		-----	GATG	TGTGCGGCCT	TGAGGCGTCA
S_siderea_	CCATCTAACG	T----	ACAC-	-----		-----	GATG	TGTGCGGCCT	TGAGGCGTCA
S_siderea_	CCATCTAACG	T----	ACAC-	-----		-----	GATG	TGTGCGGCCT	TGAGGCGTCA
U_agaricit	TCATCCAACG	C----	AC---	-----		-----	TTGAG	TGTGCGGCAC	TGAGGCGTCA
U_agaricit	TCATCCAACG	C----	AC---	-----		-----	TTGAG	TGTGCGGCAC	TGAGGCGTCA
U_agaricit	TCATCCAACG	C----	AC---	-----		-----	TTGAG	TGTGCGGCAC	TGAGGCGTCA
U_agaricit	TCATCCAACG	C----	AC---	-----		-----	TTGAG	TGTGCGGCAC	TGAGGCGTCA
U_agaricit	TCATCCAACG	C----	AC---	-----		-----	TTGAG	TGTGCGGCAC	TGAGGCGTCA
U_agaricit	TCATCCAACG	C----	AC---	-----		-----	TTGAG	TGTGCGGCAC	TGAGGCGTCA
U_agaricit	TCATCCAACG	C----	AC---	-----		-----	TTGAG	TGTGCGGCAC	TGAGGCGTCA
U_agaricit	TCATCCAACG	C----	AC---	-----		-----	TTGAG	TGTGCGGCAC	TGAGGCGTCA
U_agaricit	TCATCCAACG	C----	AC---	-----		-----	TTGAG	TGTGCGGCAC	TGAGGCGTCA
Alcyonium_	ACAAAACGTG	A----	ACGCG	CTGGCTAGTT	CGCTAGCGAG	CGCGTTTCGAT		TGAGGCGTCA	

O_franksi_	CGGC-CA---	-----CCC	AGTGGGGGCG	GCCGTGTCCC	TTGAAAGGCA	GAGAGACTCA
O_franksi_	CGGC-CA---	-----CCC	AGTGGGAGCG	GCCGTGTCCC	TTGAAAGGCA	GAGAGACTCA
O_franksi_	CGGC-CA---	-----CCC	AGTGGGAGCG	GCCGTGTCCC	TTGAAAGGCA	GAGAGACTCA
P_clivosa_	AACGCTT---	--GACGGCGA	CTCTTGGCTC	TCAATGTACC	TGGAECTCGAT	TGCAATTGTA
P_clivosa_	AACGCTT---	--GACGGCGA	CTCTTGGCTC	TCAATGTACC	TGGAECTCGAT	TGCAATTGTA
P_clivosa_	AACGCTT---	--GACGGCGA	CTCTTGGCTC	TCAATGTACC	TGGAECTCGAT	TGCAATTGTA
P_astreoid	CGGCGTGTTA	GATCGTTAGA	TAAAT--ATC	GCCGTGTCCC	TTGAAG-GAC	AGCAGCATTG
P_astreoid	CGGCGTGTTA	GATCGTTAGA	TAAAT--ATC	GCCGTGTCCC	TTGAAG-GAC	AGCAGCATTG
P_astreoid	CGGCGTGTTA	GATCGTTAGA	TAAAT--ATC	GCCGTGTCCC	TTGAAG-GAC	AGCAGCATTG
P_astreoid	CGGCGTGTTA	GATCGTTAGA	TCGTTGGATC	GCCGTGTCCC	TTGAAG-GAC	AGCAGCATTG
P_astreoid	CGGCGTGTTA	GATCGTTAGA	TAAAT--ATC	GCCGTGTCCC	TTGAAG-GAC	AGCAGCATTG
P_astreoid	CGGCGTGTTA	GATCGTTAGA	TCGTTGGATC	GCCGTGTCCC	TTGAAG-GAC	AGCAGCATTG
P_porites_	CGGCGTGAAG	AATCAGTGTA	TTAAT-GATC	GCCGTGTCCC	TTGAAG-AGC	AGCAGCAGCA
P_porites_	CGGCGTGAAG	AATCAGTGTA	TTAAT-GATC	GCCGTGTCCC	TTGAAG-AGC	AGCAGCAGCA
P_porites_	CGGCGTGAAG	AATCAGTGTA	TTAAT-GATC	GCCGTGTCCC	TTGAAG-AGC	AGCAGCAGCA
P_porites_	CGGCGTGAAG	AATCAGTGTA	TTAAT-GATC	GCCGTGTCCC	TTGAAG-AGC	AGCAGCAGCA
P_porites_	CGGCGTGAAG	AATCAGTGTA	TTAAT-GATC	GCCGTGTCCC	TTGAAG-AGC	AGCAGCAGCA
P_porites_	CGGCGTGAAG	AATCAGTGTA	TTAAT-GATC	GCCGTGTCCC	TTGAAG-AGC	AGCAGCAGCA
S_radians	CGGCCTT---	--TCTTCAA	AGAGTTGGTG	GCCGTGTCCC	TTGAAGGGAA	GGCAATACTC
S_radians	CGGCCTT---	--TCTTCAA	AGAGTTGGTG	GCCGTGTCCC	TTGAAGGGAA	GGCAATACTC
S_radians	CGGCCTT---	--TCTTCAA	AGAGTTGGTG	GCCGTGTCCC	TTGAAGGGAA	GGCAATACTC
S_radians	CGGCCTT---	--TCTTCAA	AGAGTTGGTG	GCCGTGTCCC	TTGAAGGGAA	GGCAATACTC
S_radians	CGGCCTT---	--TCTTCAA	AGAGTTGGTG	GCCGTGTCCC	TTGAAGGGAA	GGCAATACTC
S_radians	CGGCCTT---	--TCTTCAA	AGAGTTGGTG	GCCGTGTCCC	TTGAAGGGAA	GGCAATACTC
S_siderea_	CGGCCTT---	--TCTTCAA	AGAGTTGGTG	GCCGTGTCCC	TTGAAGAGAA	GGCAATACTC
S_siderea_	CGGCCTT---	--TCTTCAA	AGAGTTGGTG	GCCGTGTCCC	TTGAAGAGAA	GGCAATACTC
S_siderea_	CGGCCTT---	--TCTTCAA	AGACTTGTTG	GCCGTGTCCC	TTGAAGGGAA	GGCAATACTC
S_siderea_	CGGCCTT---	--TCTTCAA	AGAGTTGGTG	GCCGTGTCCC	TTGAAGAGAA	GGCAATACTC
S_siderea_	CGGCCTT---	--TCTTCAA	AGACTTGTTG	GCCGTGTCCC	TTGAAGGGAA	GGCAATACTC
S_siderea_	CGGCCTT---	--TCTTCAA	AGAGTTGGTG	GCCGTGTCCC	TTGAAGAGAA	GGCAATACTC
S_siderea_	CGGCCTT---	--TCTTCAA	AGACTTGTTG	GCCGTGTCCC	TTGAAGGGAA	GGCAATACTC
S_siderea_	CGGCCTT---	--TCTTCAA	AGAGTTGGTG	GCCGTGTCCC	TTGAAGAGAA	GGCAATACTC
U_agaricit	CGGCACT---	-----GCT	GGT-----AA	GCCGTGTCCC	TTGAAGAGCA	GAGAGACTCG
U_agaricit	CGGCACT---	-----GCT	GGT-----GA	GCCGTGTCCC	TTGAAGAGCA	GAGAGACTCG
U_agaricit	CGGCACT---	-----GCT	GGT-----GA	GCCGTGTCCC	TTGAAGAGCA	GAGAGACTCG
U_agaricit	CGGCACT---	-----GCT	GGT-----GA	GCCGTGTCCC	TTGAAGAGCA	GAGAGACTCG
U_agaricit	CGGCACT---	-----GCT	GGT-----GA	GCCGTGTCCC	TTGAAGAGCA	GAGAGACTCG
U_agaricit	CGGCACT---	-----GCT	GGT-----GA	GCCGTGTCCC	TTGAAGAGCA	GAGAGACTCG
U_agaricit	CGGCACT---	-----GCT	GGT-----GA	GCCGTGTCCC	TTGAAGAGCA	GAGAGACTCG
U_agaricit	CGGCACT---	-----GCT	GGT-----GA	GCCGTGTCCC	TTGAAGAGCA	GAGAGACTCG
Alcyonium_	CGGCGGC---	-----	--GATTGCTC	GTCGCGTCCT	TCCAAG----	TACAGAACGT

	1085	1095	1105	1115	1125	1135
A_cervicor	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	GCATCTTTGT
A_cervicor	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_cervicor	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	GCATCTTTGT
A_cervicor	-----	-----	A GCAGGACCG-	CCTT-AAAAA	A-T-----	-----T
A_cervicor	-----	-----	A GCAGGACCG-	CCTT-AAAAA	A-T-----	-----T
A_cervicor	-----	-----	A GCAGGACCG-	CCTT-AAAAA	A-T-----	-----T
A_cervicor	-----	-----	A GCAGGACCG-	CCTT-AAAAA	A-T-----	-----T
A_cervicor	-----	-----	A GCAGGACCG-	CCTT-AAAAA	A-T-----	-----T
A_cervicor	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_cervicor	-----	-----	A GCAGGACCG-	CCTT-AAAAA	A-T-----	-----T
A_cervicor	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_cervicor	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_cervicor	-----	-----	A GCAGGACCG-	CCTT-AAAAA	A-T-----	-----T
A_cervicor	-----	-----	A GCAGGACCG-	CCTT-AAAAA	A-T-----	-----T
A_palmata_	-----	-----	A GCAGAACCGA	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_palmata_	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_palmata_	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_palmata_	-----	-----	A GCAGGACCG-	CCTTAAAAAAA	A-T-----	-----T
A_palmata_	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_palmata_	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_palmata_	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_palmata_	-----	-----	A GCAGAACCG-	-AAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_prolifer	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	GCATCTTTGT
A_prolifer	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	GCATCTTTGT
A_prolifer	-----	-----	A GCAGGACCG-	CCTTAAAAAAA	A-T-----	-----T
A_prolifer	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_prolifer	-----	-----	G GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_prolifer	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	GCATCTTTGT
A_prolifer	-----	-----	A GCAGAACCG-	AAAA-AAAAG	G-TGAT-CAC	ACATCTTTGT
A_prolifer	-----	-----	A GCAGGACCG-	CCTTAAAAAAA	A-T-----	-----T
A_prolifer	-----	-----	A GCAGGACCG-	CCTT-AAAAA	A-T-----	-----T
D_stokesii	CCGTGGC---	CGGGCTCGGT	TCCCTGCACA	CTCGCGGAGG	C-TAAAAAAC	AATTTTGCCC
D_stokesii	CCGTGGC---	CGGGCTCGGT	TCCCTGCACA	CTCGCGGAGG	C-TAAAAAAC	AATTTTGCCC
D_stokesii	CCGTGGC---	CGGGCTCGGT	TCCCTGCACA	CTCGCGGAGG	C-TAAAAAAC	AATTTTGCCC
D_stokesii	CCGTGGC---	CGGGCTCGGT	TCCCTGCACA	CTCGCGGAGG	C-TAAAAAAC	AATTTTGCCC
D_stokesii	CCGTGGC---	CGGGCTCGGT	TCCCTGCACA	CTCGCGGAGG	C-TAAAAAAC	AATTTTGCCC
D_labyrint	CTGTTGT--A	CTCGTCTTGC	TCGAGCACG	GCAGCGGAGG	CACAAGACTT	TCCTTCGCGT
D_labyrint	CTGTTGT--A	CTCGTCTTGC	TCGAGCACG	GCAGCGGAGG	CACAGGACTT	TCCTTCGCGT

O_franksi_	CTGCGGTTGC	CGGGTTTTTC	CCGCGGCACA	CCAGCGGAGG	C-TAAAAGTG	TGACCAGTCC
O_franksi_	CTGCGGTTGC	CGGGTTTTTC	CCGCGGCACA	CCAGCGGAGG	C-TAAAAGTG	TGGCCAGTCC
O_franksi_	CTGCGGTTGC	CGGGTTTTTC	CCGCGGCACA	CCAGCGGAGG	C-TAAAAGTG	TGGCCAGTCC
P_clivosa_	AAAAGTT---	--GTTTTCG-	-----	-----GAAGG	C-TCAA-CTA	GCTTCTGTCC
P_clivosa_	AAAAGTT---	--GTTTTCG-	-----	-----GAAGG	C-TCAA-CTA	GCTTCTGTCC
P_clivosa_	AAAAGTT---	--GTTTTCG-	-----	-----GAAGG	C-TCAA-CTA	GCTTCTGTCC
P_astreoid	GACTCGC---	--ATTCTCTA	TTGAAACTTG	AGTGCGAAGG	C-TAAA----	GATGATATTT
P_astreoid	GACTCGC---	--ATTCTCTA	TTGAAACTTG	AGTGCGAAGG	C-TAAA----	GATGATATTT
P_astreoid	GACTCGC---	--ATTCTCTA	TTGAAACTTG	AGTGCGAAGG	C-TAAA----	GATGATATTT
P_astreoid	GACTCGC---	--ATTCTCTA	TTGAAACTTG	AGTGCGAAGG	C-TAAA----	GATAATATTT
P_astreoid	GACTCGC---	--ATTCTCTA	TTGAAACTTG	AGTGCGAAGG	C-TAAA----	GATGATATTT
P_astreoid	GACTCGC---	--ATTCTCTA	TTGAAACTTG	AGTGCGAAGG	C-TAAA----	GATAATATTT
P_porites_	-----GC---	--ATTCTCTA	TTGAAACTTG	AGTGTGAAGG	C-TAAA-GAT	ATTGCTATTT
P_porites_	-----GC---	--ATTCTCTA	TTGAAACTTG	AGTGCGAAGG	C-TAAA-GAT	ATTGCTATTT
P_porites_	-----GC---	--ATTCTCTA	TTGAAACTTG	AGTGCGAAGG	C-TAAA-GAT	ATTGCTATTT
P_porites_	-----GC---	--ATTCTCTA	TTGAAACTTG	AGTGCGAAGG	C-TAAA-GAT	ATTGCTATTT
P_porites_	-----GC---	--ATTCTCTA	TTGAAACTTG	AGTGCGAAGG	C-TAAA-GAT	ATTGCTATTT
P_porites_	-----GC---	--ATTCTCTA	TTGAAACTTG	AGTGCGAAGG	C-TAAA-GAT	ATTGCTATTT
S_radians	GCA--TC---	--GTGCGCGA	CCAGCACTCC	CATGCGAAGG	C-CAAA----	----ACTTCT
S_radians	GCA--TC---	--GTGCGCGA	CCAGCACTCC	CATGCGAAGG	C-CAAA----	----ACTTCT
S_radians	GCA--TC---	--GTGCGCGA	CCAGCACTCC	CATGCGAAGG	C-CAAA----	----ACTTCT
S_radians	GCA--TC---	--GTGCGCGA	CCAGCACTCC	CATGCGAAGG	C-CAAA----	----ACTTCT
S_radians	GCA--TC---	--GTGCGCGA	CCAGCACTCC	CATGCGAAGG	C-CAAA----	----ACTTCT
S_radians	GCA--TC---	--GTGCGCGA	CCAGCACTCC	CATGCGAAGG	C-CAAA----	----ACTTCT
S_siderea_	GCA--TC---	--GTGCGCGA	CCAGCACTCC	CATGCGAAGG	C-CAAA----	----AATTCT
S_siderea_	GCA--TC---	--GTGCGCGA	CCAGCACTCC	CATGCGAAGG	C-CAAA----	----AATTCT
S_siderea_	GCA--TC---	--GTGCGCGA	CCAGCACTTC	CATGCGAAGG	C-CAAA----	----AATTCT
S_siderea_	GCA--TC---	--GTGCGCGA	CCAGCACTCC	CATGCGAAGG	C-CAAA----	----AATTCT
S_siderea_	GCA--TC---	--GTGCGCGA	CCAGCACTCC	CATGCGAAGG	C-CAAA----	----AATTCT
S_siderea_	GCA--TC---	--GTGCGCGA	CCAGCACTCC	CATGCGAAGG	C-CAAA----	----AATTCT
U_agaricit	CAT-----	CGTGCGCGGT	TCCGCACCCC	CATGCGAAGG	C-TAAAAATC	CATTTGGTCC
U_agaricit	CAT-----	CGTGCGCGGT	TCCGCACCCC	CATGCGAAGG	C-TAAAAATC	CATTTGGTCC
U_agaricit	CAT-----	CGTGCGCGGT	TCCGCACCCC	CATGCGAAGG	C-TAAAAATC	CATTTGGTCC
U_agaricit	CAT-----	CGTGCGCGGT	TCCGCACCCC	CATGCGAAGG	C-TAAAAATC	CATTTGGTCC
U_agaricit	CAT-----	CGTGCGCGGT	TCCGCACCCC	CATGCGAAGG	C-TAAAAATC	CATTTGGTCC
U_agaricit	CAT-----	CGTGCGCGGT	TCCGCACCCC	CATGCGAAGG	C-TAAAAATC	CATTTGGTCC
U_agaricit	CAT-----	CGTGCGCGGT	TCCGCACCCC	CATGCGAAGG	C-TAAAAATC	CATTTGGTCC
U_agaricit	CAT-----	CGTGCGCGGT	TCCGCACCCC	CATGCGAAGG	C-TAAAAATC	CATTTGGTCC
Alcyonium_	TCAACGC---	--ACGTTCTGA	C---AACGTG	TGAACAAGGC	C-TAAGTAAC	ATTTGTGTCC

O_franksi_	TTCGC-GT--	-----	----GCC-GT	CGGGTGCCCG	CCGC-TCGAG	TCGCGGA---
O_franksi_	TTCGC-GT--	-----	----GCC-GT	CGGGTGCCCG	CCGC-TCGAG	TCGCGGA---
O_franksi_	TTCGC-GT--	-----	----GCC-GT	CGGGTGCCCG	CCGC-TCGAG	TCGCGGA---
P_clivosa_	TTACTGATAA	CAACCA--AC	CAAAGCAATT	CAATTCCAGT	TCATGGCTCC	TCTCTCTAAA
P_clivosa_	TTACTGATAA	CAACCA--AC	CAAAGCAATT	CAATTCCAGT	TCATGGCTCC	TCTCTCTAAA
P_clivosa_	TTACTGATAA	CAACCA--AC	CAAAGCAATT	CAATTCCAGT	TCATGGCTCC	TCTCTCTAAA
P_astreoid	TCCTTCGCCC	CAAAATCCAA	TAGG---AGG	AGAAATGTGG	ACAGTCCTTG	CTGAAAGAAA
P_astreoid	TCCTTCGCCC	CAAAATCCAA	TAGG---AGG	AGAAATGTGG	ACAGTCCTTG	CTGAAAGAAA
P_astreoid	TCCTTCGCCC	CAAAATCCAA	TAGG---AGG	AGAAATGTGG	ACAGTCCTCG	CTGAAACAAA
P_astreoid	TCCTTCGCCC	CAAAATCCAA	TAGGGTTAGG	AGAAATGTGG	ACAGTCCTTG	CTGAAAGAAA
P_astreoid	TCCTTCGCCC	CAAAATCCAA	TAGGGTTAGG	AGAAATGTGG	ACAGTCCTTG	CTGAAAGAAA
P_astreoid	TCCTTCGCCC	CAAAATCCAA	TAGGGTTAGG	AGAAATGTGG	ACAGTCCTTG	CTGAAAGAAA
P_porites_	TCCTTCGCTC	CAGGTTGGTT	TAGGGTTGTT	GGGTTGCTGA	AAA-----	---AGAGAAA
P_porites_	TCCTTCGCTC	CAGGTTGGTT	TAGGGTTGTT	GGGTTGCTGA	AAA-----	---AGAGAAA
P_porites_	TCCTTCGCTC	CAGGTTGGTT	TAGGGTTGTT	GGGTTGCTGA	AAA-----	---AGAGAAA
P_porites_	TCCTTCGCTC	CAGGTTGGTT	TAGGGTTGTT	GGGTTGCTGA	AAA-----	---AGAGAAA
P_porites_	TCCTTCGCTC	CAGGTTGGTT	TAGGGTTGTT	GGGTTGCTGA	AAA-----	---AGAGAAA
P_porites_	TCCTTCGCTC	CAGGTTGGTT	TAGGGTTGTT	GGGTTGCTGA	AAA-----	---AGAGAAA
S_radians	TTCCTTGGA	TGGCCGCTGT	TGGCGCCACC	TTACGACGAC	AAA-----	-----
S_radians	TTCCTTGGA	TGGCCGCTGT	TGGCGCCACC	TTACGACGAC	AAA-----	-----
S_radians	TTCCTTGGA	TGGCCGCTGT	TGGCGCCACC	TTACGACGAC	AAA-----	-----
S_radians	TTCCTTGGA	TGGCCGCTGT	TGGCGCCACC	TTACGACGAC	AAA-----	-----
S_radians	TTCCTTGGA	TGGCCGCTGT	TGGCGCCACC	TTACGACGAC	AAA-----	-----
S_siderea_	TTCCTTGGA	TGGCTGTTGT	TGGCGCCACC	TTACGACGAC	AAA-----	-----
S_siderea_	TTCCTTGGA	TGGCTGTTGT	TGGCGCCACC	TTACGACGAC	AAA-----	-----
S_siderea_	TTCCTTGGA	TGGCCGTTGT	TGGCGCCACC	TTACGACGAC	AAA-----	-----
S_siderea_	TTCCTTGGA	TGGCTGTTGT	TGGCGCCACC	TTACGACGAC	AAA-----	-----
S_siderea_	TTCCTTGGA	TGGCCGTTGT	TGGCGCCACC	TTACGACGAC	AAA-----	-----
S_siderea_	TTCCTTGGA	TGGCTGTTGT	TGGCGCCACC	TTACGACGAC	AAA-----	-----
U_agaricit	TTGGCTGG--	-----	----GTCGGT	GTACCGGAGC	GCGCGCGGAG	TCCCACG--A
U_agaricit	TTGGCTGG--	-----	----GTCGGT	GTACCGGAGC	GCGCGCGGAG	TCCCACG--A
U_agaricit	TTGGCTGG--	-----	----GTCGGT	GTACCGGAGC	GCGCGCGGAG	TCCCACG--A
U_agaricit	TTGGCTGG--	-----	----GTCGGT	GTACCGGAGC	GCGCGCGGAG	TCCCACG--A
U_agaricit	TTGGCTGG--	-----	----GTCGGT	GTACCGGAGC	GCGCGCGGAG	TCCCACG--A
U_agaricit	TTGGCTGG--	-----	----GTCGGT	GTACCGGAGC	GCGCGCGGAG	TCCCACG--A
U_agaricit	TTGGCTGG--	-----	----GTCGGT	GTACCGGAGC	GCGCGCGGAG	TCCCACG--A
U_agaricit	TTGGCTGG--	-----	----GTCGGT	GTACCGGAGC	GCGCGCGGAG	TCCCACG--A
Alcyonium_	TTGCTAGCAT	CGCTTGTCGT	TCGCGC--GT	CGACGTCAA	CAA-----	-----

D_labyrint	TGAATTTAA
D_labyrint	TGAATTTAA
D_labyrint	TGAATTTAA
D_strigosa	TGAATTTAA
D_strigosa	TGAATTTAA
D_strigosa	TGAATTTAA
D_strigosa	TGAATTTAA
D_strigosa	TGAATTTAA
F_fragum_2	TGAATTTAA
F_fragum_2	TGAATTTAA
F_fragum_2	TGAATTTAA
F_fragum_2	TGAATTTAA
F_fragum_2	TGAATTTAA
F_fragum_2	TGAATTTAA
M_cavernos	TGAATTTAA
M_cavernos	TGAATTTAA
M_cavernos	TGAATTTAA
M_cavernos	TGAATTTAA
O_annulari	TGAATTTAA
O_annulari	TGAATTTAA
O_annulari	TGAATTTAA
O_annulari	TGAATTTAA
O_annulari	TGAATTTAA
O_annulari	TGAATTTAA
O_annulari	TGAATTTAA
O_annulari	TGAATTTAA
O_annulari	TGAATTTAA
O_annulari	TGAATTTAA
O_faveolat	TGAATTTAA
O_faveolat	TGAATTTAA
O_faveolat	TGAATTTAA
O_faveolat	TGAATTTAA
O_faveolat	TGAATTTAA
O_faveolat	TGAATTTAA
O_faveolat	TGAATTTAA
O_faveolat	TGAATTTAA
O_faveolat	TGAATTTAA
O_faveolat	TGAATTTAA
O_franksi_	TGAATTTAA
O_franksi_	TGAATTTAA
O_franksi_	TGAATTTAA
O_franksi_	TGAATTTAA
O_franksi_	TGAATTTAA
O_franksi_	TGAATTTAA

O_franksi_	TGAATTTAA
O_franksi_	TGAATTTAA
O_franksi_	TGAATTTAA
P_clivosa_	CGAATTTAA
P_clivosa_	CGAATTTAA
P_clivosa_	CGAATTTAA
P_astreoid	TGAATTTAA
P_astreoid	TGAATTTAA
P_astreoid	TGAATTTAA
P_astreoid	TGAATTTAA
P_astreoid	TGAATTTAA
P_astreoid	TGAATTTAA
P_porites_	TGAATTTAA
P_porites_	TGAATTTAA
P_porites_	TGAATTTAA
P_porites_	TGAATTTAA
P_porites_	TGAATTTAA
P_porites_	TGAATTTAA
S_radians	TGAATTTAA
S_radians	TGAATTTAA
S_radians	TGAATTTAA
S_radians	TGAATTTAA
S_radians	TGAATTTAA
S_siderea_	TGAATTTAA
S_siderea_	TGAATTTAA
S_siderea_	TGAATTTAA
S_siderea_	TGAATTTAA
S_siderea_	TGAATTTAA
S_siderea_	TGAATTTAA
S_siderea_	TGAATTTAA
S_siderea_	TGAATTTAA
U_agaricit	TGAATTTAA
U_agaricit	TGAATTTAA
U_agaricit	TGAATTTAA
U_agaricit	TGAATTTAA
U_agaricit	TGAATTTAA
U_agaricit	TGAATTTAA
U_agaricit	TGAATTTAA
U_agaricit	TGAATTTAA
Alcyonium_	TGAATTTAA