

Attachment C

Christmas Tree Promotion Board

Final Research Report

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Project Title: Surveying for Oregonian slugs in Hawai'i with the goal of removing their quarantine status

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Technical report

Surveying for Oregonian slugs in Hawai'i with the goal of removing their quarantine status

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Introduction

Slugs are one of the most important pests of Christmas trees grown in the Pacific Northwest relative to load rejections in Pacific Rim destinations. Such load rejections are particularly significant in Hawai'i (http://www.oregonlive.com/news/index.ssf/2008/11/hawaii_rejects_oregongrown_chrt.html). Invasive slugs in the genus *Arion* that are present in Oregon and Washington are considered quarantine pests if they are intercepted in Hawai'i. Thus, when a shipment of trees contaminated with one of these slugs is discovered at a port of entry in Hawai'i officials reject the load, and either ship it back to Oregon/Washington or have it cleaned up in Hawai'i at the shipper's expense. Current strategies for managing slugs focus on a combination of chemical (e.g. metaldehyde) and cultural (e.g. shaking) measures. However, even with these approaches slugs continue to pose a significant economic problem for Christmas tree growers in the Pacific Northwest.

Since these management strategies are relatively ineffective, an alternative approach for mitigating issues with these slugs would be to survey for them throughout Hawai'i. Such surveys have not been completed in the past largely due to a lack of malacological expertise and consequently some of these slug species may be present on one or more islands. If these slugs are in fact established in Hawai'i then technically

they should no longer be considered a quarantine pest and their discovery on a shipment of trees from the Pacific Northwest should not lead to shipment rejection.

Thus, the objectives of this project were:

1. Survey for slugs throughout Hawai'i that are contaminant pests of Christmas trees grown in the Pacific Northwest.
2. Disseminate our survey results to the Hawai'i Dept. of Agriculture with the goal of removing the quarantine status of slug species that are established in Hawai'i.

Methods

To help identify survey sites, occurrence records of select invasive temperate gastropod species i.e. *Oxychilus*, *Ambigolimax*, *Deroceras*, and *Limax* throughout the Hawaiian Archipelago were mined from the Symbiota portal PILSBRY (<http://pilsbry.org>) and from iNaturalist (<https://www.inaturalist.org>). Our rationale for this approach was that observations of these species (which, like *Arion* are native to temperate Europe) may indicate suitable habitats for our target species. In addition, we also surveyed in anthropogenic areas where the target slug species have been collected in other U.S. states (e.g. plant nurseries, urban parks). The following is a list of our collection sites by island, their GPS co-ordinates, elevation, the dates they were surveyed, and the microhabitat where we collected gastropod specimens.

A. Oahu

Koba's Nursery Inc., Mokulama Street, Waimanalo, Honolulu County, Hawai'i, N21.3365°, W-157.7227°, elev. 36 m. August 2022. Habitat: ornamental plant nursery, under potted plants.

Mari's Garden, Makapipipi Street, Mililani, Honolulu County, Hawai'i, N21.4299°, W-158.0155°, elev. 152 m. August 2022. Habitat: ornamental plant nursery, under potted plants.

Koolau Farmers – McCully, South Beretania St., Honolulu County, Hawai'i, N21.2966°, W-157.8304°, elev. 10 m. August 2022. Habitat: ornamental plant nursery, under potted plants.

Pu'u Ohia Trail, Tantalus Drive, Honolulu County, Hawai'i, N21.3310°, W-157.8146°, elev. 492 m. August 2022. Habitat: wooded trail.

Nu'uuanu Pali, Nu'uuanu Pali Drive, Honolulu County, Hawai'i, N21.3670°, W-157.7930°, elev. 358 m. August 2022. Habitat: open grassy area.

Aiea Loop Trail, Aiea Height Drive, Honolulu County, Hawai'i, N21.3988°, W-157.8999°, elev. 336 m. August 2022. Habitat: wooded trail.

In addition to the above locations, we were denied access to sample for slugs at Glenn's Flower and Plant in Waimanalo on Oahu.

B. Maui:

Kahakapao Loop Trail, Kahakapao Road, Makawao, Maui County, Hawai'i, N20.8320°, W-156.2753°, elev. 840 m. August 2022. Habitat: mixed rainforest; under decaying wood.

Waihou Spring Trail, Olinda Road, Makawao, Maui County, Hawai'i, N20.8057°, W-156.2801°, elev. 1136 m. August 2022. Habitat: dried spring bed within mixed evergreen forest; under and inside decaying wood.

Ki Hana Nursery, South Kihei Road, Kihei, Maui County, Hawai'i, N20.7364°, W-156.4539°, elev. 3 m. August 2022. Habitat: ornamental plant nursery; under tray of plants.

Kula Botanical Gardens, Kekaulike Avenue, Kula, Maui County, Hawai'i, N20.7416°, W-156.3247°, elev. 1012 m. August 2022. Habitat: botanical garden; under small rocks.

South Maui Gardens, Auhana Road, Kihei, Maui County, Hawai'i, N20.7297°, W-156.4501°, elev. 4 m. August 2022. Habitat: ornamental plant nursery; under potted plants.

Maui Green Nursery, 28 Hea Hea Place, Wailuku, Maui County, Hawai'i, N20.9278°, W-156.5134°, elev. 58 m. August 2022. Habitat: ornamental plant nursery; under potted plants.

C. Hawai'i

Honua'ula Forest Reserve (Makāula -'O'oma section), Hawai'i County, Hawai'i, N19.7192°, W-155.9496°, elev. 980 m. September 2022; 1030-1130. Habitat: mixed forest including *Freycinetia arborea*, *Cibotium glaucum*, and *Hedychium gardnerianum*; under lava stones, litter and woody debris.

Huehue Street, Hawai'i County, Hawai'i, N19.7146°, W-155.9227°, elev. 1470 m. September 2022; 1200-1300. Habitat: mixed forest including: *Metrosideros polymorpha*, *Acacia koa*, *Leptecophylla tameiameiaae*, *Dryopteris wallichiana*; in leaf litter and stones under *Persicaria capitata* groundcover.

Kilohana Hunting Station Area, Hawai'i County, Hawai'i, N19.8104°, W-155.6276°, elev. 1770 m. September 2022; 0950-1113. Habitat: copse of *Pinus* trees surrounded by a weedy *Eragrostis atropioides* grassland; woody debris and litter.

Pu'u Huluhulu Cinder Cone, Hawai'i County, Hawai'i, N19.6876°, W-155.4657°, elev. 2022 m. September 2022; 1220-1310. Habitat: an *Acacia koa* forest with forb, fern, and grass understory; litter and woody debris.

Apela Place, Kailua-Kona, Hawai'i County, Hawai'i, N19.7035°, W-155.9656°; elev 635 m. September 2022. Habitat: suburban landscape, surface active on ornamental plant.

D. Kauai

Pihea Trail – Kalawa Rim, Kauai County, Hawai'i, N22.1499°, W-159.6228°; elev. 1227 m. September 2022. Habitat: native forest with *Metrosideros polymorpha*, *Odontosoria chinensis*, *Dicranopteris linearis*; leaf litter and woody debris.

Pu'u Hinahina Lookout, Kauai County, Hawai'i, N22.1090°, W-159.6694°; elev. 1108 m. September 2022. Habitat: forest; woody debris.

Lydgate Beach Park, Kauai County, Hawai'i, N22.0317°, W-159.3382°; elev. 7 m. September 2022. Habitat: seaside park; litter.

At each sampling site, suitable microhabitats (e.g. under potted plants, under plant trays, under litter, on weeds, under stones, in leaf litter, under woody debris) were searched for gastropods which were collected into an 8 oz plastic container lined with damp paper towel. Specimens were then either drowned overnight in pre-boiled (then cooled) water, and transferred to 100% ethanol for preservation or microwaved for 6 seconds to relax the specimens and then transferred to 75% ethanol.

On return to the laboratory, all gastropods were examined under a microscope and specimens in the genus *Arion* were set aside for molecular analysis. A reliable identification trait for slugs in this genus is the presence of an obvious breathing pore that is located anterior to the midpoint of the mantle (fleshy saddle shaped structure behind the head). The molecular analysis involved extracting DNA from each specimen separately using a Qiagen DNeasy Blood and Tissue Extraction Kit. This extracted DNA was then amplified by PCR using the universal Folmer Primers (Folmer et al., 1994) to target a ~655 bp segment of the mitochondrial COI gene. Amplicons were sequenced at the Oregon State University Center for Quantitative Life Sciences. Reads were then assembled using Geneious Prime. BLAST in Geneious was then used to identify specimens, querying the Nucleotide Collection (nr/nt) using blastn, a Max E-value of 0.05, Word Size 11, and Gap Cost 5 2.

Results

A summary of the gastropod taxa that were collected during our surveys is presented in Table 1. A total of 22 different slug and snail taxa were collected across the four islands.

Table 1. Summary of slug and snail taxa collected on Oahu, Maui, Hawai'i and Kauai.

Species	Oahu	Maui	Hawai'i	Kauai
<i>Subulina octona</i>	X			
<i>Succeinidae</i> sp.	X			
<i>Bradybaena similaris</i>	X			
<i>Veronicella cubensis</i>	X	X		
<i>Arion intermedius</i>		X	X	X
<i>Deroceras laeve</i>		X		
<i>Limax maximus</i>		X		
<i>Oxychilus alliarius</i>		X	X	X
<i>Philomycidae</i> sp.		X		
<i>Parpeas achatinaceum</i>		X		
<i>Ovachlamys fulgens</i>			X	
<i>Carychium</i> sp.			X	
<i>Achatinellidae</i> sp.			X	
<i>Parmarion martensi</i>			X	
<i>Cornu aspesum</i>			X	
<i>Ambigolimax</i> sp.			X	
<i>Limacoidea</i> sp.			X	
<i>Deroceras</i> sp.			X	X
<i>Euglandina rosea</i>				X
<i>Lissachatina fulica</i>				X
<i>Arion distinctus</i>				X
Unknown snail			X	
Number of taxa	4	7	11	6

Slugs in the genus *Arion* can be difficult to identify to species level using solely morphological traits. As a result, our morphological-based identifications for suspected *Arion* were confirmed using DNA analysis (see above). This analysis (Table 2) confirmed that we collected two species, *Arion intermedius* (Figure 1) and *Arion distinctus* (Figure 2). The former was found on Maui, Hawai'i and Kauai and the later on Hawai'i only. Specimen vouchers have been deposited into the Oregon State Arthropod Collection under curation lot OSAC_AC_2023_06_15-001-01. No *Arion* slugs were collected on Oahu.

Table 2. Results of DNA analysis using partial COI sequences to confirm the presence of *Arion intermedius* and *Arion distinctus* on Maui and Hawai'i.

Species	Island	% match	Matching Genbank Accession
<i>Arion intermedius</i>	Maui	100	MG813885
<i>Arion intermedius</i>	Maui	99.9	MG813885
<i>Arion intermedius</i>	Hawai'i	99.6	MG813885
<i>Arion intermedius</i>	Kauai	100	MG813885
<i>Arion intermedius</i>	Kauai	100	MG813885
<i>Arion distinctus</i>	Hawai'i	99.7	DQ647394
<i>Arion distinctus</i>	Hawai'i	99.9	MF544165
<i>Arion distinctus</i>	Hawai'i	99.9	EF128218

The partial COI sequences can be viewed in Appendix 1.



Figure 1. *Arion intermedius* collected on Kauai in September 2022.



Figure 2. *Arion distinctus* collected on Hawai'i in September 2022.

Discussion

Despite collecting slugs in the genus *Arion* regularly in garden centers and nurseries in multiple states on the mainland (e.g. Oregon, Washington, and California), we did not find specimens in such locations on the Hawaiian Archipelago. Similarly, Cowie et al. (2008) did not find any species of *Arion* in their surveys of horticultural facilities on the six largest Hawaiian Islands. In fact, in our study, all *Arion* specimens were collected in forest habitat (Figures 3 and 4) indicating that these taxa have established beyond highly anthropogenic habitats and also occur in more natural areas. Given that *A. intermedius* and *A. distinctus* are temperate species perhaps then it is also not surprising that we only collected these slugs at altitudes greater than 2,700ft where cooler and likely damper conditions prevail. Also, in Europe where both species are native, they are known to occur in woodlands (Rowson et al., 2014) and consequently their occurrence in such habitats on the Hawaiian Archipelago is unsurprising.

The fact that we only collected species of *Arion* on Maui, Hawai'i and Kauai but no specimens on Oahu is surprising. These slugs are heavily associated with human activity and are often introduced to new regions via trade. Oahu is the most populous island in the archipelago (according to the Hawai'i Visitors and Convention Bureau, 70% of the population live there) and is also home to the principal seaport for the State of Hawai'i (i.e. Port of Honolulu) which further adds to the mystery of their apparent absence on Oahu. Of course, it is possible that *Arion* occur in areas on Oahu where we

did not survey. Thus, in preparing this report we also queried the citizen science database, iNaturalist, for all records of *Arion* slugs on the Hawaiian Archipelago. This resulted in ten additional records, all for *A. intermedius*. There were three records for Maui, seven records for Hawai'i and again no records for Oahu. Given that Oahu is the most visited island accounting for 49.6% of all visitors (<https://www.hawaii-guide.com/hawaii-tourism-statistics>) it would be surprising that the species has not been reported if it does in fact occur there. Consequently, for now we should assume that *A. intermedius* is not present on Oahu.



Figure 3. Forest area on Kahakapao Loop Trail, Maui where specimens of *Arion intermedius* were collected.

The occurrence of *A. intermedius* on Maui, Hawai'i and Kauai and the presence of *A. distinctus* on Hawai'i will be reported to the Hawai'i Department of Agriculture and we will encourage them to use these data to review the quarantine status for *Arion* slugs detected in shipments of Christmas trees.



Figure 4. Discarded wood on Kahakapao Loop Trail, Maui under which specimens of *Arion intermedius* were found.

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References

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Folmer, O., Black, M., Hoeh, W.R., Lutz, R., and Vrijenhoek, R.C. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit 1 from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294–299.

Rowson, B., Turner, J., Anderson, R. and Symondson, B. (2014) *Slugs of Britain and Ireland. Identification, understanding and control*. FSC Publication, Telford, 136 pp.

Appendix 1. COI sequences from *Arion* specimens collected on the Hawaiian Archipelago.

Arion intermedius, Kahakapao Loop Trail, Kahakapao Road, Makawao, Maui, 840m altitude, 21st August 2022.

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GACTTTATACTTAATTTTTGGAATTTGAAGAGGTTTAGTTGGTACAGGATTATCTTTA  
CTTATTCGGTTAGAGTTAGGAACAACAGGAATCCTGACGGACGATCAATTCTTTAAT  
GTAGTTGTCACAGCTCATGCATTTGTTATAATTTTTTTTATAGTTATACCATTAATGA  
TTGGGGGGTTTGGAAATTGAATAGTTCCACTATTAATTGGAGCACCCAGATATAAGTT  
TCCCCCGGATGAATAATTTAAGATTTTGACTTCTTCCCCCATCTTTACTATTATTAAT  
TTGTTCTAGAATAGTTGAGGGAGGTGCGGGAACAGGTTGAACAGTTTACCCTCCGT  
TAAGAGGCTCTATAGCTCACAGAGGAGCTTCGGTGGATTTAGCTATTTTTTCTCTTC  
ATTTAGCGGGGATATCTTCTATTTTAGGTGCTATTAATTTTATTACTACTATTTTAAAT  
ATGCGACCTAAAGTACTTACATTAGAGCGTATAAGGTTATTTGTATGATCCATTTTA  
ATTACTGTTTTCTCCTACTCTTATCTTTACCGGTACTTGCTGGAGCCATTACTATAC  
TTTTAACAGATCGTAATTTTAATACCAGATTTTTTGATCCAGCTGGTGGGGGTGACC  
CAATTCTCTATCAGCATCTAT
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Arion intermedius, Kahakapao Loop Trail, Kahakapao Road, Makawao, Maui, 840m altitude, 21st August 2022.

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GACTTTATACTTAATTTTTGGAATTTGAAGAGGTTTAGTTGGTACAGGATTATCTTTA  
CTTATTCGGTTAGAGTTAGGAACAACAGGAATCCTGACGGACGATCAATTCTTTAAT  
GTAGTTGTCACAGCTCATGCATTTGTTATAATTTTTTTTATAGTTATACCATTAATGA  
TTGGGGGGTTTGGAAATTGAATAGTTCCACTATTAATTGGAGCACCCAGATATAAGTT  
TCCCCCGGATGAATAATTTAAGATTTTGACTTCTTCCCCCATCTTTACTATTATTAAT  
TTGTTCTAGAATAGTTGAGGGAGGTGCGGGAACAGGTTGAACAGTTTACCCTCCGT  
TAAGAGGCTCTATAGCTCACAGAGGAGCTTCGGTGGATTTAGCTATTTTTTCTCTTC  
ATTTAGCGGGGATATCTTCTATTTTAGGTGCTATTAATTTTATTACTACTATTTTAAAT  
ATGCGACCTAAAGTACTTACATTAGAGCGTATAAGGTTATTTGTATGATCCATTTTA
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ATTACTGTTTTCTCCTACTCTTATCTTTACCGGTACTTGCTGGAGCCATTACTATAC
TTTTAACAGATCGTAATTTTAATACCAGATTTTTTGATCCAGCTGGTGGGGGTGACC
CAATTCTCTATCAGCATCTATTT

Arion intermedius, Huehue Street, Kailua-Kona, Hawai'i, 1470m altitude, 5th September
2022.

GACTTTATACTTAATTTTTGGAATTTGAAGAGGTTTAGTTGGTACAGGATTATCTCTC
TTATTCGGTTTAGAGTTAGGAACAACAGGAATCCTGACGGACGATCAATTCTTTAAT
GTAGTTGTCACAGCTCATGCATTTGTTATAATTTTTTTTATAGTTATAACCATTAATGA
TTGGGGGGTTTGGAAATTGAATAGTTCCACTATTAATTGGAGCACCAGATATAAGTT
TCCCCCGGATGAATAATTTAAGATTTTGACTTCTTCCCCCATCTTTACTATTATTAAT
TTGTTCTAGAATAGTTGAGGGAGGTGCGGGAACAGGTTGAACAGTTTACCCTCCGT
TAAGAGGCTCTATAGCTCACAGAGGAGCTTCGGTGGATTTAGCTATTTTTTCTCTTC
ATTTAGCGGGGATATCTTCTATTTTAGGTGCTATTAATTTTATTACTACTATTTTTAAT
ATGCGACCTAAAGTACTTACATTAGAGCGTATAAGGTTATTTGTATGATCCATTTTA
ATTACTGTTTTCTCCTACTCTTATCTTTACCGGTACTTGCTGGAGCCATTACTATAC
TTTTAACAGATCGTAATTTTAATACCAGATTTTTTGATCCAGCTGGTGGGGGTGACC
CAATCT

Arion intermedius, Pihea Trail-Kalawa Rim, Kauai, 1227m altitude, 12th September
2022.

GACTTTATACTTAATTTTTGGAATTTGAAGAGGTTTAGTTGGTACAGGATTATCTTTA
CTTATTCGGTTAGAGTTAGGAACAACAGGAATCCTGACGGACGATCAATTCTTTAAT
GTAGTTGTCACAGCTCATGCATTTGTTATAATTTTTTTTATAGTTATAACCATTAATGA
TTGGGGGGTTTGGAAATTGAATAGTTCCACTATTAATTGGAGCACCAGATATAAGTT
TCCCCCGGATGAATAATTTAAGATTTTGACTTCTTCCCCCATCTTTACTATTATTAAT
TTGTTCTAGAATAGTTGAGGGAGGTGCGGGAACAGGTTGAACAGTTTACCCTCCGT
TAAGAGGCTCTATAGCTCACAGAGGAGCTTCGGTGGATTTAGCTATTTTTTCTCTTC
ATTTAGCGGGGATATCTTCTATTTTAGGTGCTATTAATTTTATTACTACTATTTTTAAT
ATGCGACCTAAAGTACTTACATTAGAGCGTATAAGGTTATTTGTATGATCCATTTTA
ATTACTGTTTTCTCCTACTCTTATCTTTACCGGTACTTGCTGGAGCCATTACTATAC
TTTTAACAGATCGTAATTTTAATACCAGATTTTTTGATCCAGCTGGTGGGGGTGACC
CAATT

Arion intermedius, Pihea Trail-Kalawa Rim, Kauai, 1227m altitude, 12th September
2022.

GACTTTATACTTAATTTTTGGAATTTGAAGAGGTTTAGTTGGTACAGGATTATCTTTA
CTTATTCGGTTAGAGTTAGGAACAACAGGAATCCTGACGGACGATCAATTCTTTAAT
GTAGTTGTCACAGCTCATGCATTTGTTATAATTTTTTTTATAGTTATAACCATTAATGA
TTGGGGGGTTTGGAAATTGAATAGTTCCACTATTAATTGGAGCACCAGATATAAGTT
TCCCCCGGATGAATAATTTAAGATTTTGACTTCTTCCCCCATCTTTACTATTATTAAT
TTGTTCTAGAATAGTTGAGGGAGGTGCGGGAACAGGTTGAACAGTTTACCCTCCGT
TAAGAGGCTCTATAGCTCACAGAGGAGCTTCGGTGGATTTAGCTATTTTTTCTCTTC

ATTTAGCGGGGATATCTTCTATTTTAGGTGCTATTAATTTTATTACTACTATTTTAAAT
ATGCGACCTAAAGTACTTACATTAGAGCGTATAAGGTTATTTGTATGATCCATTTTA
ATTACTGTTTTCTCCTACTCTTATCTTTACCGGTACTTGCTGGAGCCATTACTATAC
TTTTAACAGATCGTAATTTTAATACCAGATTTTTTGATCCAGCTGGTGGGGGTGACC
CAATTCTCTATCAGCATCTATTC

Arion distinctus, Huehue Street, Kailua-Kona, Hawai'i, 1470m altitude, 5th September
2022.

GGATTTGAAGCGGTTTAGTTGGTACTGGGTTATCTCTTCTAATTCGGTTAGAATTGG
GAACTACTGGAGTTCTAACAGATGATCAGTTTTTTAATGTAATTGTAAGTACTGCTCATG
CGTTTTATTATAATTTTTTTTATAGTTATACCTTTAATAATTGGTGGGTTTGGCAATTGA
ATAATCCCTATTACTAGGAGCCCCGGATATAAGTTTCCCACGAATAAATAATATA
AGGTTTTGACTACTACCGCCTTCTCTTACTATTAATTTGTTCTAGTATAGTGGAA
GGAGGGGCAGGCACTGGCTGAACTGTTTACCCCCCTCTAAGAAGTTCAATAGCTC
ATAGTGGGGCGTCAGTTGATTTAGCTATTTTTTCTTTACATTTAGCTGGAATGTCTT
CAATTTTAGGGGCAATTAATTTTATTACTACAATTTTAAACATGCGACCTAAAGTTTT
AACCTTAGAGCGAATAAGGTTATTTGTTTATCTATTTTATTACCGTATTTTTTATTA
CTACTCTCATTACCGGTACTTGCTGGAGCTATTACCATAATTACTGACAGATCGTAAT
TTTTAATAACAAGTTTTTTTATGATCCCGCAGGAGGAGGGGACCCTATTTTAT

Arion distinctus, Huehue Street, Kailua-Kona, Hawai'i, 1470m altitude, 5th September
2022.

TACCTTGTATTTACTCTTTGGAATTTGAAGCGGTTTAGTTGGTACTGGGTTATCTCT
TCTAATTCGGTTAGAATTGGGAAGTACTGGAGTTCTAACAGATGATCAGTTTTTTAA
TGTAATTGTAAGTACTGCTCATGCGTTTATTATAATTTTTTTTATAGTTATACCTTTAATAA
TTGGTGGGTTTGGCAATTGAATAATTCCTTATTACTAGGAGCCCCGGATATAAGT
TTCCCACGAATAAATAATATAAGGTTTTGACTACTACCGCCTTCTCTTACTATTAA
TTTGTCTAGTATAGTGGAAAGGAGGGGCAGGCACTGGCTGAACTGTTTACCCCCCT
CTAAGAAGTTCAATAGCTCATAGTGGGGCGTCAGTTGATTTAGCTATTTTTTCTTTA
CATTTAGCTGGAATGTCTTCAATTTTAGGGGCAATTAATTTTATTACTACAATTTTA
ACATGCGACCTAAAGTTTTAACCCTTAGAGCGAATAAGGTTATTTGTTTATCTATTTT
GATTACCGTATTTTTATTACTACTCTCATTACCGGTACTTGCTGGAGCTATTACCATA
TTACTGACAGATCGTAATTTTAATAACAAGTTTTTTTATGATCCCGCAGGAGGAGGTGAC
CCGATTTTATATCAACATTTATTT