



FINAL REPORT

Pretesting of molecular identification tests for Rhagoletis pomonella (Walsh, 1867)

Contents

1.	Introduction	2		
2.	Scope of pretesting	2		
3.	Test selection	2		
4.	Composition of samples	3		
5.	Specification of pretesting procedures	3		
	DNA extraction	3		
	Real-time PCR	3		
	Other molecular tests	3		
6.	Database inventory for sequence records	3		
	Results	4		
	Tree-based identification	5		
7.	Results	6		
8.	Discussion	7		
Арр	endix 1 - References	8		
Арр	endix 2 – <i>In silico</i> data	9		
Арр	endix 3 – Specifications and parameters for the molecular tests	24		
Арр	ppendix 4 – Real-time PCR results			

1. Introduction

The European Reference Laboratory for Insects and Mites has to select, adapt or develop reliable identification tests for the phytosanitary insect and mite species that are relevant for the European Union (included in the Commission Delegated Regulation (EU) 2019/1702 and in the EURL for Insects and Mites working programmes). One of the tasks of the EURL is to validate available diagnostic protocols before recommending their use to the National Reference Laboratories of the European Union. Pretesting of available tests is necessary to select the most reliable ones for the validation study.



Fig. 1 R. pomonella adult

Rhagoletis pomonella (Fig. 1) is, as its common name 'apple maggot fly' suggests, largely characterized by the infestation of some Rosaceae including domesticated apples (*Malus domestica*) and *Crataegus* spp., which seems to be the trait separating it from other closely related sibling species in the complex (*R. mendax, R. zephyria*, 'flowering dogwood fly' and *R. cornivora*) with hosts from the Ericaceae, Caprifoliaceae and Cornaceae family, respectively. Morphological distinction of adults is challenging, especially if information on the host plant is missing. Molecular identification employing barcoding of the *COI* locus or sequencing of other nuclear loci has so far not been able to sufficiently resolve this species complex either, excluding *R. cornivora*, which can be separated by e.g sequencing the *COI* locus (Xie *et al.* 2008).

R. pomonella is primarily found in North America, with restricted distribution in adjacent parts of Central America (Fig. 2). So far it has not been introduced to any other continents but it is a European Union regulated species, listed among the EU quarantine pests (Annex II of the Commission Implementing Regulation (EU) 2019/2072) and among the EU priority pests (Commission Delegated Regulation (EU) 2019/1702).



Fig. 2: Distribution of R. pomonella according to the EPPO global database

2. Scope of pretesting

The scope of this preliminary study was to identify molecular tests which are appropriate for the identification of *R. pomonella*. Additionally, a database inventory for sequence records should shed light on the application possibilities of barcoding as identification method.

3. Test selection

For this pest species no published specific diagnostic protocols in an international standard are available yet.

However, identification of these quarantine pests is covered in the EPPO PM 7/129 (2) DNA barcoding as an identification tool for a number of regulated pests (EPPO, 2021) (Appendix 1), which includes tests for the DNA barcoding of arthropods in general.

Specific identification tests in scientific literature for the identification of *R. pomonella i.e.*:

- Burgher-MacLellan et al. (2009): three SYBR Green PCRs targeting the COI locus
- Velez et al. (2006): three SYBR Green PCRS targeting microsatellite DNA; it has to be noted that the aim of this publication was to evaluate the usefulness of microsatellite markers for "mapping host-plant-associated adaptations in Rhagoletis that generate reproductive isolation and facilitate speciation, as well as for resolving the genetic structure and evolutionary history of fly populations".

4. Composition of samples

For pretesting of tests indicated above, DNA was extracted from parts of single adult specimens (legs) of *R. pomonella* and *Rhagoletis sp.* non-targets (see Table 1). All specimens included in this study were morphologically confirmed to the species level (pers. comm. A. Egartner, AGES). However, the sample 66/19 could only be assigned as either *R. pomonella* or *mendax*. Additionally, the condition of this sample was questionable, as *R. pomonella* samples in Europe are rare to begin with, therefore all results have to be taken with a grain of salt until further tests with fresh specimens can be conducted.

Sample	Species	Host plant	Origin	Source		
66/19	Rhagoletis pomonella/mendax	na	Michigan, US	AGES, Egartner		
2247/22	Rhagoletis cingulata	Juglans regia	Austria	AGES, Egartner		
1928/22	Rhagoletis meigenii	Juglans regia	Austria	AGES, Egartner		
1233/22	Rhagoletis alternata	Malus sp.	Austria	AGES, Egartner		
1231/22	Rhagoletis cerasi	Malus sp.	Austria	AGES, Egartner		
2552/21	Rhagoletis meigenii	Juglans regia	Austria	AGES, Egartner		

Table 1: Details on single adult specimens for pretesting *Rhagoletis* spp. identification (sample set)

*na: not available

5. Specification of pretesting procedures

DNA extraction

For DNA extraction the DNeasy Blood and Tissue (Qiagen) on *Rhagoletis* spp. legs was used. Samples indicated in Table 1 were tested undiluted and with at least one additional dilution (usually 1:20).

<u>Real-time PCR</u>

Following SYBR Green real-time PCR tests for the identification of *R. pomonella* were evaluated:

- Tests according to Burgher-MacLellan et al. (2009) targeting the COI locus
- Tests according to Velez et al. (2006) targeting microsatellite DNA

Other molecular tests

Included in EPPO PM 7/129 (2) DNA barcoding (EPPO, 2021):

• Barcoding for arthropods (Appendix 1); was tested for the identification of *R. pomonella* and related non-target species.

6. Database inventory for sequence records

DNA barcoding relies on PCR of predetermined marker genes (e.g. for the *COI* gene), DNA sequencing and comparison of those sequences to a database of reference sequences (Armstrong and Ball 2005). Applying barcoding for insect identification requires enough sequence records from the species within the genus for a reliable comparision. Not only the number, but also the genetic and geographic diversity of the records and the quality are potential issues that should taken into account.

Three different databases (NCBI GenBank, Bold and EPPO Q-Bank) were consulted for the inventory. As search parameters the genus and species name and the gene locus (*COI* and synonyms) were used. In addition the reliability of the records were checked and, if reasonable, the search was extended to other gene loci.

<u>Results</u>

Due to the extraordinary amount of sequence data deposited in the NCBI GenBank, additional restrictions have to be made to evaluate the availability and suitability of sequences, especially for additional loci.

Items: 1 to 20 of 123279

Additionally, accession descriptors such as the one depicted below make the evaluation of sequence availability difficult.

- Rhagoletis pomonella isolate G09_DW_012 cytocrhome oxidase subunit I (COI) gene, partial cds;
- 17. tRNA-Leu gene, complete sequence; and cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial

 871 bp linear DNA

 Accession: KF877743.1
 Gl: 569345324

 Protein
 PubMed
 Taxonomy

 GenBank
 FASTA
 Graphics
 PopSet

In all three consulted databases sequence records for *R. pomonella* on the *COI* locus are avaible. GenBank also offered 8 sequence records for the complete mitochondrial genome and a large number of accessions from whole genome shotgun sequencing projects. Additionally, sequences on other loci (e.g., *COII, ITS2, 12S* rDNA, *16S* rDNA, *18S* rDNA) have been deposited in the NCBI GenBank (Query date October 2022).

|--|

Gene	GenBank	Q-Bank	Bold
COI	53(13*)	11	37(28***)
COII	66(21**)	0	0
ITS2	8	0	0
125	2	0	0
165	4	0	0
185	15	0	0
Complete genome (mitochondrium)	8	0	0
Whole genome shotgun sequencing	36 796	0	0

*sequences of appropriate length for standard barcode

**sequences solely identified as COII locus

***sequences >500bp

Despite the large amount of sequence data in the NCBI GenBank, only part of the deposited *COI* sequences are of the expected length for barcoding, with many others being much longer and stretching into additional gene loci. Out of the 37 *R. pomonella* specimens listed in the Bold database, only 28 have appropriate sequences deposited, a large part of which were mined from NCBI GenBank. Regarding the geographic variation of the sequence records for *R. pomonella* in Bold, all records originate from North or Central America, with most of them being from Canada and Mexico. A similar distribution is seen in the 11 records found in EPPO Q-Bank, with the majority of them having been sampled in Mexico.

Bold Data base lists 680 specimen records for the Rhagoletis genus (about 70 species), 531 of which have barcodes. *R. cingulata* makes up the bulk of them with 115 records, followed by *R. basiola, meigenii, pomonella* and *completa*. None of the other Rhagoletis species relevant for this pretesting have any whole genome shotgun sequencing data deposited in the NCBI genbank, and only for *Rhagoletis cerasi* complete mitochondrial genome sequences are available (see Table 3). This is not relevant for the *in silico* evaluation of real-time PCRs targeting the *COI* locus, as there are sequences available for all species on this locus, but it has to be taken into account when reviewing *in silico* data for PCRs targeting microsatellite DNA.

Table 3. Number of sec	nuence records available in	NCBI GenBank for s	elected Rhagoletis species
Table 5. Nulliber of sec	juence records available in	INCDI GELIDALIK IULS	elected mildguletis species.

Species	Sequences COI	Complete mitochondrial	Whole genome
		genome	sequencing
Rhagoletis cingulata	41	0	0
Rhagoletis meigenii	39	0	0
Rhagoletis alternata	4	0	0
Rhagoletis indifferens	5	0	0
Rhagoletis cerasi	21	6	0

Tree-based identification

To evaluate the species divergences within the genus, a Neighbor Joining (NJ) tree of distance was constructed using NCBI GenBank (max. seq. diff. of 0.75). Fig 3 showcases further complications that are known to arise with the species *R. mendax* and *R. zephyria*, which are both part of the *R. pomonella* complex.



Fig 3: Neighbor joining tree for *R. pomonella* on the *COI* locus, showing the species of the *R. pomonella* locus (*R. pomonella*, *R. zephyria* and *R. mendax*) clustering

7. Results

In silico data

Burgher-MacLellan *et al.* 2009: Primer BLAST and *in silico* PCRs of the ID-1F/ID-1R/ID-3R primers indicated them as specific for *R. pomonella* complex species. Both the ID-8F and ID-9R primer BLAST led to trees where *R. pomonella* grouped with many other species of the Rhagoletis genus. *In silico* PCR on this primer pair resulted in all three species of the *R. pomonella* complex showing as potential targets, in addition to singular results of fly species belonging other families such as *Clusiodes* sp., *Lonchaeidae* sp., and *Teleopsis* sp., but no other Rhagoletis species.

Velez *et al.* 2006: All three primer pairs (P6F/P6R, P9F/P9R, P12F/P12R) showed *in silico* specificity for the *R. pomonella* complex. The *in silico* PCR resulted in exactly one hit each, which were sequences deposited by the authors. It has to be noted, that whole genome sequences are lacking for most closely related Rhagoletis species, making evaluation of such results difficult.

Details on the *in silico* data see Appendix 2.

SYBR Green real-time PCRs according to Burgher-MacLellan et al. (2009) and Velez et al. (2006)

Identification of *R. pomonella* with these SYBR Green real-time PCRs targeting the *COI* locus and microsatellite DNA proved to be difficult with the sample set used in this pretesting. As Table 4 and 5 show, unspecific signals were observed for most of the PCRs with the melting temperature being unsuitable to give further insight into the specificity in most cases. Only in the real-time PCR ID-8F/ID-9R (Burgher-MacLellan *et al.* 2009) a slight difference in melting temperature between the target sample and non-target samples could be observed.

Table 4: SYBR Green PCR results of pretesting with the sample set; results for Rhagoletis spp. according to assigned values

		Burgher-MacLellan <i>et al</i> . 2009 tests 1-3			Velez et al. 2006 tests 4-6		
Sample	Species	Test 1 (ID-8F/ID-9R)	Test 2 (ID-1F/ID-1R)	Test 3 (ID-1F/ID-3R)	Test 4 (P6F/P6R)	Test 5 (P9F/P9R)	Test 6 (P12F/P12R)
66/19	R. pomonella/mendax	+	(-)	(-)	(-)	(-)	(-)
2247/22	R. cingulata	(-)	(-)	(-)	(-)	(-)	(-)
1928/22	R. meigenii	(-)	(-)	(-)	(-)	(-)	(-)
1233/22	R. alternata	(-)	(-)	(-)	(-)	(-)	(-)
1231/22	R. cerasi	(-)	(-)	(-)	(-)	(-)	(-)
2552/21	R. meigenii	(-)	(-)	(-)	(-)	(-)	(-)

Table 5: Result summary and verdict for the eight real-time PCRs tested

Author	Test	Verdict
Burgher-MacLellan <i>et al.</i> (2009)	Test 1	<i>R. pomonella/mendax</i> does have a slightly different melting temperature than all other tested non-targets.
	Test 2	<i>R. pomonella/mendax</i> and <i>R. cerasi</i> show lower Ct values than the other samples, however with the same melting temperature.
	Test 3	<i>R. pomonella</i> and <i>R. cerasi</i> show lower Ct values than the other samples, however with the same melting temperature.
Velez <i>et al</i> . (2006)	Test 4	There is no distinction possible on the basis of either Ct or melting temperature.
(2000)	Test 5	<i>R. pomonella</i> and <i>R. cingulata</i> show lower Ct values than the other samples, however with the same melting temperature.
	Test 6	There is no distinction possible on the basis of either Ct or melting temperature.

Barcoding according to EPPO PM7/129(2)

Sequencing on the *COI* locus was able to distinguish all non-targets of the sample set used in this pretesting (Tab. 6). The target sample could be identified down to the *Rhagoletis pomonella* complex, which includes *R. mendax* and *R. zephyria*. Additionally, the sequence quality did not meet the expected quality criteria.

Sample	Assigned value	Result of pretesting (LepF/R)
66/19	Rhagoletis pomonella/mendax	Rhagoletis pomonella complex
2247/22	Rhagoletis cingulata	Rhagoletis cingulata
1928/22	Rhagoletis meigenii	Rhagoletis meigenii
1233/22	Rhagoletis alternata	Rhagoletis alternata
1231/22	Rhagoletis cerasi	Rhagoletis cerasi
2552/21	Rhagoletis meigenii	Rhagoletis meigenii

Table 6: Results of barcoding with the pretesting sample set

8. Discussion

Molecular identification of *Rhagoletis pomonella* pretested in this study highlighted some of the difficulties that arise with this species complex. *In silico* data (database inventory, primer blast, including fast minimum evolution tree analysis, and *in silico* PCR) already indicated the impossibility of resolving the species within the *R. pomonella* complex with the tests included in this pretesting, and occasionally led to doubts in regards to the specificity of the tests as well.

The *in silico* specificity of the tests according to Velez *et al.* (2006) targeting microsatellite DNA could not be evaluated, as there seem to be no sequences available for the non-target Rhagoletis species included in this study on these gene loci.

For one real-time PCR targeting the *COI* locus (Test1 according to Burgher-MacLellan *et al.* 2009) *R. pomonella/mendax* was distinguishable via differences in melting temperature. None of the other real-time PCRs could be confirmed as suitable for the identification of *R. pomonella* in this pretesting. Nevertheless, in Test2 and 3, significantly lower Ct values were achieved for *R. pomonella/mendax* and *R. cerasi*, which could be an indication of these tests not being exclusive for all tested Rhagoletis species. It has to be noted however, that due to the limited availability of *R. pomonella* specimens, the target in this pretesting was of questionable condition.

COI barcoding could be confirmed as suitable to identify targets down to the level of *Rhagoletis pomonella* complex, but distinction among species in this complex remains difficult.

Further studies will have to be conducted both with an extended sample set for non-targets and more target specimens of sufficient quality. Special attention will have to be given to Rhagoletis cerasi specimen, as well as sufficient representation of all species included in the Rhagoletis pomonella complex (*R. pomonella*, *R. mendax*, *R. zephyria*, *R. cornivora*).

Participating staff:

Experts/ Supervisors: Richard Gottsberger, Helga Reisenzein Technical staff/ Operators: Claudia Heiss, Chiara Pohn

Date: December 12th, 2022

dism sin

Helga Reisenzein EURL Deputy Director

Ridord L. fattiler

Richard Gottsberger Scientific Project Leader – Molecular Unit

EPPO (2021). EPPO standards PM 7/129 (2) DNA barcoding as an identification tool for a number of regulated pests. Bulletin OEPP/EPPO Bulletin, 51 (1): 100–143.

Hajibabaei, M., Janzen, D. H., Burns, J. M., Hallwachs, W., & Hebert, P. D. (2006). DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences, 103(4), 968-971.

Burgher-MacLellan, K. L., Gaul, S., MacKenzie, K., & Vincent, C. (2009). The use of real-time PCR to identify blueberry maggot (Diptera: Tephritidae, Rhagoletis mendax) from other Rhagoletis species and in lowbush blueberry fruit (Vaccinium angustifolium). Acta Horticulturae International Society of Horticultural Sciences, 810, 265-274.

Velez, S., Taylor, M. S., Noor, M. A. F., Lobo, N. F., & Feder, J. L. (2006). Isolation and characterization of microsatellite loci from the apple maggot fly Rhagoletis pomonella (Diptera: Tephritidae). Molecular Ecology Notes, 6(1), 90-92.

Xie, X., Michel, A. P., Schwarz, D., Rull, J., Velez, S., Forbes, A. A., Aluja, M., Feder, J. L. (2008) Radiation and divergence in the *Rhagoletis pomonella* species complex: inferences from DNA sequence data. J Evol Biol. 21(3), 900-913.

In silico data PCR assay 1

EPPO (2021). EPPO standards PM 7/129 (2) DNA barcoding as an identification tool for a number of regulated pests. Bulletin OEPP/EPPO Bulletin, 51 (1): 100–143.

Hajibabaei, M., Janzen, D. H., Burns, J. M., Hallwachs, W., & Hebert, P. D. (2006). DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences, 103(4), 968-971.

Search was restricted to Rhagoletis pomonella

Fast Minimum Evolution tree for LepF

	PREDICTED: Rhagoletis pomonella D-aspartate oxidase (LOC118744484), mRNA
	 viciQuery _soc₂s Rhapoletis pomonella isolate mex 7 cvtochrome c oxidase subunit I (COI) gene. partial cds: mitochondrial
	PREDICTED: Rhagoletis pomonella uncharacterized LOC118748537 (LÓČ118748537), ncRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC118748537 (LÓČ118748537), ncRNA Prediction of the second secon
	PREDICTED: Rhagoletis pomonella membrane metallo-endopepidase-like 1 (LOC118/3/025), mRNA PREDICTED: Phagoletis nononella nortein virilizet-like (LOC118743236), mRNA
	PREDICTED: Rhagoletis pomonella protein virilizer-like (LOC118743229), mRNA
	● PREDICTED: Rhagoletis pomonella acetyl-CoA carboxylase (LOC118742753), transcript variant X3, mRNA PREDICTED: Rhagoletic pomonella 43 kDp researcher acesoritation periodic (LOC11874500), transcript variant X2, mRNA
	PREDICTED: Magoletis pomonella cullin-2 (LOC118735585), transcript variant XI, mRNA
	PREDICTED: Rhagoletis pomonella cullin-2 (LOC118735585), transcript variant X2, mRNA PREDICTED: Rhagoletis pomonella cullin-2 (LOC118735484), uservitati ACC118735484), uservitati ACC118735484
	PREDICTED: Rhagoleus pomonella 43 KDa recentor-associated protein of the synapse homolog (LOC118745600), transcript variant X1, mRNA
	¹ Rhagoletis pomonella isolate RP cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial
	PREDICTED: Rhagoletis pomonella terminal uridylyltransferase Tailor-like (LOC118/4524/), transcript variant X3, mRNA
	PREDICTED: Rhagoletis pomonella terminal uridylyltransferase Tailor-like (LOC118745247), transcript variant XI, mRNA
	PREDICTED: Rhagoletis pomonella twitchin (LOC118745768), mRNA PREDICTED: Propositis pomonella understatistication (LOC118740110), mRNA
Ť	PREDICTED: Magoletis pomonella putative nuclease DE HABBII (LOC11872809), partial mRNA
	PREDICTED: Rhagoletis pomonella iron-sulfur cluster co-chaperone protein HscB (LOC118747785), mRNA
	Khagotetis pomonella isolate mex. 3 cytochrome c oxidase subuniti (COI) gene, partial cds; mitochondriai R hagotetis pomonella isolate USA 01 mitochondrion. comblete genome
	Rhagoletis pomonel la mitochondrion, complete genome
	Rhagoletis pomonella mitochondrion, complete genome
	A Ragoletis pomonella mitochondrion, complete genome
	Rhagoletis pomonella vioucher Q004 1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial
	PREDICTED: Rhagoletis promonella ATP-cirate synthase (LOC118743253), transcript variant X3, mRNA
	PREDICTED: Rhagoletis pomonella ATP-cirate synthase (LOCI18743253), transcript variant X2, mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOCI18747753 (LOCI18747753), neRNA
	PREDICTED: Rhagoletis pomonella arf-GAP domain and FG repeat-containing protein 1-like (LOC118751330), transcript variant X1, mRNA
	YPREDICTED: Rhagoletis pomonella artícal putative nuclease HARBII (LOC118/32053), partial mRNA
	PREDICTED: Rhagoletis pomonella arf-GAP domain and FG repeat-containing protein 1-like (LOC118751330), transcript variant X5, mRNA
	9 PREDICTED: Rhagoletis pomonella mitochondrial sodium/calcium exchanger protein-like (LOC118752048), mRNA 9 PREDICTED: Rhagoletis pomonella art/GAP domain and FG repeat-containing mortein Like (LOC118752048), mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC118755820 (LOC118755820), mRNA
	PREDICTED: Rhagoletis pomonella polyadenylate-binding protein (LOC118744348), transcript variant X2, mRNA DEDICTED: Rhagoletis pomonella garing/theorem a costain kinase tousided like 2 (OC11873350), transcript variant X5 mRNA
	PREDICTED: Ringoletis pointorella serine/interpotein kinase tousied-like 2 (LOC118735350), transcript variant X4, mRNA
	PREDICTED: Rhagoletis pomonella serine/threonine-protein kinase tousled-like 2 (LOC118733350), transcript variant X3, mRNA
	PREDICTED: Rhagoletis pomorella serine/interporte in kinase tousted-like 2 (LOC118735350), transcript variant X2, mRVA
	PREDICTED: Rhagoletis pomonella transmembrane protein 50B (LOC118748018), mRNA
	PREDICTED: Rhagoletis pomonella populaenvalenvalenvalenvalenvalenvalenvalenv
	PREDICTED: Rhagoletis pomonella uncharacterized LOCI 18736363 (LOCI 18736363), mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC118/35488 (LOC118/35488), mRNA RPEDICTED: Rhagoletis pomonella uncharacterized LOC118/48571 (LOC118/48571), transcript variant X2, mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC118748571 (LOC118748571), transcript variant X1, mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC118/49021 (LOC118/49021), mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC118/44930 (LOC118/44930), mRNA
	PREDICTED: Rhagoletis pomonella ubiquitin conjugation factor E4 A (LOC118735428), mRNA
	PREDICTED: Rhagoletis pomonella sialin (LOC118744905), mRNA
	PREDICTED: Rhagoletis pononella neuroligin-4, Y-linked (LOC11875032), interva
	PREDICTED: Rhagoletis pomonella uncharacterized LOC118756725 (LOC118756725), partial mRNA PREDICTED: Nuclearing and an entering of the president and the
	PREDICTED: Rhagoletis pomonella beta-1,3-galactos/transferase 6 (LOC118735962), transferit X2, misc RNA
	PREDICTED: Rhagoletis pomonella beta-1,3-galactosyltransferase 6 (LOCU18735962), transcript variant X1, mRNA
	PREDICTED: knagoletis pomonelia uncharacterized protein 50 omolog (LOC118/32/18), partiai mKNA
	PREDICTED: Rhagoletis pomonella DNA-directed RNA polymerase II subunit RPB1 (LOC118751915), mRNA
	PREDICTED: Rhagoletis pomonella dichyl-dinkostkologi saccharide-protein givosvitransferase subunii STT3 (LOC118/33230). mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC118743252 (LOC118743252), mRNA
	PREDICTED: Rhagoletis pornonella transcription initiation factor TFIID subunit 2-like (LOC118/45962), mRNA
	PREDICTED: Rhagoletis pomonella protein ANTAGONIST OF LIKE HETEROCHROMATIN PROTEIN 1-like (LOC118751634), mRNA
	PREDICTED: Rhagoletis pomonella uncharected RNA polymerase II subunit RPB1 (LOC118751917), mRNA PREDICTED: Rhagoletis pomonella uncharecterized IOC118757436 (IOC118757436) mRNA
	PREDICTED: Rhagoletis pomonella zinc finger protein 431-like (LOC118752716), mRNA
	PKEDICTED: Rhagoletis pomonella urcharacterized IOCI1875547 (IOCU1875513), transcript variant X6, mRNA PREDICTED: Rhagoletis pomonella urcharacterized IOC11875547 (IOCU1875547), uRNA
	PREDICTED: Rhagoletis pomonella mitochondrial uncoupling protein 4 (LOC118733850), transcript variant X5, misc_RNA
	PREDICTED: Rhagoletis pomonella uncheracirad I/OCI18733850, transcript variant X2, misc_RNA
	FRENCH ED: Rhagoletis pomonella euronal acelylcholine receptor suburit alpha-7 (LOCH18735498), mRNA
	PREDICTED: Rhagoletis pormonella notes submit albea types 5 (LOCUB72494), mRNA
	PREDICTED: Rhagelets pomorie an protessorie submit apine types (100-110/2200), inkt/vk PREDICTED: Rhagelets pomorie la myosin-VIII (LOC118/32567), mRNA
	* PREDICTED: Rhagoletis pomonella esterase B1-like (LOC118741534), mRNA

PREDICTED: Rhagoletis pomonella uncharacterized LOC118737017 (LOC118737017, mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC118737016 (LOC118737016) mRNA
PREDICTED: Rhagoletis pomonella uncharacterized LOC(1874592 (LOC(1874592), uRNA SREDICTED: Rhagoletis pomonella uncharacterized LOC(18745222), uRNA
PREDICTED: Rhagoletis pomonella venom metalloproteinase 3 (LOC118744526), transcript variant X1, mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC118757059 (LOC118757059), partial mRNA PREDICTED: Rhagoletis pomonella protein hi-ti tai shao (LOC118757395), mRNA PREDICTED: Rhagoletis pomonella SNAPIN protein homolog (LOC118757395), mRNA PREDICTED: Rhagoletis pomonella phospholipase A-2-activating protein-like (LOC11874452), transcript variant X3, mRNA PREDICTED: Rhagoletis pomonella molecular differentiation 3-like (LOC11873762), mRNA PREDICTED: Rhagoletis pomonella MATH and LRR domain-containing protein PFE0570w (LOC118746161), transcript variant X4, m PREDICTED: Rhagoletis pomonella MATH and LRR domain-containing protein PFE0570w (LOC11874611), transcript variant X4, m PREDICTED: Rhagoletis pomonella MATH and LRR domain-containing protein PFE0570w (LOC11874611), transcript variant X4, m PREDICTED: Rhagoletis pomonella Indiculin-like (LOC11874683, transcript variant X2, mRNA PREDICTED: Rhagoletis pomonella Indiracterized LOC118746631, transcript variant X4, m PREDICTED: Rhagoletis pomonella Indiracterized LOC118746631, transcript variant X4, m PREDICTED: Rhagoletis pomonella Indiracterized LOC118746631, transcript variant X4, m PREDICTED: Rhagoletis pomonella Indiracterized LOC118746631, transcript variant X4, m PREDICTED: Rhagoletis pomonella Indiracterized LOC118746631, transcript variant X4, m PREDICTED: Rhagoletis pomonella MATH and LRR domain-containing protein PFE0570w (LOC118746101), transcript variant X4, m PREDICTED: Rhagoletis pomonella Indiracterized LOC118746631, transcript variant X4, m PREDICTED: Rhagoletis pomonella Indiracterized LOC118746631, transcript variant X4, m PREDICTED: Rhagoletis pomonella uncharacterized LOC118746631, transcript variant X4, m PREDICTED: Rhagoletis pomonella uncharacterized LOC118746631, transcript variant X4, m PREDICTED: Rhagoletis pomonella uncharacterized LOC118746631, transcript variant X4, m PREDICTED: Rhagoletis p
files 6 leaves
 Thes 13 teaves Rhagoletis pomonella isolate S8P1H111 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial PREDICTED: Rhagoletis pomonella MATH and LRR domain-containing protein PFE0570w (LOC118746161), transcript variant X5, m PREDICTED: Rhagoletis pomonella MATH and LRR domain-containing protein PFE0570w like (LOC118746547), mRNA PREDICTED: Rhagoletis pomonella MATH and LRR domain-containing protein PFE0570w like (LOC118746547), mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC118748683 (LOC118748683), transcript variant X1, mRNA
 PREDICTED: Rhagoletis pomonella uncharacterizzal LOCI IB74683 (LOCI IB74683), the LOCI IB74613), transcript variant X1, mRNA PREDICTED: Rhagoletis pomonella MATH and LRR domain-containing protein PEED570w (LOCI IB746161), transcript variant X2, m PREDICTED: Rhagoletis pomonella MATH and LRR domain-containing protein PEED570w (LOCI IB746161), transcript variant X2, m PREDICTED: Rhagoletis pomonella MATH and LRR domain-containing protein PEED570w (LOCI IB746161), transcript variant X1, mR. PREDICTED: Rhagoletis pomonella uncharacterizzal LOCI IB737151 (LOCI IB737151), transcript variant X1, mRNA PREDICTED: Rhagoletis pomonella uncharacterizzal LOCI IB737713 (LOCI IB737151), transcript variant X2, mRNA PREDICTED: Rhagoletis pomonella uncharacterizzal LOCI IB737713 (LOCI IB737151), transcript variant X3, mRNA PREDICTED: Rhagoletis pomonella uncharacterizzal LOCI IB7374520, transcript variant X3, mRNA PREDICTED: Rhagoletis pomonella venom metalloproteinase 3 (LOCI IB744520), transcript variant X3, mRNA PREDICTED: Rhagoletis pomonella venom metalloproteinase 3 (LOCI IB744520), transcript variant X3, mRNA PREDICTED: Rhagoletis pomonella venom metalloproteinase 3 (LOCI IB744520), transcript variant X6, mRNA PREDICTED: Rhagoletis pomonella venom metalloproteinase 3 (LOCI IB744520), transcript variant X6, mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOCI IB736501 (LOCI IB7365050), mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOCI IB736501 (LOCI IB7365050), mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOCI IB736501 (LOCI IB7365050), mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOCI IB736501 (LOCI IB7356150), mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOCI IB736501 (LOCI IB7356150), mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOCI IB7365
PREDICTED: Rhagdetis pomonella homeolox protein prospero-like (LOC118747291), transcript variant X9, mRNA PREDICTED: Rhagdetis pomonella homeolox protein prospero-like (LOC118747291), transcript variant X9, mRNA PREDICTED: Rhagdetis pomonella homeolox protein prospero-like (LOC118747291), transcript variant X9, mRNA PREDICTED: Rhagdetis pomonella homeolox protein prospero-like (LOC118747291), transcript variant X9, mRNA
PREDICTED: Rhagoletis pomonella homeobox protein prospero-like (LOC118747291), transcript variant X1, mRNA SPREDICTED: Rhagoletis pomonella homeobox protein prospero-like (LOC118747291), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella homeobox protein prospero-like (LOC118747291), transcript variant X7, mRNA PREDICTED: Rhagoletis pomonella homeobox protein prospero-like (LOC118747291), transcript variant X7, mRNA
 PREDICTED: Rhagoletis pomonella homeobox protein prospero-like (LOC118747291), transcript variant X3, mRNA PREDICTED: Rhagoletis pomonella RNA-binding protein Musashi homolog Rbp6-like (LOC118746341), mRNA PREDICTED: Rhagoletis pomonella venom metalloproteinase 3 (LOC11874656), transcript variant X8, mRNA
* PREJECTED: Rhagoletis pomonella protein spit2 (LOC118/36353), LoC11875633), partial RNA
 PREDICTED: Rhagoletis pomonella coronin-7 (LOC118733976), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella coronin-7 (LOC118733976), transcript variant X3, mRNA PREDICTED: Rhagoletis pomonella coronin-7 (LOC118733976), transcript variant X2, mRNA
PREDICTED: Rhagoletis pomonella KX506-binding protein 2 (LOC118742997), transcript variant X2, mRNA PREDICTED: Rhagoletis pomonella FK506-binding protein 2 (LOC118742997), transcript variant X2, mRNA PREDICTED: Rhagoletis pomonella uncharacterized protein XIAA0513 (LOC11873559), transcript variant X4, mRNA
flies 7 leaves

In silico data PCR assay 2-4

Burgher-MacLellan, K. L., Gaul, S., MacKenzie, K., & Vincent, C. (2009). The use of real-time PCR to identify blueberry maggot (Diptera: Tephritidae, Rhagoletis mendax) from other Rhagoletis species and in lowbush blueberry fruit (Vaccinium angustifolium). Acta Horticulturae International Society of Horticultural Sciences, 810, 265-274.

Search was restricted to Rhagoletis

Rhagoletis basiola isolate WA_A5 cytochrome c oxidase subunit I (COI) gene, partial cds: mitochondrial	
Rhagoletis basiola voucher BIOUGI9851-D07 cvtochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis basiola voucher BIOUG19690-G10 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis basiola voucher BIOUG22954-G06 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
⁸ Rhagoletis basiola voucher BIOUG23579-D01 cytochrome oxidase subunit 1 (CO1) gene, partial cds; mitochondrial	
Rhagoletis basiola voucher BIOUG322/4-E09 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis basiola isolate wA A / cylochrome oxidase subunit 1 (COI) gene, partial cus; mitochondrial	
Rhapoletis basiola voucher BIOUG22549-G06 cyclochrome oxidase subunit 1 (COI) gene, partial cds, mitochondrial	
Rhagoletis basiola voucher BIOUG11603-C03 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis basiola voucher BIOUG11923-F03 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis basiola isolate WA A1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	
Rhagoletis basiola voucher BIOUG04485-A04 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
* Phagoletis basiola voucher BIOUG005442-D12 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhapoletis basiola voucher BIOUG0592-050 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis basiola voucher BIOUG04877-G03 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
🛔 Rhağoletis basiola voucher BIOUG01544-B12 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis basiola isolate Rbas671816 cytochrome oxidase subunit 1 (CO1) gene, partial cds; mitochondrial	
Reagolatis basiola voucher BIOUG11/0-F05 cytochrome oxidase subunit 1 (COI) gene, partial cdc, mitochondrial	
Rhapoletis basiola voucher BIOUGI 1603-G11 cvytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis basiola voucher BIOUG05447-E09 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis basiola voucher BIOUG04485-B04 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Phagoletis basiola voucher BIOUG08227-A09 cytochrome oxidase subunit 1 (COI) gene, promoter region and partial cds; mito	chond
Rhagoletis basiola voucher BIOUG08718-E10 cytochrome oxidase subunit 1 (COI) gene, promoter region and partial cds; mito Phylicia de la subunitation and partial cds; mito	chondr.
Pintagoretis basioia voucner 08BBDIP-0955 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial Dispers on BCD DiAA E7554 voucher BIOUCCCAN> 10BBCDIP-3132 citachrome oxidase subunit 1 (COD) some merial cds;	mitoc
Repeated by DOLD AREAS Volume DIOCOCCATV TO DOLD 1-3-52 cytochrome oxnase sublimit 1 (COI) gene, partial cds;	rial
Rhagoletis basiola voucher BIOUGCAN> 10BBCDIP-1727 cytochrome oxidase subunit 1 (COI) gene, partial eds. intochond	rial
Rhagoletis basiola voucher BIOUG <can>10BBCDIP-1223 cytochrome oxidase subunit 1 (COI) gene, partial cds: mitochond</can>	rial
Rhagoletis basiola voucher UOG:DEBU:01009033 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	
Rhagoletis basiola voucher UOG:DEBU:00173517 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	
7 ICIQUery 184841 P Dispaty is associate and a strategy of the state of the strategy of the s	
PReagoreus zepnyna isolaie wny / cylochrome c oxidase subumi 1 (Col) gene, partial cus; mitochondrial PREDICTED: Rhaooletis zenbyria uncharacterized LOC1083810951 (DCC108381095) mRNA	
PREDICTED: Rhagoletis zephyria chitinase-like (LOC108353980), partial mRNA	
flies 6 leaves	
flies 2 leaves	
PREDICTED: Rhagoletis pomonella uncharacterized LOC118735876 (LOC118735876), transcript variant X6, mRNA	
PREDICTED: Rhagoletis pomonella uncharacterized LOC118735876 (LOC118735876), transcript variant X5, mRNA	
PREDICTED: Rhagoletis pomonella uncharacterized LOC118735876 (LOC118735876), transcript variant X4, mRNA	
Bhagoletis zephyria isolate MN_A4 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	
Rhagoletis pomonella isolate MN_19 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	
Rhagoletis pomonella isolate MI A3 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	
Reagoletis pomonella isolate mex ⁻³ cytochrome c'oxidase subunit I (COI) gene, partial cds; mitochondrial	
Rhagoletis mendax isolate 090517 3 cvtochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	
Rhagoletis mendax isolate 090517 ⁻² cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	
Rhagoletis mendax isolate 090517-1 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	
Rhagoletis cornivora isolate 051816 2 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	
Rhagoletis comivora isolate 051810 1 cytochrome c oxtrasse submit 1 (CO1) gene, partial cus; mitochondrial	
Rhagoletis pomoneria vouener MFQCDQ HEF050+02 cytoenrone oxidase subunit 1 (COI) gene, partial cds, initochondrial	
Rhagoletis combridi isolate MV A4 cytochrome axidase subunit 1 (COI) gene, partial cds: mitochondrial	
Rhagoletis pomonella isolate MI A3 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis mendax isolate MI_A4 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis pomonella voucher RPMe4 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1
Knagoletis pomonetia voucner RPMe3 cytochrome oxidase auburit I (COI) gene, partial cds; mitochondrial Rhandatis nomenalla voucher RDMe3 cytochrome oxidase auburit I (COI) gene, partial cds; mitochondrial	
Transpore is pomonenta voucher RPMe2 cytoenrome oxidase subunit i (COI) gene, partial cds, infloednodrial	
Rhagoletis pomonella voucher BIOIG21829-603 cytochrome oxidase subunit (COI) gene, partial cds: mitochondrial	
Rhagoletis sp. BIOUG28473-A02 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1
Rhagoletis zephyria isolate MN_A4 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
Rhagoletis mendax isolate MI A4 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	
w Knagotetts pomonella isolate USA UI mitochondrion, complete genome	1
Rhagorets pononenta isotate Ni A3 cytochronie oxidase subunit i (CO) gene, partiai cus, initochondriai	
Rhagoletis zeplyria mitochondrion, complete genome	1
Rhagoletis zephyria mitochondrion, complete genome	
PRhagoletis zephyria mitochondrion, complete genome	1
Rhagoletis zephyria mitochondrion, complete genome	
w Rhagoietis zepnyria mitochondrion, complete genome	1
Knagoreus mendax imicochondrion, compiète genome Bhagoletis noncolla mitochondrion, compiète conome	
King Jones Politorena internolation, complete genome	
Phone intes 7 reaves	1
Transports pomonenta isolate Sor 211212 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1
Rhagoletis pomonella isolate SSP11211 Cytochrome oxidase subunit 1 (CO1) gene, partial cds, mitochondrial	
Rhagoletis pomonella isolate S8P1H112 cytochrome oxidase subunit I (COI) gene, partial cds: mitochondrial	1
Rhagoletis pomonella isolate S8P1H111 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	
Rhagoletis pomonella isolate RP cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	
Rhagoletis pomonella voucher BIOUG <can>:10BBCDIP-1722 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocher Rhagoletis pomonella voucher BIOUG<can>:10BBCDIP-1722 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocher Rhagoletis pomonella voucher BIOUG<can>:10BBCDIP-1722 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocher Rhagoletis pomonella voucher BIOUG<can>:10BBCDIP-1722 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocher Rhagoletis pomonella voucher BIOUG<can>:10BBCDIP-1722 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocher Rhagoletis pomonella voucher BIOUG<can>:10BBCDIP-1722 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocher Rhagoletis pomonella voucher BIOUG<can>:10BBCDIP-1722 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocher Rhagoletis pomonella voucher BIOUG<can>:10BBCDIP-1722 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocher Rhagoletis pomonella voucher BIOUG<can>:10BBCDIP-1722 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocher Rhagoletis pomonella voucher BIOUG<can>:10BBCDIP-1722 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocher Rhagoletis pomonella voucher BIOUG</can></can></can></can></can></can></can></can></can></can>	ndrial
Knagoletis pomonella isolate FF628 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial Bhagoletis pomonella viendere O(WU) oxidenergie origidade subunit I (COI) gene, partial cds; mitochondrial	
v knagoteus pomonena vouener Q004 i cytoenrome oxidase subunit (COI) gene, partial cas, mitoehondrial Rhaodetis mendav vouener Q004 i cytoenrome oxidase subunit (COI) gene, partial cas, mitoehondrial	
Rhagoletis mendax voucher 0005_1 cytochrone oxidase subjinit 1 (COI) gene, partial cus, intochondrial	

Fast Minimum Evolution tree for ID-9R:

Rhagoletis indifferens isolate WA. A 1 cytochrome c oxid Rhagoletis electromorpha isolate MT_42 cytochrome c oxidas estimation of the solate MT_42 cytochrome c oxidas Rhagoletis berberis isolate WA. A 2 cytochrome c oxidas Rhagoletis berberis isolate WA. A 2 cytochrome c oxidas estimation of the solate MT_42 cytochrome c oxidas estimation of the solate MT_42 cytochrome c oxidas rhagoletis juniperina isolate CA 1 cytochrome c oxidas estimation of the solate cytochrome coxidas estimation of the solate cytochrome coxidas estimation of the solate cytochrome oxidas estimation estimation of the solate solate the solate cytochrome oxidas estimation estimation of the solate solate the solate the solate solate solate estimation of the solate solate the solate solate solate solate estimation the solate solate solate estimation	Anse subuni 1 (CO) gene, partial cis; mischondrial skikas subuni 1 (CO) gene, partial cis; mischondrial skikas subuni 1 (CO) gene, partial cis; mischondrial se subuni 1 (CO) gene, partial cis; mischondrial date subuni 1 (CO) gene, partial cis; mischondrial mine oxidase subuni 1 (CO) gene, partial cis; mischondrial mine cis; mischondrial mine cis; mischondrial mine cis; mischondrial mine cis; mischondrial if gene, partial cis; mischondrial if gene,

Fast Minimum Evolution tree for ID-1F:

Affies | 15 leaves
Rhagoletis zephyria haplotype 17 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, c.,
Rhagoletis zephyria haplotype 16 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, c.,
Rhagoletis zephyria haplotype 15 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, c.,
Rhagoletis zephyria haplotype 13 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, c.,
Rhagoletis zephyria haplotype 13 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, c.,
Rhagoletis mendax haplotype 10 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co.
Rhagoletis mendax haplotype 9 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co.
Rhagoletis pomonella haplotype 7 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co.
Rhagoletis mendax haplotype 7 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co.
Rhagoletis pomonella haplotype 5 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co.
Rhagoletis pomonella haplotype 5 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co.
Rhagoletis pomonella haplotype 4 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co..
Rhagoletis zephyria haplotype 3 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co..
Rhagoletis zephyria haplotype 4 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co..
Rhagoletis zephyria haplotype 3 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co..
Rhagoletis pomonella isolate USA 01 mitochondrion, complete genome
Rhagoletis pomonella isolate B08 A 013 cytocrhome oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gen.
Rhagoletis pomonella isolate G09 MF 013 cytocrhome ox flies | 15 leaves Rhagoletis zephyria mitochondrion, complete genome Rhagoletis mendax mitochondrion, complete genome Rhagoletis pomonella mitochondrion, complete genome Rhagoletis pomonella mitochondrion, complete genome Prinagoletis pomonella mitochondrion, complete genome
 Prinagoletis pomonella mitochondrion, complete genome
 Prinagoletis pomonella mitochondrion, complete genome
 Prinagoletis pomonella initochondrion, complete genome
 Prinagoletis pomonella isolate Rp II-TX HMG cytochrome oxidase subunit II (COII) gene, partial cds, mitochondrial
 Prinagoletis pomonella isolate Rp II-TX HMG cytochrome oxidase subunit II (COII) gene, partial cds, mitochondrial
 Prinagoletis pomonella isolate Rp II-TX4 cytochrome oxidase subunit II (COI) gene, partial cds, mitochondrial
 Prinagoletis pomonella isolate Rp II-TX4 cytochrome oxidase subunit II (COI) gene, partial cds, mitochondrial
 Prinagoletis pomonella isolate Rp II-TX4 cytochrome oxidase subunit II (COI) gene, partial cds, mitochondrial
 Prinagoletis pomonella isolate RSSPA20 cytochrome oxidase subunit II (COI) gene, partial cds, mitochondrial
 Prinagoletis pomonella voucher RSU303212 cytochrome oxidase subunit II (COI) gene, partial cds, mitochondrial
 Prinagoletis pomonella voucher RSU303212 cytochrome oxidase subunit II (COI) gene, partial cds, mitochondrial
 Prinagoletis pomonella voucher RSU303212 cytochrome oxidase subunit II (COI) gene, partial cds, mitochondrial
 Prinagoletis pomonella voucher RSU303212 cytochrome oxidase subunit II (COI) gene, partial cds, mitochondrial
 Prinagoletis mendax isolate NSSPA20 cytochrome oxidase subunit II (COI) gene, partial cds, mitochondrial
 Prinagoletis mendax isolate RSI300 cytochrome oxidase subunit II (COI) gene, partial cds, mitochondrial
 Prinagoletis mendax isolate PA1300 cytochrome oxidase subunit II (COI) gene, partial cds, Rhagoletis pomonella mitochondrion, complete genome Rhagoletis pomonella mitochondrion, complete genome Icl|Query_346781

Page 13/27

Fast Minimum Evolution tree for ID-1R:

a flies 15 leaves
Rhagoletis zephyria haplotype 15 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, c
Rhagoletis zephyria haplotype 14 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, c
Rhagoletis zepityria haplotype 15 cytochrome c oxidase subunit 1 (COX1) gene, partial cus; tKNA-Leu gene, c
Rhapoletis mendax hanfotype 12 cytochrome c oxidase subunit 1 (COX1) gene, partial cds: tRVA-Leu gene, ca.
Rhagoletis mendax haplotype 10 cytochrome c oxidase subunit 1 (COX1) gene, partial cds: tRNA-Leu gene, co.
Rhagoletis mendax haplotype 9 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co
Phagoletis pomonella haplotype 8 cytochrome c oxidase subunit 1 (COXI) gene, partial cds; tRNA-Leu gene, c.
PRhagoletis zephyria haplotype 7 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co
PRhagoletis mendax haplotype 6 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co
Rhagoletis pomonella haplotype 5 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, c.
Rhagoletis zephyria haplotype 4 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; tRNA-Leu gene, co
Rhagoletis zepityria haplotype 5 cytochrome c oxidase subunit 1 (COA1) gene, partial cds, tRVA-Leu gene, co
Rhagoletis zepityia haplotype 2 cytochrome c oxidase subunit 1 (COX1) gene, partial cds: tRNA-Leu gene, co.
Rhagoletis pomonella cytochrome oxidase subunit II (COII) gene, partial cds: mitochondrial
Rhagoletis pomonella isolate USA 01 mitochondrion, complete genome
Rhagoletis pomonella isolate B08_A_013 cytocrhome oxidase subunit I (COI) gene, partial cds; tRNA-Leu gen.
Rhagoletis pomonella isolate G09 MF_013 cytocrhome oxidase subunit I (COI) gene, partial cds; tRNA-Leu g
Rhagoletis pomonella isolate G08 BB 017 cytocrhome oxidase subunit I (COI) gene, partial cds; IRNA-Leu ge.
Rhagoletis pomonella isolate M06 1 012 cytocrhome oxidase subunit 1 (COI) gene, partial cds; tRNA-Leu gen.
Rhagoletis pomonella isolate B07 SC 009 cylocrhome oxidase subunit I (COI) gene, partial cds; IRNA-Leu ge.
Phagoletis pomonena isorate bus A 022 cylocrnome oxidase subunit i (COI) gene, paruai cus; tRIVA-Leu gen.
Rhagoletis zentyria mitochondrion, complete genome
Rhagoletis zephyria mitochondrion, complete genome
Rhagoletis zephyria mitochondrion, complete genome
PRhagoletis zephyria mitochondrion, complete genome
PRhagoletis zephyria mitochondrion, complete genome
Rhagoletis mendax mitochondrion, complete genome
Knagoletis pomonella mitochondrion, complete genome
Rhagoletis pomonella mitochondrion, complete genome
Rhagoletis pomonella mitochondrion, complete genome
Rhagoletis ponionenia mitochondrion, complete genome
= Rhagoletis pomonella isolate Rn H NY3 cytochrome oxidase subunit II (COII) sene, partial cds: mitochondrial
Rhagoletis pomonella isolate Rp H TX HNG cvtochrome oxidase subunit II (COII) eene, partial cds: mitocho.
Rhagoletis pomonella isolate Rp-H-NY5 cvtochrome oxidase subunit II (COII) gene, partial cds; mitochondrial
Rhagoletis pomonella isolate Rp-H-NY2 cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial
Rhagoletis pomonella isolate Rp_H_NY4 cytochrome oxidase subunit II (COII) gene, partial cds, mitochondrial
Rhagoletis aff. pomonella 'flowering dogwood fly' cytochrome oxidase subunit I (COI) gene, partial cds; tRNA
Rhagoletis aff. pomonella 'flowering dogwood fly' haplotype 2 cytochrome oxidase subunit I (COI) gene, partia.
Rhagoletis pomonella cytochrome oxidase subunit I (COI) gene, partial cds; tRNA-Leu gene, complete sequenc.
Rhagoletis pomonella voucher Rp1 cytochrome c oxidase subunit 2 gene, partial cds; mitochondrial Bhagoletis nomenella voucher Rp102022012 artichrome oxidase subunit 1 (COID, aena, nortical eds) mitochon
Rhagoletis pomonella vouchel PSO 95052912 cytochnome oxidase subunit in (COI) gene, partai dis, intodutori, Rhagoletis pomonella isolate NSSPA20 cytochnome oxidase subunit in (COI) gene, partai dis, intodutori,
Rhagoletis mendax isolate NSMP13 cytochrome oxidase subunit II (COII) gene, partial cds: mitochondrial
Rhagoletis mendax isolate NSSML5 cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial
Rhagoletis mendax isolate NSSMA14 cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial
Rhagoletis zephyria isolate PA1300 cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial
Rhagoletis zephyria isolate PA2900 cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial
Rhagoletis mendax isolate PA1 cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial
Rhagoletis mendax isolate PA1300 cytochrome oxidase subunit II (COII) gene, partial cos; mitochondrial
Rhagoletis mendax isolate ML ortgebrome evidese subunit il (COII) gene, partial cus; mitochondrial
Rhagoletis mendax isolate will cytochrone oxidase submit il (COI) gene, partal cus, initochronean al Bhagoletis aff pomonella 'flowering dogwood fly' isolate GA cytochrome oxidase submit II (COII) gene, parti
Rhagoletis pomonella isolate NY18013 cytochrome oxidase subunit II (COII) gene partial cds: mitochondrial
Rhagoletis pomonella isolate TX2100 cvtochrome oxidase subunit II (COII) gene, partial cds: mitochondrial
Rhagoletis pomonella isolate MIA4011 cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial
Rhagoletis pomonella isolate NY1 cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial
Rhagoletis pomonella isolate MC6030 cytochrome oxidase subunit II (COII) gene, partial cds; mitochondrial
Rhagoletis pomonella isolate CP2 cytochrome oxidase subunit II gene, partial cds; mitochondrial
Phagotetis pomotetia isotate CP5 cytochrome oxidase subunit II gene, partial cds; mitochondrial Phagotetis pomotelia isotate SU autochrome oxidase subunit II gene, partial cds; mitochondrial
Rhagowis pointeria isotate Si cytochrone oxidate submit il gene, partal cus, miochondrial
Rhagoletis pomorella isolate SI3 cytochrome oxidase submit II gene, partial cds, micochondrial
Rhagoletis aff, pomonella JLF-2002 isolate Cfloridaa 8 cvtochrome oxidase subunit 1 gene, partial cds: tRNA-
Rhagoletis pomonella isolate PomTXH2 3 cytochrome oxidase subunit I gene, partial cds; tRNA-Leu gene, co
PRhagoletis pomonella isolate CB1 cytochrome oxidase subunit II gene, partial cds; mitochondrial
Rhagoletis mendax cytochrome oxidase subunit I gene, partial cds; tRNA-Leu gene, complete sequence; and cy.
Rhagoietts nr. mendax JJS-1996 cytochrome oxidase subunit I (COI) gene, partial cds; IRNA-Leu (trnL) gene, Blaedelsts neuroalle isoleto Ma vilia de utechnose autidate in the state of the stateo
Knagoretis pomonelia isolate MCXTI12 + Cytochrome oxidase subunit I gene, partial cds; IKNA-Leu gene, conti Bhagolatis pomonalla isolate MCS outpolynome oxidase subunit I and outpolynome oxidase activity in a subunit II and outpolynome oxidase subunit I gene, partial cds; IKNA-Leu gene, conti Representation of the subunit I and outpolynome oxidase subunit I gene, partial cds; IKNA-Leu gene, conti I and outpolynome oxidate and the subunit I and outpolynome oxidase subunit I gene, partial cds; IKNA-Leu gene, continue oxidase subunit I gene, partial cds; IKNA-Leu gene, continue oxidase subunit I and outpolynome oxidase subunit I gene, partial cds; IKNA-Leu gene, continue oxi
Reasonable is nonnella isolate MCJ cytochrome ovidase subunit i and cytochrome ovidase subunit il genes, parti
Rhagoletis pomonella isolate MC2 cytochrome oxidase subunit I and cytochrome oxidase subunit II genes, parti
Rhagoletis mendax isolate Rmendax5 6 cytochrome oxidase subunit I gene, partial cds: IRNA-Leu gene, compl.
Rhagoletis zephyria cytochrome oxidase subunit I (COI) gene, partial cds; tRNA-Leu (trnL) gene, complete sea.
PRhagoletis pomonella isolate PomMexHiH5_1 cytochrome oxidase subunit I gene, partial cds; tRNA-Leu gene
Rhagoletis pomonella isolate PomMexHiHI4 8 cytochrome oxidase subunit I gene, partial cds; tRNA-Leu gen
Rhagoletis pomonella isolate PomMexHiH2_3 cytochrome oxidase subunit I gene, partial cds; tRNA-Leu gene,
Rhagoletis pomonella isolate MexHII cytochrome oxidase subunit I gene, partial cds; tRNA-Leu gene, complet.
Rhagoletis pomonella isolate PomMIA45ma 4 cylochrome oxidase subunit I gene, partial cds; IRNA-Leu gene,
Tragoretis pomoretia isolate Pomirivi ri19 cytochrome oxidase subunit i gene, partial cds; tKNA-Leu gene, co.
Rhagoletis pomorella cytochrome oxidase subunit II gene, partial ces, intVA-Leu gene, compete semicore and
♦ Icl[Query 349771]

Fast Minimum Evolution tree for ID-3R:

flies | 12 leaves

In silico PCR for primers ID-8F/ID-9R:

>MH998965.1 Rhagoletis zephyria isolate MN_A4 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial

product length Forward primer Template	= 206 1 381	TGGAGGGGCATCTGTTGA	18 398	
Reverse primer Template	1 586	TAATGGCTCCTGCTAATACT	IGGT	23 564

>MH998964.1 Rhagoletis pomonella isolate MN_19 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial

```
product length = 206
Forward primer 1 TGGAGGGGCATCTGTTGA 18
Template 378 ..... 395
Reverse primer 1 TAATGGCTCCTGCTAATACTGGT 23
Template 583 ..... 561
```

>MH998963.1 Rhagoletis pomonella isolate MI_A3 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial

product length	= 206			
Forward primer	1	TGGAGGGGCATCTGTTGA 18	8	
Template	378		95	
Reverse primer	1	TAATGGCTCCTGCTAATACTG	GT	23
Template	583		• •	561

>MH998960.1 Rhagoletis mendax isolate 090517_3 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial

```
product length = 206
Forward primer 1 TGGAGGGGCATCTGTTGA 18
Template 375 ..... 392
Reverse primer 1 TAATGGCTCCTGCTAATACTGGT 23
Template 580 ..... 558
```

>JF873670.1 Clusiodes melanostoma voucher BIOUG:10JSROW-1054 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial

product length	= 206			
Forward primer	1	TGGAGGGGCATCTGTTGA	18	
Template	367	•••••	384	
Reverse primer	1	TAATGGCTCCTGCTAATAC	TGGT	23
Template	572			550

>KP978468.1 Teleopsis pallifacies voucher RMNH.INS.555138 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial

product length Forward primer Template	= 206 1 367	TGGAGGGGCATCTGTTGA	18 384	
Reverse primer Template	1 572	TAATGGCTCCTGCTAATACT	IGGT	23 550

>KR395792.1 Clusiodes orbitalis voucher BIOUG12107-B07 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial

>KM939719.1 Lonchaeidae sp. BOLD:AAP8893 voucher BIOUG05093-F07 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial

product length Forward primer Template	= 206 1 367	TGGAGGGGCATCTGTTGA	18 384	
Reverse primer Template	1 572	TAATGGCTCCTGCTAATACT	GGT	23 550

>KM929290.1 Clusiodes clandestinus voucher BIOUG05088-H06 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial

product length Forward primer Template	= 206 1 366	TGGAGGGGCATCTGTTGA	18 383	
Reverse primer Template	1 571	TAATGGCTCCTGCTAATACT	IGGT	23 549

>MN443940.1 Rhagoletis zephyria mitochondrion, complete genome

In silico PCR ID-1F/ID-1R:

In silico PCR ID-1F/ID-3R:

Reverse primer 1 GATTTAGACGACCAGGAGTTC 21 Template 3587 3567

>MN443940.1 Rhago	oletis zej	ohyria mitochondrion, complete genome	>MN443940.1 Rhagoletis zephyria mitochondrion, complete genome
product length Forward primer Template	= 152 1 3463	GACGGATTTCGATTATTAGATGT 23 	product length = 151 Forward primer 1 GACGGATTTCGATTATTAGATGT 23 Template 3463
Reverse primer Template	1 3614	TGATTTAGACGACCAGGAGTT 21 	Reverse primer 1 GATTTAGACGACCAGGAGTTC 21 Template 3613 3593
>MN443939.1 Rhago	oletis me	endax mitochondrion, complete genome	>MN443939.1 Rhagoletis mendax mitochondrion, complete genome
product length Forward primer Template	= 152 1 3463	GACGGATTTCGATTATTAGATGT 23 	product length = 151 Forward primer 1 GACGGATTTCGATTATTAGATGT 23 Template 3463
Reverse primer Template	1 3614	TGATTTAGACGACCAGGAGTT 21 	Reverse primer 1 GATTTAGACGACCAGGAGTTC 21 Template 3613 3593
>MN443938.1 Rhago	oletis po	monella mitochondrion, complete genome	>MN443938.1 Rhagoletis pomonella mitochondrion, complete genome
product length Forward primer Template	= 152 1 3463	GACGGATTTCGATTATTAGATGT 23 	product length = 151 Forward primer 1 GACGGATTTCGATTATTAGATGT 23 Template 3463
Reverse primer Template	1 3614	TGATTTAGACGACCAGGAGTT 21 	Reverse primer 1 GATTTAGACGACCAGGAGTTC 21 Template 3613 3593
			>MN443937.1 Rhagoletis pomonella mitochondrion, complete genome

In silico data PCR assay 5-7

Velez, S., Taylor, M. S., Noor, M. A. F., Lobo, N. F., & Feder, J. L. (2006). Isolation and characterization of microsatellite loci from the apple maggot fly Rhagoletis pomonella (Diptera: Tephritidae). Molecular Ecology Notes, 6(1), 90-92.

Search was restricted to Rhagoletis

Fast Minimum Evolution tree for P6_F

PREDICTED: Rhagoletis pomonella uncharacterized LOC118733109 (LOC118733109), mRNA
 PREDICTED: Rhagoletis zephyria protein msa (LOC108354983), transcript variant X1, mRNA PREDICTED: Rhagoletis pomorella SET domain-containing protein SmydA-8 (LOC118741326), transcript variant X1, mRNA PREDICTED: Rhagoletis zephyria ejaculatory bulb-specific protein 3 (LOC108371789), transcript variant X1, mRNA PREDICTED: Rhagoletis zephyria ejaculatory bulb-specific protein 3 (LOC108371789), transcript variant X1, mRNA PREDICTED: Rhagoletis zephyria ejaculatory bulb-specific protein 3 (LOC108371789), transcript variant X1, mRNA PREDICTED: Rhagoletis zephyria 405 rhosomal protein S54 (LOC108371789), transcript variant X3, mRNA PREDICTED: Rhagoletis pomorella ejaculatory bulb-specific protein 3-like (LOC118742245), transcript variant X3, mRNA PREDICTED: Rhagoletis pomorella ejaculatory bulb-specific protein 3-like (LOC118742245), transcript variant X2, mRNA PREDICTED: Rhagoletis pomorella ejaculatory bulb-specific protein 3-like (LOC118742245), transcript variant X1, mRNA PREDICTED: Rhagoletis pomorella ejaculatory bulb-specific protein 3-like (LOC118742245), transcript variant X1, mRNA PREDICTED: Rhagoletis pomorella ejaculatory bulb-specific protein 3-like (LOC118742245), transcript variant X1, mRNA PREDICTED: Rhagoletis zephyria zinc finger protein 675-like (LOC108379680), transcript variant X4, mRNA PREDICTED: Rhagoletis zephyria zinc finger protein 675-like (LOC118739680), transcript variant X2, mRNA PREDICTED: Rhagoletis zephyria zinc finger protein 675-like (LOC118739680), transcript variant X1, mRNA PREDICTED: Rhagoletis pomorella zinc finger protein 675-like (LOC118739880), transcript variant X1, mRNA PREDICTED: Rhagoletis pomorella zinc finger protein 675-like (LOC118739880), transcript variant X1, mRNA PREDICTED: Rhagoletis pomorella zinc finger protein 675-like (LOC118739880), transcr
e lies 2 leaves
ΦPREDICTED: Rhagoletis zephyria mas-related protein Rap-2a (LOC108374750), partial mRNA ΦPREDICTED: Rhagoletis zephyria peptide transporter family 1-like (LOC108356063), mRNA ΦPREDICTED: Rhagoletis zephyria protein vellow (LOC108350665), mRNA ΦPREDICTED: Rhagoletis zephyria motein vellow (LOC108350665), mRNA ΦPREDICTED: Rhagoletis zephyria multidrug resistance-associated protein 4-1ike (LOC108381523), partial mRNA ΦPREDICTED: Rhagoletis zephyria zutein finger protein 476-like (LOC108381472), mRNA ΦPREDICTED: Rhagoletis zephyria zutei finger and SCAN domain-containing protein 22-like (LOC108381471), partial mRNA ΦPREDICTED: Rhagoletis zephyria zutei finger and SCAN domain-containing protein 22-like (LOC108381471), partial mRNA
PREDICTED: Rhagoletiz zeptyra large neutral amino acids transporter small subunit 2-like (LOC108380983), partial mRNA
PREDICTED: Rhagoletis zephyria zinc finger protein 215-like (LOC108380911), mRNA fire 12 leaves
PPREDICTED: Rhagoletis zephyria inositol hexakisphosphate and diphosphoinositol-pentakisphosphate kinase-like (LOC10836511 PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X3, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X2, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X2, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide transporter family 1 (LOC108363674), transcript variant X1, mRNA PPREDICTED: Rhagoletis zephyria peptide tra
 PREDICTED: Rhagoletis zephyria myeloid zinc finger 1 (LOC108379117), transcript variant X2, mRNA PREDICTED: Rhagoletis zephyria myeloid zinc finger 1 (LOC108379117), transcript variant X1, mRNA PREDICTED: Rhagoletis pomonella mitogen-activated protein kinase kinase (kinase (kinase (kinase (kinase kinase (LOC118754421), transcript variant X2, mRNA PREDICTED: Rhagoletis pomonella myeloid zinc finger 1 (LOC118379374), transcript variant X2, mRNA
 PREDICTED: Rhagoletis pomonella myeloid zinc finger 1 (LOC118739374), transcript variant X1, mRNA PREDICTED: Rhagoletis pomonella trypsin-1-like (LOC118754297), transcript variant X1, mRNA PREDICTED: Rhagoletis pomonella serine-rich adhesin for platelets (LOC118748764), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella serine-rich adhesin for platelets (LOC118748764), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella serine-rich adhesin for platelets (LOC118748764), transcript variant X4, mRNA
PPEDICTED: Rhagoletis pomonella serine-rich adhesin for platelets (LOC118748764), transcript variant X2, mRNA PPEDICTED: Rhagoletis pomonella serine-rich adhesin for platelets (LOC118748764), transcript variant X1, mRNA PPEDICTED: Rhagoletis pomonella clathrin heavy chain (LOC118748697), transcript variant X1, mRNA PPEDICTED: Rhagoletis pomonella clathrin heavy chain (LOC118748697), transcript variant X7, mRNA PPEDICTED: Rhagoletis pomonella clathrin heavy chain (LOC118748697), transcript variant X7, mRNA

This is a large set of the set of
• PREDUCTED: Rhagoletis pomonella maternal protein tudor (LOC118743100), transcript variant X2, mRNA • PREDUCTED: Rhagoletis pomonella maternal protein tudor (LOC118743100), transcript variant X4, mRNA • PREDUCTED: Rhagoletis pomonella activated Cde42 kinase-like (LOC118742986), transcript variant X4, mRNA • PREDUCTED: Rhagoletis pomonella activated Cde42 kinase-like (LOC118742986), transcript variant X4, mRNA • PREDUCTED: Rhagoletis pomonella activated Cde42 kinase-like (LOC118742986), transcript variant X4, mRNA • PREDUCTED: Rhagoletis pomonella activated Cde42 kinase-like (LOC118742986), transcript variant X4, mRNA • PREDUCTED: Rhagoletis pomonella activated Cde42 kinase-like (LOC118742986), transcript variant X4, mRNA • PREDUCTED: Rhagoletis pomonella activated Cde42 kinase-like (LOC118742986), transcript variant X4, mRNA • PREDUCTED: Rhagoletis pomonella activated Cde42 kinase-like (LOC118742986), transcript variant X4, mRNA • PREDUCTED: Rhagoletis pomonella activated Cde42 kinase-like (LOC118742986), transcript variant X4, mRNA • PREDUCTED: Rhagoletis pomonella activater (LOC118742986), transcript variant X4, mRNA PREDUCTED: Rhagoletis pomonella activater (LOC1187418741876), transc
PREDICTED: Rhagoletis pomonella activated Cdc42 kinase-like (LOC118742986), transcript variant X2, mRNA PREDICTED: Rhagoletis pomonella activated Cdc42 kinase-like (LOC118742986), transcript variant X2, mRNA PPREDICTED: Rhagoletis pomonella nuclear pore complex protein DDB G0274915 (LOC118742307), transcript variant X2, mRNA PREDICTED: Rhagoletis pomonella nuclear pore complex protein DDB G0274915 (LOC118742307), transcript variant X1, mRNA PREDICTED: Rhagoletis pomonella nuclear pore complex protein DDB G0274915 (LOC118742307), transcript variant X1, mRNA PREDICTED: Rhagoletis pomonella trypsin-l-like (LOC118741264), transcript variant X3, mRNA PREDICTED: Rhagoletis pomonella united and trypsin-l-like (LOC118742307), transcript variant X3, mRNA
PREDICTED: Rhagoletis pointone ita Ministri-Interprotein (Np204 LIOC118736962), transcript variant X2, mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC118736962, transcript variant X1, mRNA PREDICTED: Rhagoletis pomonella 3-ketoacyl-CoA thiolase, mitochondrial-like (LOC118736961), partial mRNA PREDICTED: Rhagoletis pomonella 3-ketoacyl-CoA thiolase, mitochondrial-like (LOC118736964), mRNA Ties [2] lawse
PREDICTED: Rhagoletis pomonella DDB1- and CUL4-associated factor 8 (LOC118747816), mRNA FIGS [2] leaves PleQuery 84263 Figs [5] leaves
PREDICTED: Rhagoletis pomonella molybdenum cofactor sulfurase 3-like (LOC118753631), partial mRNA PREDICTED: Rhagoletis pomonella ucharacterized LOC118752544), mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC118748079, mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC118748079, mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC11874821), transcript variant X2, mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC11874821), transcript variant X2, mRNA
* FRED/CTED, Riagoreus pontonena protein meneo (EOC110/47021), transcript Variant A1, mRIVA



In silico PCR P6 F/R

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGTCAGAGTGCGGCAAAAGT	20	60.18	50.00	5.00	1.00
Reverse primer	CGGTAGACCTCAGGCTGATAG	21	59.12	57.14	7.00	3.00
Products on target	emplates					
>AY734890.1 Rhage	etis pomonella clone 6 microsatellite seq	uence				
product length	= 162					
product length Forward primer	= 162 1 AGTCAGAGTGCGGCAAAAGT 20					
product length Forward primer Template	= 162 1 AGTCAGAGTGCGGCAAAAGT 20 119138	8				
product length Forward primer Template	= 162 1 AGTCAGAGTGCGGCAAAAGT 20 119 138	8				
product length Forward primer Template Reverse primer	= 162 1 AGTCAGAGTGCGGCAAAAGT 20 119 134 1 CGGTAGACCTCAGGCTGATAG 2:	8				

Fast Minimum Evolution tree for P9_F



PREDICTED: Rhagoletis pomor	hella F-actin-uncapping protein LRRC16A (LOC118742510), transcript variant X2, mRNA hella F-actin-uncapping protein LRRC16A (LOC118742510), transcript variant X1, mRNA
Rhapoletis ponnella clone 9 mi Rhapoletis ponnella clone 9 mi	crosstellite sequence
PREDICTED: Rhagoleti	s pomonella uncharacterized LOC118743497 (LOC118743497), transcript variant X2, ncRNA
PREDICTED: Rhago PREDICTED: Rhago	letis pomonella uncharacterized LOC118743497 (LOC118743497), transcript variant X1, ncRNA
	igoletis zephyria DNA topoisomerase 2-like (LOC10855/31/), partial mKNA
PREDICTED: Rha	goletis zephyria putative nuclease HARBII (LOC108370409), transcript variant XI, mRNA
PREDICTED: Rha	goletis zephyria uncharacterized LOC108359014 (LOC108359014), transcript variant X3, mRNA
PREDICTED: Rh	goletis zephyria uncharacterized LOC108359014 (LOC108359014), transcript variant X2, mRNA
PREDICTED: Rha	igoletis zephyria uncharacterized LOC108559014 (LOC108559014), transcript variant X1, mKNA
PREDICTED: Rha	goletis zepinyia uncharacterized LOCI18749064 (LOCI18749064), ncRNA
PREDICTED: Rha	goletis pomonella uncharacterized LOC118745636 (LOC118745636), mRNA
PREDICTED: Rh PREDICTE	goletis pomonella transcriptional regulator ovo (LOC118747445), transcript variant X2, mRNA
	goieus pomonena transcriptional regulator ovo (LOC118/4/445), transcripti variant X1, mKNA
a lites 15 leaves	
PREDICTED: Rha	igoletis zephyria very long-chain specific acyl-CoA denydrogenase, mitochondrial-like (LOC108554045), partial m poletis pomonella uncharzoterizzed LOC118746417 (LOC118746417), nePNA
PREDICTED: Rha	goletis pomonella caldesmon-like (LOC118739453), transcript variant X10, mRNA
SPREDICTED: Rha	goletis pomonella chromatin modification-related protein eaf-1 (LOC118748081), mRNA
PREDICTED: Rha	goletis zephyria probable cation-transporting ATPase 13A3 (LOC108362043), transcript variant X3, mRNA
REDICTED: Ria	goletis zephyria direnaria terizeti Eoc 106539872 (Eoc 106539872), mrtva peoletis zephyria chromatin modification-related protein eaf-1-like (LoC108374926), mRNA
PREDICTED: Rha	goletis pomonella uncharacterized LOC118755272 (LOC118755272), mRNA
PREDICTED: Rh PREDICTE	igoletis pomonella uncharacterized LOC118742711 (LOC118742711), transcript variant X1, mRNA
PREDICTED: Rha	igoletis pomonella uncharacterized LOC118/42/11 (LOC118/42/11), transcript variant X2, misc. KNA
PREDICTED: Rha	goletis pomonella caldesmon-like (LOC116739453), transcript variant XS, mRNA
PREDICTED: Rha	goletis pomonella caldesmon-like (LOC118739453), transcript variant X7, mRNA
PREDICTED: Rh	goletis pomonella caldesmon-like (LOC118739453), transcript variant X6, mRNA
PREDICTED: Rha	igoretis pomonella caldesmon-like (LOC118739453), transcript variant X3, mKNA
	goletis pomonella caldesmon-like (LOC118739453), transcript variant X3, mRNA
PREDICTED: Rha	goletis pomonella caldesmon-like (LOC118739453), transcript variant X2, mRNA
PREDICTED: Rha	igoletis pomonella caldesmon-like (LOC118/39455), transcript variant X1, mKNA volatis pomonella unchara citarizza 10 CC118/324540, de NNA
PREDICTED: Rha	goletis pomonella uncharacterized LOC11873474 (LOC118747344), mRNA
PREDICTED: Rha	goletis zephyria probable cation-transporting ATPase 13A3 (LOC108362043), transcript variant X1, mRNA
PREDICTED: Rha DEEDICTED: Rha	goletis zephyria probable peroxisomal acyl-coenzyme A oxidase 1 (LOC108356675), partial mRNA
PREDICTED. Rha	goletis zephyria protassium voltage-gated channel protein Shaw-like (LOC108354797), partial mRNA
PREDICTED: Rha	goletis zephyria uncharacterized LOC108370978 (LOC108370978), partial mRNA
PREDICTED: Rh	goletis zephyria uncharacterized LOC108370351 (LOC108370351), mRNA
PREDICTED: Rha	goletis zephylia znie carboxypephilase-nike (LOC 1005005)-4), intrvA jeoletis pomonella probable cation-transporting ATPase 13A3 (LOC 118745766), transcript variant X4, mRNA
PREDICTED: Rha	goletis pomonella probable cation-transporting ATPase 13A3 (LOC118745766), transcript variant X3, mRNA
PREDICTED: Rh	igoletis pomonella probable cation-transporting ATPase 13A3 (LOC118745766), transcript variant X2, mRNA
APREDICTED: Rha	goletis pendera provale carbon antiporting AFrase 15A5 (COC116745)(00), transcript variant X2, mRNA
PREDICTED: Rha	goletis zephyria probable cation-transporting ATPase 13A3 (LOC108362043), transcript variant X2, mRNA
PREDICTED: Rh	goletis zephyria uncharacterized LOC108380863 (LOC108380863), transcript variant X1, mRNA
PREDICTED: Ria PREDICTED: Ria	igoletis zephyria putative protein TPKAL (LOC1083/9630), partiai mKNA pooletis zephyria iranscription factor SPT20 homolog (LOC108378631), mRNA
PREDICTED: Rha	goletis zephyria uncharacterized LOC108360163 (LOC108360163), mRNA
PREDICTED: Rha PREDICTED: Rha	goletis zephyria solute carrier family 12 member 9 (LOC108358898), transcript variant X2, mRNA
PREDICTED: Ria	igoletis zepnyria duai specificity tyrosine-pnospnorylation-regulated kinase 2-like (LOC108558554), mkNA woletis pomonella uncharacterized LOC118757071 (LOC118757071), transcript variant X2 mRNA
PREDICTED: Rha	goletis pomonella LMBR1 domain-containing protein 2 homolog (LOC118752788), partial mRNA
PREDICTED: Rha	goletis pomonella uncharacterized LOC118757071 (LOC118757071), transcript variant X1, mRNA
PREDICTED: Rha	igoletis pomonella uncharacterized LOC118/56499 (LOC118/56499), mRNA volatis pomonella uncharacterized LOC118/56499 (LOC118/56499), transcript variant X2, pPNA
PREDICTED: Rha	goletis pomonella nutative sodium-coupled neutral amino acid transporter 10 (LOC118743446), transcript variant
PREDICTED: Rha	goletis pomonella uncharacterized LOC118757069 (LOC118757069), transcript variant X1, mRNA
PREDICTED: Rha	goletis pomonella IDLSRF-like peptide (LOC118744113), transcript variant X1, mRNA
PREDICTED: Rha	igoletis pomonella nuclear exosome regulator NRDE2-like (LOC118/32200), mRNA
PREDICTED: Rha	goletis pomonella uncharacterized LOC118751046 (LOC118751046), partial mRNA
PREDICTED: Rha	goletis pomonella probable peroxisomal acyl-coenzyme A oxidase 1 (LOC118743224), transcript variant X2, mRN.
	noletis nomonella prohable perovisomal acvi-coenzyme & ovidase 1 (LOC118743224), transcrint variant X1, mRN
PREDICTED: Rha	goletis pomonella protein bunched, class 2/F/G isoform-like (LOC118741876), mRNA
SPREDICTED: Rha	goletis pomonella uncharacterized LOC118741531 (LOC118741531), mRNA
PREDICTED: Rha	igoletis zephyria procioting enzyme-like (LOC108355730), partial mKNA isoletis zephyria peritrophin-48-like (LOC108365741), mRNA
PREDICTED: Rha	igoletis pomonella modular serine protease-like (LOC118754313), mRNA
	goletis pomonella modular serine protease-like (LOC118753781), transcript variant X2, mRNA
PREDICTED: Rha	agoletis pomonella modular serine protease-like (LOC118753781), transcript variant X1, mRNA
PREDICTED: Rha	igoreus pomonena unenaracierized LOC118/50289 (LOC118/50289), partial mKNA igoreus pomonella uneharacterized LOC118756309 (LOC118756309), mRNA
PREDICTED: Rha	igoletis zephyria LMBR1 domain-containing protein 2 homolog (LOC108358730), transcript variant X2. mRNA
PREDICTED: Rha	igoletis pomonella uncharacterized LOC118755133 (LOC118755133), mRNA
PREDICTED: Rha	igoletis zephyria uncharacterized LOC108373356 (LOC108373356), mRNA
PREDICTED: Rha	goletis pomonella LMBR1 domain-containing protein 2 homolog (LOC108558750), transcript variant X1, mKNA
PREDICTED: Rha	igoletis pomonella putative sodium-coupled neutral amino acid transporter 10 (LOC118743446), transcript variant
PREDICTED: Rha	goletis pomonella IDLSRF-like peptide (LOC118744113), transcript variant X2, mRNA

In silico PCR P9_F/R

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CGGCAGGTAAATGACCAAAA	20	56.61	45.00	5.00	0.00
Reverse primer	GCAATGACCGTTGGCTATTA	20	56.20	45.00	4.00	3.00

Products on target templates

>AY734893.1 Rhagoletis pomonella clone 9 microsatellite sequence

	PREDICTED: Rhagoletis pomonella asparagine synthetase [glutamine-hydrolyzing] 2-like (LOC118747703), mRNA
	PREDICTED: Rhagoletis pomonella asparagine synthetase [glutamine-hydrolyzing] 2-like (LOC118747702), mRNA
Ť	PREDICTED: Rhagoletis pomonella uncharacterized LOC118/43035 (LOC118/43035), transcript variant X1, mRNA
	- PREDICTED: Rhagoletis pomonella pared box protein Avio (LOC118/45021), transcript variant X1, mRNA
	PREDICTED: Rhagoletis pomonella protein cheress (LOC118742230), mRNA
	PREDICTED: Rhagoletis pomonella trichalose phoenbate phoenbatea B (I OC118738700), maiscript variant A+, mixtvA
	PREDICTED: Rhapoletis pomonella unabascentizzed LOCIX42556 (LOCIX8742556), transcript variant X1 mRNA
	Filies 3 leaves
	DEDICTEDI Diagolatis nomonalla contaurin-commo-1A (LOC119735543), transcrint variant V2, mDNA
	Relación de la vera
	Ines 0 leaves
	PREDICTED: Rhagoletis pomonella paired box profein Pax-6 (LOCI18/45621), transcript variant X2, mRNA
	PREDICTED: Rhagoleus pomonella uncharacterized LOC118/42556 (LOC118/42556), transcript variant A2, mRINA
	PREDICTED: Rhagoleus pomorena centaurin-gamma-rA (LOC116/353-3), transcript variant A3, mKNA
	PREDICTED: Phagoletis pomonella uncharacterized LOC118738766 (LOC118738766), transcript variant X2, mRNA
	PREDICTED: Rhapiletis pomonella uncharacterized LOCI18738766 (LOCI18738766), transcript variant X2, mRVA
	PREDICTED: Rhagoletis pomonella ATPase H(+)-transporting accessory protein 2-like (LOC118737530), mRNA
	PREDICTED: Rhagoletis pomonella centaurin-gamma-1A (LÖC118735543), transcript variant X1, mRNA
	PRhagoletis cerasi clone Ree mic Rp12 microsatellite sequence
	PRhagoletis pomonella clone PS12 microsatellite sequence
	PREDICTED: Rhagoletis pomonella mucin-5AC (LOC118738195), mRNA
	PREDICTED: Rhagoletis zephyria F-box/WD repeat-containing protein 7 (LOC108359500), transcript variant X2, mRNA
	PREDICTED: Rhagoletis zepnyria F-box/w D repeat-containing protein / (LOC108359500), transcript variant X1, mKNA
	PREDICTED: Rhagoleus pomonella P-box/WD repeat-containing protein 7 (LOC118/4/053), transcript variant X2, mRNA
	PREDICTED: Rhaoletis pomorella uncharacterized I OC118740479 (I OC11874079) mRNA
	PREDICTED: Rhagoletis zephyria uncharacterized LOC108382403 (LOC108382403), ncRNA
	PREDICTED: Rhagoletis pomonella protein LSM14 homolog A-like (LOC118755689), partial mRNA
	PREDICTED: Rhagoletis pomonella innexin inx5 (LOC118747758), mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC118749171 (LOC118749171), ncRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC118744518 (LOC118744518), mRNA
	PREDICTED: Rhagoletis zephyria serine-rich adhesin for platelets-like (LOC108376306), mRNA
	PREDICTED: Rhagoletis zephyria N-acetylgalactosamine kinase (LOC1083/3683), transcript variant X9, mRNA
	PREDICTED: Rhagoletis zephyria N-acetylgalactosamine kinase (LOC1083/3083), transcript variant X8, mRNA
	PREDICTED: Rhagoletis zephyria N-acetylgalactosamine kinase (LOC1085/5085), transcript Variant X/, mRNA DELOTEDICTED: Bhagoletis zephyria N-acetylgalactosamine kinase (LOC1085/5085), transcript Variant X/, mRNA
	#PREDICTED: Rhagoletis zephyna Iv-acetylgalactosamine kinase (LOC108373683) transcript variant X5, mRNA
	PREDICTED: Rhapoletis zephyria N-acetylgalactosamine kinase (LOC108373683), transcript variant X4, mRNA
	PREDICTED: Rhagoletis zephyria N-acetylgalactosamine kinase (LOC108373683), transcript variant X3, mRNA
	PREDICTED: Rhagoletis zephyria N-acetylgalactosamine kinase (LOC108373683), transcript variant X2, mRNA
	PREDICTED: Rhagoletis zephyria N-acetylgalactosamine kinase (LOC108373683), transcript variant X1, mRNA
	🚝 flies 8 leaves
	PREDICTED: Rhagoletis pomonella protein serrate (LOC118737496), transcript variant X2, mRNA
	PREDICTED: Rhagoletis pomonella protein serrate (LOC118737496), transcript variant X1, mRNA
	PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC118745690), transcript variant X10, mRNA
	PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC118745690), transcript variant X8, mRNA
	PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC118/45090), transcript variant X/, mRNA
	PREDICTED: Rhagoleus pomonella transcriptional activator cubitus interruptus (LOC118/43050), transcript variant X3, mRNA
	PREDICTED: Phagoletis pomorella transcriptional activator cubitus interruptus (LOC118/45050), transcriptivariant X1, mRNA
	PREDICTED: Rhagoletis pomorella caliscriptional activator contrastingenergy (IOC1187-5050), taliscriptional Art, niktva
	PREDICTED: Rhagoletis pomonella neuroligin-2 (IOCI18735173), mRNA
	PREDICTED: Rhagoletis pomonella myb-like protein Q (LOC118747697), mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC118747546 (LOC118747546), mRNA
	PREDICTED: Rhagoletis pomonella protein 5NUC-like (LOC118744350), transcript variant X3, mRNA
	PREDICTED: Rhagoletis pomonella protein 5NUC-like (LOC118744350), transcript variant X2, mRNA
	PREDICTED: Rhagoletis pomonella protein SNUC-like (LOC118744350), transcript variant X1, mRNA
	T FREDICI ED: Rhagoleus pomonella uransmemorane protein 39A-A (LOC118/4420/), mKNA
	PREDICTED: Rhagoletis pomorella retinol dehvdrogenase 13-like (I OC118742554) transcrint variant X5 mRNA
	PREDICTED: Rhagoletis pomonella retinol dehydrogenase 13-like (LOC118742554), transcript variant X1, mRNA
	PREDICTED: Rhagoletis pomonella DNA polymerase iota (LOC118737676), transcript variant X3, mRNA
	PREDICTED: Rhagoletis pomonella DNA polymerase iota (LOC118737676), transcript variant X2, mRNA
	PREDICTED: Rhagoletis pomonella DNA polymerase iota (LOC118737676), transcript variant X1, mRNA
	PREDICTED: Rhagoletis pomonella enolase (LOC118740678), mRNA
	PREDICTED: Knagoletis pomonella Iilamin-A (LOC118/5/3854), mRNA
	PREDUCED: Knagoleus pomonella uncharacterized LOC118/39/29 (LOC118/39/29), mRNA DREDUCED: Physiolatic pomonella uncharacterized LOC118/355500, martial mRNA
	PREDICTED: Rhagoleus politolella prefutophil ⁺⁴⁰⁻ IRC(LOC110/53050), partial IRKINA
	PREDICTED: Rhagoletis pomorella integratementary mucin C. 1-like (I OC118755290), partial mRNA
	* PREDICTED: Rhagoletis pomonella integumentary mucin C.1-like (LOC118732763), partial mRNA
	PREDICTED: Rhagoletis zephyria msx2-interacting protein-like (LOC108370706), partial mRNA
	PREDICTED: Rhagoletis zephyria multidrug resistance-associated protein 4-like (LOC108354614), partial mRNA
	PREDICTED: Rhagoletis zephyria beta-mannosidase-like (LOC108377167), mRNA
	PKEDICI ED: Knagoletis zephyria ER degradation-enhancing alpha-mannosidase-like protein 2 (LOC108376188), mRNA [https://www.csub.com/
	Viciouery 08822
	MARKET HE TENT A METHODAL AND A
	PREDICTED: Rhagoletis zepnyra uncharacterized LOC108501552 (LOC108501552), ncRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC 108501552 (LOC 108501552), ICKNA PREDICTED: Rhagoletis pomonella uncharacterized LOC118741403 (LOC118741403), mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus internutus (LOC118745600), transcript variant X9 mRNA
	PREDIC 1 ED: Rnagoletis zepnyrta uncharacterized LOC 108/61322 (LOC 108/61322), netwo PREDICTED: Rnagoletis pomonella uncharacterized LOC 118/314403 (LOC 118/31403), mRNA PREDICTED: Rnagoletis pomonella transcriptional activator cubitus interruptus (LOC 118/345690), transcript variant X9, mRNA PREDICTED: Rnagoletis pomonella transcriptional activator cubitus interruptus (LOC 118/345690), transcript variant X6, mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC 108361352 (LOC 108361352, DRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC 118741403 (LOC 118741403), mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X9, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X6, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X6, mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC 108361352 (LOC 108361352, LOC 108361352, LOC 108361352) PREDICTED: Rhagoletis pomonella uncharacterized LOC 118741403 (LOC 118741403), mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X9, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X6, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X4, mRNA
	 PREDICTED: Rhagoletis pomonella uncharacterized LOC 108501552 (LOC 108501552), n.EKVA PREDICTED: Rhagoletis pomonella uncharacterized LOC 118741403 (LOC 108501526), n.EKVA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X9, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X6, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X2, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X2, mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC 108501552 (LOC 108501552), nervA PREDICTED: Rhagoletis pomonella uncharacterized LOC 1185414403 (neRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X9, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X6, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118745690), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella nuclear pore complex protein DDB G0274915-like (LOC 118755912), partial mRNA PREDICTED: Rhagoletis pomonella salivary gue protein Sg2-3-like (LOC 118732831), partial mRNA
	PREDICTED: Rhagoletis pomonella uncharacterized LOC 108/301322 (LOC 108/301324), nervA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118/34690), transcript variant X9, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118/345690), transcript variant X6, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118/345690), transcript variant X6, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118/345690), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella transcriptional activator cubitus interruptus (LOC 118/345690), transcript variant X4, mRNA PREDICTED: Rhagoletis pomonella nuclear pore complex protein DDB (602/24)15.ike (LOC 118/35512), partial mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC 118/32383 (LOC 118/32383), partial mRNA PREDICTED: Rhagoletis pomonella uncharacterized LOC 118/32383 (LOC 118/32383), partial mRNA

Fast Minimum Evolution tree for P12_R



In silico PCR P12_F/R

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GGGTGTTCATGGTAGTTGTAGAT	23	57.90	43.48	4.00	2.00
Reverse primer	ACTAGTAAAGGAAAGGCGCAAT	22	58.05	40.91	6.00	2.00

Products on target templates

>JX870459.1 Rhagoletis cerasi clone Rce_mic_Rp12 microsatellite sequence

```
product length = 224
Forward primer 1 GGGTGTTCATGGTAGTTGTAGAT 23
Template 229 ..... 207
Reverse primer 1 ACTAGTAAAGGAAAGGCGCAAT 22
Template 6 ..... 27
```

>AY734896.1 Rhagoletis pomonella clone PS12 microsatellite sequence

```
product length = 250
Forward primer 1 GGGTGTTCATGGTAGTTGTAGAT 23
Template 1 ..... 23
Reverse primer 1 ACTAGTAAAGGAAAGGCGCAAT 22
Template 250 ..... 229
```

Specification of the PCR Assay 1 (COI Barcoding according to EPPO PM7/129(2))

Name of the primer incl. sequence, literature reference, fragment length in bp:

LepF: 5'- ATTCAACCAATCATAAAGATATTGG-3' LepR: 5'- TAAACTTCTGGATGTCCAAAAAAAATCA-3'

Literature: Hajibabaei, M., Janzen, D. H., Burns, J. M., Hallwachs, W., & Hebert, P. D. (2006). DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences, 103(4), 968-971.

Fragment length: 709bp

PCR - Parameters:

Thermocyler used: Biometra T3000 Thermal cycler

Mastermix: 5x HOT FIREPol® Master Mix, Solis Biodyne:

Composition:		Final concentration:
	Volume per reaction μ l	
Water	6	
Mastermix	2	1x
Primer1:	0,5	0,5μM
Primer2:	0,5	0,5μM
Σ	9	
DNA	1	

PCR conditions:

	°C	Duration (min., sec.)	Nr. of Cycles
Start	95	15 min	1
Denaturation	95	45 sec	5
Annealing	44	45 sec	
Extension	72	45 sec	
Denaturation	95	45 sec	35
Annealing	49	45 sec	
Extension	72	45 sec	
Final extension	72	7 min	1
Cooling	15	8	

Specification of the PCR Assay 2-4 (SYBR Green real-time PCRs according to Burgher-McLellan et al. 2009)

Name of the primer incl. sequence, literature reference, fragment length in bp:

ID-8 F: 5'- TGGAGGGGCATCTGTTGA-3' ID-9R: 5'- TAATGGCTCCTGCTAATACTGGT-3' ID-1 F: 5'- GACGGATTTCGATTATTAGATGT-3' ID-1 R: 5'- TGATTTAGACGACCAGGAGTT-3' ID-3 R: 5'- GATTTAGACGACCAGGAGTTC-3'

Burgher-MacLellan, K. L., Gaul, S., MacKenzie, K., & Vincent, C. (2009). The use of real-time PCR to identify blueberry maggot (Diptera: Tephritidae, Rhagoletis mendax) from other Rhagoletis species and in lowbush blueberry fruit (Vaccinium angustifolium). Acta Horticulturae International Society of Horticultural Sciences, 810, 265-274.

Fragment length: ID-8F/ID-9R: 206bp ID-1F/ID-1R: 152bp ID-1F/ID-3R: 151bp

PCR - Parameters:

Analytic Jena qTower³ G (230 V) with accompanying software, Bio Molecular Systems Magnetic Induction Cycler (MIC) with accompanying software.

Mastermix: HOT FIREPol[®] EvaGreen[®] qPCR Mix (Solis Biodyne)

Composition:		Final concentration:
	Volume per reaction µl	
Water	6	
Mastermix	2	1x
Primer1:	0,5	0.5μΜ
Primer2:	0,5	0.5μΜ
Σ	9	
DNA	1	

PCR conditions ID-8F/ID-9R:

Step	°C	Duration (min., sec.)	Nr. of Cycles
Start	95	12 min	1
Denaturation	95	15 sec	45
Annealing	60	30 sec	
Extension and fluorescence reading	72	30 sec	
Melting curve	72-95	0.1°C/s	Stepwise increment

PCR conditions ID-1F/ID-1R:

Step	°C	Duration (min., sec.)	Nr. of Cycles
Start	95	12 min	1
Denaturation	95	15 sec	45
Annealing	50	30 sec	
Extension and fluorescence reading	72	30 sec	
Melting curve	72-95	0.1°C/s	Stepwise increment

PCR conditions ID-1F/ID-3R

Step	°C	Duration (min., sec.)	Nr. of Cycles
Start	95	12 min	1
Denaturation	95	15 sec	45
Annealing	58	30 sec	
Extension and fluorescence reading	72	30 sec	
Melting curve	72-95	0.1°C/s	Stepwise increment

Specification of the PCR Assay 5-7 (SYBR Green real-time PCRs according to Velez et al. 2006)

Name of the primer incl. sequence, literature reference, fragment length in bp:

P12_F: 5'- GGGTGTTCATGGTAGTTGTAGAT-3' P12_R: 5'- ACTAGTAAAGGAAAGGCGCAAT-3' P6_F: 5'- AGTCAGAGTGCGGCAAAAGT -3' P6_R: 5'- CGGTAGACCTCAGGCTGATAG -3' P9_F: 5'- CGGCAGGTAAATGACCAAAA -3' P9_R: 5'- GCAATGACCGTTGGCTATTA -3'

Velez, S., Taylor, M. S., Noor, M. A. F., Lobo, N. F., & Feder, J. L. (2006). Isolation and characterization of microsatellite loci from the apple maggot fly Rhagoletis pomonella (Diptera: Tephritidae). Molecular Ecology Notes, 6(1), 90-92.

Fragment length: P12_F/R: 250bp P6_F/R: 155bp P9_F /R: 156bp

PCR - Parameters:

Analytic Jena qTower³ G (230 V) with accompanying software, Bio Molecular Systems Magnetic Induction Cycler (MIC) with accompanying software.

Mastermix: HOT FIREPol[®] EvaGreen[®] qPCR Mix (Solis Biodyne)

Composition:		Final concentration:
	Volume per reaction μ l	
Water	6	
Mastermix	2	1x
Primer1:	0,5	0.5μΜ
Primer2:	0,5	0.5μΜ
Σ	9	
DNA	1	

PCR conditions:

Step	°C	Duration (min., sec.)	Nr. of Cycles
Start	95	12 min	1
Denaturation	95	15 sec	45
Annealing	56	30 sec	
Extension and fluorescence reading	72	30 sec	
Melting curve	72-95	0.1°C/s	Stepwise increment

Appendix 4 – Real-time PCR results

Ct values for each of the samples

Author	Burgher-MacLellan <i>et al</i> . (2009)			Velez <i>et al</i> . 2006		
Primer	ID-8F/ID-9R	ID-1F/ID-1R	ID-1F/ID-3R	P6F/P6R	P9F/P9R	P12F/P12R
Target	R. pomonella	R. pomonella	R. pomonella	R. pomonella	R. pomonella	R. pomonella
66/19	21.08	20.22	20.61	28.44	27.75	28.58
2247/22	16.05	34.23	30.18	24.48	26.46	30.14
1928/22	21.59	32.68	29.88	24.49	33.11	24.26
1233/22	19.16	33.27	28.58	29.01	35.82	30.50
1231/22	17.32	25.00	22.96	29.74	38.97	26.31
2552/21	22.56	34.31	31.77	26.20	34.39	27.07

Melting temperature (Tm) for each of the samples

Author	Burgher-MacLellan <i>et al</i> . (2009)			Velez <i>et al</i> . 2006		
Primer	ID-8F/ID-9R	ID-1F/ID-1R	ID-1F/ID-3R	P6F/P6R	P9F/P9R	P12F/P12R
Target	R. pomonella	R. pomonella	R. pomonella	R. pomonella	R. pomonella	R. pomonella
66/19	80.55/79.54	79.97/78.85	79.89/78.89	-/83.26	-/84.14	-/-
2247/22	78.79/78.81	79.84/-	79.81/79.78	83.96/84.38	84.53/84.53	-/-
1928/22	78.64/78.74	79.73/80.08	80.00/79.89	84.49/84.50	-/-	85.53/85.67
1233/22	77.95/77.95	79.28/76.35	79.03/79.44	-/-	-/-	-/75.12
1231/22	78.37/78.43	78.98/79.11	79.17/79.09	-/-	-/-	84.29/84.29
2552/21	78.42/78.54	79.67/79.67	79.91/78.99	84.33/-	84.08/-	85.39/85.56