Optimized DNA extraction and library preparation for minute arthropods: Application to target enrichment in chalcid wasps used for biocontrol

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Astrid Cruaud[1*], Sabine Nidelet[1], Pierre Arnal[1, 2], Audrey Weber[3], Lucian Fusu[4], Alex Gumovsky[5], John Huber[6], Andrew Polaszek[7] and Jean-Yves Rasplus[1]

[1] CBGP, INRA, CIRAD, IRD, Montpellier SupAgro, Univ Montpellier, Montpellier, France

[2] ISYEB–UMR 7205 MNHN, CNRS, UPMC, EPHE, Sorbonne Universités, Paris, France

[3] AGAP, INRA, CIRAD, Montpellier SupAgro, Univ Montpellier, Montpellier, France.

[4] Faculty of Biology, Alexandru Ioan Cuza University, Iasi, Romania

[5] Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kiev, Ukraine

[6] Natural Resources Canada, c/o Canadian National Collection of Insects, Ottawa, Canada

[7] Department of Life Sciences, Natural History Museum, London, UK

*corresponding author: astrid.cruaud@inra.fr

running headline = target-enrichment in minute wasps

Abstract

Enriching subsets of the genome prior to sequencing allows focusing effort on regions that are relevant to answer specific questions. As experimental design can be adapted to sequence many samples simultaneously, using such approach also contributes to reduce cost. In the field of ecology and evolution, target enrichment is increasingly used for genotyping of plant and animal species or to better understand the evolutionary history of important lineages

through the inference of statistically robust phylogenies. Limitations to routine target enrichment by research laboratories are both the complexity of current protocols and low input DNA quantity. Thus, working with tiny organisms such as micro-arthropods can be challenging. Here, we propose easy to set up optimisations for DNA extraction and library preparation prior to target enrichment. Prepared libraries were used to capture 1432 Ultra-Conserved Elements (UCEs) from microhymenoptera (Chalcidoidea), which are among the tiniest insects on Earth and the most commercialized worldwide for biological control purposes. Results show no correlation between input DNA quantities (1.8-250ng, 0.4 ng with an extra whole genome amplification step) and the number of sequenced UCEs. Phylogenetic inferences highlight the potential of UCEs to solve relationships within the families of chalcid wasps, which has not been achieved so far. The protocol (library preparation + target enrichment), allows processing 96 specimens in five working days, by a single person, without requiring the use of expensive robotic molecular biology platforms, which could help to generalize the use of target enrichment for minute specimens.

Key words : Chalcidoidea, library construction, low DNA quantity, micro-arthropods, target enrichment, UCEs.

INTRODUCTION

Enriching subsets of the genome prior to sequencing (target enrichment, Mamanova *et al.* 2010) allows effort to be concentrated on genomic regions that are relevant to answer specific research questions. Using this approach also contributes to reducing cost, as experimental design can be adapted to sequence many samples simultaneously. In the fields of ecology and evolution, target enrichment has been used for genotyping or phylogenomics of plant and animal species (Gasc *et al.* 2016; Lemmon & Lemmon 2013), to characterize phenotypic traits (e.g., Muraya *et al.* 2015) or to explore microbial ecosystems (Gasc & Peyret 2018).

However, routine target enrichment by research laboratories is limited both by the complexity of current protocols, and by input DNA quantity that may be very low for some minute species (e.g. micro-arthropods < 1mm) and /or old /rare (museum) specimens. Indeed, current protocols (DNA extraction, library preparation, target-enrichment) are time consuming and require handling expertise. They have been initially developed to work with large amounts of input DNA (e.g., vertebrates or large/medium size insects; Faircloth *et al.* 2015; McCormack *et al.* 2013) and include many purification steps that increase DNA loss. Working on hyper-diverse groups of microarthropods is challenging, as it requires one to perform the extraction on i) a large number of specimens/species to be representative of the overall diversity of the group, without the possibility of using pipetting robots that increase DNA loss, ii) single individuals because species complexes are frequent (Al Khatib *et al*. 2014; Kenyon *et al*. 2015; Mottern & Heraty 2014), iii) the whole insect without destruction for vouchering and often prior to species identification, iv) rare species that have been collected once and may be represented in collection by a few specimens or only one specimen and, sometimes, v) old and dry museum specimens used for species description (types).

In this study, we propose optimised protocols for DNA extraction and library preparation for target enrichment purposes, as well as a custom pipeline to analyse the sequence data obtained. We used these protocols and customised pipeline to capture and analyse Ultra Conserved Elements (UCEs) in minute wasps, the chalcids (Insecta: Hymenoptera: Chalcidoidea, Heraty *et al.* 2013; Noyes 2018), that are key components of terrestrial ecosystems. Chalcids are key models for basic and applied research. With an estimated diversity of more than 500,000 species these microhymenoptera have colonised almost all extant terrestrial habitats. Many of them develop as parasitoids of arthropod eggs, larvae or pupae. As such, they are both key regulators of the populations of many other arthropod species in natural ecosystems and are increasingly used worldwide as biocontrol agents (e.g., Consoli *et al.* 2010; Heraty 2009). A few of them, especially *Nasonia* (Pteromalidae) or *Trichogramma* (Trichogrammatidae) species are also used as model systems to answer challenging questions about sex determination, genetics of speciation, host-symbiont interactions or behavioural ecology (e.g., Pinto *et al.* 1991; Stouthamer *et al.* 1990; Werren & Loehlin 2009). Chalcidoidea has undergone a spectacular radiation resulting in a huge diversity of morphologies and sizes (Gibson *et al.* 1999; Heraty *et al.* 2013), but are generally small insects (< 2 mm long). Among them, *Kikiki huna* Huber (Mymaridae) at 158 µm long is the smallest winged insect currently known and the wingless male of *Dicopomorpha echmepterygis* Mockford at 139 µm is the smallest insect currently known (Huber & Noyes 2013). Notably, most species used for biological control, belonging mainly to five families (Aphelinidae, Encyrtidae, Eulophidae, Mymaridae and Trichogrammatidae) are among the tiniest wasps on earth (< 1mm).

Their small size, huge diversity and widespread morphological convergence make chalcid wasps difficult to identify to species by non-expert taxonomists, which limits their use in

biological control. Attempts have been made to resolve the phylogeny of the whole superfamily (Heraty *et al.* 2013; Munro *et al.* 2011) or a few families (Burks *et al*. 2011; Chen *et al*. 2004; Cruaud *et al*. 2012; Desjardins *et al*. 2007; Janšta *et al*. 2017; Owen *et al*. 2007) but none has succeeded. Indeed, the few markers that could be targeted with Sanger sequencing were not informative enough to solve deeper relationships. A study based on transcriptome data (3,239 single-copy genes) obtained from 37 species of chalcids and 11 outgroups also failed to solve relationships within the superfamily (Peters *et al.* 2018). As only a representative sampling in both markers and taxa will allow one to draw accurate conclusions on the history of this hyperdiverse group, target enrichment approaches appear relevant. More specifically, targeting UCEs and their flanking regions that have been proven useful to solve ancient and recent divergences (Faircloth *et al.* 2012; Smith *et al.* 2014) seems pertinent. Indeed, a set of probes has been developed to target UCEs in Hymenoptera (Faircloth *et al.* 2015). This set and an enriched one (Branstetter *et al.* 2017c) were successfully used to solve the phylogeny of a few groups of ants, wasps and bees for which the amount of DNA was not limiting (Blaimer *et al.* 2015; Blaimer *et al.* 2016a; Blaimer *et al.* 2016b; Bossert *et al.* 2017; Branstetter *et al.* 2017a; Branstetter *et al.* 2017b; Jesovnik *et al.* 2017; Prebus 2017; Ward & Branstetter 2017). Thus, contributing to the global effort to solve the Hymenoptera tree of life while addressing the challenge of the phylogeny of chalcid wasps seemed sound.

Here, we provide a detailed description of the optimised protocol for DNA extraction and library preparation, followed by a description of the phylogenetic trees obtained through target enrichment of UCEs from 96 species belonging to seven families and one subfamily of chalcids used for biological control (Aphelinidae, Azotidae, Encyrtidae, Eulophidae, Mymaridae, Pteromalidae: Eunotinae, Signiphoridae, Trichogrammatidae) as well as three

120 outgroups in Mymmaromatidae, the putative sister group to Chalcidoidea (Gibson *et al.* 2007;
121 Heraty *et al.* 2013).
122
123 **MATERIALS AND METHODS**
124
125 **Sampling**
126 Samples were taken from the personal collections of the co-authors of this paper, or borrowed
127 from the Queensland Museum (Australia) or the Australian National Insect Collection,
128 Canberra. Details of the samples included in the analysis are presented in Table S1. Most
129 specimens sampled in the field were placed directly into ethanol for storage. On average,
130 specimens spent 3.5 years in ethanol before being processed (maximum storage time in
131 alcohol = 34 years). Two specimens were critical point dried 25 or 34 years ago. UCE data for
132 three specimens were retrieved from a previous study (Branstetter *et al.* 2017a): *Euplectrus*
133 sp. (empirical data); *Copidosoma floridanum* and *Trichogramma pretiosum* (*in silico*
134 extraction of UCEs from genomes).
135
136 **DNA extraction**
137 DNA was extracted using the Qiagen DNeasy Blood and Tissue kit. All extractions were
138 conducted without destruction of the specimens' external (and certain internal) structures,
139 with digestion and lysis of just the soft tissues. In this way, actual or potential type specimens
140 are preserved. An often-essential feedback to the morphology is also preserved which is
141 critical in this difficult group. The following modifications were made to manufacturer's
142 protocol. Samples were incubated overnight in an Eppendorf thermomixer (temperature =
143 56°C, mixing frequency = 300 rpm). To increase DNA yield, two successive elutions (50 µL
144 each) were performed with heated buffer AE (56°C) and an incubation step of 15 minutes

145 followed by centrifugation (8000 rpm for 1 minutes at room temperature). Eppendorf
146 microtubes LoBind 1,5ml were used for elution and to store DNA at -20°C until library
147 preparation. DNA was quantified with a Qubit® 2.0 Fluorometer (Invitrogen). The final
148 version of the DNA extraction protocol is available as an additional file 1. Vouchers were
149 deposited at CBGP, Montferrier-sur-Lez, France or returned to their owner.
150
151 **Whole genome amplification**
152 DNA extracted from two specimens was subjected to ethanol precipitation and whole genome
153 amplification (WGA) using the GenomiPhi™ V2 DNA Amplification kit (GE Healthcare) as
154 described in Cruaud *et al.* (in press). 1µl of concentrated DNA was used as input (i.e. 4 ng or
155 0.4 ng, Table S1).
156
157 **Library preparation.**
158 Our starting point was the protocol described in http://ultraconserved.org and Faircloth *et al.*
159 (2015). The final goal was to obtain a standardized protocol that could be implemented by one
160 person and which, in 5 working days, would made possible the manual preparation of
161 libraries and the capture of UCEs from 96 samples in parallel. Steps by steps optimisations
162 were made specially to remove time-consuming purification steps and different reagents were
163 tested. The final version of the protocol is available as additional file 2. Briefly, input DNA
164 was sheared to a size of ca 400 bp using the Bioruptor® Pico (Diagenode). End repair, 3'-end
165 adenylation, adapters ligation and PCR enrichment were then performed with the NEBNext
166 Ultra II DNA Library prep kit for Illumina (NEB). We used barcoded adapters that contained
167 amplification and Illumina sequencing primer sites, as well as a nucleotide barcode of 5 or 6
168 bp long for sample identification (additional file 3). Pools of 16 samples were made at
169 equimolar ratio. We enriched each pool using the 2749 probes designed by Faircloth *et al.*

(2015) using MYbaits kits (MYcroarray, Inc.). We followed manufacturer's protocol (MYbaits, user manual version 3, http://www.mycroarray.com/pdf/MYbaits-manual-v3.pdf). The hybridization reaction was run for 24h at 65°C. Post enrichment amplification was performed on beads with the KAPA Hifi HotStart ReadyMix. The enriched libraries were quantified with Qubit, an Agilent Bioanalizer and qPCR with the Library Quantification Kit - Illumina/Universal from KAPA (KK4824). They were then pooled at equimolar ratio. Paired-end sequencing (2*300bp) was performed on an Illumina Miseq platform at UMR AGAP (Montpellier, France) to get longer flanking regions and, as a consequence, more information to differentiate closely related species.

Raw data cleaning

The analytical workflow is summarized in figure S1. In the next paragraph, chosen parameter values (different from default value) are provided between parentheses. Quality control checks were performed on raw sequence data with FastQC v.0.11.2 (Andrews 2010). Quality filtering and adapter trimming were performed with Trimmomatic-0.36 (Bolger *et al.* 2014) (LEADING:20 TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:180, with PrefixPE/1= AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT and PrefixPE/2 = CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT). Overlapping reads were merged using FLASH-1.2.11 (-M 300) (Magoc & Salzberg 2011). Demultiplexing was performed using a bash custom script (no mismatch in barcode sequences was allowed, additional file 4). Assembly of cleaned reads was performed using CAP3 (-i 25 -o 25 -s 400) (Huang & Madan 1999). The 2749 probes designed by Faircloth *et al.* (2015) were assembled into non-overlapping UCEs (hereafter called reference UCEs, n=1432, additional files 5 and 6) using Geneious 8.1.8 (Kearse *et al.* 2012) and contigs

were aligned to this set of reference UCEs using LASTZ Release 1.02.00 (Harris 2007). Contigs that aligned with more than one reference UCE and different contigs that aligned with the same reference UCE were filtered out using Geneious 8.1.8.

Data analysis

UCEs for which sequences were available for more than 25% of the taxa were kept in the next steps of the analysis. Alignments were performed with MAFFT v7.245 (Katoh & Standley 2013) (-linsi option). Ambiguously aligned blocks were removed using Gblock_0.91b with relaxed constrains (-t=d -b2=b1 -b3=10 -b4=2 -b5=h) (Talavera & Castresana 2007). The final data set was analysed using supermatrix approaches and coalescent-based summary methods. Two gene tree reconciliation approaches were used: ASTRAL-III v5.6.1 (Zhang *et al.* 2018), which computes the phylogeny that agrees with the largest number of quartet trees induced by the set of input gene trees and ASTRID (Vachaspati & Warnow 2015) which takes a set of gene trees, computes a distance matrix (*ca* sum of number of edges in the path between two samples divided by the number of gene trees in which the two samples are represented) and infers a phylogeny from this distance matrix. Following recommendations for incomplete distance matrices, BioNJ was used to compute the phylogeny. Individual trees were inferred from each UCE using raxmlHPC-PTHREADS-AVX (Stamatakis 2014) (version 8.2.4; -f a -x 12345 -p 12345 -# 100 -m GTRGAMMA). ASTRAL and ASTRID analyses were performed with 100 multi-locus bootstrapping (MLBS, site-only resampling (Seo 2008)). Phylogenetic trees were estimated from the concatenate, unpartitioned data set using Maximum Likelihood (ML) approaches as implemented in RAxML and IQTREE v1.6.4 (Nguyen *et al.* 2015). For the RAxML analysis, a rapid bootstrap search (100 replicates) followed by a thorough ML search (-m GTRGAMMA) was performed. For the IQTREE analysis, a ML search with the best-fit substitution model automatically selected was

220 performed with branch supports assessed with ultrafast bootstrap (Minh *et al.* 2013) and SH-
221 aLRT test (Guindon *et al.* 2010) (1000 replicates).
222 Summary statistics for all data sets (alignment length, number of samples, number of variable
223 sites, number of parsimony informative sites etc.) were calculated using AMAS (Borowiec
224 2016). Tree annotation was performed with TreeGraph 2.13 (Stöver & Müller 2010).
225 Linear correlation between the number of UCEs and the quantity of DNA used to build the
226 library was tested with the Pearson's correlation coefficient in R (R Core Team 2015).
227 Analyses were performed on a Dell PowerEdge T630 with 10 Intel Xeon E5-2687 dual-core
228 CPUs (3.1 GHz, 9.60 GT/s), 125 Go RAM and 13 To hard drive and on the Genotoul Cluster
229 (INRA, Toulouse).
230
231 **RESULTS**
232 Optimisations made for DNA extraction are detailed in the additional file 1. The final version
233 of the library preparation protocol is available as additional files 2 and 3. Hereafter, the range
234 of values provided between parentheses refers to the range of data that fall between the 2.5th
235 percentile (LB=lower bound) and 97.5th percentile (UP = upper bound). Table S1 contains
236 sequencing information for all samples. The median amount of input DNA was 25ng
237 (LB=1.8ng; UP=250ng). An average of 76,330 reads (cleaned and merged) per sample was
238 obtained (LB=3,359; UP=348,326). The average number of contigs was 3,454 (LB=546;
239 UP=14,012) and the average sequencing depth was 18X (LB=3X; UP=44.0X). The average
240 number of UCEs obtained per sample after filtering of problematic contigs was 687 (LB=193-
241 UP=1,082) with a length comprised between LB=315 and UP=816bp (mean=603bp).
242 Figure 1 and S2 show the variation of the number of UCEs obtained with regard to the
243 amount of input DNA. No significant correlation was observed (Pearson's correlation
244 coefficient = 0.096, p-value = 0.36).

The final dataset (25%-complete matrix; i.e. at least 25 taxa on the 99 should have a sequence to keep the locus in the analysis) comprised 1,139 UCEs and 340,286bp (missing data = 47.0 %; parsimony informative sites = 72.3 %; GC-content = 42.6%).

Specimens retrieved from a previous study (Branstetter *et al.* 2017a) that were either represented by empirical data (*Euplectrus* sp.) or UCEs extracted from published genomes (*Copidosoma floridanum* and *Trichogramma pretiosum*) displayed a number of UCEs comparable to what was obtained for other specimens. Their placement in the trees (Figures 2; S3-S5) was in accordance with their morphology. Whatever the method used (supermatrix approaches versus coalescent-based summary methods), all families, except for Aphelinidae, were recovered as monophyletic with high support. Aphelinids were split into three groups: 1) a monophyletic Aphelininae + Eretmocerinae; 2) Coccophaginae; 3) *Cales* sp. (Calesinae). The position of *Cales* was ambiguous. *Cales* was either recovered as sister to Trichogrammatidae (RAxML, low support) or as a lineage distinct from all other chalcidoids (all other analyses). Except for Mymaridae that was strongly placed as sister to all other Chalcidoidea in all analyses, the tree backbone remained poorly resolved. Statistical support was much higher within families. In all analyses Azotidae clustered with Signiphoridae, with strong support.

DISCUSSION

To our knowledge this study is the second after Sproul and Maddison (2017) to demonstrate success in library preparation from such low input using commercial kits, and the first to report successful sequencing of >1000 low copy genes in 96 specimens in parallel, from such low input and processing time. Our optimisations differ from what was proposed by Sproul and Maddison (2017). First, we tried to optimize DNA extraction itself by using overnight lysis with gentle mixing to preserve fragile specimens, heated elution buffer and increased

incubation time before elution. Second, instead of increasing the number of time-consuming purification steps we decreased them. It is noteworthy that in this library only two historical specimens were included. This may have masked challenges posed by adapter dimers (Burrell *et al.* 2015; Sproul & Maddison 2017; Tin *et al.* 2014) that led Sproul and Maddison (2017) to add a second bead clean-up prior to library amplification. However, we have already used this protocol on hundreds of chalcid and moth species, including historical specimens that were processed the same way as fresh ones and we never had such an issue. Finally, instead of increasing the number of amplification cycles prior to target enrichment, we used a new generation mastermix including a hot start, processive and high-fidelity polymerase (NEBNext Ultra II Q5 Master Mix). Our protocol also allows one to back-up DNA at several steps that allows for multiple attempts without delay in case the first attempt fails. Finally, sequencing was performed on a MiSeq to get longer flanking regions and, as a consequence, more information to differentiate closely related species.

The protocol was successfully used on minute chalcid wasps widely used for biological control purposes. Up to 1165 valid UCEs were captured from 25ng DNA (median amount of DNA used for this study). No correlation was observed between the quantity of DNA used for library preparation and the number of captured UCEs. The average number of captured UCEs validated by our quality control workflow was 687, and 685 valid UCEs were captured from a tiny aphelinid (1.8ng of input DNA). The number of UCEs obtained per individual appears to drop within the basal clades (i.e., Mymaridae and Trichogrammatidae, Figure S2), a result probably linked to the relatively long branches observed in these groups, and that could reduce the efficiency of the probes that were designed from the genome of *Nasonia* (Faircloth *et al.* 2015). Trees were well resolved at the family level, with high statistical support, showing the potential of UCEs to solve long-standing taxonomic issues. However, the tree

295 backbone remained unresolved, a pattern that confirms the rapid diversification of the group
296 (Heraty *et al.* 2013; Peters *et al.* 2018). Understanding the evolutionary history of the group
297 was not the purpose of this paper. Indeed, only a representative sampling in both markers and
298 taxa as well as cutting-edge data analysis will allow drawing accurate conclusions. By
299 providing suitable tools for a fast, easy and affordable acquisition of data, this paper is a first
300 step.
301
302 Interestingly, as it has been shown previously on RADseq data (Cruaud *et al.* in press), WGA
303 does not seem to bias the results even when input DNA used for the WGA is below the
304 recommendation on the manufacturer kit (here 0.4 and 4 ng when the Genomiphi kit V2
305 requires 10 ng). This further increases the possibilities opened by our protocol as input DNA
306 as low as 0.2-0.3 ng may be used when an extra WGA step is included after DNA extraction
307 (Cruaud *et al.* in press). It is noteworthy that reducing the amount of DNA required for library
308 preparation allows one to use extracted DNA for different approaches in parallel (RADseq,
309 amplicon, Shotgun etc.). This also allows one to send DNA back to museums from which
310 specimens were borrowed, for archival purposes. We definitely agree with Sproul and
311 Maddison (2017) who emphasize how important it is not to waste DNA obtained from
312 irreplaceable specimens (whether fresh or historical). It is even more important to capitalize
313 on existing collections as collecting samples for large-scale studies may be more and more
314 difficult, given that many countries have imposed restrictive access regulations, even to
315 academic researchers, to reduce the risk of supposed biopiracy (Divakaran Prathapan *et al.*
316 2018).
317
318 All the elements discussed above indicate that this protocol may be of great help to
319 reconstruct phylogenetic hypotheses in multiple groups of tiny arthropods, e.g., springtails,

sandflies, lice, whiteflies, mites, etc. Coupled or not with WGA, some steps of our protocol may also contribute to simplify and hasten construction of other reduced-representation libraries (RAD-Seq, ddRAD-Seq, etc). These methods may then be used to analyse species relationships, hybridization or population genomics (Cruaud *et al.* 2014; Eaton & Ree 2013; Emerson *et al.* 2010; Gagnaire *et al.* 2013; Hipp *et al.* 2014) on minute arthropods or on endangered species using non-invasive DNA sampling (Vila *et al.* 2009).

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DATA ACCESSIBILITY

Demultiplexed reads are available as a NCBI Sequence Read Archive (ID#XXX)

AUTHOR CONTRIBUTIONS

Designed the study: AC, JYR; contributed samples and identifications: AC, LF, AG, JH, AP,

JYR; optimized protocol: SN with the help of AC, JYR, PA; performed lab work: SN with the

help of AC, JYR, LF; sequenced libraries: AW; analysed data: AC, JYR; drafted the manuscript: AC, JYR. All authors commented on the manuscript.

TABLES AND FIGURES

Figure 1. Variation of the number of UCEs with regard to the amount of input DNA (ng).

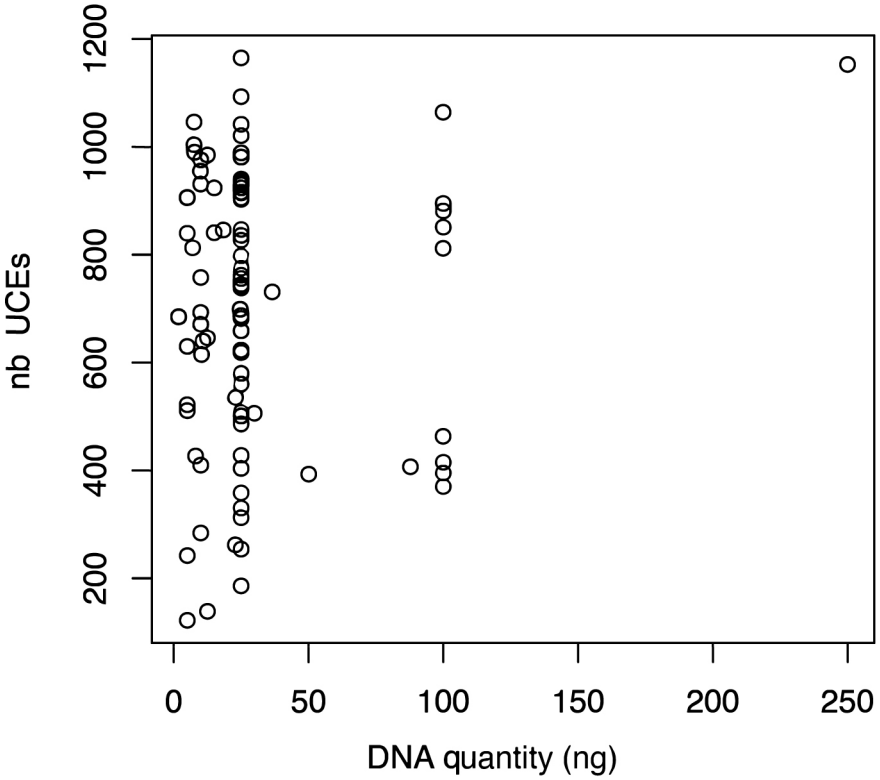
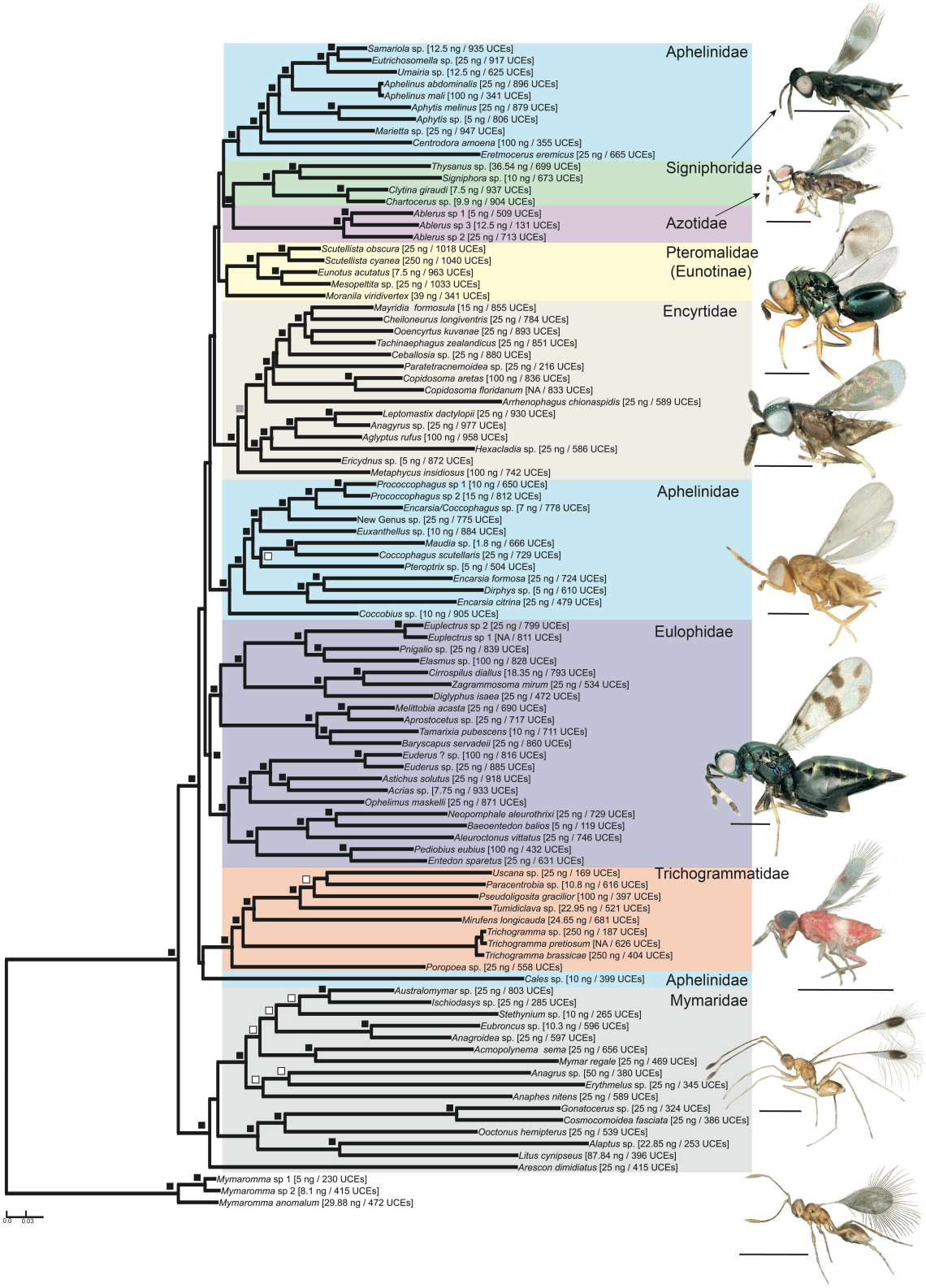


Figure 2. RAxML tree obtained from the analysis of the 25%-complete data set.
Black squares indicate node supported with RAxML BP = 100, IQTREE aLRT = 100 / BP = 100 and ASTRAL/ASTRID BP > 75. Grey square indicates node with RAxML BP = 100, IQTREE aLRT = 100 / BP = 100 and ASTRAL BP > 75. White squares indicate nodes with RAxML BP > 95 and IQTREE aLRT > 80 / BP > 95. The DNA quantity used to build the library as well as the number of UCEs analysed for each sample is given in brackets. Photos ©J.-Y. Rasplus. Scale bars = 500 µm. IQTREE, ASTRAL and ASTRID trees are available in figures S3-S5.



Supplementary material to:

Optimised DNA extraction and library preparation for minute arthropods: application to target enrichment in chalcid wasps used for biocontrol.

Astrid Cruaud[1*], Sabine Nidelet[1], Pierre Arnal[1, 2], Audrey Weber[3], Lucian Fusu[4], Alex Gumovsky[5], John Huber[6], Andrew Polaszek[7] and Jean-Yves Rasplus[1]

Additional file 1: Optimized protocol for DNA extraction

Optimisations were done to the DNeasy Blood & Tissue Kit (250) protocol (Qiagen)

Consumables

Tweezers and fine brush/or forceps to manipulate specimens; 1 pot with bleach at a 1:10 dilution with osmosis cleaned water; 1 pot with osmosis cleaned water to wash tweezers/forceps, cup, paper towels, sterile gloves, 50ml Falcon™ tube**,** 1.5ml Safe-lock tubes (Eppendorf), 1,5ml Microtubes DNA LoBind (Eppendorf).

---------day 1 / afternoon---------

● Label 1.5ml safe-lock tubes with sample codes. Close caps. Put under UV light for 10 min.

● Equilibrate frozen specimens to room temperature before processing.

● Dry specimens on clean paper towel to remove EtOH. Place specimen in tube. Clean forceps to avoid contamination between 2 different specimens: bleach, then water, then dry with paper towel.

● Add 200µl buffer ATL (Qiagen) to each tube.
(NB DNeasy® Blood & Tissue Handbook uses 180µl; we use 200µl to improve diffusion of the DNA from the specimen)

● Add 20µl proteinase K to each tube (or prepare a master mix of buffer ATL + proteinase K and add master mix to tubes.)

● Vortex + Pulse spin.
(NB if the specimens are fragile, i.e. old and dry mounted collection specimens, dispense the master mix of buffer ATL + proteinase K to each tube and then add specimens; or dispense specimens to tubes and then add the master mix. Do not vortex at all to avoid damage to specimens and place the tubes directly in the thermomixer after ensuring that the specimens are in the liquid.)

● Place tubes in thermomixer (Eppendorf) at 56°C and 300 rpm overnight.

---------day 2 / morning---------

● Remove tubes from thermomixer.
● Vortex + pulse spin.
(NB if working with fragile specimens do not vortex. Pulse spin, transfer all the liquid to a new labelled tube leaving the specimen behind and then proceed to the next step.)

(DNeasy® Blood & Tissue Handbook, p. 15, recommends using carrier DNA or RNA for samples containing less than 5 µg of DNA. Though this was not used for this publication, poly (A) RNA (2 µl of 2 µg/µl) is added to the lysate before the next step by one of us (LF) for routine DNA purification from small or old specimens. This should not interfere with library preparation as it was used for example by Sproul & Maddison (2017) as recommended in the QIAamp DNA Micro Kit by Qiagen.)

● Add 200µl buffer AL to each tube (kit Qiagen).
(DNeasy® Blood & Tissue Handbook, p. 19, 29 recommends adding RNase A if RNA-free genomic DNA is required. We do not use it because the copurified RNA is not interfering with target enrichment anyway and because RNase A also degrades DNA; see Donà & Houseley (2015).)

A white precipitate may appear that will dissolve during incubation at 70°C.
● Vortex + pulse spin.
● Incubate tubes in thermomixer at 70°C and 300 rpm for 10 minutes.
● During that time, prepare DNeasy mini spin Qiagen columns and 1.5 ml DNA LoBind Eppendorf tubes (label tubes with sample codes).
(NB LoBind tubes minimize DNA loss during storage by reducing sample-to-surface binding.)

● Aliquot buffer AE in Eppendorf tubes and heat at 56°C in thermomixer (heated buffer will improve the release of DNA from the resin.)

● Add 200µl absolute ethanol (not provided with the Qiagen kit) to each tube.
(NB only high purity ethanol should be used; cheap ethanol may contain traces of other chemicals that will interfere with the solubilisation of the DNA from the membrane in the last step.)

● Vortex + Pulse spin.
● Pipette the liquid (including precipitate) from each tube and transfer into a DNeasy mini spin Qiagen column placed in a 2 ml collection tube (Qiagen kit).
● Centrifuge columns + collection tubes at 6000 x g (8000 rpm) for 1 minute; discard the flow-through and collection tubes. Keep the columns.
● Place spin columns in new collection tubes.
● Add 500µl buffer AW1 (kit Qiagen).
● Centrifuge at 6000 x g (8000rpm) for 1 minute; discard the flow-through and collection tubes. Keep the columns.
● Place spin columns in new collection tubes.
● Add 500µl buffer AW2 (kit Qiagen).
● Centrifuge at 20,000 x g (14,000 rpm) for 3 minutes to dry the columns.
● Rotate columns to 180 degrees in their collection tubes and centrifuge again at 20000 xg (14000rpm) for 3 minutes (this will make sure the column is well dried).
● Place dried spin columns in 1.5ml LoBind tubes that you previously labelled.
● Add 50µl of heated AE buffer. **Deposit buffer right in the middle of the resin.**
● Incubate 15 min at room temperature **(meanwhile, reheat buffer AE.)**
● Centrifuge at 6000 x g (8000 rpm) for 1 minute.
● Rotate columns to 180 degrees and centrifuge again at 6000 x g (8000 rpm) for 1 minute.
● Remove columns + tubes from the centrifuge.
● Add again 50µl of heated buffer **right in the middle of the resin.**
● Incubate 15 min at room temperature.
● Centrifuge at 6000 x g (8000 rpm) for 1 minute at room temperature.
● Rotate columns to 180 degrees and centrifuge again at 6000 x g (8000 rpm) for 1 minute.
● DNA (ca 2 x 50µl) is ready for use.

● Add distilled water to the extracted specimens and incubate at room temperature for 30 min.

(NB water is used to eliminate the residual salts left by the buffers. Otherwise they usually crystallise on the specimen upon storage in ethanol.)

● Remove water and replace with ethanol. Keep specimens in the dark in a freezer until mounting.
(NB if the specimens are stored at room temperature under ambient light they will become sun-bleached very quickly, much quicker than unextracted specimens.)

● Use a critical point dryer, hexamethyldisilazane, acetone or amyl acetate to dry the specimens before mounting. Do not air dry as they will collapse.

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Additional file 2: Optimized protocol for library preparation

1. DNA quantification

Qubit® 2.0 Fluorometer (Invitrogen); dsDNA HS Assay Kit.

2. DNA normalization and shearing

Bioruptor ® Pico, sonication bath-based rotor 12 samples in parallel.

Transfer 100µl of input DNA in tubes adapted to the Bioruptor ® Pico that will be used for DNA shearing (Diagenode tubes 0.5ml). [now we shear 30ng DNA in routine but you can use as little as 1.8 ng, see main text]

Set up the following program for DNA shearing to obtain a mean fragment size of ca 400pb:

15sec ON / 90sec OFF for 8 cycles.

You can STOP here for up to 1 week (store tubes in a freezer at -20°C).

The NEBNext Ultra II DNA Library prep kit for Illumina by NEB will be used for End repair, A tailing, Adapter ligation and PCR enrichment of adaptor-ligated DNA.

3. End repair + A tailing

To keep a backup, only 50 µl of the sheared DNA is used [you may want to re-concentrate DNA on beads in 50 µl when you have a small amount i.e. < 5 ng]

Transfer 50 µl of sheared DNA into a strip / plate.

Thaw NEBNext Ultra II End Prep Reaction Buffer. Vortex + pulse spin.

ATTENTION: Pull out NEBNext Ultra II End Prep Enzyme Mix out of freezer just before use. LIGHT vortex + pulse spin.

Master Mix:	x1 reaction	x 96 reactions
NEBNext Ultra II End Prep Enzyme Mix	3 µl	288 µl
NEBNext Ultra II End Prep Reaction Buffer	7 µl	672 µl
Master mix to add to each tube	10 µl	

Vortex master mix gently + pulse spin.

- Add 10µl of master mix to tubes containing 50µl of sheared DNA.
- Vortex (**mix well.)**
- Pulse spin.
- Place in a thermocycler. **Enable heated lid** and run the following program:
  30 min at 20°C
  30 min at 65°C
  Hold at 4°C.

PROCEED WITHOUT DELAY TO ADAPTER LIGATION

4. Adapter ligation and cleanup

See **additional file 3** for adapter sequences and hybridization.

Thaw adapters (1.5µM**)**. Centrifuge to avoid cross contamination. Open carefully.

For 96 samples use the following tagging scheme:

P1-1	P1-9	P1-1	P1-9	P1-1	P1-9	P1-1	P1-9	P1-1	P1-9	P1-1	P1-9
P1-2	P1-10	P1-2	P1-10	P1-2	P1-10	P1-2	P1-10	P1-2	P1-10	P1-2	P1-10
P1-3	P1-11	P1-3	P1-11	P1-3	P1-11	P1-3	P1-11	P1-3	P1-11	P1-3	P1-11
P1-4	P1-12	P1-4	P1-12	P1-4	P1-12	P1-4	P1-12	P1-4	P1-12	P1-4	P1-12
P1-5	P1-13	P1-5	P1-13	P1-5	P1-13	P1-5	P1-13	P1-5	P1-13	P1-5	P1-13
P1-6	P1-14	P1-6	P1-14	P1-6	P1-14	P1-6	P1-14	P1-6	P1-14	P1-6	P1-14
P1-7	P1-15	P1-7	P1-15	P1-7	P1-15	P1-7	P1-15	P1-7	P1-15	P1-7	P1-15
P1-8	P1-16	P1-8	P1-16	P1-8	P1-16	P1-8	P1-16	P1-8	P1-16	P1-8	P1-16
P2-1		P2-2		P2-3		P2-4		P2-5		P2-6	

Thaw NEBNext Ultra II Ligation master mix. Light vortex + Pulse spin.

Thaw NEBNext Ligation enhancer. Light Vortex + pulse spin.

Master Mix:	x1 reaction	x 96 reactions
NEBNext Ultra II Ligation master mix	30 µl	2880 µl
NEBNext Ligation enhancer	1 µl	96 µl
Master mix to add to each tube	31 µl	

Vortex (mix well) + pulse spin

- Add 31 µl of master mix to tubes containing the 60 µl end prep reaction mixture obtained in the previous step.
- Add 1.25 µl of P1 adapters (1.5µM) and 1.25 µl of P2 adapters (1.5 µM) to each tube.
- Vortex **(mix well.)**
- Pulse spin.
- Place in a thermocycler. **Disable heated lid** and run the following program:
  15 min at 20°C.

(NB samples can be stored overnight at -20°C.)

Clean-up adaptor ligated DNA (remove dimers of adapters):

Purification was conducted with the Agencourt AMPure XP Purification system (Beckman Coulter) and a ALPAQUA magnet plate.

Pull Ampure beads out of fridge. Make sure they are at room temperature (RT) before use.

- Vortex Ampure beads.
- Add 0.8 vol. of Ampure beads for 1 vol. of DNA (i.e. 74.8 µl of beads for 93.5 µl of DNA => vol. tot. = 168.3 µl).

- Vortex + pulse spin.
- Incubate for **5min** at RT.
- Move strip to magnetic rack and wait for **2 to 5min**.
- Remove and discard supernatant without disturbing beads.
- Do not remove tubes from magnetic rack and add **200 µl of 70 % ethanol** without disturbing beads (use freshly made 70% ethanol to ensure accurate concentration.)
- Wait for **30s** (leave cap open.)
- Remove and discard supernatant without disturbing beads.
- Add again **200 µl of 70 % ethanol**.
- Wait for **30s** (leave cap open.)
- Remove and discard supernatant without disturbing beads.
- **Make sure to remove all traces of ethanol** (use a 10µl micropipette if necessary.)
- Dry for **5 min** (leave cap open.)
- Do not remove tubes from magnetic rack and add **15µl of buffer EB**.
- Close cap.
- Remove strip from magnetic rack.
- Vortex + pulse spin.
- Wait for **2min**.
- Move strip back to magnetic rack and wait for **2 to 5min**.
- Transfer the **15µl** of DNA to a new strip.

5. PCR enrichment of adaptor-ligated DNA

Thaw NEBNext Ultra II Q5 Master Mix. Homogenize by pipetting + Pulse spin. DO NOT VORTEX. Leave on Ice.

Master Mix:	x 1 reaction	x 96 reactions
NEBNext Ultra II Q5 Master Mix	25 µl	2400 µl
Primer F (10µM)	2 µl	192 µl
Primer R (10µM)	2 µl	192 µl
Master mix to add to each tube	29 µl	

With
Primer F = AATGATACGGCGACCACCG*A
Primer R = CAAGCAGAAGACGGCATACG*A
(synthesized by IDT (Integrated DNA Technologies, Inc.), HPLC Purification, * = Phosphorothioate Bond.)

Vortex + pulse spin master mix

- Add 29 µl of master mix to tubes containing the 15 µl of adaptor ligated DNA.
- Vortex.
- Pulse spin.
- Place in a thermocycler. **Enable heated lid** and run the following program:

		15 cycles			
Time	30s	10s	75s	5min	∞
Temperature	98°C	98°C	65°C	65°C	4°C

6. Quantification, equimolar pooling and re-concentration

Qubit - quantify 2 µl of each library.

In our experience, a clean-up step of the librairies is not necessary.

Prepare 6 pools of 16 samples in 1.5ml LoBind tubes.

Pool 100 ng of each library (you want a final quantity of 100-500ng DNA per pool after concentration to fit with the requirements of the MYbaits® protocol.)

On beads Concentration of DNA (Agencourt AMPure XP Purification system (Beckman Coulter)). Concentration of DNA is required, as MyBaits require 7 µl of starting material.

Remove Ampure beads from fridge. Make sure they are at room temperature (RT) before use.

- Vortex Ampure beads.
- Add 0.8 vol. of Ampure beads for 1 vol. of DNA.
- Vortex + pulse spin.
- Incubate for **5min** at RT.
- Move tubes to magnetic rack and wait for **2 to 5 min**.
- Remove and discard supernatant without disturbing beads.
- Do not remove tubes from magnetic rack and add **200 µl of 70 % ethanol** without disturbing beads (use freshly made 70% EtOH to ensure accurate concentration.)
- Wait for **30s** (leave cap open.)
- Remove and discard supernatant without disturbing beads.
- Add again **200 µl of 70 % ethanol**.
- Wait for **30s** (leave cap open.)
- Remove and discard supernatant without disturbing beads.
- **Make sure to remove all traces of Ethanol** (use a 10µl micropipette if necessary.)
- Dry for **5 min** (leave cap open.)
- Do not remove tubes from magnetic rack and add **9µl** of buffer EB.
- Close cap.
- Remove tubes from magnetic rack.
- Vortex + spin pulse.
- Wait for **2min**.
- Move strip back to magnetic rack and wait for **2 to 5min**.
- Transfer the **9µl** of DNA to a new 1.5ml LoBind tube.

Qubit quantify 1 µl of each library (dilution 1:2).

Check library profile on an Agilent Bioanalyzer with a High Sensitivity DNA Analysis Kit (load 1µl of library dilution 1:2).

7. Capture of UCEs

UCEs were captured on each pool using the probes designed by Faircloth et al. (2015) and synthetisized by MYbaits®. Capture was performed with the MYbaits® kit following manufacturer's instruction (http://www.mycroarray.com/pdf/MYbaits-manual-v3.pdf).

8. PCR Enrichment of captured libraries and final clean-up

At this step of the process we have obtained libraries and Streptavidin beads mixed in 30 µl of 10mM Tris-Cl, 0.05% TWEEN®-20 solution (pH 8.0 – 8.5).

Here we want to release the bead-bound UCE-enriched library from the baits and amplif the resulting fragments.

PCR enrichment of the captured fragment is performed on beads with the following mix.
We performed 2 PCR reactions for each pool of samples.

Post-capture PCR Master Mix:	Final concentration	x1
H2O up	-	5µl
2X KAPA Hifi HotStart ReadyMix	1X	25µl
Primer F (10µM)	500nM	2.5µl
Primer R (10µM)	500nM	2.5µl
Enriched Library (on-bead)	-	15µl
Master mix to add to each well		50µl

With
Primer F = AATGATACGGCGACCACCG*A
Primer R = CAAGCAGAAGACGGCATACG*A
(synthesized by IDT (Integrated DNA Technologies, Inc.), HPLC Purification, * = Phosphorothioate Bond)

Program:		14 cycles				
Step	Activation	Denaturation	Annealing	Extension	Final Extension	End
Time	2min	20sec	30sec	45sec	5min	∞
Temperature	98°C	98°C	65°C	72°C	72°C	10°C

Remove Streptavidin beads:

Pool the 2 PCR products obtained for each pool of samples in DNA LoBind tubes (1.5ml.)
Move the 6 tubes to a magnetic rack and wait for 2 to 5min.
Transfer supernatant to new tubes.

Final Cleanup (Agencourt AMPure XP Purification system (Beckman Coulter)

Remove Ampure beads from fridge. Make sure they are at room temperature (RT) before use.

- Vortex Ampure beads.
- Add 1V of Ampure beads for 1V of DNA (ie 100 µl of beads for 100 µl of DNA.)
- Vortex + pulse spin.
- Incubate for **5min** at RT.
- Move tubes to magnetic rack and wait for **2 to 5min**.
- Remove and discard supernatant without disturbing beads.

- Do not remove tubes from magnetic rack and add **200 µl of 70 % ethanol** without disturbing beads (use freshly made 70% EtOH to ensure accurate concentration.)
- Wait for **30s** (leave cap open.)
- Remove and discard supernatant without disturbing beads.
- Add again **200 µl of 70 % ethanol**.
- Wait for **30s** (leave cap open.)
- Remove and discard supernatant without disturbing beads.
- **Make sure to remove all traces of ethanol** (use a 10µl micropipette if necessary.)
- Dry for **5 min** (leave cap open.)
- Do not remove tubes from magnetic rack and add **25µl of buffer EB**.
- Close cap.
- Remove tubes from magnetic rack.
- Vortex + pulse spin.
- Wait for **2min**.
- Move strip back to magnetic rack and wait for **2 to 5min**.
- Transfer the **25µl** of DNA to new LoBind tubes.

9. Final quantification and equimolar pooling prior to sequencing

- Qubit Quantify 2µl of each.
- Check library profile on an Agilent Bioanalyzer with a High Sensitivity DNA Analysis Kit (1µl undiluted library).
- Perform KAPA quantification.
- Equimolar pooling of the 6 librairies.

References cited:

Faircloth, B.C., Branstetter, M.G., White, N.D., S.G., B., 2015. Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. Molecular Ecology Resources 15, 489-501.

MYbaits® kit, Instruction manual: http://www.mycroarray.com/pdf/MYbaits-manual-v3.pdf

NEBNext® Ultra™ DNA Library Prep Kit for Illumina®, Instruction manual: https://www.neb.com/-/media/catalog/Datacards%20or%20Manuals/manualE7370.pdf

Additional file 3

Adapters (Ultramer Oligos, TruGrade synthesis, Standard Desalting) were synthesized by IDT (Integrated DNA Technologies, Inc.) /5Phos/ = 5' Phosphorylation; * = Phosphorothioate Bond, F=forward, R=reverse).

Adapter were hybridized as follows:

25µM oligo preparation:

- Thaw 100µM oligos. Vortex and spin well.
- Pool each pair of oligos (F/R) in DNA LoBind tubes (1.5ml) as follows:

	Initial Concentration	Final Concentration
P1_F or P2_F	100µM	25µM
P1_R or P2_R	100µM	25µM
AB Buffer	10X	1X
Ultra Pure H2O		QS 100µl

- Vortex and spin well the oligo's pools and split each pool in tow PCR tubes (0.2ml)

Adapters hybridization:

- Place the PCR tubes in a thermocycler:

Program:		15 cycles (with -1.7°C/cycle)	40 cycles (with -1°C/cycle)	
Time	2min	1min	1min	∞
Temperature	95°C	95°C	69°C	10°C

- Vortex and spin well the tubes.
- Qubit quantify 2µl of diluted adapters (1/20e.)
- Adjust adapters concentration to 1.5µM if necessary and make aliquots.
- Store the aliquots at -20°C.

Adapters (1.5µM) were stored in tube strips for usage comfort.

#id	barcode	sequence
P1-1_F	AAGTG	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGTG*T
P1-2_F	ACAAT	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTACAAT*T
P1-3_F	CTAGA	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCTAGA*T
P1-4_F	GATAG	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATAG*T
P1-5_F	GCCTC	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCTC*T
P1-6_F	TGTGT	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTGT*T
P1-7_F	GTATT	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTATT*T
P1-8_F	CGGAC	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGGAC*T
P1-9_F	AAACAA	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAAACAA*T
P1-10_F	ACGTCA	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTACGTCA*T
P1-11_F	CGTCTC	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGTCTC*T
P1-12_F	CCCAAA	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCCCAAA*T
P1-13_F	GTTCGG	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTCGG*T
P1-14_F	TGCCCA	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCCCA*T
P1-15_F	TTGGGC	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGGGC*T
P1-16_F	TACGAG	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTACGAG*T
P1-1_R	AAGTG	/5Phos/CACTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-2_R	ACAAT	/5Phos/ATTGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-3_R	CTAGA	/5Phos/TCTAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-4_R	GATAG	/5Phos/CTATCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-5_R	GCCTC	/5Phos/GAGGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-6_R	TGTGT	/5Phos/ACACAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-7_R	GTATT	/5Phos/AATACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-8_R	CGGAC	/5Phos/GTCCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-9_R	AAACAA	/5Phos/TTGTTTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T

P1-10_R	ACGTCA	/5Phos/TGACGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-11_R	CGTCTC	/5Phos/GAGACGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-12_R	CCCAAA	/5Phos/TTTGGGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-13_R	GTTCGG	/5Phos/CCGAACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-14_R	TGCCCA	/5Phos/TGGGCAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-15_R	TTGGGC	/5Phos/GCCCAAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P1-16_R	TACGAG	/5Phos/CTCGTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT*T
P2-1_R	ACCTA	CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTACCTA*T
P2-2_R	TAGGTC	CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTTAGGTC*T
P2-3_R	CGTAC	CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTCGTAC*T
P2-4_R	GTACGA	CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTGTACGA*T
P2-5_R	AGTCA	CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTAGTCA*T
P2-6_R	GACTCG	CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCTGACTCG*T
P2-1_F	ACCTA	/5Phos/TAGGTAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCAGAACAA
P2-2_F	TAGGTC	/5Phos/GACCTAAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCAGAACAA
P2-3_F	CGTAC	/5Phos/GTACGAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCAGAACAA
P2-4_F	GTACGA	/5Phos/TCGTACAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCAGAACAA
P2-5_F	AGTCA	/5Phos/TGACTAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCAGAACAA
P2-6_F	GACTCG	/5Phos/CGAGTCAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCGATCAGAACAA

Additional file 4

```
#scripts tested on a Linux ditribution (Debian/Ubuntu)

#===============
#demultiplexing
#===============

#you must create a barcode.txt file in which you store the barcodes and the sample codes
#see example below (field separator = tab)
#column1 = sample code (avoid special characters except underscore)
#column2 = barcode P1 (forward read) + T (complement of the A added after shearing) [see additional file 2 for barcode sequences]
#column3 = barcode P2 (reverse read) + T (complement of the A added after shearing)
#column4 = reverse complement of P2-barcode (required for demultiplexing)
#column5 = term (required by the script)


sample  barcode_P1     barcode_P2     barcode_P2_rev term

JRAS07752_0101 AAGTGT  ACCTAT  ATAGGT  term

GDEL06009_0101 ACAATT  ACCTAT  ATAGGT  term

JRAS07754_0101 CTAGAT  ACCTAT  ATAGGT  term

JRAS07766_0101 GATAGT  ACCTAT  ATAGGT  term

GDEL07010_0101 GCCTCT  ACCTAT  ATAGGT  term


#ATTENTION IF YOU HAVE CREATED YOUR barcode.txt FILE IN YOUR PERSONNAL COMPUTER (e.g. excel ...)
#Remove potential troublesome end of lines characters before used

mac2unix barcodes.txt          #this will change format from Mac native to full UNIX
dos2unix barcodes.txt          #this will change format from Windows to UNIX
sed -i '/^$/d' barcodes.txt    #this will remove extra spaces introduced by Mac


# A perfect match between the barcode sequence and the read is required to keep the read

#cd to datadir (in which the reads merged by FLASH are stored)

#use the barcodes.txt file to generate grep commands to find occurrences of barcodes at the beginning and end of each reads
#and redirect output to fastq files named after your sample codes

awk 'BEGIN{FS="\t";RS="\n"} {if (NR>1){print "grep -B 1 -i \047^"$2"\.*"$4"\$\047 input.extendedFrags.fastq > "$1"_fq"}}'
/AbsolutePathTo/barcodes.txt |bash

# remove barcodes from beginning / end of reads

awk 'BEGIN{FS="\t";RS="\n"} {if (NR>1){print "sed -i \047s/^"$2"//g\;s/"$4"\$//g\047 "$1"_fq"}}' /AbsolutePathTo/barcodes.txt |bash
```

Additional file 5

>locus0001
CGAAACTCGCAAGTGCAGAAATTTGATAGAGGTTAGTATATAAGACCGCGAGTCGAGGAGTTATTTTGGAAGTGTGTGCT
AGTGATGGCACAGCTGAACATTGATCGCTAACTAGTAGATATATGGCAGATCTGCTCAGTGGGTGTTGTCACGTGGTCGC
TTCAAGTAGCTTGTGCTCGGCTCCCGATGCGCCTACAAACCGAGGCCTGTCTCGTGGCCCCTGGCACCTGTAGGCCCGCA
CGGTCGAGTCTTCACGTCGCTCCTCGAACTACCGCGATTTCCAAATTGGGGGGCAATCGAATAGGTCAGACGAGGTAATT
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Additional file 6

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locus0003	uce-1366_p1	uce-1366_p2				
locus0004	uce-1022_p1	uce-1022_p2				
locus0005	uce-1019_p1	uce-1019_p2				
locus0006	uce-1015_p1	uce-1015_p2				
locus0007	uce-1013_p1	uce-1013_p2				
locus0008	uce-1008_p1	uce-1008_p2				
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locus0158	uce-1257_p1	uce-1257_p2
locus0159	uce-237_p1	uce-237_p2
locus0160	uce-235_p1	uce-235_p2
locus0161	uce-233_p1	uce-233_p2
locus0162	uce-228_p1	uce-228_p2
locus0163	uce-217_p1	uce-217_p2
locus0164	uce-200_p1	uce-200_p2
locus0165	uce-199_p1	uce-199_p2
locus0166	uce-182_p1	uce-182_p2
locus0167	uce-180_p1	uce-180_p2

locus0168	uce-170_p1	uce-170_p2
locus0169	uce-1249_p1	uce-1249_p2
locus0170	uce-157_p1	uce-157_p2
locus0171	uce-148_p1	uce-148_p2
locus0172	uce-143_p1	uce-143_p2
locus0173	uce-139_p1	uce-139_p2
locus0174	uce-115_p1	uce-115_p2
locus0175	uce-110_p1	uce-110_p2
locus0176	uce-104_p1	uce-104_p2
locus0177	uce-98_p1	uce-98_p2
locus0178	uce-87_p1	uce-87_p2
locus0179	uce-86_p1	uce-86_p2
locus0180	uce-1248_p1	uce-1248_p2
locus0181	uce-79_p1	uce-79_p2
locus0182	uce-78_p1	uce-78_p2
locus0183	uce-77_p1	uce-77_p2
locus0184	uce-75_p1	uce-75_p2
locus0185	uce-74_p1	uce-74_p2
locus0186	uce-68_p1	uce-68_p2
locus0187	uce-67_p1	uce-67_p2
locus0188	uce-64_p1	uce-64_p2
locus0189	uce-59_p1	uce-59_p2
locus0190	uce-54_p1	uce-54_p2
locus0191	uce-1244_p1	uce-1244_p2
locus0192	uce-49_p1	uce-49_p2
locus0193	uce-44_p1	uce-44_p2
locus0194	uce-43_p1	uce-43_p2
locus0195	uce-41_p1	uce-41_p2
locus0196	uce-34_p1	uce-34_p2
locus0197	uce-29_p1	uce-29_p2
locus0198	uce-11_p1	uce-11_p2
locus0199	uce-9_p1	uce-9_p2
locus0200	uce-1387_p1	uce-1387_p2
locus0201	uce-666_p1	uce-666_p2

locus0202	uce-1243_p1	uce-1243_p2		
locus0203	uce-1080_p1	uce-1080_p2		
locus0204				
locus0205	uce-1237_p1	uce-1237_p2		
locus0206	uce-456_p1	uce-456_p2	uce-457_p1	uce-457_p2
locus0207	uce-1235_p1	uce-1235_p2		
locus0208	uce-1233_p1	uce-1233_p2		
locus0209	uce-1232_p1	uce-1232_p2		
locus0210	uce-1225_p1	uce-1225_p2		
locus0211	uce-1213_p1	uce-1213_p2		
locus0212	uce-1212_p1	uce-1212_p2		
locus0213	uce-1211_p1	uce-1211_p2		
locus0214	uce-1200_p1	uce-1200_p2		
locus0215	uce-1188_p1	uce-1188_p2		
locus0216	uce-1182_p1	uce-1182_p2		
locus0217	uce-1164_p1	uce-1164_p2	uce-1165_p1	uce-1165_p2
locus0218	uce-1181_p1	uce-1181_p2		
locus0219	uce-1160_p1	uce-1160_p2		
locus0220	uce-1151_p1	uce-1151_p2		
locus0221	uce-1149_p1	uce-1149_p2		
locus0222	uce-1147_p1	uce-1147_p2		
locus0223	uce-1146_p1	uce-1146_p2		
locus0224	uce-1134_p1	uce-1134_p2		
locus0225	uce-1131_p1	uce-1131_p2		
locus0226	uce-1128_p1	uce-1128_p2		
locus0227	uce-1124_p1	uce-1124_p2		
locus0228	uce-419_p1	uce-419_p2	uce-420_p1	uce-420_p2
locus0229	uce-1110_p1	uce-1110_p2		
locus0230	uce-1099_p1	uce-1099_p2		
locus0231	uce-1088_p1	uce-1088_p2		
locus0232	uce-1074_p1	uce-1074_p2		
locus0233	uce-1066_p1	uce-1066_p2		
locus0234	uce-1065_p1	uce-1065_p2		
locus0235	uce-1052_p1	uce-1052_p2		

locus0236	uce-1051_p1	uce-1051_p2		
locus0237	uce-1050_p1	uce-1050_p2		
locus0238	uce-1049_p1	uce-1049_p2		
locus0239	uce-1115_p1	uce-1115_p2	uce-1116_p1	uce-1116_p2
locus0240	uce-1036_p1	uce-1036_p2		
locus0241	uce-1029_p1	uce-1029_p2		
locus0242	uce-1025_p1	uce-1025_p2		
locus0243	uce-996_p1	uce-996_p2		
locus0244	uce-991_p1	uce-991_p2		
locus0245	uce-990_p1	uce-990_p2		
locus0246	uce-988_p1	uce-988_p2		
locus0247	uce-987_p1	uce-987_p2		
locus0248	uce-976_p1	uce-976_p2		
locus0249	uce-975_p1	uce-975_p2		
locus0250	uce-311_p1	uce-311_p2	uce-312_p1	uce-312_p2
locus0251	uce-962_p1	uce-962_p2		
locus0252	uce-960_p1	uce-960_p2		
locus0253	uce-958_p1	uce-958_p2		
locus0254	uce-955_p1	uce-955_p2		
locus0255	uce-948_p1	uce-948_p2		
locus0256	uce-942_p1	uce-942_p2		
locus0257	uce-937_p1	uce-937_p2		
locus0258	uce-930_p1	uce-930_p2		
locus0259	uce-920_p1	uce-920_p2		
locus0260	uce-914_p1	uce-914_p2		
locus0261	uce-1100_p1	uce-1100_p2	uce-1101_p1	uce-1101_p2
locus0262	uce-907_p1	uce-907_p2		
locus0263	uce-906_p1	uce-906_p2		
locus0264	uce-905_p1	uce-905_p2		
locus0265	uce-901_p1	uce-901_p2		
locus0266	uce-897_p1	uce-897_p2		
locus0267	uce-892_p1	uce-892_p2		
locus0268	uce-877_p1	uce-877_p2		
locus0269	uce-866_p1	uce-866_p2		

locus0270	uce-864_p1	uce-864_p2				
locus0271	uce-858_p1	uce-858_p2				
locus0272	uce-1310_p1	uce-1310_p2	uce-1311_p1	uce-1311_p2		
locus0273	uce-852_p1	uce-852_p2				
locus0274	uce-851_p1	uce-851_p2				
locus0275	uce-848_p1	uce-848_p2				
locus0276	uce-846_p1	uce-846_p2				
locus0277	uce-836_p1	uce-836_p2				
locus0278	uce-835_p1	uce-835_p2				
locus0279	uce-831_p1	uce-831_p2				
locus0280	uce-828_p1	uce-828_p2				
locus0281	uce-823_p1	uce-823_p2				
locus0282	uce-820_p1	uce-820_p2				
locus0283	uce-83_p1	uce-83_p2	uce-84_p1	uce-84_p2		
locus0284	uce-816_p1	uce-816_p2				
locus0285	uce-812_p1	uce-812_p2				
locus0286	uce-787_p1	uce-787_p2				
locus0287	uce-778_p1	uce-778_p2				
locus0288	uce-764_p1	uce-764_p2				
locus0289	uce-751_p1	uce-751_p2				
locus0290	uce-750_p1	uce-750_p2				
locus0291	uce-745_p1	uce-745_p2				
locus0292	uce-739_p1	uce-739_p2				
locus0293	uce-720_p1	uce-720_p2				
locus0294	uce-729_p1	uce-729_p2	uce-730_p1	uce-730_p2	uce-731_p1	uce-731_p2
locus0295	uce-970_p1	uce-970_p2	uce-971_p1	uce-971_p2		
locus0296	uce-719_p1	uce-719_p2				
locus0297	uce-710_p1	uce-710_p2				
locus0298	uce-707_p1	uce-707_p2				
locus0299	uce-702_p1	uce-702_p2				
locus0300	uce-690_p1	uce-690_p2				
locus0301	uce-661_p1	uce-661_p2				
locus0302	uce-654_p1	uce-654_p2				
locus0303	uce-650_p1	uce-650_p2				

locus0304	uce-649_p1	uce-649_p2		
locus0305	uce-644_p1	uce-644_p2		
locus0306	uce-164_p1	uce-164_p2	uce-165_p1	uce-165_p2
locus0307	uce-643_p1	uce-643_p2		
locus0308	uce-638_p1	uce-638_p2		
locus0309	uce-615_p1	uce-615_p2		
locus0310	uce-614_p1	uce-614_p2		
locus0311	uce-607_p1	uce-607_p2		
locus0312	uce-601_p1	uce-601_p2		
locus0313	uce-594_p1	uce-594_p2		
locus0314	uce-589_p1	uce-589_p2		
locus0315	uce-582_p1	uce-582_p2		
locus0316	uce-577_p1	uce-577_p2		
locus0317	uce-890_p1	uce-890_p2	uce-891_p1	uce-891_p2
locus0318	uce-576_p1	uce-576_p2		
locus0319	uce-554_p1	uce-554_p2		
locus0320	uce-548_p1	uce-548_p2		
locus0321	uce-542_p1	uce-542_p2		
locus0322	uce-541_p1	uce-541_p2		
locus0323	uce-537_p1	uce-537_p2		
locus0324	uce-535_p1	uce-535_p2		
locus0325	uce-522_p1	uce-522_p2		
locus0326	uce-512_p1	uce-512_p2		
locus0327	uce-507_p1	uce-507_p2		
locus0328	uce-972_p1	uce-972_p2	uce-973_p1	uce-973_p2
locus0329	uce-504_p1	uce-504_p2		
locus0330	uce-501_p1	uce-501_p2		
locus0331	uce-496_p1	uce-496_p2		
locus0332	uce-495_p1	uce-495_p2		
locus0333	uce-488_p1	uce-488_p2		
locus0334	uce-486_p1	uce-486_p2		
locus0335	uce-476_p1	uce-476_p2		
locus0336	uce-460_p1	uce-460_p2		
locus0337	uce-455_p1	uce-455_p2		

locus0338	uce-449_p1	uce-449_p2		
locus0339	uce-283_p1	uce-283_p2	uce-284_p1	uce-284_p2
locus0340	uce-446_p1	uce-446_p2		
locus0341	uce-437_p1	uce-437_p2		
locus0342	uce-432_p1	uce-432_p2		
locus0343	uce-427_p1	uce-427_p2		
locus0344	uce-407_p1	uce-407_p2		
locus0345	uce-395_p1	uce-395_p2		
locus0346	uce-394_p1	uce-394_p2		
locus0347	uce-393_p1	uce-393_p2		
locus0348	uce-392_p1	uce-392_p2		
locus0349	uce-390_p1	uce-390_p2		
locus0350	uce-209_p1	uce-209_p2	uce-210_p1	uce-210_p2
locus0351	uce-379_p1	uce-379_p2		
locus0352	uce-374_p1	uce-374_p2		
locus0353	uce-367_p1	uce-367_p2		
locus0354	uce-366_p1	uce-366_p2		
locus0355	uce-360_p1	uce-360_p2		
locus0356	uce-359_p1	uce-359_p2		
locus0357	uce-342_p1	uce-342_p2		
locus0358	uce-334_p1	uce-334_p2		
locus0359	uce-329_p1	uce-329_p2		
locus0360	uce-327_p1	uce-327_p2		
locus0361	uce-1455_p1	uce-1455_p2	uce-1456_p1	uce-1456_p2
locus0362	uce-307_p1	uce-307_p2		
locus0363	uce-303_p1	uce-303_p2		
locus0364	uce-301_p1	uce-301_p2		
locus0365	uce-299_p1	uce-299_p2		
locus0366	uce-297_p1	uce-297_p2		
locus0367	uce-291_p1	uce-291_p2		
locus0368	uce-278_p1	uce-278_p2		
locus0369	uce-267_p1	uce-267_p2		
locus0370	uce-265_p1	uce-265_p2		
locus0371	uce-258_p1	uce-258_p2		

locus0372	uce-1389_p1	uce-1389_p2	uce-1390_p1	uce-1390_p2		
locus0373	uce-257_p1	uce-257_p2				
locus0374	uce-253_p1	uce-253_p2				
locus0375	uce-251_p1	uce-251_p2				
locus0376	uce-250_p1	uce-250_p2				
locus0377	uce-236_p1	uce-236_p2				
locus0378	uce-219_p1	uce-219_p2				
locus0379	uce-213_p1	uce-213_p2				
locus0380	uce-212_p1	uce-212_p2				
locus0381	uce-195_p1	uce-195_p2				
locus0382	uce-189_p1	uce-189_p2				
locus0383	uce-80_p1	uce-80_p2	uce-81_p1	uce-81_p2		
locus0384	uce-185_p1	uce-185_p2				
locus0385	uce-173_p1	uce-173_p2				
locus0386	uce-171_p1	uce-171_p2				
locus0387	uce-162_p1	uce-162_p2				
locus0388	uce-155_p1	uce-155_p2				
locus0389	uce-150_p1	uce-150_p2				
locus0390	uce-144_p1	uce-144_p2				
locus0391	uce-136_p1	uce-136_p2				
locus0392	uce-132_p1	uce-132_p2				
locus0393	uce-131_p1	uce-131_p2				
locus0394	uce-1153_p1	uce-1153_p2	uce-1154_p1	uce-1154_p2		
locus0395	uce-122_p1	uce-122_p2				
locus0396	uce-121_p1	uce-121_p2				
locus0397	uce-117_p1	uce-117_p2				
locus0398	uce-116_p1	uce-116_p2				
locus0399	uce-114_p1	uce-114_p2				
locus0400	uce-108_p1	uce-108_p2				
locus0401	uce-100_p1	uce-100_p2				
locus0402	uce-99_p1	uce-99_p2				
locus0403	uce-96_p1	uce-96_p2				
locus0404	uce-60_p1	uce-60_p2				
locus0405	uce-755_p1	uce-755_p2	uce-756_p1	uce-756_p2	uce-757_p1	uce-757_p2

locus0406	uce-247_p1	uce-247_p2	uce-248_p1	uce-248_p2
locus0407	uce-55_p1	uce-55_p2		
locus0408	uce-53_p1	uce-53_p2		
locus0409	uce-48_p1	uce-48_p2		
locus0410	uce-46_p1	uce-46_p2		
locus0411	uce-38_p1	uce-38_p2		
locus0412	uce-35_p1	uce-35_p2		
locus0413	uce-33_p1	uce-33_p2		
locus0414	uce-27_p1	uce-27_p2		
locus0415	uce-21_p1	uce-21_p2		
locus0416	uce-20_p1	uce-20_p2		
locus0417	uce-281_p1	uce-281_p2	uce-282_p1	uce-282_p2
locus0418	uce-18_p1	uce-18_p2		
locus0419	uce-17_p1	uce-17_p2		
locus0420	uce-10_p1	uce-10_p2		
locus0421	uce-6_p1	uce-6_p2		
locus0422	uce-5_p1	uce-5_p2		
locus0423	uce-3_p1	uce-3_p2		
locus0424	uce-225_p1	uce-225_p2		
locus0425	uce-2_p1	uce-2_p2		
locus0426	uce-1508_p1	uce-1508_p2		
locus0427	uce-1500_p1	uce-1500_p2		
locus0428	uce-1255_p1	uce-1255_p2	uce-1256_p1	uce-1256_p2
locus0429	uce-1492_p1	uce-1492_p2		
locus0430	uce-1487_p1	uce-1487_p2		
locus0431	uce-1485_p1	uce-1485_p2		
locus0432	uce-1481_p1	uce-1481_p2		
locus0433	uce-1475_p1	uce-1475_p2		
locus0434	uce-1468_p1	uce-1468_p2		
locus0435	uce-1466_p1	uce-1466_p2		
locus0436	uce-1465_p1	uce-1465_p2		
locus0437	uce-1462_p1	uce-1462_p2		
locus0438	uce-1461_p1	uce-1461_p2		
locus0439	uce-1270_p1	uce-1270_p2	uce-1271_p1	uce-1271_p2

locus0440	uce-1460_p1	uce-1460_p2		
locus0441	uce-1446_p1	uce-1446_p2		
locus0442	uce-1443_p1	uce-1443_p2		
locus0443	uce-1429_p1	uce-1429_p2		
locus0444	uce-1410_p1	uce-1410_p2		
locus0445	uce-1409_p1	uce-1409_p2		
locus0446	uce-1407_p1	uce-1407_p2		
locus0447	uce-1378_p1	uce-1378_p2		
locus0448	uce-1376_p1	uce-1376_p2		
locus0449	uce-1372_p1	uce-1372_p2		
locus0450	uce-667_p1	uce-667_p2	uce-668_p1	uce-668_p2
locus0451	uce-1352_p1	uce-1352_p2		
locus0452	uce-1351_p1	uce-1351_p2		
locus0453	uce-1350_p1	uce-1350_p2		
locus0454	uce-1348_p1	uce-1348_p2		
locus0455	uce-1346_p1	uce-1346_p2		
locus0456	uce-1335_p1	uce-1335_p2		
locus0457	uce-1333_p1	uce-1333_p2		
locus0458	uce-1329_p1	uce-1329_p2		
locus0459	uce-1326_p1	uce-1326_p2		
locus0460	uce-1319_p1	uce-1319_p2		
locus0461	uce-417_p1	uce-417_p2	uce-418_p1	uce-418_p2
locus0462	uce-1318_p1	uce-1318_p2		
locus0463	uce-1315_p1	uce-1315_p2		
locus0464	uce-1307_p1	uce-1307_p2		
locus0465	uce-1296_p1	uce-1296_p2		
locus0466	uce-1286_p1	uce-1286_p2		
locus0467	uce-1284_p1	uce-1284_p2		
locus0468	uce-1282_p1	uce-1282_p2		
locus0469	uce-1281_p1	uce-1281_p2		
locus0470	uce-1280_p1	uce-1280_p2		
locus0471	uce-1279_p1	uce-1279_p2		
locus0472	uce-61_p1	uce-61_p2	uce-62_p1	uce-62_p2
locus0473	uce-1253_p1	uce-1253_p2		

locus0474	uce-1252_p1	uce-1252_p2		
locus0475	uce-1247_p1	uce-1247_p2		
locus0476	uce-1242_p1	uce-1242_p2		
locus0477	uce-1240_p1	uce-1240_p2		
locus0478	uce-1239_p1	uce-1239_p2		
locus0479	uce-1231_p1	uce-1231_p2		
locus0480	uce-1228_p1	uce-1228_p2		
locus0481	uce-1220_p1	uce-1220_p2		
locus0482	uce-1218_p1	uce-1218_p2		
locus0483	uce-439_p1	uce-439_p2	uce-440_p1	uce-440_p2
locus0484	uce-1217_p1	uce-1217_p2		
locus0485	uce-1215_p1	uce-1215_p2		
locus0486	uce-1214_p1	uce-1214_p2		
locus0487	uce-1203_p1	uce-1203_p2		
locus0488	uce-1201_p1	uce-1201_p2		
locus0489	uce-1199_p1	uce-1199_p2		
locus0490	uce-1196_p1	uce-1196_p2		
locus0491	uce-1193_p1	uce-1193_p2		
locus0492	uce-1184_p1	uce-1184_p2		
locus0493	uce-1175_p1	uce-1175_p2		
locus0494	uce-444_p1	uce-444_p2	uce-445_p1	uce-445_p2
locus0495	uce-1169_p1	uce-1169_p2		
locus0496	uce-1163_p1	uce-1163_p2		
locus0497	uce-1162_p1	uce-1162_p2		
locus0498	uce-1148_p1	uce-1148_p2		
locus0499	uce-1144_p1	uce-1144_p2		
locus0500	uce-1136_p1	uce-1136_p2		
locus0501	uce-1133_p1	uce-1133_p2		
locus0502	uce-1127_p1	uce-1127_p2		
locus0503	uce-1121_p1	uce-1121_p2		
locus0504	uce-1120_p1	uce-1120_p2		
locus0505	uce-1470_p1	uce-1470_p2	uce-1471_p1	uce-1471_p2
locus0506	uce-1119_p1	uce-1119_p2		
locus0507	uce-1111_p1	uce-1111_p2		

locus0508	uce-1106_p1	uce-1106_p2				
locus0509	uce-1104_p1	uce-1104_p2				
locus0510	uce-1095_p1	uce-1095_p2				
locus0511	uce-1094_p1	uce-1094_p2				
locus0512	uce-1093_p1	uce-1093_p2				
locus0513	uce-1083_p1	uce-1083_p2				
locus0514	uce-1077_p1	uce-1077_p2				
locus0515	uce-1076_p1	uce-1076_p2				
locus0516	uce-1412_p1	uce-1412_p2	uce-1413_p1	uce-1413_p2	uce-1414_p1	uce-1414_p2
locus0517	uce-1000_p1	uce-1000_p2	uce-1001_p1	uce-1001_p2		
locus0518	uce-1063_p1	uce-1063_p2				
locus0519	uce-1061_p1	uce-1061_p2				
locus0520	uce-1058_p1	uce-1058_p2				
locus0521	uce-1053_p1	uce-1053_p2				
locus0522	uce-1048_p1	uce-1048_p2				
locus0523	uce-1037_p1	uce-1037_p2				
locus0524	uce-1034_p1	uce-1034_p2				
locus0525	uce-1031_p1	uce-1031_p2				
locus0526	uce-1030_p1	uce-1030_p2				
locus0527	uce-1026_p1	uce-1026_p2				
locus0528	uce-1043_p1	uce-1043_p2	uce-1044_p1	uce-1044_p2		
locus0529	uce-1024_p1	uce-1024_p2				
locus0530	uce-1020_p1	uce-1020_p2				
locus0531	uce-1016_p1	uce-1016_p2				
locus0532	uce-1012_p1	uce-1012_p2				
locus0533	uce-1010_p1	uce-1010_p2				
locus0534	uce-998_p1	uce-998_p2				
locus0535	uce-993_p1	uce-993_p2				
locus0536	uce-989_p1	uce-989_p2				
locus0537	uce-985_p1	uce-985_p2				
locus0538	uce-984_p1	uce-984_p2				
locus0539	uce-286_p1	uce-286_p2	uce-287_p1	uce-287_p2		
locus0540	uce-978_p1	uce-978_p2				
locus0541	uce-963_p1	uce-963_p2				

locus0542	uce-956_p1	uce-956_p2		
locus0543	uce-952_p1	uce-952_p2		
locus0544	uce-950_p1	uce-950_p2		
locus0545	uce-949_p1	uce-949_p2		
locus0546	uce-941_p1	uce-941_p2		
locus0547	uce-940_p1	uce-940_p2		
locus0548	uce-936_p1	uce-936_p2		
locus0549	uce-934_p1	uce-934_p2		
locus0550	uce-703_p1	uce-703_p2	uce-704_p1	uce-704_p2
locus0551	uce-925_p1	uce-925_p2		
locus0552	uce-923_p1	uce-923_p2		
locus0553	uce-918_p1	uce-918_p2		
locus0554	uce-915_p1	uce-915_p2		
locus0555	uce-899_p1	uce-899_p2		
locus0556	uce-898_p1	uce-898_p2		
locus0557	uce-886_p1	uce-886_p2		
locus0558	uce-875_p1	uce-875_p2		
locus0559	uce-873_p1	uce-873_p2		
locus0560	uce-870_p1	uce-870_p2		
locus0561	uce-1039_p1	uce-1039_p2	uce-1040_p1	uce-1040_p2
locus0562	uce-856_p1	uce-856_p2		
locus0563	uce-850_p1	uce-850_p2		
locus0564	uce-849_p1	uce-849_p2		
locus0565	uce-837_p1	uce-837_p2		
locus0566	uce-833_p1	uce-833_p2		
locus0567	uce-830_p1	uce-830_p2		
locus0568	uce-824_p1	uce-824_p2		
locus0569	uce-819_p1	uce-819_p2		
locus0570	uce-811_p1	uce-811_p2		
locus0571	uce-803_p1	uce-803_p2		
locus0572	uce-622_p1	uce-622_p2	uce-623_p1	uce-623_p2
locus0573	uce-789_p1	uce-789_p2		
locus0574	uce-786_p1	uce-786_p2		
locus0575	uce-777_p1	uce-777_p2		

locus0576	uce-767_p1	uce-767_p2		
locus0577	uce-997_p1	uce-997_p2		
locus0578	uce-754_p1	uce-754_p2		
locus0579	uce-753_p1	uce-753_p2		
locus0580	uce-752_p1	uce-752_p2		
locus0581	uce-747_p1	uce-747_p2		
locus0582	uce-746_p1	uce-746_p2		
locus0583	uce-376_p1	uce-376_p2	uce-377_p1	uce-377_p2
locus0584	uce-738_p1	uce-738_p2		
locus0585	uce-737_p1	uce-737_p2		
locus0586	uce-736_p1	uce-736_p2		
locus0587	uce-722_p1	uce-722_p2		
locus0588	uce-718_p1	uce-718_p2		
locus0589	uce-716_p1	uce-716_p2		
locus0590	uce-714_p1	uce-714_p2		
locus0591	uce-709_p1	uce-709_p2		
locus0592	uce-708_p1	uce-708_p2		
locus0593	uce-692_p1	uce-692_p2		
locus0594	uce-1448_p1	uce-1448_p2	uce-1449_p1	uce-1449_p2
locus0595	uce-689_p1	uce-689_p2		
locus0596	uce-688_p1	uce-688_p2		
locus0597	uce-678_p1	uce-678_p2		
locus0598	uce-675_p1	uce-675_p2		
locus0599	uce-673_p1	uce-673_p2		
locus0600	uce-672_p1	uce-672_p2		
locus0601	uce-665_p1	uce-665_p2		
locus0602	uce-663_p1	uce-663_p2		
locus0603	uce-657_p1	uce-657_p2		
locus0604	uce-655_p1	uce-655_p2		
locus0605	uce-1488_p1	uce-1488_p2	uce-1489_p1	uce-1489_p2
locus0606	uce-645_p1	uce-645_p2		
locus0607	uce-641_p1	uce-641_p2		
locus0608	uce-634_p1	uce-634_p2		
locus0609	uce-633_p1	uce-633_p2		

locus0610	uce-629_p1	uce-629_p2		
locus0611	uce-627_p1	uce-627_p2		
locus0612	uce-625_p1	uce-625_p2		
locus0613	uce-624_p1	uce-624_p2		
locus0614	uce-611_p1	uce-611_p2		
locus0615	uce-608_p1	uce-608_p2		
locus0616	uce-1186_p1	uce-1186_p2	uce-1187_p1	uce-1187_p2
locus0617	uce-596_p1	uce-596_p2		
locus0618	uce-595_p1	uce-595_p2		
locus0619	uce-590_p1	uce-590_p2		
locus0620	uce-588_p1	uce-588_p2		
locus0621	uce-581_p1	uce-581_p2		
locus0622	uce-574_p1	uce-574_p2		
locus0623	uce-566_p1	uce-566_p2		
locus0624	uce-560_p1	uce-560_p2		
locus0625	uce-553_p1	uce-553_p2		
locus0626	uce-550_p1	uce-550_p2		
locus0627	uce-765_p1	uce-765_p2	uce-76_p1	uce-76_p2
locus0628	uce-724_p1	uce-724_p2	uce-725_p1	uce-725_p2
locus0629	uce-543_p1	uce-543_p2		
locus0630	uce-540_p1	uce-540_p2		
locus0631	uce-539_p1	uce-539_p2		
locus0632	uce-536_p1	uce-536_p2		
locus0633	uce-529_p1	uce-529_p2		
locus0634	uce-526_p1	uce-526_p2		
locus0635	uce-524_p1	uce-524_p2		
locus0636	uce-521_p1	uce-521_p2		
locus0637	uce-517_p1	uce-517_p2		
locus0638	uce-515_p1	uce-515_p2		
locus0639	uce-894_p1	uce-894_p2	uce-895_p1	uce-895_p2
locus0640	uce-513_p1	uce-513_p2		
locus0641	uce-511_p1	uce-511_p2		
locus0642	uce-509_p1	uce-509_p2		
locus0643	uce-508_p1	uce-508_p2		

locus0644	uce-505_p1	uce-505_p2		
locus0645	uce-503_p1	uce-503_p2		
locus0646	uce-500_p1	uce-500_p2		
locus0647	uce-491_p1	uce-491_p2		
locus0648	uce-480_p1	uce-480_p2		
locus0649	uce-479_p1	uce-479_p2		
locus0650	uce-25_p1	uce-25_p2	uce-26_p1	uce-26_p2
locus0651	uce-475_p1	uce-475_p2		
locus0652	uce-472_p1	uce-472_p2		
locus0653	uce-8_p1	uce-8_p2		
locus0654	uce-441_p1	uce-441_p2		
locus0655	uce-422_p1	uce-422_p2		
locus0656	uce-409_p1	uce-409_p2		
locus0657	uce-404_p1	uce-404_p2		
locus0658	uce-403_p1	uce-403_p2		
locus0659	uce-400_p1	uce-400_p2		
locus0660	uce-391_p1	uce-391_p2		
locus0661	uce-585_p1	uce-585_p2	uce-586_p1	uce-586_p2
locus0662	uce-389_p1	uce-389_p2		
locus0663	uce-388_p1	uce-388_p2		
locus0664	uce-386_p1	uce-386_p2		
locus0665	uce-383_p1	uce-383_p2		
locus0666	uce-380_p1	uce-380_p2		
locus0667	uce-375_p1	uce-375_p2		
locus0668	uce-373_p1	uce-373_p2		
locus0669	uce-370_p1	uce-370_p2		
locus0670	uce-364_p1	uce-364_p2		
locus0671	uce-363_p1	uce-363_p2		
locus0672	uce-1264_p1	uce-1264_p2	uce-1265_p1	uce-1265_p2
locus0673	uce-350_p1	uce-350_p2		
locus0674	uce-347_p1	uce-347_p2		
locus0675	uce-344_p1	uce-344_p2		
locus0676	uce-343_p1	uce-343_p2		
locus0677	uce-340_p1	uce-340_p2		

locus0678	uce-338_p1	uce-338_p2		
locus0679	uce-332_p1	uce-332_p2		
locus0680	uce-328_p1	uce-328_p2		
locus0681	uce-326_p1	uce-326_p2		
locus0682	uce-325_p1	uce-325_p2		
locus0683	uce-1370_p1	uce-1370_p2	uce-1371_p1	uce-1371_p2
locus0684	uce-321_p1	uce-321_p2		
locus0685	uce-317_p1	uce-317_p2		
locus0686	uce-316_p1	uce-316_p2		
locus0687	uce-315_p1	uce-315_p2		
locus0688	uce-313_p1	uce-313_p2		
locus0689	uce-310_p1	uce-310_p2		
locus0690	uce-309_p1	uce-309_p2		
locus0691	uce-308_p1	uce-308_p2		
locus0692	uce-305_p1	uce-305_p2		
locus0693	uce-302_p1	uce-302_p2		
locus0694	uce-1418_p1	uce-1418_p2	uce-292_p1	
locus0695	uce-293_p1	uce-293_p2		
locus0696	uce-288_p1	uce-288_p2		
locus0697	uce-274_p1	uce-274_p2		
locus0698	uce-271_p1	uce-271_p2		
locus0699	uce-261_p1	uce-261_p2		
locus0700	uce-260_p1	uce-260_p2		
locus0701	uce-259_p1	uce-259_p2		
locus0702	uce-256_p1	uce-256_p2		
locus0703	uce-254_p1	uce-254_p2		
locus0704	uce-243_p1	uce-243_p2		
locus0705	uce-1363_p1		uce-1364_p1	uce-1364_p2
locus0706	uce-239_p1	uce-239_p2		
locus0707	uce-234_p1	uce-234_p2		
locus0708	uce-232_p1	uce-232_p2		
locus0709	uce-230_p1	uce-230_p2		
locus0710	uce-229_p1	uce-229_p2		
locus0711	uce-226_p1	uce-226_p2		

locus0712	uce-224_p1	uce-224_p2		
locus0713	uce-206_p1	uce-206_p2		
locus0714	uce-205_p1	uce-205_p2		
locus0715	uce-201_p1	uce-201_p2		
locus0716	uce-362_p1	uce-362_p2		uce-705_p2
locus0717	uce-191_p1	uce-191_p2		
locus0718	uce-186_p1	uce-186_p2		
locus0719	uce-181_p1	uce-181_p2		
locus0720	uce-174_p1	uce-174_p2		
locus0721	uce-172_p1	uce-172_p2		
locus0722	uce-169_p1	uce-169_p2		
locus0723	uce-167_p1	uce-167_p2		
locus0724	uce-160_p1	uce-160_p2		
locus0725	uce-159_p1	uce-159_p2		
locus0726	uce-146_p1	uce-146_p2		
locus0727		uce-15_p2	uce-16_p1	uce-16_p2
locus0728	uce-145_p1	uce-145_p2		
locus0729	uce-130_p1	uce-130_p2		
locus0730	uce-129_p1	uce-129_p2		
locus0731	uce-127_p1	uce-127_p2		
locus0732	uce-126_p1	uce-126_p2		
locus0733	uce-125_p1	uce-125_p2		
locus0734	uce-120_p1	uce-120_p2		
locus0735	uce-118_p1	uce-118_p2		
locus0736	uce-95_p1	uce-95_p2		
locus0737	uce-88_p1	uce-88_p2		
locus0738	uce-178_p1	uce-178_p2	uce-179_p1	uce-179_p2
locus0739		uce-943_p2	uce-944_p1	uce-944_p2
locus0740	uce-73_p1	uce-73_p2		
locus0741	uce-72_p1	uce-72_p2		
locus0742	uce-71_p1	uce-71_p2		
locus0743	uce-70_p1	uce-70_p2		
locus0744	uce-69_p1	uce-69_p2		
locus0745	uce-65_p1	uce-65_p2		

locus0746	uce-52_p1	uce-52_p2		
locus0747	uce-47_p1	uce-47_p2		
locus0748	uce-45_p1	uce-45_p2		
locus0749	uce-42_p1	uce-42_p2		
locus0750	uce-202_p1	uce-202_p2	uce-203_p1	
locus0751	uce-39_p1	uce-39_p2		
locus0752	uce-28_p1	uce-28_p2		
locus0753	uce-23_p1	uce-23_p2		
locus0754	uce-22_p1	uce-22_p2		
locus0755	uce-13_p1	uce-13_p2		
locus0756	uce-1223_p1	uce-1223_p2		
locus0757	uce-734_p1	uce-734_p2		
locus0758	uce-1510_p1	uce-1510_p2		
locus0759	uce-1497_p1	uce-1497_p2		
locus0760	uce-1495_p1	uce-1495_p2		
locus0761	uce-1017_p1	uce-1017_p2	uce-1018_p1	
locus0762	uce-1484_p1	uce-1484_p2		
locus0763	uce-1478_p1	uce-1478_p2		
locus0764	uce-1476_p1	uce-1476_p2		
locus0765	uce-1472_p1	uce-1472_p2		
locus0766	uce-1467_p1	uce-1467_p2		
locus0767	uce-1464_p1	uce-1464_p2		
locus0768	uce-1463_p1	uce-1463_p2		
locus0769	uce-1457_p1	uce-1457_p2		
locus0770	uce-1452_p1	uce-1452_p2		
locus0771	uce-1450_p1	uce-1450_p2		
locus0772		uce-1355_p2	uce-1356_p1	uce-1356_p2
locus0773	uce-1442_p1	uce-1442_p2		
locus0774	uce-1439_p1	uce-1439_p2		
locus0775	uce-1438_p1	uce-1438_p2		
locus0776	uce-1433_p1	uce-1433_p2		
locus0777	uce-1431_p1	uce-1431_p2		
locus0778	uce-1428_p1	uce-1428_p2		
locus0779	uce-1427_p1	uce-1427_p2		

locus0780	uce-1423_p1	uce-1423_p2		
locus0781	uce-1422_p1	uce-1422_p2		
locus0782	uce-1421_p1	uce-1421_p2		
locus0783		uce-1435_p2	uce-1436_p1	uce-1436_p2
locus0784	uce-1420_p1	uce-1420_p2		
locus0785	uce-1417_p1	uce-1417_p2		
locus0786	uce-1415_p1	uce-1415_p2		
locus0787	uce-1408_p1	uce-1408_p2		
locus0788	uce-1403_p1	uce-1403_p2		
locus0789	uce-1399_p1	uce-1399_p2		
locus0790	uce-1397_p1	uce-1397_p2		
locus0791	uce-1392_p1	uce-1392_p2		
locus0792	uce-1383_p1	uce-1383_p2		
locus0793	uce-1382_p1	uce-1382_p2		
locus0794		uce-578_p2	uce-579_p1	uce-579_p2
locus0795	uce-1381_p1	uce-1381_p2		
locus0796	uce-1380_p1	uce-1380_p2		
locus0797	uce-1379_p1	uce-1379_p2		
locus0798	uce-1375_p1	uce-1375_p2		
locus0799	uce-1361_p1	uce-1361_p2		
locus0800	uce-1360_p1	uce-1360_p2		
locus0801	uce-1359_p1	uce-1359_p2		
locus0802	uce-1358_p1	uce-1358_p2		
locus0803	uce-1349_p1	uce-1349_p2		
locus0804	uce-1328_p1	uce-1328_p2		
locus0805	uce-813_p1	uce-813_p2	uce-814_p1	
locus0806	uce-1322_p1	uce-1322_p2		
locus0807	uce-1314_p1	uce-1314_p2		
locus0808	uce-1313_p1	uce-1313_p2		
locus0809	uce-1308_p1	uce-1308_p2		
locus0810	uce-1305_p1	uce-1305_p2		
locus0811	uce-1298_p1	uce-1298_p2		
locus0812	uce-1293_p1	uce-1293_p2		
locus0813	uce-1275_p1	uce-1275_p2		

locus0814	uce-1274_p1	uce-1274_p2		
locus0815	uce-1266_p1	uce-1266_p2		
locus0816	uce-1505_p1	uce-1505_p2	uce-1506_p1	
locus0817	uce-1262_p1	uce-1262_p2		
locus0818	uce-1260_p1	uce-1260_p2		
locus0819	uce-1259_p1	uce-1259_p2		
locus0820	uce-1245_p1	uce-1245_p2		
locus0821	uce-1241_p1	uce-1241_p2		
locus0822	uce-1236_p1	uce-1236_p2		
locus0823	uce-1234_p1	uce-1234_p2		
locus0824	uce-1222_p1	uce-1222_p2		
locus0825	uce-1219_p1	uce-1219_p2		
locus0826	uce-1208_p1	uce-1208_p2		
locus0827		uce-1250_p2	uce-1251_p1	uce-1251_p2
locus0828	uce-1207_p1	uce-1207_p2		
locus0829	uce-1194_p1	uce-1194_p2		
locus0830	uce-1191_p1	uce-1191_p2		
locus0831	uce-1178_p1	uce-1178_p2		
locus0832	uce-1177_p1	uce-1177_p2		
locus0833	uce-1174_p1	uce-1174_p2		
locus0834	uce-1173_p1	uce-1173_p2		
locus0835	uce-1161_p1	uce-1161_p2		
locus0836	uce-1156_p1	uce-1156_p2		
locus0837	uce-1155_p1	uce-1155_p2		
locus0838	uce-994_p1	uce-994_p2	uce-995_p1	
locus0839	uce-1142_p1	uce-1142_p2		
locus0840	uce-1140_p1	uce-1140_p2		
locus0841	uce-1139_p1	uce-1139_p2		
locus0842	uce-1135_p1	uce-1135_p2		
locus0843	uce-1130_p1	uce-1130_p2		
locus0844	uce-1129_p1	uce-1129_p2		
locus0845	uce-1125_p1	uce-1125_p2		
locus0846	uce-1114_p1	uce-1114_p2		
locus0847	uce-1113_p1	uce-1113_p2		

locus0848	uce-1112_p1	uce-1112_p2		
locus0849	uce-1299_p1	uce-1299_p2	uce-1300_p1	uce-1300_p2
locus0850		uce-1157_p2	uce-1158_p1	uce-1158_p2
locus0851	uce-1109_p1	uce-1109_p2		
locus0852	uce-1108_p1	uce-1108_p2		
locus0853	uce-1098_p1	uce-1098_p2		
locus0854	uce-1097_p1	uce-1097_p2		
locus0855	uce-1092_p1	uce-1092_p2		
locus0856	uce-1091_p1	uce-1091_p2		
locus0857	uce-1086_p1	uce-1086_p2		
locus0858	uce-1078_p1	uce-1078_p2		
locus0859	uce-1073_p1	uce-1073_p2		
locus0860	uce-1072_p1	uce-1072_p2		
locus0861		uce-592_p2	uce-593_p1	uce-593_p2
locus0862	uce-1062_p1	uce-1062_p2		
locus0863	uce-1060_p1	uce-1060_p2		
locus0864	uce-1055_p1	uce-1055_p2		
locus0865	uce-1047_p1	uce-1047_p2		
locus0866	uce-1046_p1	uce-1046_p2		
locus0867	uce-1038_p1	uce-1038_p2		
locus0868	uce-1014_p1	uce-1014_p2		
locus0869	uce-1011_p1	uce-1011_p2		
locus0870	uce-1006_p1	uce-1006_p2		
locus0871	uce-992_p1	uce-992_p2		
locus0872		uce-1056_p2	uce-1057_p1	uce-1057_p2
locus0873	uce-983_p1	uce-983_p2		
locus0874	uce-982_p1	uce-982_p2		
locus0875	uce-981_p1	uce-981_p2		
locus0876	uce-969_p1	uce-969_p2		
locus0877	uce-968_p1	uce-968_p2		
locus0878	uce-965_p1	uce-965_p2		
locus0879	uce-964_p1	uce-964_p2		
locus0880	uce-961_p1	uce-961_p2		
locus0881	uce-959_p1	uce-959_p2		

locus0882	uce-954_p1	uce-954_p2		
locus0883	uce-1002_p1	uce-1002_p2	uce-1003_p1	
locus0884	uce-945_p1	uce-945_p2		
locus0885	uce-939_p1	uce-939_p2		
locus0886	uce-938_p1	uce-938_p2		
locus0887	uce-935_p1	uce-935_p2		
locus0888	uce-933_p1	uce-933_p2		
locus0889	uce-929_p1	uce-929_p2		
locus0890	uce-922_p1	uce-922_p2		
locus0891	uce-911_p1	uce-911_p2		
locus0892	uce-910_p1	uce-910_p2		
locus0893	uce-902_p1	uce-902_p2		
locus0894	uce-322_p1	uce-322_p2	uce-323_p1	
locus0895	uce-900_p1	uce-900_p2		
locus0896	uce-896_p1	uce-896_p2		
locus0897	uce-881_p1	uce-881_p2		
locus0898	uce-880_p1	uce-880_p2		
locus0899	uce-879_p1	uce-879_p2		
locus0900	uce-878_p1	uce-878_p2		
locus0901	uce-876_p1	uce-876_p2		
locus0902	uce-872_p1	uce-872_p2		
locus0903	uce-867_p1	uce-867_p2		
locus0904	uce-863_p1	uce-863_p2		
locus0905		uce-726_p2	uce-727_p1	uce-727_p2
locus0906	uce-862_p1	uce-862_p2		
locus0907	uce-860_p1	uce-860_p2		
locus0908	uce-847_p1	uce-847_p2		
locus0909	uce-845_p1	uce-845_p2		
locus0910	uce-843_p1	uce-843_p2		
locus0911	uce-842_p1	uce-842_p2		
locus0912	uce-839_p1	uce-839_p2		
locus0913	uce-838_p1	uce-838_p2		
locus0914	uce-834_p1	uce-834_p2		
locus0915	uce-827_p1	uce-827_p2		

locus0916		uce-853_p2	uce-854_p1	uce-854_p2
locus0917	uce-821_p1	uce-821_p2		
locus0918	uce-818_p1	uce-818_p2		
locus0919	uce-809_p1	uce-809_p2		
locus0920	uce-799_p1	uce-799_p2		
locus0921	uce-797_p1	uce-797_p2		
locus0922	uce-793_p1	uce-793_p2		
locus0923	uce-792_p1	uce-792_p2		
locus0924	uce-791_p1	uce-791_p2		
locus0925	uce-790_p1	uce-790_p2		
locus0926	uce-783_p1	uce-783_p2		
locus0927	uce-451_p1	uce-451_p2	uce-452_p1	
locus0928	uce-780_p1	uce-780_p2		
locus0929	uce-776_p1	uce-776_p2		
locus0930	uce-775_p1	uce-775_p2		
locus0931	uce-771_p1	uce-771_p2		
locus0932	uce-769_p1	uce-769_p2		
locus0933	uce-766_p1	uce-766_p2		
locus0934	uce-763_p1	uce-763_p2		
locus0935	uce-758_p1	uce-758_p2		
locus0936	uce-748_p1	uce-748_p2		
locus0937	uce-741_p1	uce-741_p2		
locus0938	uce-762_p1	uce-762_p2		
locus0939	uce-728_p1	uce-728_p2		
locus0940	uce-717_p1	uce-717_p2		
locus0941	uce-706_p1	uce-706_p2		
locus0942	uce-701_p1	uce-701_p2		
locus0943	uce-696_p1	uce-696_p2		
locus0944	uce-695_p1	uce-695_p2		
locus0945	uce-694_p1	uce-694_p2		
locus0946	uce-693_p1	uce-693_p2		
locus0947	uce-686_p1	uce-686_p2		
locus0948	uce-683_p1	uce-683_p2		
locus0949	uce-1502_p1	uce-1502_p2		

locus0950	uce-682_p1	uce-682_p2		
locus0951	uce-677_p1	uce-677_p2		
locus0952	uce-676_p1	uce-676_p2		
locus0953	uce-670_p1	uce-670_p2		
locus0954	uce-669_p1	uce-669_p2		
locus0955	uce-664_p1	uce-664_p2		
locus0956	uce-662_p1	uce-662_p2		
locus0957	uce-659_p1	uce-659_p2		
locus0958	uce-652_p1	uce-652_p2		
locus0959	uce-651_p1	uce-651_p2		
locus0960	uce-1453_p1	uce-1453_p2	uce-1454_p1	uce-1454_p2
locus0961	uce-1482_p1	uce-1482_p2		
locus0962	uce-647_p1	uce-647_p2		
locus0963	uce-637_p1	uce-637_p2		
locus0964	uce-636_p1	uce-636_p2		
locus0965	uce-618_p1	uce-618_p2		
locus0966	uce-617_p1	uce-617_p2		
locus0967	uce-613_p1	uce-613_p2		
locus0968	uce-605_p1	uce-605_p2		
locus0969	uce-604_p1	uce-604_p2		
locus0970	uce-600_p1	uce-600_p2		
locus0971	uce-598_p1	uce-598_p2		
locus0972	uce-1474_p1	uce-1474_p2		
locus0973	uce-591_p1	uce-591_p2		
locus0974	uce-583_p1	uce-583_p2		
locus0975	uce-573_p1	uce-573_p2		
locus0976	uce-570_p1	uce-570_p2		
locus0977	uce-569_p1	uce-569_p2		
locus0978	uce-568_p1	uce-568_p2		
locus0979	uce-565_p1	uce-565_p2		
locus0980	uce-559_p1	uce-559_p2		
locus0981	uce-546_p1	uce-546_p2		
locus0982	uce-545_p1	uce-545_p2		
locus0983	uce-1473_p1	uce-1473_p2		

locus0984	uce-538_p1	uce-538_p2
locus0985	uce-534_p1	uce-534_p2
locus0986	uce-532_p1	uce-532_p2
locus0987	uce-530_p1	uce-530_p2
locus0988	uce-527_p1	uce-527_p2
locus0989	uce-525_p1	uce-525_p2
locus0990	uce-520_p1	uce-520_p2
locus0991	uce-519_p1	uce-519_p2
locus0992	uce-516_p1	uce-516_p2
locus0993	uce-510_p1	uce-510_p2
locus0994	uce-1469_p1	uce-1469_p2
locus0995	uce-502_p1	uce-502_p2
locus0996	uce-493_p1	uce-493_p2
locus0997	uce-489_p1	uce-489_p2
locus0998	uce-473_p1	uce-473_p2
locus0999	uce-450_p1	uce-450_p2
locus1000	uce-448_p1	uce-448_p2
locus1001	uce-443_p1	uce-443_p2
locus1002	uce-442_p1	uce-442_p2
locus1003	uce-438_p1	uce-438_p2
locus1004	uce-433_p1	uce-433_p2
locus1005	uce-4_p1	uce-4_p2
locus1006	uce-423_p1	uce-423_p2
locus1007	uce-421_p1	uce-421_p2
locus1008	uce-412_p1	uce-412_p2
locus1009	uce-411_p1	uce-411_p2
locus1010	uce-408_p1	uce-408_p2
locus1011	uce-402_p1	uce-402_p2
locus1012	uce-399_p1	uce-399_p2
locus1013	uce-396_p1	uce-396_p2
locus1014	uce-387_p1	uce-387_p2
locus1015	uce-368_p1	uce-368_p2
locus1016	uce-1447_p1	uce-1447_p2
locus1017	uce-365_p1	uce-365_p2

locus1018	uce-361_p1	uce-361_p2
locus1019	uce-355_p1	uce-355_p2
locus1020	uce-351_p1	uce-351_p2
locus1021	uce-341_p1	uce-341_p2
locus1022	uce-298_p1	uce-298_p2
locus1023	uce-290_p1	uce-290_p2
locus1024	uce-285_p1	uce-285_p2
locus1025	uce-279_p1	uce-279_p2
locus1026	uce-276_p1	uce-276_p2
locus1027	uce-1445_p1	uce-1445_p2
locus1028	uce-273_p1	uce-273_p2
locus1029	uce-270_p1	uce-270_p2
locus1030	uce-266_p1	uce-266_p2
locus1031	uce-263_p1	uce-263_p2
locus1032	uce-255_p1	uce-255_p2
locus1033	uce-252_p1	uce-252_p2
locus1034	uce-249_p1	uce-249_p2
locus1035	uce-245_p1	uce-245_p2
locus1036	uce-244_p1	uce-244_p2
locus1037	uce-238_p1	uce-238_p2
locus1038	uce-1444_p1	uce-1444_p2
locus1039	uce-222_p1	uce-222_p2
locus1040	uce-221_p1	uce-221_p2
locus1041	uce-208_p1	uce-208_p2
locus1042	uce-207_p1	uce-207_p2
locus1043	uce-204_p1	uce-204_p2
locus1044	uce-197_p1	uce-197_p2
locus1045	uce-194_p1	uce-194_p2
locus1046	uce-192_p1	uce-192_p2
locus1047	uce-188_p1	uce-188_p2
locus1048	uce-175_p1	uce-175_p2
locus1049	uce-1434_p1	uce-1434_p2
locus1050	uce-168_p1	uce-168_p2
locus1051	uce-163_p1	uce-163_p2

locus1052	uce-161_p1	uce-161_p2		
locus1053	uce-156_p1	uce-156_p2		
locus1054	uce-154_p1	uce-154_p2		
locus1055	uce-153_p1	uce-153_p2		
locus1056	uce-152_p1	uce-152_p2		
locus1057	uce-142_p1	uce-142_p2		
locus1058	uce-141_p1	uce-141_p2		
locus1059	uce-128_p1	uce-128_p2		
locus1060	uce-1432_p1	uce-1432_p2		
locus1061	uce-124_p1	uce-124_p2		
locus1062	uce-119_p1	uce-119_p2		
locus1063	uce-113_p1	uce-113_p2		
locus1064	uce-111_p1	uce-111_p2		
locus1065	uce-109_p1	uce-109_p2		
locus1066	uce-107_p1	uce-107_p2		
locus1067	uce-106_p1	uce-106_p2		
locus1068	uce-103_p1	uce-103_p2		
locus1069	uce-102_p1	uce-102_p2		
locus1070	uce-97_p1	uce-97_p2		
locus1071	uce-1493_p1	uce-1493_p2	uce-1494_p1	uce-1494_p2
locus1072	uce-1424_p1	uce-1424_p2		
locus1073	uce-93_p1	uce-93_p2		
locus1074	uce-92_p1	uce-92_p2		
locus1075	uce-91_p1	uce-91_p2		
locus1076	uce-90_p1	uce-90_p2		
locus1077	uce-82_p1	uce-82_p2		
locus1078	uce-63_p1	uce-63_p2		
locus1079	uce-58_p1	uce-58_p2		
locus1080	uce-57_p1	uce-57_p2		
locus1081	uce-51_p1	uce-51_p2		
locus1082	uce-50_p1	uce-50_p2		
locus1083	uce-1419_p1	uce-1419_p2		
locus1084	uce-40_p1	uce-40_p2		
locus1085	uce-37_p1	uce-37_p2		

locus1086	uce-36_p1	uce-36_p2
locus1087	uce-32_p1	uce-32_p2
locus1088	uce-31_p1	uce-31_p2
locus1089	uce-24_p1	uce-24_p2
locus1090	uce-14_p1	uce-14_p2
locus1091	uce-12_p1	uce-12_p2
locus1092	uce-1504_p1	uce-1504_p2
locus1093	uce-1499_p1	uce-1499_p2
locus1094	uce-1416_p1	uce-1416_p2
locus1095	uce-1496_p1	uce-1496_p2
locus1096	uce-1486_p1	uce-1486_p2
locus1097	uce-1483_p1	uce-1483_p2
locus1098	uce-1479_p1	uce-1479_p2
locus1099	uce-1451_p1	uce-1451_p2
locus1100	uce-1440_p1	uce-1440_p2
locus1101	uce-1426_p1	uce-1426_p2
locus1102	uce-1425_p1	uce-1425_p2
locus1103	uce-1406_p1	uce-1406_p2
locus1104	uce-1398_p1	uce-1398_p2
locus1105	uce-1405_p1	uce-1405_p2
locus1106	uce-1394_p1	uce-1394_p2
locus1107	uce-1393_p1	uce-1393_p2
locus1108	uce-1377_p1	uce-1377_p2
locus1109	uce-1374_p1	uce-1374_p2
locus1110	uce-1373_p1	uce-1373_p2
locus1111	uce-1365_p1	uce-1365_p2
locus1112	uce-1362_p1	uce-1362_p2
locus1113	uce-1353_p1	uce-1353_p2
locus1114	uce-1340_p1	uce-1340_p2
locus1115	uce-1334_p1	uce-1334_p2
locus1116	uce-1401_p1	uce-1401_p2
locus1117	uce-1331_p1	uce-1331_p2
locus1118	uce-1327_p1	uce-1327_p2
locus1119	uce-1323_p1	uce-1323_p2

locus1120	uce-1321_p1	uce-1321_p2
locus1121	uce-1317_p1	uce-1317_p2
locus1122	uce-1316_p1	uce-1316_p2
locus1123	uce-1306_p1	uce-1306_p2
locus1124	uce-1304_p1	uce-1304_p2
locus1125	uce-1303_p1	uce-1303_p2
locus1126	uce-1301_p1	uce-1301_p2
locus1127	uce-1396_p1	uce-1396_p2
locus1128	uce-1297_p1	uce-1297_p2
locus1129	uce-1283_p1	uce-1283_p2
locus1130	uce-1277_p1	uce-1277_p2
locus1131	uce-1272_p1	uce-1272_p2
locus1132	uce-1269_p1	uce-1269_p2
locus1133	uce-1268_p1	uce-1268_p2
locus1134	uce-1267_p1	uce-1267_p2
locus1135	uce-1254_p1	uce-1254_p2
locus1136	uce-1238_p1	uce-1238_p2
locus1137	uce-1230_p1	uce-1230_p2
locus1138	uce-1391_p1	uce-1391_p2
locus1139	uce-1229_p1	uce-1229_p2
locus1140	uce-1227_p1	uce-1227_p2
locus1141	uce-1216_p1	uce-1216_p2
locus1142	uce-1209_p1	uce-1209_p2
locus1143	uce-1206_p1	uce-1206_p2
locus1144	uce-1205_p1	uce-1205_p2
locus1145	uce-1197_p1	uce-1197_p2
locus1146	uce-1192_p1	uce-1192_p2
locus1147	uce-1190_p1	uce-1190_p2
locus1148	uce-1189_p1	uce-1189_p2
locus1149	uce-1369_p1	uce-1369_p2
locus1150	uce-1183_p1	uce-1183_p2
locus1151	uce-1180_p1	uce-1180_p2
locus1152	uce-1176_p1	uce-1176_p2
locus1153	uce-1171_p1	uce-1171_p2

locus1154	uce-1167_p1	uce-1167_p2
locus1155	uce-1166_p1	uce-1166_p2
locus1156	uce-1159_p1	uce-1159_p2
locus1157	uce-1126_p1	uce-1126_p2
locus1158	uce-1117_p1	uce-1117_p2
locus1159	uce-1107_p1	uce-1107_p2
locus1160	uce-1368_p1	uce-1368_p2
locus1161	uce-1105_p1	uce-1105_p2
locus1162	uce-1102_p1	uce-1102_p2
locus1163	uce-1096_p1	uce-1096_p2
locus1164	uce-1085_p1	uce-1085_p2
locus1165	uce-1084_p1	uce-1084_p2
locus1166	uce-1082_p1	uce-1082_p2
locus1167	uce-1079_p1	uce-1079_p2
locus1168	uce-1075_p1	uce-1075_p2
locus1169	uce-1071_p1	uce-1071_p2
locus1170	uce-1069_p1	uce-1069_p2
locus1171	uce-1367_p1	uce-1367_p2
locus1172	uce-1067_p1	uce-1067_p2
locus1173	uce-1064_p1	uce-1064_p2
locus1174	uce-1059_p1	uce-1059_p2
locus1175	uce-1045_p1	uce-1045_p2
locus1176	uce-1035_p1	uce-1035_p2
locus1177	uce-1033_p1	uce-1033_p2
locus1178	uce-1032_p1	uce-1032_p2
locus1179	uce-1028_p1	uce-1028_p2
locus1180	uce-1027_p1	uce-1027_p2
locus1181	uce-1023_p1	uce-1023_p2
locus1287	uce-1_p1	
locus1182	uce-1009_p1	
locus1183	uce-101_p2	
locus1184	uce-1021_p1	
locus1186	uce-105_p2	
locus1185	uce-1054_p2	

locus1187	uce-1068_p2
locus1188	uce-1070_p2
locus1189	uce-1081_p1
locus1190	uce-1087_p1
locus1191	uce-1089_p2
locus1192	uce-1090_p1
locus1193	uce-1103_p1
locus1194	uce-1118_p2
locus1197	uce-112_p1
locus1195	uce-1122_p2
locus1196	uce-1123_p1
locus1198	uce-1132_p2
locus1199	uce-1137_p2
locus1200	uce-1138_p2
locus1201	uce-1141_p1
locus1202	uce-1143_p2
locus1203	uce-1145_p1
locus1204	uce-1150_p1
locus1205	uce-1152_p1
locus1206	uce-1168_p2
locus1207	uce-1170_p2
locus1208	uce-1172_p1
locus1209	uce-1179_p2
locus1210	uce-1185_p2
locus1211	uce-1195_p2
locus1212	uce-1198_p2
locus1213	uce-1202_p2
locus1214	uce-1204_p1
locus1215	uce-1210_p2
locus1216	uce-1221_p2
locus1217	uce-1224_p1
locus1218	uce-1226_p2
locus1219	uce-123_p1
locus1220	uce-1246_p2

locus1221	uce-1263_p1
locus1222	uce-1276_p2
locus1223	uce-1285_p2
locus1224	uce-1288_p1
locus1225	uce-1289_p2
locus1226	uce-1290_p2
locus1227	uce-1291_p1
locus1228	uce-1292_p1
locus1229	uce-1294_p1
locus1230	uce-1295_p1
locus1231	uce-1302_p1
locus1232	uce-1309_p1
locus1233	uce-1312_p1
locus1234	uce-1320_p1
locus1239	uce-133_p2
locus1235	uce-1332_p2
locus1236	uce-1336_p2
locus1237	uce-1338_p1
locus1238	uce-1339_p1
locus1242	uce-134_p2
locus1240	uce-1341_p1
locus1241	uce-1347_p2
locus1245	uce-135_p1
locus1243	uce-1354_p1
locus1244	uce-1357_p2
locus1246	uce-137_p1
locus1251	uce-138_p2
locus1247	uce-1384_p2
locus1248	uce-1385_p1
locus1249	uce-1386_p2
locus1250	uce-1388_p2
locus1252	uce-1395_p1
locus1256	uce-140_p2
locus1253	uce-1400_p1

locus1254	uce-1402_p1
locus1255	uce-1404_p2
locus1257	uce-1411_p2
locus1258	uce-1430_p2
locus1259	uce-1437_p1
locus1260	uce-1441_p2
locus1261	uce-1458_p1
locus1262	uce-1459_p2
locus1264	uce-147_p2
locus1263	uce-1477_p1
locus1265	uce-1480_p2
locus1269	uce-149_p1
locus1266	uce-1490_p1
locus1267	uce-1491_p2
locus1268	uce-1498_p1
locus1270	uce-1501_p1
locus1271	uce-1503_p2
locus1272	uce-1507_p2
locus1273	uce-1509_p2
locus1274	uce-151_p1
locus1275	uce-158_p2
locus1276	uce-166_p1
locus1277	uce-176_p2
locus1278	uce-177_p2
locus1279	uce-183_p1
locus1280	uce-184_p2
locus1281	uce-187_p2
locus1286	uce-19_p2
locus1282	uce-190_p2
locus1283	uce-193_p1
locus1284	uce-196_p2
locus1285	uce-198_p1
locus1288	uce-211_p1
locus1289	uce-214_p2

locus1290	uce-215_p1
locus1291	uce-216_p2
locus1292	uce-218_p1
locus1293	uce-220_p2
locus1294	uce-223_p1
locus1295	uce-227_p2
locus1296	uce-231_p2
locus1297	uce-246_p2
locus1298	uce-262_p1
locus1299	uce-264_p1
locus1300	uce-268_p1
locus1301	uce-272_p2
locus1302	uce-277_p2
locus1303	uce-280_p1
locus1304	uce-289_p2
locus1305	uce-292
locus1306	uce-294_p1
locus1309	uce-30_p1
locus1307	uce-300_p2
locus1308	uce-306_p1
locus1310	uce-314_p2
locus1311	uce-318_p1
locus1312	uce-324_p1
locus1313	uce-331_p2
locus1314	uce-336_p2
locus1315	uce-346_p1
locus1316	uce-352_p1
locus1317	uce-353_p2
locus1318	uce-354_p1
locus1319	uce-356_p2
locus1320	uce-357_p1
locus1321	uce-358_p1
locus1322	uce-371_p1
locus1323	uce-372_p2

locus1324	uce-378_p1
locus1325	uce-381_p1
locus1326	uce-382_p2
locus1327	uce-384_p2
locus1328	uce-385_p2
locus1329	uce-398_p1
locus1330	uce-405_p1
locus1331	uce-410_p1
locus1332	uce-414_p1
locus1333	uce-425_p1
locus1334	uce-426_p2
locus1335	uce-434_p2
locus1336	uce-435_p1
locus1337	uce-447_p2
locus1338	uce-454_p2
locus1339	uce-462_p2
locus1340	uce-463_p2
locus1341	uce-464_p1
locus1342	uce-469_p1
locus1343	uce-474_p1
locus1344	uce-477_p2
locus1345	uce-482_p1
locus1346	uce-484_p1
locus1347	uce-485_p2
locus1348	uce-487_p2
locus1349	uce-490_p1
locus1350	uce-494_p2
locus1351	uce-549_p2
locus1352	uce-555_p2
locus1353	uce-558_p1
locus1356	uce-56_p2
locus1354	uce-562_p2
locus1355	uce-563_p1
locus1357	uce-572_p1

locus1358	uce-575_p1
locus1359	uce-587_p2
locus1360	uce-599_p1
locus1361	uce-602_p1
locus1362	uce-603_p1
locus1363	uce-612_p2
locus1364	uce-619_p2
locus1365	uce-626_p1
locus1366	uce-628_p1
locus1367	uce-639_p2
locus1368	uce-648_p1
locus1369	uce-653_p2
locus1371	uce-66_p1
locus1370	uce-660_p2
locus1372	uce-671_p1
locus1373	uce-674_p2
locus1374	uce-680_p1
locus1375	uce-681_p1
locus1376	uce-685_p1
locus1377	uce-697_p1
locus1378	uce-698_p2
locus1394	uce-7_p1
locus1379	uce-700_p1
locus1380	uce-705
locus1381	uce-712_p1
locus1382	uce-732_p2
locus1383	uce-733_p1
locus1384	uce-740_p1
locus1385	uce-742_p1
locus1386	uce-760_p1
locus1387	uce-768_p2
locus1388	uce-782_p2
locus1389	uce-788_p1
locus1390	uce-794_p1

locus1391	uce-795_p2
locus1392	uce-796_p1
locus1393	uce-798_p1
locus1395	uce-802_p1
locus1396	uce-806_p2
locus1397	uce-807_p2
locus1398	uce-808_p2
locus1399	uce-810_p2
locus1400	uce-817_p2
locus1401	uce-829_p2
locus1404	uce-85_p1
locus1402	uce-855_p1
locus1403	uce-857_p1
locus1405	uce-865_p2
locus1406	uce-868_p2
locus1407	uce-869_p1
locus1408	uce-871_p1
locus1409	uce-874_p1
locus1410	uce-883_p1
locus1411	uce-884_p2
locus1412	uce-885_p1
locus1413	uce-887_p2
locus1414	uce-889_p1
locus1415	uce-89_p1
locus1416	uce-903_p2
locus1417	uce-904_p2
locus1418	uce-908_p1
locus1419	uce-917_p1
locus1420	uce-919_p1
locus1421	uce-921_p2
locus1422	uce-924_p1
locus1423	uce-926_p1
locus1424	uce-94_p1
locus1425	uce-951_p1

locus1426	uce-967_p2
locus1427	uce-974_p1
locus1428	uce-977_p1
locus1429	uce-979_p2
locus1430	uce-980_p2
locus1431	uce-986_p2
locus1432	uce-999_p2

Table S1

sample code	source	collection year	Family	genus	species	preservative method	identified by	WGA	q of input DNA (ng) for library preparation	nb raw reads	nb contigs	average contig depth	average length of UCEs	nb UCEs after filtering	nb UCEs in the 25%-complete matrix
JRAS07732_0101	this study	2017	Azotidae	Ablerus	sp 1	96% EtOH	Polaszek A.	no	5	4536	800	4.3	447.5	522	509
JRAS07826_0189	this study	2009	Azotidae	Ablerus	sp 2	96% EtOH	Delvare G.	no	25	29290	3157	6.0	532.5	739	713
LFMM00018_0689	this study	2015	Azotidae	Ablerus	sp 3	96% EtOH	Rasplus J.Y.	no	12.5	11157	982	2.7	265.5	139	131
JRAS06864_0689	this study	2016	Mymaridae	Acmopolynema	sema	96% EtOH	Rasplus J.Y.	no	25	61132	2536	21.6	725.6	682	656
JRAS07790_0401	this study	2012	Eulophidae	Acrias	sp.	96% EtOH	Rasplus J.Y.	no	7.75	33085	1497	20.7	550.0	990	933
JRAS07597_0189	this study	2017	Encyrtidae	Aglyptus	rufus	96% EtOH	Rasplus J.Y.	no	100	391096	10490	24.0	816.5	1064	958
JRAS07777_0189	this study	2015	Mymaridae	Alaptus	sp.	96% EtOH	Huber J.	no	22.85	23725	1168	17.2	697.3	262	253
JRAS07824_0189	this study	2011	Eulophidae	Aleuroctonus	vittatus	96% EtOH	Delvare G.	no	25	28486	2174	9.6	553.7	775	746
JRAS07787_0189	this study	2010	Mymaridae	Anagroidea	sp.	96% EtOH	Huber J.	no	25	51511	1931	25.3	665.8	619	597
JRAS07625_0389	this study	2015	Mymaridae	Anagrus	sp.	alcohol	Rasplus J.Y.	no	50	20265	1288	13.4	509.9	393	380
GDEL06009_0101	this study	-	Encyrtidae	Anagyrus	sp.	96% EtOH	Delvare G.	no	25	108502	2198	30.2	645.9	1042	977
JRAS07782_0189	this study	2015	Mymaridae	Anaphes	nitens	96% EtOH	Huber J.	no	25	110238	2097	49.8	784.3	623	589
JSTR01594_0189	this study	2015	Aphelinidae	Aphelinus	abdominalis	96% EtOH	Rasplus J.Y.	no	25	57538	2867	17.9	667.8	940	896
JRAS07609_0289	this study	2017	Aphelinidae	Aphelinus	mali	96% EtOH	Rasplus J.Y.	no	100	60941	2647	13.7	602.5	370	341
JSTR01583_0189	this study	2015	Aphelinidae	Aphytis	melinus	96% EtOH	Rasplus J.Y.	no	25	94733	3052	29.1	671.6	932	879
JRAS07736_0101	this study	2017	Aphelinidae	Aphytis	sp.	96% EtOH	Polaszek A.	no	5	30646	2557	10.7	586.5	840	806
JRAS07817_0189	this study	2005	Eulophidae	Aprostocetus	sp.	96% EtOH	Delvare G.	no	25	26019	2785	6.7	540.0	738	717
JRAS07776_0189	this study	2014	Mymaridae	Arescon	dimidiatus	96% EtOH	Huber J.	no	25	18469	2268	6.5	555.2	428	415
JRAS07827_0189	this study	2007	Encyrtidae	Arrhenophagus	chionaspidis	96% EtOH	Delvare G.	no	25	49555	1625	28.9	554.6	620	589
JRAS07815_0189	this study	2015	Eulophidae	Astichus	solutus	96% EtOH	Rasplus J.Y.	no	25	90220	2374	35.9	645.9	981	918
JRAS07786_0189	this study	2010	Mymaridae	Australomymar	sp.	96% EtOH	Huber J.	no	25	23748	2011	10.5	533.2	836	803
JRAS07766_0101	this study	2015	Eulophidae	Baeoentedon	balios	96% EtOH	Polaszek A.	no	5	852	165	2.6	336.0	122	119
JRAS07837_0189	this study	2001	Eulophidae	Baryscapus	servadeii	96% EtOH	Auger M.A.	no	25	76712	3364	20.8	703.4	903	860
JRAS07739_0101	this study	2017	Aphelinidae	Cales	sp.	96% EtOH	Polaszek A.	no	10	11919	1575	3.6	481.9	410	399
LFMM00028_0189	this study	2015	Encyrtidae	Ceballosia	sp.	96% EtOH	Fusu L.	no	25	78255	2425	30.1	685.3	930	880
JRAS07600_0389	this study	2015	Aphelinidae	Centrodora	amoena	96% EtOH	Rasplus J.Y.	no	100	442931	16406	18.1	837.0	395	355
JRAS07600_0201	this study	2015	Signiphoridae	Chartocerus	sp.	96% EtOH	Rasplus J.Y.	no	9.9	33997	1582	20.4	625.4	955	904
LFMM00028_0289	this study	2015	Encyrtidae	Cheiloneurus	longiventris	96% EtOH	Fusu L.	no	25	81297	8009	5.5	528.8	827	784
JRAS07593_0101	this study	2017	Eulophidae	Cirrospilus	diallus	96% EtOH	Rasplus J.Y.	no	18.35	51149	2387	19.7	449.2	846	793
JRAS07820_0189	this study	-	Signiphoridae	Clytina	giraudi	96% EtOH	Delvare G.	no	7.5	85324	4148	16.7	726.4	1004	937

sample code	source	collection year	Family	genus	species	preservative method	identified by	WGA	q of input DNA (ng) for library preparation	nb raw reads	nb contigs	average contig depth	average length of UCEs	nb UCEs after filtering	nb UCEs in the 25%-complete matrix
JRAS07742_0101	this study	2017	Aphelinidae	Coccobius	sp.	96% EtOH	Polaszek A.	no	10	111940	2913	37.2	687.7	976	905
JSTR00692_0189	this study	2014	Aphelinidae	Coccophagus	scutellaris	96% EtOH	Rasplus J.Y.	no	25	51925	2740	16.8	627.1	756	729
JRAS07595_0189	this study	2017	Encyrtidae	Copidosoma	aretas	96% EtOH	Rasplus J.Y.	no	100	134565	6815	11.3	718.1	895	836
GENO00013_0101	Branstetter et al. 2017	-	Encyrtidae	Copidosoma	floridanum			no	NA	NA	NA	NA	NA	NA	833
JRAS07868_0189	this study	2002	Mymaridae	Cosmocomoidea	fasciata	96% EtOH	Triapitsyn S.	no	25	106354	2757	36.2	767.7	404	386
JRAS07832_0189	this study	2012	Eulophidae	Diglyphus	isaea	96% EtOH	Rasplus J.Y.	no	25	71682	2947	22.6	718.4	508	472
JRAS07727_0101	this study	2017	Aphelinidae	Dirphys	sp.	96% EtOH	Polaszek A.	no	5	41322	2206	16.8	610.3	630	610
JRAS06480_2689	this study	2015	Eulophidae	Elasmus	sp.	96% EtOH	Rasplus J.Y.	no	100	115944	2600	29.2	777.8	881	828
JSTR01585_0189	this study	2015	Aphelinidae	Encarsia	citrina	96% EtOH	Rasplus J.Y.	no	25	82030	6402	11.0	612.4	501	479
JSTR00475_0189	this study	2013	Aphelinidae	Encarsia	formosa	96% EtOH	Rasplus J.Y.	no	25	51437	1753	27.5	654.0	745	724
JRAS07747_0101	this study	2017	Aphelinidae	Encarsia/Coccophagus	sp.	96% EtOH	Polaszek A.	no	7	74566	4502	14.6	639.7	813	778
JRAS07830_0189	this study	2010	Eulophidae	Entedon	sparetus	96% EtOH	Delvare G.	no	25	48985	3293	12.9	615.8	659	631
JSTR00476_0189	this study	2013	Aphelinidae	Eretmocerus	eremicus	96% EtOH	Rasplus J.Y.	no	25	60775	3504	15.5	687.3	687	665
LFMM00028_0301	this study	2015	Encyrtidae	Ericydnus	sp.	96% EtOH	Fusu L.	no	5	58772	5503	5.5	551.9	906	872
JRAS07780_0189	this study	1983	Mymaridae	Erythmelus	sp.	96% EtOH	Huber J.	no	25	19917	1843	9.0	560.4	358	345
JRAS07856_0101	this study	-	Mymaridae	Eubroncus	sp.	96% EtOH	Gumovsky A.	no	10.3	24380	1330	16.2	641.3	615	596
JRAS07829_0189	this study	2007	Eulophidae	Euderus	sp.	96% EtOH	Delvare G.	no	25	59521	1782	31.5	669.9	915	885
JRAS07575_0389	this study	2017	Eulophidae	Euderus ?	sp.	96% EtOH	Rasplus J.Y.	no	100	51724	1697	19.5	641.5	851	816
JRAS07602_1001	this study	2015	Pteromalidae	Eunotus	acutatus	96% EtOH	Rasplus J.Y.	no	7.5	27785	1989	11.9	528.4	1046	963
BRAN00055_0101	Branstetter et al. 2017	2013	Eulophidae	Euplectrus	sp 1			no	NA	NA	NA	NA	NA	NA	811
JRAS06864_1089	this study	2016	Eulophidae	Euplectrus	sp 2	96% EtOH	Rasplus J.Y.	no	25	143918	3104	44.4	749.2	847	799
ANIC00100_0199	this study	1992	Aphelinidae	Eutrichosomella	sp.	dried	Rasplus J.Y.	no	25	42124	1671	23.6	409.2	975	917
JRAS07740_0101	this study	2017	Aphelinidae	Euxanthellus	sp.	96% EtOH	Polaszek A.	no	10	71869	2781	23.9	632.2	931	884
JRAS07784_0189	this study	2014	Mymaridae	Gonatocerus	sp.	96% EtOH	Huber J.	no	25	17096	2101	6.5	544.9	330	324
JRAS07894_0101	this study	-	Encyrtidae	Hexacladia	sp.	96% EtOH	Rasplus J.Y.	no	25	15294	1388	8.0	336.5	621	586
JRAS07785_0189	this study	2014	Mymaridae	Ischiodasys	sp.	96% EtOH	Huber J.	no	25	61925	2242	25.9	576.9	312	285
JSTR01580_0189	this study	2015	Encyrtidae	Leptomastix	dactylopii	96% EtOH	Rasplus J.Y.	no	25	52771	4013	11.5	581.7	989	930
JRAS07625_0289	this study	2015	Mymaridae	Litus	cynipseus	96% EtOH	Rasplus J.Y.	no	87.84	16966	1102	9.0	581.7	407	396
JRAS07825_0189	this study	2015	Aphelinidae	Marietta	sp.	96% EtOH	Delvare G.	no	25	224864	5027	43.2	775.4	1021	947

sample code	source	collection year	Family	genus	species	preservative method	identified by	WGA	q of input DNA (ng) for library preparation	nb raw reads	nb contigs	average contig depth	average length of UCEs	nb UCEs after filtering	nb UCEs in the 25%-complete matrix
JRAS07895_0101	this study	-	Aphelinidae	Maudia	sp.	DNA	Polaszek A.	no	1.8	28055	1834	13.2	585.8	685	666
LFMM00027_0301	this study	2015	Encyrtidae	Mayridia	formosula	96% EtOH	Fusu L.	no	15	161521	3895	39.5	782.8	924	855
JRAS07818_0189	this study	2006	Eulophidae	Melittobia	acasta	96% EtOH	Delvare G.	no	25	134156	7216	15.3	708.9	742	690
JRAS07823_0189	this study	2008	Pteromalidae	Mesopeltita	sp.	96% EtOH	Rasplus J.Y.	no	25	195850	7280	22.8	778.1	1165	1033
JRAS07597_0489	this study	2017	Encyrtidae	Metaphycus	insidiosus	96% EtOH	Rasplus J.Y.	no	100	151469	4634	20.4	797.0	812	742
JRAS07597_0501	this study	2017	Trichogrammatidae	Mirufens	longicauda	96% EtOH	Rasplus J.Y.	no	24.65	51012	2247	20.9	624.7	699	681
ANIC00046_0189	this study	1983	Pteromalidae	Moranila	viridivertex	dried	Rasplus J.Y.	no	39	2654	521	3.0	211.9	359	341
JRAS07603_0889	this study	2015	Mymaridae	Mymar	regale	alcohol	Rasplus J.Y.	no	25	73337	5242	12.2	658.9	486	469
JRAS07601_0189	this study	2015	Mymarommatidae	Mymaromma	anomalum	-	Rasplus J.Y.	no	29.88	216429	3064	48.4	888.6	506	472
JRAS07882_0189	this study	2014	Mymarommatidae	Mymaromma	sp 1	96% EtOH	Fusu L.	no	5	2175	292	6.3	303.1	242	230
JRAS07883_0189	this study	2014	Mymarommatidae	Mymaromma	sp 2	96% EtOH	Fusu L.	no	8.1	15163	590	24.5	513.0	427	415
JRAS07828_0189	this study	2006	Eulophidae	Neopomphale	aleurothrixi	96% EtOH	Delvare G.	no	25	54638	3024	16.0	601.0	762	729
LFMM00003_0189	this study	2015	Aphelinidae	New Genus	sp.	96% EtOH	Mitroiu M.D.	no	25	21302	1737	10.6	570.8	798	775
JRAS07781_0189	this study	2015	Mymaridae	Ooctonus	hemipterus	96% EtOH	Huber J.	no	25	58731	2189	24.6	662.6	560	539
JSTR00645_0189	this study	2013	Encyrtidae	Ooencyrtus	kuvanae	96% EtOH	Delvare G.	no	25	37148	2186	15.7	527.7	936	893
JRAS07816_0189	this study	2005	Eulophidae	Ophelimus	maskelli	96% EtOH	Delvare G.	no	25	36855	4103	6.0	511.6	916	871
JRAS07871_0101	this study	2012	Trichogrammatidae	Paracentrobia	sp.	96% EtOH	Gumovsky A.	no	10.8	18770	1126	14.6	502.7	640	616
JRAS07313_0289	this study	2017	Encyrtidae	Paratetracnemoidea	sp.	96% EtOH	Rasplus J.Y.	no	25	91306	7326	9.5	335.1	254	216
JRAS07600_0489	this study	2015	Eulophidae	Pediobius	eubius	70% EtOH	Rasplus J.Y.	no	100	95338	5132	11.5	654.2	463	432
JRAS07594_0101	this study	2017	Eulophidae	Pnigalio	sp.	96% EtOH	Rasplus J.Y.	no	25	75129	2304	30.9	564.9	905	839
JRAS07859_0101	this study	2015	Trichogrammatidae	Poropoea	sp.	96% EtOH	Gumovsky A.	no	25	39892	2835	11.8	526.8	580	558
JRAS07738_0101	this study	2017	Aphelinidae	Prococcophagus	sp 1	96% EtOH	Polaszek A.	no	10	12301	1842	5.2	440.8	671	650
JRAS07744_0101	this study	2017	Aphelinidae	Prococcophagus	sp 2	96% EtOH	Polaszek A.	no	15	34952	1995	15.5	647.8	841	812
JRAS07625_0489	this study	2015	Trichogrammatidae	Pseudoligosita	gracilior	alcohol	Rasplus J.Y.	no	100	87834	4029	12.5	757.1	415	397
JRAS07730_0101	this study	2017	Aphelinidae	Pteroptrix	sp.	96% EtOH	Polaszek A.	no	5	7325	980	5.9	480.7	511	504

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JRAS07752_0101	this study	-	Aphelinidae	Samariola	sp.	96% EtOH	Polaszek A.	no	12.5	38874	1848	19.3	538.5	985	935
JRAS06497_0201	this study	2015	Pteromalidae	Scutellista	cyanea	96% EtOH	Rasplus J.Y.	no	250	180452	7376	22.1	761.6	1153	1040
JRAS07626_0389	this study	2015	Pteromalidae	Scutellista	obscura	96% EtOH	Rasplus J.Y.	no	25	31944	1774	16.3	604.1	1093	1018
JRAS07789_0189	this study	2014	Signiphoridae	Signiphora	sp.	96% EtOH	Rasplus J.Y.	no	10	26963	2907	7.4	416.2	693	673
JRAS07788_0189	this study	2014	Mymaridae	Stethynium	sp.	96% EtOH	Huber J.	no	10	61669	2076	27.9	636.1	284	265
JRAS07884_0189	this study	2014	Encyrtidae	Tachinaephagus	zealandicus	96% EtOH	Fusu L.	no	25	191956	9167	18.7	704.2	924	851
GDEL07010_0101	this study	-	Eulophidae	Tamarixia	pubescens	96% EtOH	Delvare G.	no	10	31963	3399	27.0	672.8	758	711
JRAS07628_0189	this study	2014	Signiphoridae	Thysanus	sp.	alcohol	Fusu L.	no	36.54	147810	11231	6.0	589.3	731	699
TRIC00029_0199	this study	2014	Trichogrammatidae	Trichogramma	brassicae	96% EtOH	Rasplus J.Y.	yes (0.4 ng input)	250	340094	15682	20.0	816.6	425	404
GENO00028_0101	Branstetter et al. 2017	-	Trichogrammatidae	Trichogramma	pretiosum	-		no	NA	NA	NA	NA	NA	NA	626
TRIC00001_0199	this study	-	Trichogrammatidae	Trichogramma	sp.	96% EtOH	Rasplus J.Y.	yes (4 ng input)	250	353266	16954	18.8	688.5	206	187
JRAS06864_1401	this study	2016	Trichogrammatidae	Tumidiclava	sp.	96% EtOH	Rasplus J.Y.	no	22.95	29994	1630	16.2	618.1	535	521
LFMM00029_0101	this study	2015	Aphelinidae	Umairia	sp.	96% EtOH	Rasplus J.Y.	no	12.5	28887	3133	4.5	457.4	646	625
JRAS07858_0189	this study	2014	Trichogrammatidae	Uscana	sp.	96% EtOH	Gumovsky A.	no	25	126025	3420	34.8	544.5	186	169
JRAS03390_0389	this study	2011	Eulophidae	Zagrammosoma	mirum	96% EtOH	Rasplus J.Y.	no	25	6729	859	6.5	407.1	560	534

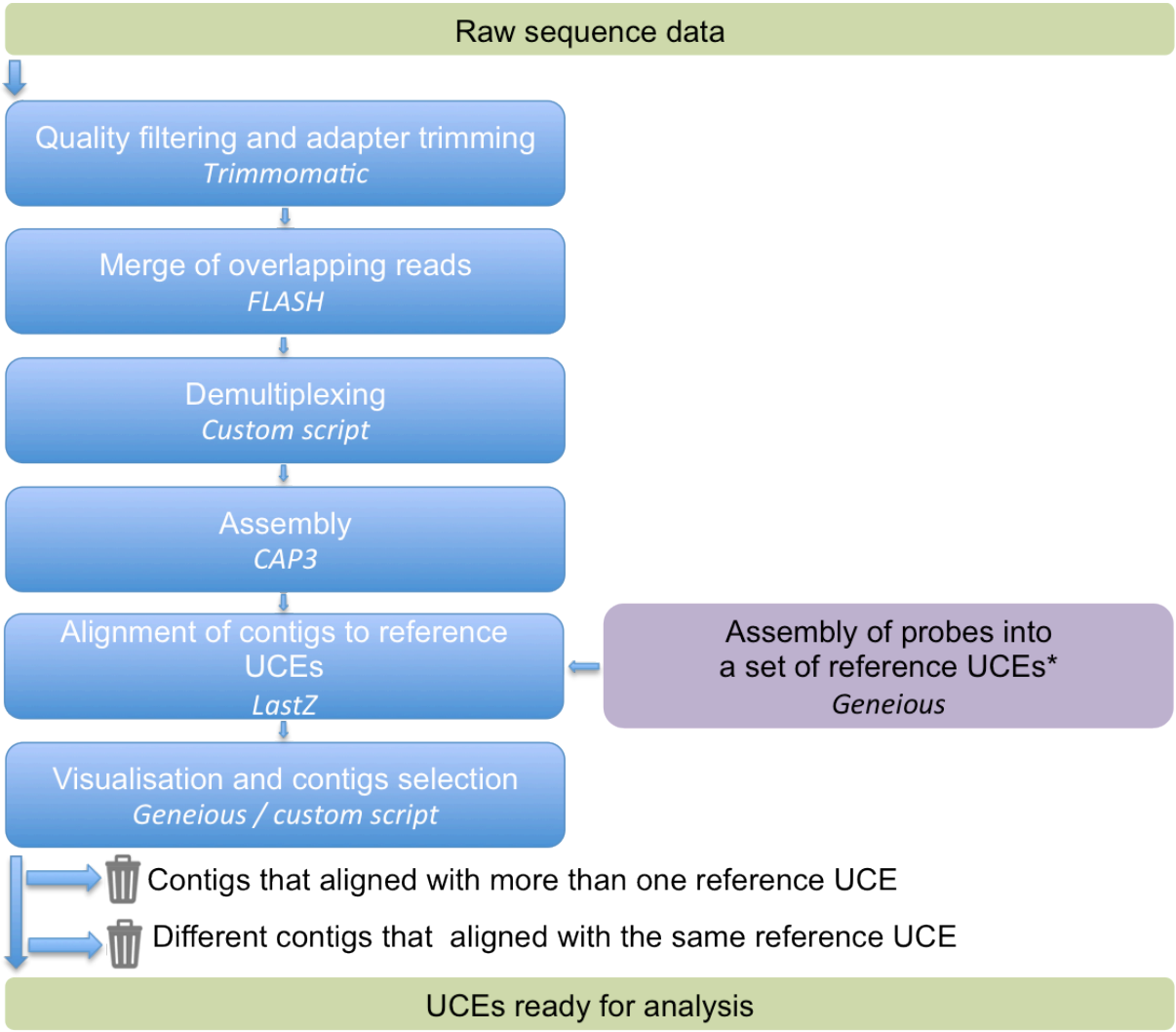
Cited reference

Branstetter MG, Danforth BN, Pitts JP, Faircloth BC, Ward PS, Buffington ML, Gates MW, Kula RR, and Brady SG. 2017.
Phylogenomic Insights into the Evolution of Stinging Wasps and the Origins of Ants and Bees. Current Biology 27:1019-1025. 10.1016/j.cub.2017.03.027

Figure S1

Analytical workflow – Step 1. Pre-processing of the data: from raw reads to raw UCEs

*The 2749 probes designed by Faircloth et al. (2015) were first assembled into a set of non-overlapping loci using Geneious. This set of reference UCE loci (N=1432) was then used to align sample-specific contigs obtained with CAP3. The reference UCEs are available in additional file 4.



Reference cited:
Faircloth, B.C., Branstetter, M.G., White, N.D., S.G., B., 2015. Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. Molecular Ecology Resources 15, 489-501.

Figure S2

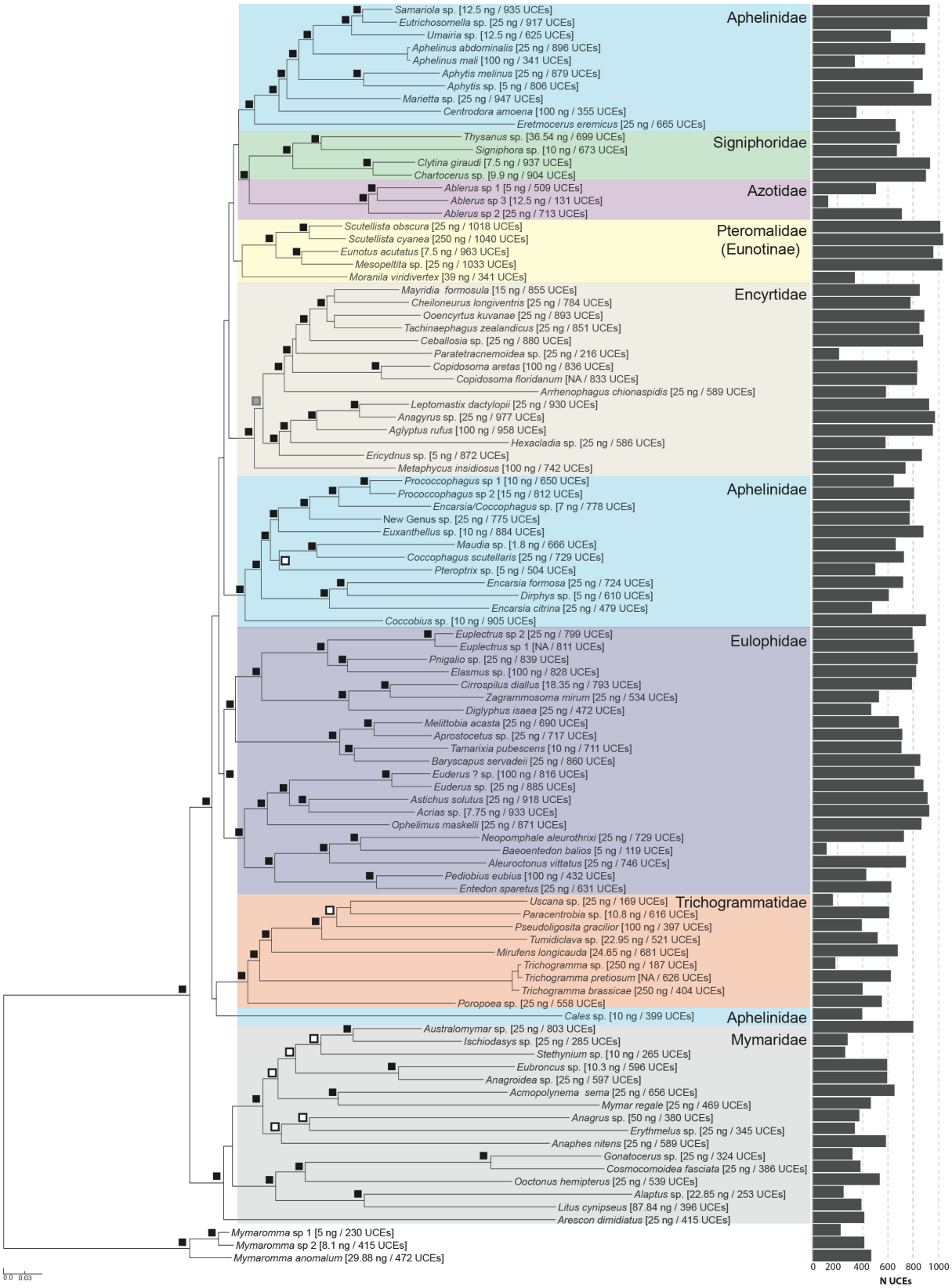


Figure S3

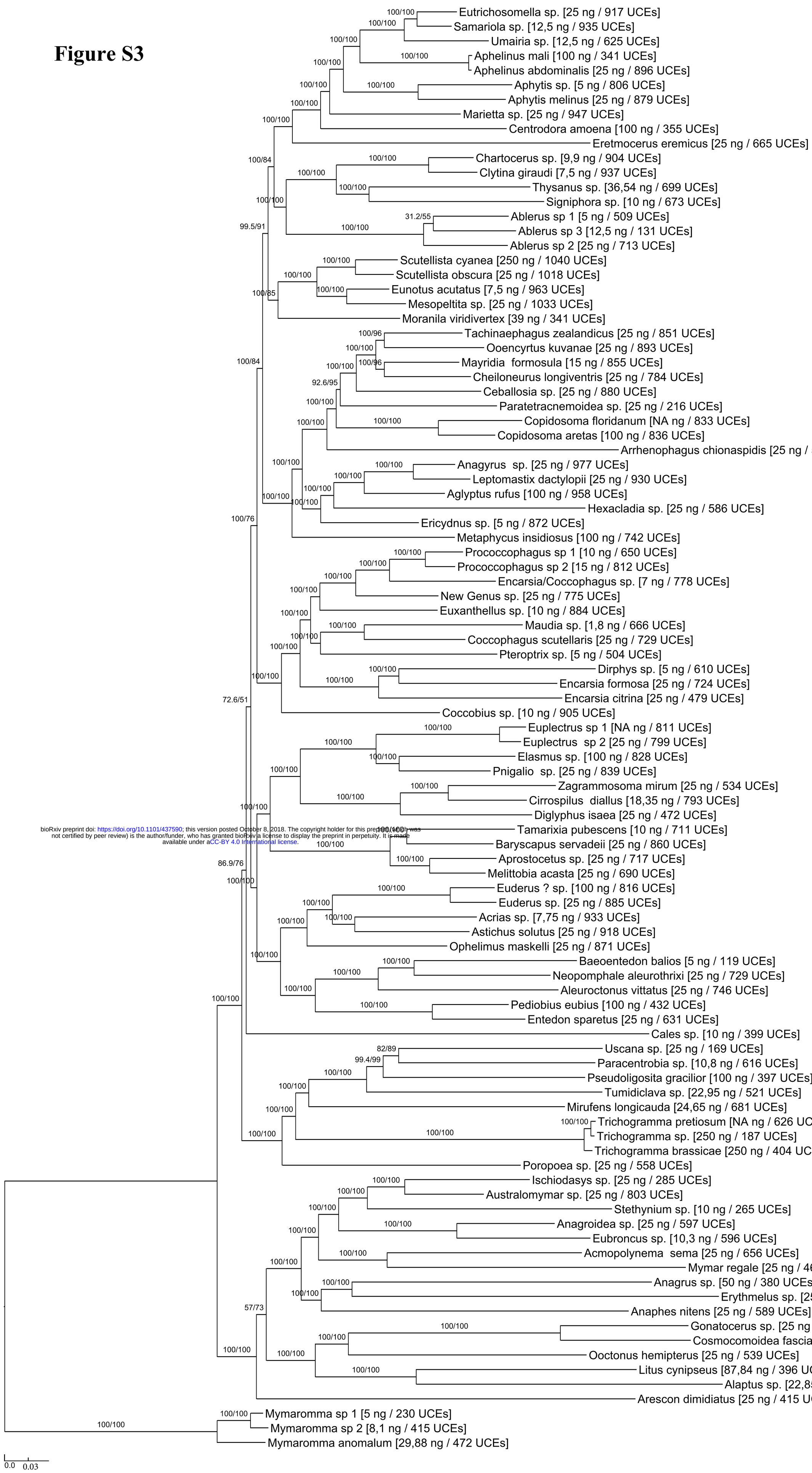


Figure S4

Figure S5