# **Supporting Information**

# **Supplementary data 1**: DNA extraction protocol for QIAGEN DNeasy Blood & Tissue Kit

Kit: QIAGEN DNeasy Blood & Tissue Kit (250) (product no. 69506)

BEFORE STARTING: Preheat the incubator to 55–56 °C. Before using some of the buffers provided in the kit for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to get a working solution. Per sample prepare one column, two collection-tubes, and one final tube. Prepare glass with distilled water for used pestles. Prepare 100% ethanol.

1. Place about 50 mg of sample in a 1.5-ml tube. Add 180 µl Buffer ATL. Grind sample using a sterile plastic pestle (clean pestle for each sample).
2. Add 20 µl proteinase K, vortex briefly and spin down. Incubate at 56 °C on the shaker for 1–3 h.
3. For each sample, mix 200 µl buffer AL with 200 µl 100% ethanol.
4. Vortex samples for 15 s and spin down. Add 400 µl AL/ethanol mixture to each sample and vortex briefly.
5. Centrifuge for 2 min at 10000 ***g***. Transfer the clear lysate into a DNeasy Mini spin column.
6. Centrifuge for 1 min at 6000 ***g***. Discard flow-through and transfer the column to a new collection tube.
7. Add 500 µl buffer AW1 and centrifuge for 1 min at 6000 ***g***. Discard flow-through and transfer the column to a new collection tube.
8. Add 500 µl buffer AW2 and centrifuge for 3.5 min at maximum ***g***. Discard flow-through.
9. Place the column in a new 1.5-ml micro-centrifuge tube and pipette 50-200 µl buffer AE directly onto the membrane.
10. Incubate the columns at room temperature for 2 min.
11. Centrifuge at 10000 ***g*** for 1 min.
12. Store the DNA extractions at –20 °C.

# **Table S1** PCR protocol for COI amplification with insect DNA

|  |  |  |  |
| --- | --- | --- | --- |
|  | Temperature (°C) | Time | Cycle number |
| Initial denaturation | 95 | 3 min |  |
| Denaturation | 95 | 30 s | 40 |
| Annealing | 55 | 30 s |
| Extension | 72 | 50 s |
| Final extension | 72 | 5 min |  |

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# **Table S2** List of primers used for PCR amplification of a 658 bp, 313 bp and 157 bp COI amplicon

|  |  |  |
| --- | --- | --- |
| Primer name | Sequence 5' - 3' | Reference |
| ZBJ-ArtF1c1 | AGA TAT TGG AAC WTT ATA TTT TAT TTT TGG | Zeale et al., 2011 |
| ZBJ-ArtR2c1 | WAC TAA TCA ATT WCC AAA TCC TCC | Zeale et al., 2011 |
| dgLCO-14903 | GGT CAA CAA ATC ATA AAG AYA TYG G | Meyer, 2003 |
| dgHCO-21982,3 | TAA ACT TCA GGG TGA CCA AAR AAY CA | Meyer, 2003 |
| mICOIintF2 | GGW ACW GGW TGA ACW GTW TAY CCY CC | Leray et al., 2013 |

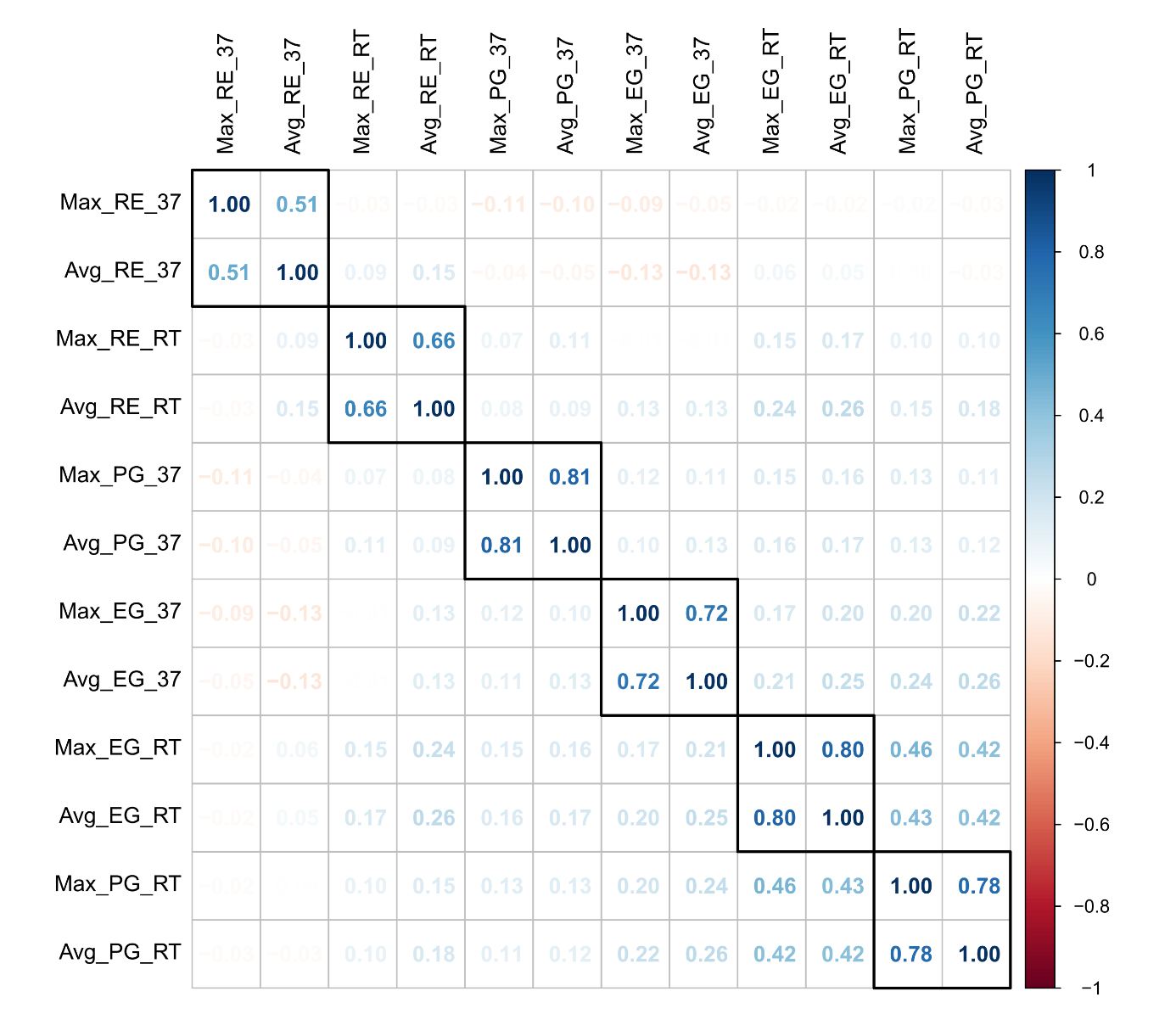
1157 bp amplicon

2313 bp amplicon

3658 bp amplicon

# **Table S3** ANOVA results for the relationship between maximum fragment size (response variable) and concentration, extraction date, and temperature for specimens kept in either propylene glycol, ethylene glycol, or Renner solution

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Preservative | Variable | F | d.f. | P |
| Propylene glycol | Concentration | 93.709 | 1,211 | <0.001 |
|  | Extraction date | 0.601 | 1,211 | 0.44 |
|  | Temperature | 13.349 | 1,211 | 0.003 |
|  | Concentration\*temperature | 34.956 | 1,211 | <0.001 |
| Ethylene glycol | Concentration | 118.501 | 1,211 | <0.001 |
|  | Extraction date | 8.877 | 1,211 | 0.003 |
|  | Temperature | 66.777 | 1,211 | <0.001 |
|  | Concentration\*temperature | 85.749 | 1,211 | <0.001 |
| Renner solution | Concentration | 0.008 | 1,211 | 0.93 |
|  | Extraction date | 0.525 | 1,211 | 0.47 |
|  | Temperature | 12.027 | 1,211 | <0.001 |
|  | Concentration\*temperature | 0.012 | 1,211 | 0.91 |

**Figure S1** Kendall correlation graph for the maximum (Max) and average (Avg) fragment size of propylene glycol (PG), ethylene glycol (EG), and Renner solution (RE) at room temperature (RT) and 37 °C (37). For all preservatives at both temperatures average and maximum fragment size are strongly correlated.

# **Figure S2** Average fragment size of samples kept in 100, 75, 50, and 25% concentration of propylene glycol, ethylene glycol, or Renner solution on nine extraction dates on every third day with a total experiment time of 27 days and the negative control (NC). The average fragment size of the positive control is indicated by the dashed line. Fragment lengths ≥50000 bp are condensed in the Fragment Analyser system output. The y-axis is transformed logarithmical for better visualisation.

