# DNA Extraction Sri Bulan Musmiah G352110101

**EKSTRAKSI DNA**
Sri Bulan Musmiah G352110101

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<td>Mitochondrial control region Total genome</td>
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Total DNA was isolated from milk samples by a phenol/chloroform method, followed by ethanol precipitation according to Sambrook et al. (1989). Cheese samples were cut into small pieces, and total DNA from cheese matrix was obtained by performing
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DNA from reference strain of bacteria were prepared from pure culture according to the guanidium extraction procedure described by Pitcher et al. (1989).
Muscle: Individuals with opened and closed shells were
| tissue preserved from each beach in 80% ethanol. Muscle tissue was extracted from the middle and apex region (c. 1mm²) of the foot and cleaned with ethanol (75%) to remove sand, detritus or external organic matter. DNA extraction was performed with the Qiagen DNeasy kit, with slight modification to increase the concentration of DNA, i.e. we used 150 µl of elution buffer. |  |  |
the mitochondrial COI gene

total genome
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| 16S mitochondrial ribosomal DNA fragments | total genome |
Snake
Egg yolks, slough skin and non-invasively sampled (NIS) muscle, blood, and faecal material.
Field collected carcasses, road kills and foetal samples were directly frozen at -20°C or preserved in 95% ethanol. Ancient ethanol preserved tissue (A.E.P.T.; collected pre-1907 to 1969) consisted of museum samples. Non-viable grass snake eggs, located in compost heaps, were preserved at -20°C. Sloughed skins, mostly collected at the entrance of identified adder domains, were preserved dry at room temperature for up to two years. An additional fresh slough was obtained from an adder observed in the process of ecdysis. Snake faeces (associated with sloughs) collected from a range of UK sites were either immediately frozen at -20°C or preserved in 95% ethanol. A 10 µl blood sample, obtained by caudal extraction from an adder and stored in 90 µl of Seutin's buffer (Seutin et al., 1991) at room temperature, was collected as a positive PCR control. Sloughed skin required a rehydration step to remove impurities prior to DNA extraction. A fragment (1-2 cm²) of slough was rinsed in 1 ml of ddwater at 55 °C in a rocking incubator for 4-6 h, and repeated for a further 8-12 h. DNA extraction was performed on these rehydrated samples, egg yolks (approximately 0.2 cm³), NIS muscle (1 cm³) and blood (5 µl in Seutin's buffer) using QiagenDNeasy tissue extraction kit. Faecal material was extracted using QIAamp® DNA stool mini kit.
500 and 750 bp mtDNA

total genome
| The soft tissue, stomach and pyloric caecae |  |
Upon collection, specimens were cleaned in seawater, immediately frozen on dry ice, and subsequently stored at -20°C. The soft tissue, stomach and pyloric caeca, of each starfish was removed and separated from the skeleton. The DNA was extracted using AxyPrep multi source Genomic DNA Mini-Prep Kit.
As a blood sample was available only from the proband, clipped fingernail samples were obtained from 10 of the other 12 members instead. Genomic DNA was extracted from the fingernails using a buffer solution containing urea, DDT and proteinase K, as reported previously. Briefly, clipped fingernails were once frozen in liquid nitrogen and crushed into fine powder using Multi-beads Shocker™ (Yasui Kikai, Osaka, Japan). The nail powder was lysed in a urea-lysis solution (2 M urea; 0.5 %SDS; 10 mM Tris-HCl, pH 7.5; 0.1 M EDTA) containing 1 mg/ml proteinase K and 40 mM DDT at 55 °C overnight. Nail DNA was extracted with phenol/chloroform, and precipitated with ethanol and sodium acetate. Precipitated nail DNA was dissolved again in extraction buffer (0.5 % SDS; 10 mM Tris-HCl, pH 7.5; 0.1 M EDTA) containing 1 mg/ml proteinase K, and incubated at 55 °C overnight. DNA was purified again as above, and was suspended in 30 µl of 1x TE buffer.
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The first set DNA, blood of subjects which agreed consent obtained by phlebotomized and shipped to laboratory in Iowa City. The DNA from a 15 ml sodium citrate tube of blood was obtained using the cold protein precipitation method (Lahiri and Nurnberger 1991). The second set of DNA sample which was derived from saliva (SD for saliva derived). DNA from these caretaker-samples was processed using materials and methods using Oragene™ kits (DNA Genetek, Canada).

Subjects rinsed their mouths, then deposited 4 ml of saliva into the Oragene sample container after receiving instructions from a trained research assistant.

Both types of DNA samples were then quantified spectrophotometrically, then stored at -20°C until use.


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Fragments 200 to 300 bp in size of the mitochondrial DNA cytochrome b gene

Total genome - fragmented genome
Milk and curd
Total DNA was isolated from milk samples by a phenol/chloroform method, followed by ethanol precipitation according to Sambrook et al. (1989). Cheese samples were cut into small pieces, and total DNA from cheese matrix was obtained by performing phenol/chloroform as described above.

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