High Quality DNA Extraction for Advanced Genetic Analysis: Final Report

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Introduction

Advances in genomic technologies are being applied to marine mammals to understand kinship, population structure and taxonomy in unprecedented detail (e.g., Cammen et al., 2016). Such techniques require high quality and quantity of DNA to ensure good results.

The 2020 Hector's and Māui Dolphin Threat Management Plan (TMP) and 2021 Hector's and Māui Dolphin Research Strategy highlights the importance of understanding and maintaining connectivity between subpopulations. This project aimed to extract high quality DNA from some of the vulnerable subpopulations of Hector's dolphins identified in the 2020 TMP and subsequent research strategy. In particular we focus on Māui dolphin (*Cephalorhynchus hectori maui*) and Hector's dolphin (*C. h. hectori*) samples the southern South Island (Te Wae Wae and Toi Toi Bays), Kaikōura, Golden Bay and Queen Charlotte Sound.

Methods

Sample collection

Hector's dolphins that have been received by the New Zealand Cetacean Tissue Archive (NZCeTA) between 2012 and February 2022, i.e., the time period since the previous analyses (Hamner et al., 2012), were identified from the archive's database. These samples are a combination of biopsy samples, primarily from Queen Charlotte Sound, and stranding samples sent in by Department of Conservation – Te Papa Atawhai (DOC) rangers or Prof. Wendi Roe, Massey University as part of the necropsy protocols.

DNA Extraction and sex identification

Samples were stored in 70%-95% ethanol prior to DNA extraction. A small section of tissue (approximately 2 x 2 x 2 mm) was sub-sampled and cut into pieces which resembled grains of sand. The tissue was digested with proteinase K followed by total cellular DNA extraction using a standard phenol/chloroform/isoamyl (PCI) protocol (Sambrook et al., 1989) which had been modified for small samples (Baker et al., 1998) or with the DNeasy kit.

Sex was identified for each sample using a multiplexed PCR protocol which amplified fragments of the *sry* and ZFX/ZFY genes (Aasen & Medrano, 1990; Gilson et al., 1998). For each PCR reaction, 1 µL of DNA stock was used initially. If this did not produce a PCR product, DNA stock was diluted (5 uL DNA: 45 µL

Qiagen EB buffer) and sex identification PCR was reattempted with 1 µL of diluted DNA. If a sample still did not amplify, the sample was processed with the Zymo one step PCR inhibitor removal kit to improve the PCR success rate. The PCR products were visualised using gel electrophoresis to determine the sex of each sample (Figure 1).

Assessment of DNA quality and quantity

DNA was visualised with gel electrophoresis to determine DNA quality (2 uL DNA mixed with 2 uL gel red and loading dye and run on a 1% agarose gel; Figure 1). The gel picture was used to categorise DNA quality into:
(1) High molecular weight (HMW) DNA, with a single bright band indicating DNA was extracted on average >10 kb long. This is typically seen in well-preserved biopsy samples.
(2) Smear: with a HMW band extracted as above, but also a smear of DNA in the lower molecular weight (LMW) range, indicating some degradation of sample. This is typically seen in fresh stranding samples.
(3) LMW DNA: gel picture shows that only LMW DNA (<5 kb) was extracted, indicating the sample has undergone degradation.

Categories (1) and (2) are suitable for use in genomic applications whereas category (3) is likely to work for some applications (e.g., biparentally inherited microsatellite loci) but not for high throughput genomic sequencing.

DNA quantity was measured using spectrophotometry with the Nanodrop 2000 (Thermofisher) or using fluorometry with the Qubit broad range DNA quantification kit (Thermofisher).

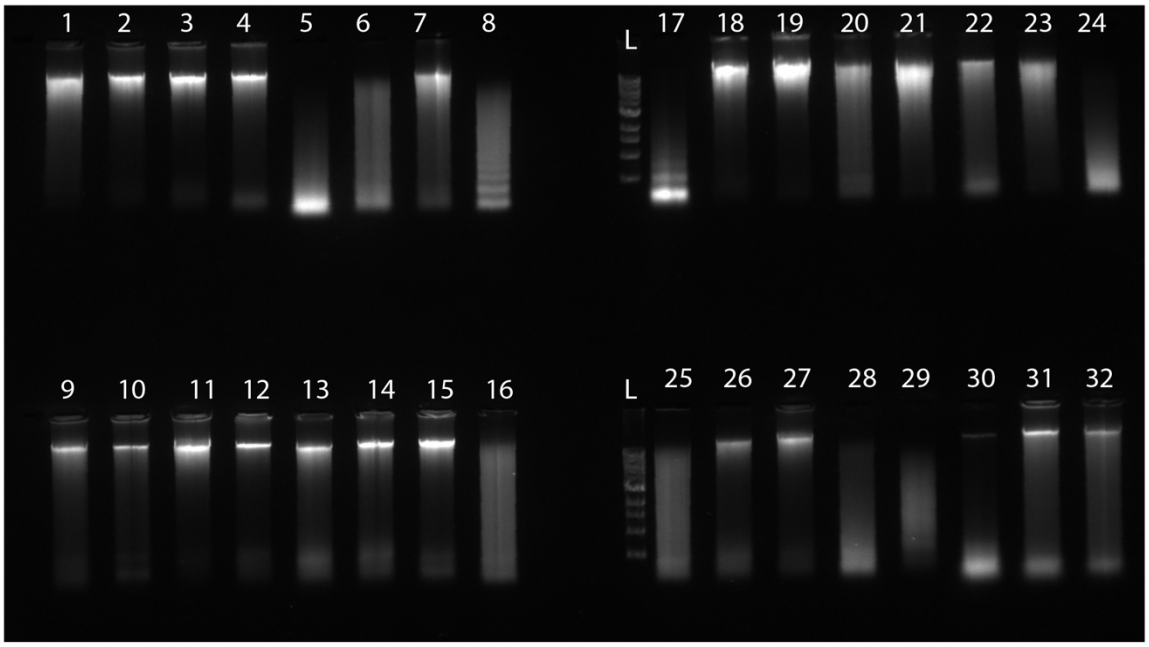

Figure 1: Image from gel electrophoresis used to assess DNA quality. High molecular weight (HMW) examples include samples 1-4 and 18 and 19. HMW smear examples include samples 16 and 25. Low molecular weight (LMW) examples include samples 5 and 24. The 1 kb ladder is indicated with L.

Table 1: Overview of Hector's dolphin samples included in the project based on sampling region. DOC ID = DOC stranding code assigned for Hector's & Māui dolphins. HMW = high molecular weight, LMW = low molecular weight (see Figure 1 for examples).

[1] Includes mana whenua Ngāti Toa Rangatira/ Te Ātiawa o Te Waka-a-Māui/ Ngāti Apa ki te Rā Tō/ Rangitāne o Wairua/ Ngāti Kuia/ Ngāti Rārua/ Ngāti Kōata/ Ngāti Tama ki Te Tau Ihu
[2] Includes mana whenua Ngāti Toa Rangatira/ Te Ātiawa o Te Waka-a-Māui/ Rangitāne o Wairua/ Ngāti Kuia/ Ngāti Kōata

Species code	U-code, DOC ID	Date stranded	Location	Region	Sex	Iwi/Hapu	Quantity: ng/ul	DNA quality
Che12TM01	U12-250, H225	24/Aug/2012	Between Taupata and Billy King Creek	Tasman	F	Te Tau Ihu Iwi[1]	97	HMW
Che12TM02	U12-246, H227	12/Nov/2012	Seaford, Golden Bay	Tasman	F	Te Tau Ihu Iwi[1]	28	HMW
Che13SO01	U13-091, H238	8/Mar/2013	Freshwater Basin - Milford Sound	Southland	M	Ngāi Tahu	29	smear
Che14TM01	U14-138, U14-198, H251	30/Oct/2014	Pakawau Beach, Golden Bay	Tasman	M	Te Tau Ihu Iwi[1]	77	HMW
Che15TM01	U15-005	9/Jan/2015	Rocks Road, Nelson	Tasman	F	Te Tau Ihu Iwi[1]	122	LMW
Che15TM02	U15-006, U15-161, H253	11/Jan/2015	Waimea Inlet, Nelson	Tasman	M	Te Tau Ihu Iwi[1]	124	smear
Che15SO01	U15-159, H254	23/Feb/2015	Colac Bay	Southland	F	Ngāi Tahu	35	HMW
Che16QCS01	U16-042	13/Jun/2016	Queen Charlotte Sound	Marlborough	F	Te Tau Ihu Iwi[2]	119	smear
Che16QCS03	U16-043	13/Jun/2016	Queen Charlotte Sound	Marlborough	M	Te Tau Ihu Iwi[2]	30	smear
Che16QCS04	U16-044	13/Jun/2016	Queen Charlotte Sound	Marlborough	M	Te Tau Ihu Iwi[2]	32	HMW
Che16QCS05	U16-045	13/Jun/2016	Queen Charlotte Sound	Marlborough	F	Te Tau Ihu Iwi[2]	18	smear
Che16QCS06	U16-051	13/Jun/2016	Queen Charlotte Sound	Marlborough	F	Te Tau Ihu Iwi[2]	47	HMW
Che16QCS07	U16-047	13/Jun/2016	Queen Charlotte Sound	Marlborough	M	Te Tau Ihu Iwi[2]	59	HMW
Che16QCS09	U16-049, U16-052	13/Jun/2016	Queen Charlotte Sound	Marlborough	M	Te Tau Ihu Iwi[2]	36	smear
Che16QCS13	U16-053	13/Jun/2016	Queen Charlotte Sound	Marlborough	F	Te Tau Ihu Iwi[2]	91	smear
Che16QCS14	U16-054	13/Jun/2016	Queen Charlotte Sound	Marlborough	M	Te Tau Ihu Iwi[2]	39	HMW
Che14KK05	U17-096, H260	15/Dec/2016	Old Beach Road	Kaikōura	F	Ngāi Tahu	18	HMW
Che18TM01	U18-069, H269	4/Mar/2018	Rabbit Island	Tasman	M	Te Tau Ihu Iwi[1]	460	smear
Che18KK01	U18-071, H272	6/Apr/2018	Kaikoura	Kaikōura	M	Ngāi Tahu	143	smear

Che18KK02	U18-066, H271	22/Apr/2018	South Bay Kaikoura	Kaikōura	F	Ngāi Tahu	36	No visible DNA
Che19SO01	U19-056, H285	12/Dec/2019	Milford Sound	Southland	F	Ngāi Tahu	31	No visible DNA
Che21QCS01	NA	19/Oct/2021	Queen Charlotte Sound	Marlborough	F	Te Tau Ihu Iwi[2]	157	smear

Table 2: DNA assessment of Māui dolphin genetic samples, based on quantification (assessed by fluorometry) and quality (assessed by gel). Samples are shown by Individual ID, and for those dolphins sampled in more than one year, assessment is given for a representative sample for each year, shown by Survey ID. As the 2015-2016 samples have been analysed using ddRADSeq already, a subset (indicated by *) will be rerun to ensure data comparability across studies and information is given here only for reference where available. N/A = not analysed. Several samples had small tissue sample that did not provide sufficient DNA for ddRADSeq. HMW = high molecular weight, LMW = low molecular weight, whereas one sample ran poorly on the gel (gel error) (see Figure 1 for examples).

Individual ID	Survey ID						Individual ID	
	2010	2011	2015	2016	2020	2021	Quantity:ng/ul	Quantity:ng/ul
Chem15NZ11					20NZ39		N/A	N/A
Chem15NZ11					20NZ43		N/A	HMW
Chem15NZ11						21NZ11	33	HMW
Chem15NZ16					20NZ35		39	smear
Chem15NZ16						21NZ31	38	HMW
Chem15NZ17					20NZ50		50	HMW
Chem15NZ22						21NZ05	32	HMW
Chem15NZ25					20NZ11		20	Gel error
Chem15NZ28					20NZ22		35	HMW
Chem15NZ28						21NZ23	27	HMW
Chem15NZ31					20NZ06		43	HMW
Chem15NZ31						21NZ34	39	HMW
Chem15NZ33					20NZ34		29	smear
Chem15NZ39					20NZ31		33	HMW
Chem15NZ39						21NZ19	27	HMW
Chem15NZ44						21NZ10	60	HMW
Chem15NZ45					20NZ40		55	smear
Chem16NZ47					20NZ24		47	HMW

Chem18NZ03							47	No visible DNA
Chem18NZ04							51	HMW
Chem20NZ02					20NZ02		33	HMW
Chem20NZ02						21NZ26	32	HMW
Chem20NZ05					20NZ30		Not enough tissue for ddRAD	
Chem20NZ05						21NZ06	11	No visible DNA
Chem20NZ07					20NZ07		25	HMW
Chem20NZ08					20NZ49		30	HMW
Chem20NZ08						21NZ09	20	HMW
Chem20NZ09					20NZ10		21	HMW
Chem20NZ12					20NZ14		41	HMW
Chem20NZ13					20NZ13		55	HMW
Chem20NZ16					20NZ16		49	HMW
Chem20NZ18					20NZ18		65	HMW
Chem20NZ20					20NZ20		23	HMW
Chem20NZ25					20NZ27		165	smear
Chem20NZ25						21NZ08	31	HMW
Chem20NZ26					20NZ26		42	smear
Chem20NZ26						21NZ22	29	HMW
Chem20NZ29					20NZ29		32	smear
Chem20NZ29						21NZ18	35	HMW
Chem20NZ36					20NZ37		24	smear
Chem20NZ36						21NZ27	33	HMW
Chem20NZ42					20NZ45		41	HMW
Chem20NZ47					20NZ48		34	smear
Chem21NZ02						21NZ17	72	HMW

Chem21NZ04						21NZ14	75	HMW
Chem21NZ07						21NZ07	63	HMW
Chem21NZ20						21NZ20	58	HMW
Chem21NZ25						21NZ25	51	HMW
Chem21NZ35							Not enough tissue for ddRAD	
NI10-01	NI10-01						56	HMW
NI10-02	NI10-02						54	HMW
NI10-03	NI10-03						23	HMW
NI10-04	NI10-12						18	HMW
NI10-05	NI10-05						35	HMW
NI10-05	NI10-07						48	HMW
NI10-05		NI11-03					51	HMW
NI10-05		NI11-04					55	HMW
NI10-06	NI10-06						Not enough tissue for ddRAD	
NI10-06		NI11-13					32	HMW
NI10-09	NI10-09						108	HMW
NI10-10	NI10-10						50	HMW
NI10-11	NI10-11						45	HMW
NI10-11		NI11-05					57	HMW
NI10-13	NI10-13						113	HMW
NI10-13		NI11-02					10	HMW
NI10-16	NI10-16						20	No visible DNA
NI10-16		NI11-07					Not enough tissue for ddRAD	
NI10-16						21NZ24	27	HMW
NI10-17	NI10-17						13	HMW
NI10-17		NI11-06					18	HMW

NI10-20	NI10-20						48	HMW
NI10-20					20NZ21		29	smear
NI10-20						21NZ28	33	HMW
NI10-21	NI10-23						10	HMW
NI10-21		NI11-18					36	HMW
NI10-24	NI10-24						51	HMW
NI10-24	NI10-37						Not enough tissue for ddRAD	
NI10-24		NI11-11					Not enough tissue for ddRAD	
NI10-24					20NZ23		13	HMW
NI10-25	NI10-25						15	HMW
NI10-26	NI10-26						58	HMW
NI10-26					20NZ32		26	smear
NI10-27	NI10-27						16	HMW
NI10-27		NI11-31					48	HMW
NI10-28	NI10-28						29	HMW
NI10-28		NI11-29					53	HMW
NI10-32	NI10-32						7	HMW
NI10-33	NI10-33						31	HMW
NI10-35	NI10-35						15	HMW
NI10-35		NI11-10					110	HMW
NI11-01		NI11-01					95	HMW
NI11-09		NI11-09					90	HMW
NI11-09						21NZ13	44	HMW
NI11-14		NI11-14					41	HMW
NI11-14					20NZ01		37	HMW
NI11-17		NI11-17					Not enough tissue for ddRAD	

NI11-20		NI11-20					33	HMW
NI11-20					20NZ15		52	HMW
NI11-21		NI11-21					65	HMW
NI11-23		NI11-23					37	HMW
NI11-24		NI11-24					Not enough tissue for ddRAD	
NI11-25		NI11-25					85	HMW
NI11-28		NI11-28					36	HMW
NI11-30		NI11-30					72	HMW
NI11-33		NI11-33					48	HMW
NI37		NI11-26					70	HMW
NI45		NI11-19					104	HMW
NI56	NI10-31						82	HMW
NI56		NI11-12					62	HMW
NI69	NI10-36						40	HMW
NI69						21NZ32	43	HMW
NI70		NI11-15					54	HMW
NI73	NI10-30						60	HMW
NI74	NI10-15						18	HMW

Results and Discussion

A total of 25 Hector's dolphin samples from stranded or biopsied animals were received by NZCeTA from Southland, Kaikōura, Golden Bay and Queen Charlotte Sound up until February 2022. This represents 22 individuals, based on genotypes matches. Notably, a stranding from 2017 was confirmed as a genetic profile match to a 2014 biopsy sample of a Hector's dolphin from Kaikoura. All but three of 22 individuals had HMW DNA (HMW or smear category) and all had DNA concentrations of > 20 ng/uL, providing a good basis for future advanced genomic work

It is worth noting that of these 25 samples, two duplicate genotypes identified were samples received by NZCeTA separately from DOC and from Massey University from the same dolphin. During the process of this contract and related contracts, we have come across five instances where duplicate samples have been received in this way since 2012. It was not always possible to reconcile these based on metadata provided, although the genetic matches prompted us to review metadata available for all Hector's and Māui samples held where our analysis indicated potential genotype matches. There were also several samples received without metadata that would be useful for population structure analyses (e.g., stranding or bycatch location) for which we have requested information held by DOC. We suggest that a centralised database, shared by DOC, Massey University and University of Auckland for keeping track of the types of samples sent to and held by the different institutes, as well as their sample IDs and metadata, would ensure complete and accurate tracking of samples and data in future. This is particularly critical for the Hector's and Māui dolphins.

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