Data Article

The mixed liver and heart transcriptome dataset of the New Zealand brushtail possum, *Trichosurus vulpecula*

Daniel J. White, Katherine Trought, Brian Hopkins

Manaaki Whenua Landcare Research, Lincoln, 7608, New Zealand
School of Biological Sciences, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

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**Abstract**

New Zealand suffers greatly from invasive mammal predators including rats, stoats, feral cats and possums all of which not only damage or prey on New Zealand’s unique terrestrial biodiversity, but also have huge impact on NZ’s economy as many of these pests act as vectors of disease to farm and game animals. As such, the NZ government has invested nearly $90 m to support an ambitious plan to make the country predator free by 2050. Although there are adequate means to control invasive predator populations, it is widely agreed that current technologies are not sufficient for total eradication and that improved technologies are required. The Achilles Heel approach is one such developmental technology that attempts to exploit variation in the genes of target species that are vital to key physiological or cellular pathways within the body, such that interference with these genes will cause a species-specific death without the harmful effects on the environment and non-targets species that the current suite of control agents engender. Interference could either be through species-specific gene knock-down using such agents as siRNA and/or the use of species-selective chemical toxicants specifically developed against these targets. To assist with identifying species-specific gene targets in the New Zealand brushtail possum (*Trichosurus vulpecula*)

*Corresponding author. School of Biological Sciences, University of Western Australia, 35 Stirling Highway, Crawley, WA, 6009, Australia.
E-mail address: daniel.white@uwa.edu.au (D.J. White).

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we have assembled and annotated a possum mixed heart and liver transcriptome.

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1. Data

Data presented in this article include raw reads for two paired-end libraries generated from mRNA which was isolated from heart and liver tissue of a male New Zealand brushtail possum, *Trichosurus vulpecula*. The BioSample accession numbers for the heart and liver paired-end libraries are SAMN11044341 and SAMN11044342, respectively. These raw reads were quality checked and filtered and then *de novo* assembled into a raw transcriptome. This raw transcriptome was improved by merging open reading frames and predicting protein domains, referred to as the refined transcriptome. This refined transcriptome has been deposited at Manaaki Whenua Landcare Research’s data repository, DataStore, with doi https://doi.org/10.7931/nzcx-1176. Any non-mammalian transcripts were screened out before this final clean transcriptome was functionally annotated. The clean transcriptome
represents the New Zealand brushtail possum combined heart and liver transcriptome and has been deposited as a Transcriptome Shotgun Assembly project at DDBJ/EMBL/GenBank under the accession GHKB00000000. The overall BioProject ID is PRJNA525264.

2. Experimental design, materials and methods

2.1. Tissue samples

Tissue from the heart and liver were obtained from an adult male brushtail possum (*Trichosurus vulpecula*) immediately following euthanasia. This animal was euthanised for a different project at Manaaki Whenua Landcare Research, following all appropriate animal ethics guidelines. Small segments of tissue (5 mm cubed) were placed into 1.7 ml microcentrifuge tubes containing 1 ml DNA/RNA shield™ (Zymo Research, CA, USA) and stored at −80 °C until required for RNA extraction.

2.2. RNA isolation

RNA was extracted from the tissue samples using the Easy-spin Total RNA Extraction Kit (iNtRON Biotechnology Inc, Gyeonggi-do, South Korea) following a modified version of the manufacturer’s instructions. Briefly, 50 mg of tissue was added to 700 µl lysis buffer. The sample was homogenised in a bead mill (Tissuelyser II, Qiagen Inc, CA USA) with a 3-mm stainless steel ball at a frequency of 30/second for 60 seconds. Samples were processed from this point onward following the manufacturer’s instructions until the elution step, where an additional wash step of 700 µl 80% ethanol was placed in the spin column to remove any additional salts. The spin column was dried for 2 minutes at 16,000×g and the RNA eluted in 50 µl ultra-pure water. The eluted RNA was treated with the RNase-free Dnase set (Qiagen Inc, CA USA) using the protocol outlined in Appendix E of the RNeasy mini handbook (DNase Digestion of RNA before RNA Cleanup (Qiagen Inc, CA USA)). The purified RNA was eluted in a final volume of 50 µl ultrapure water. RNA quantity and quality were assessed using the LabChip GX Touch HT (PerkinElmer Inc, MA, USA) and 2.5–7.5 µg total RNA was loaded into RNASTable tubes (Biomatrica Inc, San Diego, USA). The RNA was stabilised and dried as per the manufacturer’s instructions and shipped at ambient temperature for library preparation and transcriptome sequencing to Macrogen, South Korea. 100 base-pair (bp) strand specific paired-end mRNA libraries were made for each tissue type and sequenced on one lane of an Illumina HiSeq 2500 platform.

2.3. Bioinformatic analysis

Raw paired-end reads were quality checked using FASTQC v0.11.8 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc).

Adapters were trimmed from the 3’ end of reads using CUTADAPT v1.16 [2], then quality filtered in SOLEXAQA++ v3.1.7 based on base-pair quality (p set to 0.01) and minimum read length of 25 bp [3]. After filtering any reads lacking their pair were removed, so only properly paired reads were included

| Table 1 |
| NZ brushtail possum raw transcriptome assembly statistics. |

| No. libraries | 2 — heart tissue, liver tissue |
| Total no. bases assembled | 201,171,876 |
| No. transcripts | 203,458 |
| N50 | 2451 nt |
| Ex90N50 | 3055 nt |
| Ex83N50a | 3381 nt |
| Shortest contig | 176 nt |
| Longest contig | 18,175 nt |
| No. transcripts in transcriptome after merging predicted ORFs, and pfam domain and peptide sequence homology | 66,610 |

a Ex83N50 is the optimal ExN50 value.
in the assembly. As no reference genome existed for the brushtail possum at the time of assembly, a *de novo* assembly was done, using reads from both the heart and liver tissue paired-end libraries in TRINITY v2.8.4 with the SS_lib_type flag set to RF [4]. Assessment of the assembly was made by calculating N50, read content of the assembly and ExN50, which is a measurement of N50 at desired percentages of total expressed transcripts, using TRINITY [5]. N50 values can be driven downwards by overabundance of small incomplete transcripts beyond the optimal ExN50 value, and E90N50 is a commonly chosen value for quality assessment. Assembly statistics are provided in Table 1.

TRANSDECODER v5.5.0 [5] was run to select the best transcripts from the raw transcriptome. First, open reading frames (ORFs) from the same gene were merged into the longest representative open reading frame. This set of transcripts were then processed by gene prediction software and screened against the SwissProt database using the blastp option in BLAST v2.6.0 (evalue threshold set to 1e-5) and the Pfam database using HMMER v3.1 with default parameters to detect protein domains [6,7]. This generated a refined transcriptome which was purged of possible non-mammal contaminants by screening for homology to the mammal subset of the non-redundant nr database at Genbank using blastx-fast (which uses a word length of 6) in Blast2Go Pro, with the evalue threshold set to 1e-3 [8,9]. Any transcripts that did not find a match were excluded from further analysis, and the retained transcripts were considered the final, clean NZ brushtail possum transcriptome (see Table 2). Due to less information available in databases for marsupial mammals, search criteria were left at default values to prevent excluding potential possum proteins.

For annotation and characterisation of the clean transcriptome, a new project was set up in Blast2Go Pro and the final sub-set of transcripts were screened once more against the mammalian subset of the nr database at Genbank. This time regular blastx (word length of 3) and an evalue threshold of 1e-3, as well as running a functional analysis using InterProScan, were run in Blast2Go Pro [8,9]. InterProScan predicts domains and important sites based on numerous databases and prediction algorithms and classifies proteins into families. CDD and Pfam resources were used to search for domains [10,11],

### Table 2
**NZ brushtail possum clean transcriptome assembly statistics.**

| No. transcripts | 50,484 |
|-----------------|-------|
| Total length of transcripts | 62,738,637 nt |
| Mean length | 1242 nt |
| N50 | 1737 nt |
| Shortest contig | 258 nt |
| Longest contig | 15,819 nt |

Fig. 1. A summary of blastx, InterProScan, mapping and annotation results for 50,484 contigs in the final clean NZ brushtail possum transcriptome.
Panther was used to identify families and associate proteins with function [12], Prosite was used to identify families based on sites and patterns [13] and Superfamily was used to predict functional domains [14]. Of the 50,484 transcripts in the clean transcriptome 50,477 (100%) had blastx hits and 47,150 (93%) had IPS hits (Fig. 1). A summary of transcript function based on gene ontology level 2 terms for biological process, cellular component and molecular function is provided in Fig. 2.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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