DDRAD-SEQ LIBRARIES PREPARATION AND SEQUENCING

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Genomic DNA from liver and muscle samples were extracted with DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA), following the manufacturer's recommendations, except for the DNA elution step where we used double distilled water (ddH_20) instead of elution buffer. Genomic DNA from skin and dry muscle samples were extracted following the same protocol as fresh tissues but with some modifications before the digestion step. In a sterile environment the hairs were removed from the skin samples. Afterwards, both the skin and dry muscle samples were hydrated for three days with ddH_20, replacing the water every 24 hours. After the hydration, the material was washed twice with 1X STE buffer (Bi *et al.* 2013), and then cut into small pieces to facilitate the digestion. During the digestion step I added 1 mM of dithiotreitol (DTT), a reducing agent, in 20 μL of volume per sample (Rohland & Hofreiter 2007). Extracted DNA was quantified by Qubit fluorometer (Life Technologies, Grand Island, NY, USA), and it was diluted with ddH_20 or concentrated in the SpeedVac Concentrator (ThermoFisher Scientific, Waltham, MA, USA) at 43º C (medium temperature) to reach the concentration of 17.6 ng/μL.

We followed the protocol from Peterson *et al.* (2012) for the preparation of genomic libraries (see Material and Methods for details about the number of samples and libraries) using the ddRAD-Seq technique. In this approach 300 ng of genomic DNA (*i.e.*, 17 μL of extracted DNA) were cut in variable-sized fragments, using two restriction enzymes: *Eco*-RI and *Mse*-I. The resulting solution was cleaned with commercial Ampure XP Beads (Beckman Coulter, Brea, CA, USA) and quantified in Qubit fluorometer (Life Technologies, Grand Island, NY, USA). Then, I used 50 ng of fragmented DNA in a volume of 33 μL per sample for the ligation step, in which the ends of the fragmented DNA were bonded to the Illumina adapters and a unique barcode per sample. After the reaction samples were

pooled together and the solution was cleaned again with commercial Ampure XP Beads (Beckman Coulter, Brea, CA, USA). DNA fragments were automatically selected by size (between 350 and 450 bp) through Pippin Prep (Sage Science, Beverly, MA, USA) and selected fragments were amplified by PCR. The libraries were cleaned with the beads again, quantified and sequenced. All libraries were sequenced in three lanes of HiSeq2500 (Illumina, San Diego, CA, USA) according to instructions of the manufacturer to generate 150 base pairs, single-end reads in the Hospital for Sick Children (Toronto, Ontario, Canada).

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