Capture of the Canine Exome Using the Nextera® Exome Enrichment Kit

Contributed by members of the Animal Health Trust, UK.

Introduction

Whole human exome enrichment tools have been used successfully to analyze non-human primate exomes, such as chimpanzee and macaque.[1,2] To determine whether human and canine genomes are sufficiently homologous for exome capture, researchers at the Animal Health Trust applied the Nextera Exome Enrichment Kit to canine DNA samples. Exome sequencing on the MiSeq® benchtop sequencing system demonstrated that human and canine genomes contain sufficient homology, enabling successful enrichment of the canine exome using the Nextera kit. This method also presents a unique opportunity to investigate diseases that are common to dogs and humans[3] without resorting to costly, customized tools.

Experimental Design

The Nextera Exome Enrichment Kit offers an integrated sample preparation and enrichment method, complementing the streamlined MiSeq workflow. In this study, one canine genomic DNA sample and a control human DNA sample were enriched using the Nextera Exome Enrichment Kit. Each enrichment was performed independently to avoid potential capture and sequencing bias toward the human DNA sample. Captured libraries were quantified and pooled for sequencing on the MiSeq system. Automated cluster generation and a 2 × 35 bp sequencing run was carried out within a few hours, without any further user intervention. Primary data analysis was performed on the instrument using MiSeq Reporter software and the Integrative Genomics Viewer provided by the Broad Institute.[4] Basic alignments were performed with the NCBI Basic Local Alignment Search Tool (BLAST).

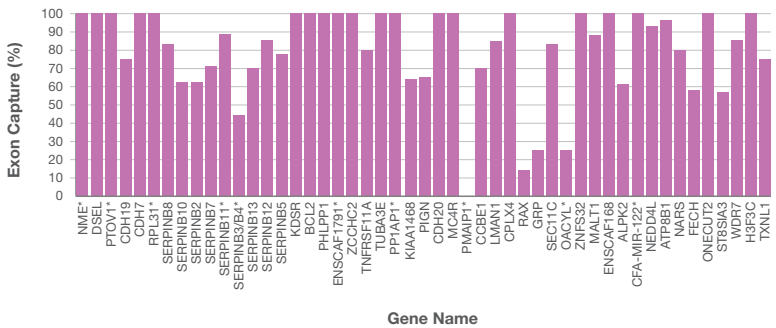
Results

Exome sequencing on the MiSeq system generated a data set of 0.8 Gb in total, with 0.4 Gb of data contributed from each sample. This coverage was sufficient to evaluate the effectiveness of the Nextera Exome Enrichment Kit with canine DNA. Due to the small size of the data set, if a read depth greater than 3× coverage was achieved across each entire exon, exon capture was considered successful. Exon capture percentages per gene are shown in Figure 1. A visual scan across a region of the canine genome 10 Mb in length determined capture efficiency.

In total, this region contains 490 exons across 50 genes, 79% of which were captured in this study. The *RPGRIP1* gene, a candidate gene for retinitis pigmentosa, typically demonstrates variable levels of homology to human *RPGRIP1* at the exon level. [5] Researchers analyzed the *RPGRIP1* gene to gauge the approximate homology required to capture canine exons. The results illustrated in Figure 2 indicate that exons with ≥ 80% homology are successfully captured, with a success rate of 85% (11 of 13 exons). For exons with < 80% homology, capture was less successful, with a success rate of 18% (2 of 11 exons). Exon size also likely influenced capture success, as larger exons often correlate to higher success rates.

To determine whether the Nextera Exome Enrichment Kit captures disease-causing mutations in canine DNA, researchers investigated 18 previously identified loci associated with disease. Of the loci investigated, 16 of them (89%) were successfully captured and sequenced (Table 1). These results provide support for future studies in canine genetics and disease research.

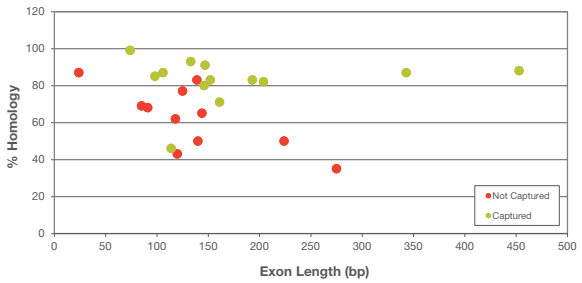
Figure 1: Exon Capture across Region of Canine Genome



The Nextera Exome Enrichment Kit provides sufficient exon capture across a region of the canine chromosome 1 that is 10 Mb in length. The only gene with no exons captured is the canine homologue of *PMAIP1*, likely due to low homology levels or specificity to the human exome.

*As the canine specificity of these genes has not yet been confirmed in the RefSeq database, the names presented here indicate homologous genes found in other species.

Figure 2: Canine Exon Capture Success



Canine exon capture success is dependent on homology to human exons, demonstrated here in the *RPGRIP1* gene. Exons with ≥ 80% homology were successfully captured using the Nextera Exome Enrichment Kit. *RPGRIP1* homology between canine and human genomes was calculated using information available in the Ensembl genome browser.[6]

Conclusions

The data presented here illustrate that the Nextera Exome Enrichment Kit can be used to enrich a large portion of exons in the canine genome. Combined with rapid sequencing on the MiSeq platform, the easy-to-use Nextera Exome Enrichment Kit provides an opportunity to study inherited diseases in canine and other mammalian species.

Learn More

For questions regarding this application note, contact Oliver Forman at oliver.forman@aht.org.uk. To learn more about the MiSeq system or the Nextera Exome Enrichment Kit, visit www.illumina.com/miseq or www.illumina.com/products/nextera_exome_enrichment_kit.ilmn.

References

1. Vallender EJ. (2011) Expanding whole-exome resequencing into non-human primates. Genome Biol 12: R87.
2. Jin X, He M, Ferguson B, Meng Y, Ouyang L, et al. (2012) An effort to use human-based exome capture methods to analyze chimpanzee and macaque exomes. PLoS One 7: e40637.
3. Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, et al. (2005) Genome sequence, comparative analysis, and haplotype structure of the domestic dog. Nature 438: 803–19.
4. www.broadinstitute.org/igv
5. Mellersh CS, Boursnell ME, Pettitt L, Ryder EJ, Holmes NG, et al. (2006) Canine *RPGRIP1* mutation establishes cone-rod dystrophy in miniature longhaired dachshunds as a homologue of human Leber's congenital amaurosis. Genomics 88: 293–301.
6. uswest.ensembl.org/index.html

Table 1: Capture of Disease-Causing Mutations

Phenotype	Gene	Depth	Additional Read Pairs Across Locus
Bobtail	T	3	Yes
Neuronal ceroid ipofuscinosis (NCL)	CLN5	4	Yes
Canine leukocyte adhesion deficiency (CLAD)	ITGB2	19	Yes
Congenital stationary night blindness	RPE65	1*	Yes
Canine coat	FGF5	0**	No
Fucosidosis	FUCA1	3	Yes
Hyperuricosuria and hyperuricemia	SLC2A9	3	Yes
Pyruvate dehydrogenase phosphatase (PDP1) deficiency	PDP1	2	Yes
Phosphofructokinase deficiency	PFKM	0*	Yes
Canine factor VII deficiency	F7	15	Yes
von Willebrand Disease	VWF	9	Yes
Yellow coat	MC1R	10	Yes
Progressive retinal atrophy in golden retrievers	SLC4A3	3	Yes
Rod-cone degeneration	C2ORF71	1**	No
L-2-hydroxyglutaric aciduria	L2HGDH	1*	Yes
Hereditary cataracts	HSF4	24	Yes
Curly coat syndrome	FAM83H	2	Yes
Multidrug resistance 1	ABCB1	0*	Yes

*Despite low reported depth indicating that the mutation position was not sequenced, these genes had 6–9 read pairs on either side of the mutation, resulting in adequate capture of the region.

**These genes were not sufficiently captured, likely due to low homology or specificity to the canine genome.

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Pub. No. 770-2013-020 Current as of 05 June 2013

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