Design and performance of the Twist Alliance Canine Exome for comparative genomic and oncology research

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INTRODUCTION

Pet dogs are a powerful natural model system for cancer and other human diseases. The importance of dogs for comparative and translational cancer research continues to grow because they overcome limitations inherent in other model systems (*1–3*). Dogs spontaneously develop many of the same cancers as humans, with clinical, pathological, and genomic similarities to human cancers (*2*). They develop these cancers while sharing our environment and in the context of an intact immune system. The clinical course of dog cancers is often accelerated, reflecting their shorter lifespans. As a result, clinical trials in pet dogs can be performed more quickly and with more flexibility than in humans, and benefit both dog and human cancer patients.

The dog model offers unique potential to advance cancer genomics and precision medicine, as many human and dog tumors of the same histologic types (including lymphoma(*4*, *5*), osteosarcoma(*6–9*), glioma(*10*), melanoma(*11*, *12*), mammary tumors(*13*), and hemangiosarcoma (angiosarcoma)(*14*, *15*)) share similar underlying somatic alterations (*14*, *15*). Driver mutations of interest for the development and testing of targeted therapies are the same in dogs and humans at the gene and amino acid levels (*4*, *15–17*). Thus, comparative dog cancer studies can investigate, at genome-scale, spontaneously occurring cancer mutations and how they influence clinical outcomes, including response to therapeutics. Cost-effective genomic assays with sufficient breadth and depth of coverage, and that include targets important in human and dog cancers, are essential for realizing the full value of dogs as a comparative model.

The Twist Alliance Canine Exome is an off-the-shelf solution to exome sequencing that supports comparative genomic and comparative oncology studies in dogs. It enables cost-effective deep sequencing essential for identifying both germ-line and somatic variants, is compatible with existing workflows, and is priced competitively.

DESIGN

The Twist Alliance Canine Exome was designed by researchers from the Vertebrate Genomics group at the Broad Institute, the Cummings School of Veterinary Medicine at Tuft University and the UMass Chan Medical School. It was developed in collaboration with the Genomics Platform at the Broad Institute. The Bioinformatics team at Twist Bioscience designed baits to capture the regions included in the design.

The Twist Alliance Canine Exome is designed to be useful for both oncology and non-oncology focused studies. It captures the exons of nearly all protein-coding genes annotated in either the human and dog genomes. It also captures non-coding regions of the dog genome that are orthologous to those targeted in by the Broad's Human Somatic Exome Capture, and of particular interest to cancer researchers. The orthologous regions of the dog genome were defined using the LiftOver tool(*18*, *19*).

This panel targets:

- 20,257 genes derived from the Ensembl canFam3.1 canine gene annotation (Release 99) (*20*, *21*)
- Human exome-targeted regions without annotated 1:1 canine orthologs
- Human cancer-specific targets (hg19)
- Selected gene promoter sites and UTRs

In total, the Twist Alliance Canine Exome design covers approximately 36.6 Mb of target regions using roughly 40.5 Mb of probe territory.

PERFORMANCE

Pilot project: cell-free DNA

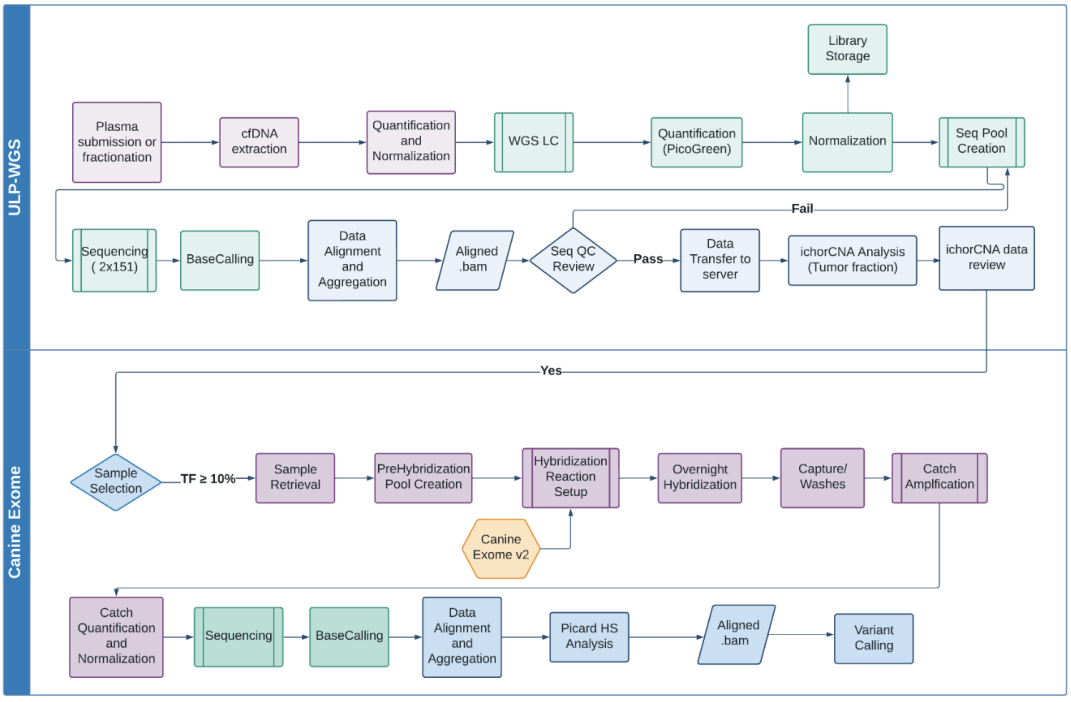

Figure 1. Workflow schema for pilot project using Canine Exome v2.

Twelve cell-free DNA libraries which had been processed according to the Genomics Platform at the Broad Institute's cell-free DNA workflow and underwent ultra low-pass whole genome sequencing (ULPWGS) were selected for a pilot project evaluating the new Twist Alliance Canine Exome (**Figure 1**). These samples were isolated from plasma collected from dogs with various cancer types, which were found on analysis with ichorCNA (*22*) to have a tumor fraction of ≥ 10%.

The libraries were processed according to the Twist Alliance Canine Exome workflow. Initially, cfDNA input was normalized to approximately 10 ng in 50 uL of TE buffer

(10mM Tris HCl 1mM EDTA, pH 8.0) according to picogreen quantification. Library preparation was performed using a commercially available kit provided by KAPA Biosystems (KAPA HyperPrep Kit with Library Amplification product KK8504) and IDT's duplex UMI adapters. Unique 8-base dual index sequences embedded within the p5 and p7 primers (purchased from IDT) were added during PCR. Enzymatic clean-ups were performed using Beckman Coulter AMPure XP beads with elution volumes reduced to 30µL to maximize library concentration. The resulting libraries were quantified and pooled prior to selection. The library pool along with the Twist Alliance Canine Exome probe set, manufactured by Twist BioScience, were used as input into the xGEn Hyb and Wash kit (IDT). Post capture, libraries were sequenced on the Illumina HiSeq X platform using a 2x151 bp run. Data analysis, including alignment to the reference genome, marking duplicate reads, and running the Picard hybsel tools was performed in the cloud pipeline using the bait and target interval files based on the CanFam3.1 reference build.

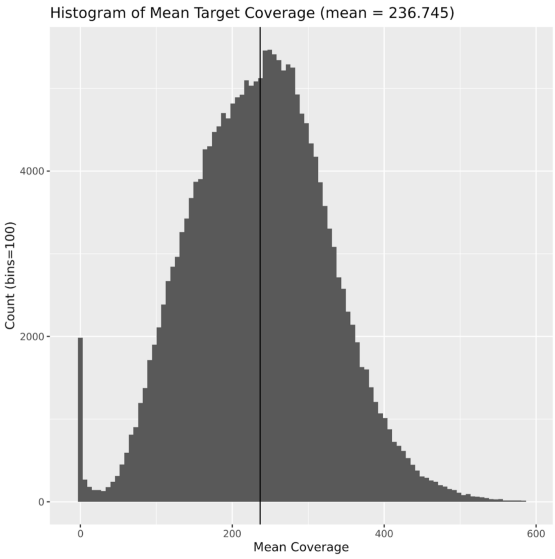

Figure 2. Mean target coverage in cfDNA pilot experiment

Cell-free DNA coverage

The Genomics Platform at the Broad Institute performed Bait QC Coverage Analysis on the pilot project data to evaluate the performance of the newly designed exome panel (**Figure 2**). We calculated per-base coverage for the twelve dog exome samples using GATK-4.1.9.0 DepthOfCoverage, filtering out bases with base quality < 20, reads with mapping quality <20, and loci with "N" in the reference. From the per-base coverage, we then calculated mean coverage per target. To evaluate the panel's performance, we subset targets by their coverage relative to the overall mean coverage among all targets, designating targets below 20% overall mean target coverage as being low-covered. For a well-performing panel, we look for <3% low-covered targets and a smooth histogram of target coverage with few 0x coverage targets. For this panel, only 3,367 / 206,018 (1.63%) of targets were low-covered, however, we saw a spike in the histogram at 0x, with 1199 targets having no coverage. Looking into these uncovered targets, we found that the majority (994/1199) mapped to loci that were identical to other regions in the genome and therefore were assigned mapping quality 0 and filtered out by GATK DepthOfCoverage.

We reevaluated these zero coverage targets in the newly released CanFam4 genome(*23*). Using PicardTools' SamToFastq, the twelve bam files were reverted to FASTQ, then aligned to both canFam3 and canFam4 using bwa mem(*24*) with default options. The bed file containing the target regions used for the exome design was converted from canFam3 to canfam4 using LiftOver with default options. In total, 205,524 regions successfully lifted over, and 504 intervals failed to lift over. Bedtools coverage(*25*) was used to count the intervals with zero coverage of all bases. On average, 88.3% of regions not covered in canFam3.1 were covered in canFam4.

Cell-free DNA capture metrics

The Twist Bioinformatics team performed an analysis of capture metrics from the pilot project using the Genomics Platform at the Broad Institute's hybridization protocol. Overall the performance of Twist Alliance Canine Exome panel exceeded expectations for a non-human panel, with most metrics higher than the expected values for the Twist Human Exome 2.0 (**Table 1**).

To normalize the performance metrics, Twist bioinformatics downsampled the 12 cfDNA samples sequenced in the pilot project to 150x reads. The average fold enrichment was 40x, yielding an average on-target rate of 94%, showing not only high efficiency of target enrichment, but also very high specificity to those targets. As expected for cfDNA samples, some of the metrics were highly impacted by the amount of input DNA, quality, and sample preparation. Therefore some of these metrics are quite variable in the pilot samples. HS library size, the estimated number of unique library molecules captured and sequenced, ranged from 85 million - 281 million. Percent duplication was inversely correlated to library size, and was on average 7.5% with a range of 3 - 10%. Fold 80, a measure of coverage uniformity, ranged from 1.4 - 2.0 with a corresponding mean target coverage >30x at 76 - 89%. On average, the percentage of targets with zero coverage was 1%.

Sample	HS Library Size	Mean Target Coverage	On-Target	Fold Enrichment	Fold 80	PCT Target Bases >30X	Duplicates	Zero-coverage Targets
Sample_1	281,344,402	51.58	93.90%	41.98	1.78	80.00%	3.34%	1.10%
Sample_2	115,595,829	50.69	93.80%	39.65	1.41	88.98%	7.68%	0.93%
Sample_3	113,875,619	52.24	93.90%	40.74	1.69	82.71%	7.81%	1.01%
Sample_4	105,764,992	51.85	93.80%	40.81	1.79	79.20%	8.35%	0.98%
Sample_5	109,469,797	51.88	93.70%	41.52	2.00	75.89%	8.10%	1.01%
Sample_6	84,816,642	50.73	93.80%	40.81	1.75	79.09%	10.24%	0.95%
Sample_7	112,682,000	49.77	93.50%	41.90	1.56	84.46%	7.85%	1.11%
Sample_8	98,585,653	52.09	93.40%	40.62	1.63	83.81%	8.89%	1.05%
Sample_9	94,625,683	50.06	93.70%	41.21	1.67	80.81%	9.27%	1.05%
Sample_10	95,614,589	52.49	93.70%	38.34	1.50	88.17%	9.15%	0.86%
Sample_11	220,226,507	54.82	94.00%	40.70	1.83	81.06%	4.22%	1.01%
Sample_12	180,932,820	54.86	93.90%	41.15	2.03	77.72%	5.07%	1.02%
Average	**134,461,211**	**51.92**	**93.80%**	**40.79**	**1.72**	**81.80%**	**7.50%**	**1.00%**
Twist Human Exome 2.0	319,389,408	65.30	93%	39	1.28	97.68%	4.2%	0.7%

Table 1. Cell-free DNA pilot sequencing metrics.

Genomic DNA pilot

Twist performed a second pilot of the panel run on control gDNA derived from two commercially-available canine cell lines (BioChain Institute, Inc.), one male (Lot: C5016490), and one female (Lot: B306066), with four replicates of each cell line. The full Twist library prep and hybridization workflow(s) were utilized to generate performance metrics to use as a benchmark. Briefly, Twist Library Preparation EF 2.0 was used following the published protocol(*26*). The libraries were then taken into the Fast Hyb (*27*) or Standard Hyb (v2) (*28*) workflows following the protocol for an 8-plex reaction. Final captured libraries were run on a NextSeq550, 2x74, HO flow cell with 5% PhiX. All samples were downsampled to 150X raw reads for normalization and run through the analysis pipeline.

Table 2 shows a summary of Picard performance metrics. Importantly, fold 80 and HS library size were greatly improved in this follow-up experiment. We believe this can be replicated for cfDNA, FFPE, or other hard to work with samples when there is enough input DNA to reach the protocol recommendations.

	gDNA (mean)		**cfDNA (mean)**	**Twist Human Exome 2.0**
Metrics (150X DS)	Twist EF2.0 LP w/ Std Hyb v2	Twist EF2.0 w/ Fast Hyb	The Genomics Platform at the Broad Institute LP + Hyb	Twist EF2.0 w/ Std Hyb v2
HS Library Size	430M	422M	135M	319M
Mean Target Coverage	63.83	74.92	51.92	65.3
On-Target	85.93%	92.92%	93.80%	93%
Fold 80	1.34	1.43	1.72	1.28
PCT Target Bases >30X	94.31%	92.97%	81.80%	97.68%
Duplicates	3.82%	4.34%	7.50%	4.2%

Table 2. Genomic DNA pilot capture metrics.

Summary

The Twist Alliance Canine Exome panel will enable researchers from across the globe to undertake powerful comparative and veterinary dog genomics studies at a fraction of the price due to commercialization. By facilitating canine comparative studies, we hope to speed discovery and translation of novel findings into both the veterinary and human clinics.

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ADDITIONAL INFORMATION

Twist Alliance Canine Exome product page: https://www.twistbioscience.com/products/ngs/alliance-panels#tab-4

Webinar: https://www.twistbioscience.com/resources/webinar/developing-blood-biopsy-technology-using-new-canine-exome-capture

Capture design: https://www.twistbioscience.com/resources/data-files/twist-alliance-canine-exome-405-mb-bed-file

References

1. J. D. Schiffman, M. Breen, Comparative oncology: what dogs and other species can teach us about humans with cancer. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370** (2015), doi:10.1098/rstb.2014.0231.
2. H. L. Gardner, J. M. Fenger, C. A. London, Dogs as a Model for Cancer. *Annu Rev Anim Biosci*. **4**, 199–222 (2016).
3. A. K. LeBlanc, C. N. Mazcko, Improving human cancer therapy through the evaluation of pet dogs. *Nat. Rev. Cancer*.

20, 727–742 (2020).

4. I. Elvers, J. Turner-Maier, R. Swofford, M. Koltookian, J. Johnson, C. Stewart, C.-Z. Zhang, S. E. Schumacher, R. Beroukhim, M. Rosenberg, R. Thomas, E. Mauceli, G. Getz, F. D. Palma, J. F. Modiano, M. Breen, K. Lindblad-Toh, J. Alföldi, Exome sequencing of lymphomas from three dog breeds reveals somatic mutation patterns reflecting genetic background. *Genome Res.* **25**, 1634–1645 (2015).

5. K. R. Bushell, Y. Kim, F. C. Chan, S. Ben-Neriah, A. Jenks, M. Alcaide, D. Fornika, B. M. Grande, S. Arthur, R. D. Gascoyne, C. Steidl, R. D. Morin, Genetic inactivation of TRAF3 in canine and human B-cell lymphoma. *Blood*. **125**, 999–1005 (2015).

6. M. Paoloni, S. Davis, S. Lana, S. Withrow, L. Sangiorgi, P. Picci, S. Hewitt, T. Triche, P. Meltzer, C. Khanna, Canine tumor cross-species genomics uncovers targets linked to osteosarcoma progression. *BMC Genomics*. **10**, 625 (2009).

7. S. Sakthikumar, I. Elvers, J. Kim, M. L. Arendt, R. Thomas, J. Turner-Maier, R. Swofford, J. Johnson, S. E. Schumacher, J. Alföldi, E. Axelsson, C. G. Couto, W. C. Kisseberth, M. E. Pettersson, G. Getz, J. R. S. Meadows, J. F. Modiano, M. Breen, M. Kierczak, K. Forsberg-Nilsson, V. D. Marinescu, K. Lindblad-Toh, SETD2 Is Recurrently Mutated in Whole-Exome Sequenced Canine Osteosarcoma. *Cancer Res.* **78**, 3421–3431 (2018).

8. H. L. Gardner, K. Sivaprakasam, N. Briones, V. Zismann, N. Perdigones, K. Drenner, S. Facista, R. Richholt, W. Liang, J. Aldrich, J. M. Trent, P. G. Shields, N. Robinson, J. Johnson, S. Lana, P. Houghton, J. Fenger, G. Lorch, K. A. Janeway, C. A. London, W. P. D. Hendricks, Canine osteosarcoma genome sequencing identifies recurrent mutations in DMD and the histone methyltransferase gene SETD2. *Commun Biol*. **2**, 266 (2019).

9. S. Das, R. Idate, D. P. Regan, J. S. Fowles, S. E. Lana, D. H. Thamm, D. L. Gustafson, D. L. Duval, Immune pathways and TP53 missense mutations are associated with longer survival in canine osteosarcoma. *Commun Biol*. **4**, 1178 (2021).

10. S. B. Amin, K. J. Anderson, C. E. Boudreau, E. Martinez-Ledesma, E. Kocakavuk, K. C. Johnson, F. P. Barthel, F. S. Varn, C. Kassab, X. Ling, H. Kim, M. Barter, C. C. Lau, C. Y. Ngan, M. Chapman, J. W. Koehler, J. P. Long, A. D. Miller, C. R. Miller, B. F. Porter, D. R. Rissi, C. Mazcko, A. K. LeBlanc, P. J. Dickinson, R. A. Packer, A. R. Taylor, J. H. Rossmeisl Jr, K. D. Woolard, A. B. Heimberger, J. M. Levine, R. G. W. Verhaak, Comparative Molecular Life History of Spontaneous Canine and Human Gliomas. *Cancer Cell*. **37**, 243–257.e7 (2020).

11. W. P. D. Hendricks, V. Zismann, K. Sivaprakasam, C. Legendre, K. Poorman, W. Tembe, N. Perdigones, J. Kiefer, W. Liang, V. DeLuca, M. Stark, A. Ruhe, R. Froman, N. S. Duesbery, M. Washington, J. Aldrich, M. W. Neff, M. J. Huentelman, N. Hayward, K. Brown, D. Thamm, G. Post, C. Khanna, B. Davis, M. Breen, A. Sekulic, J. M. Trent, Somatic inactivating PTPRJ mutations and dysregulated pathways identified in canine malignant melanoma by integrated comparative genomic analysis. *PLOS Genetics*. **14** (2018), p. e1007589.

12. K. Wong, L. van der Weyden, C. R. Schott, A. Foote, F. Constantino-Casas, S. Smith, J. M. Dobson, E. P. Murchison, H. Wu, I. Yeh, D. R. Fullen, N. Joseph, B. C. Bastian, R. M. Patel, I. Martincorena, C. D. Robles-Espinoza, V. Iyer, M. L. Kuijjer, M. J. Arends, T. Brenn, P. W. Harms, G. A. Wood, D. J. Adams, Cross-species genomic landscape comparison of human mucosal melanoma with canine oral and equine melanoma. *Nat. Commun.* **10**, 353 (2019).

13. T.-M. Kim, I. S. Yang, B.-J. Seung, S. Lee, D. Kim, Y.-J. Ha, M.-K. Seo, K.-K. Kim, H. S. Kim, J.-H. Cheong, J.-H. Sur, H. Nam, S. Kim, Cross-species oncogenic signatures of breast cancer in canine mammary tumors. *Nat.*

Commun. **11**, 3616 (2020).

14. G. Wang, M. Wu, M. A. Maloneyhuss, J. Wojcik, A. C. Durham, N. J. Mason, D. B. Roth, Actionable mutations in canine hemangiosarcoma. *PLoS One*. **12**, e0188667 (2017).

15. K. Megquier, J. Turner-Maier, R. Swofford, J.-H. Kim, A. L. Sarver, C. Wang, S. Sakthikumar, J. Johnson, M. Koltookian, M. Lewellen, M. C. Scott, A. J. Graef, L. Borst, N. Tonomura, J. Alfoldi, C. Painter, R. Thomas, E. K. Karlsson, M. Breen, J. F. Modiano, I. Elvers, K. Lindblad-Toh, Comparative genomics reveals shared mutational landscape in canine hemangiosarcoma and human angiosarcoma. *Mol. Cancer Res.* (2019), doi:10.1158/1541-7786.MCR-19-0221.

16. M. Tagawa, N. Tambo, M. Maezawa, M. Tomihari, K.-I. Watanabe, H. Inokuma, K. Miyahara, Quantitative analysis of the BRAF V595E mutation in plasma cell-free DNA from dogs with urothelial carcinoma. *PLoS One*. **15**, e0232365 (2020).

17. L. Rodrigues, J. Watson, Y. Feng, B. Lewis, G. Harvey, K. Megquier, L. Lambert, A. Miller, C. Lopes, S. Zhao, Shared hotspot mutations in spontaneously arising cancers position dog as an unparalleled comparative model for precision therapeutics. *bioRxiv* (2021), p. 2021.10.22.465469.

18. L. R. Nassar, G. P. Barber, A. Benet-Pagès, J. Casper, H. Clawson, M. Diekhans, C. Fischer, J. N. Gonzalez, A. S. Hinrichs, B. T. Lee, C. M. Lee, P. Muthuraman, B. Nguy, T. Pereira, P. Nejad, G. Perez, B. J. Raney, D. Schmelter, M. L. Speir, B. D. Wick, A. S. Zweig, D. Haussler, R. M. Kuhn, M. Haeussler, W. J. Kent, The UCSC Genome Browser database: 2023 update. *Nucleic Acids Res.* **51**, D1188–D1195 (2023).

19. R. M. Kuhn, D. Haussler, W. J. Kent, The UCSC genome browser and associated tools. *Brief. Bioinform.* **14**, 144–161 (2013).

20. M. P. Hoeppner, A. Lundquist, M. Pirun, J. R. S. Meadows, N. Zamani, J. Johnson, G. Sundström, A. Cook, M. G. FitzGerald, R. Swofford, E. Mauceli, B. T. Moghadam, A. Greka, J. Alföldi, A. Abouelleil, L. Aftuck, D. Bessette, A. Berlin, A. Brown, G. Gearin, A. Lui, J. P. Macdonald, M. Priest, T. Shea, J. Turner-Maier, A. Zimmer, E. S. Lander, F. di Palma, K. Lindblad-Toh, M. G. Grabherr, An improved canine genome and a comprehensive catalogue of coding genes and non-coding transcripts. *PLoS One*. **9**, e91172 (2014).

21. F. Cunningham, J. E. Allen, J. Allen, J. Alvarez-Jarreta, M. R. Amode, I. M. Armean, O. Austine-Orimoloye, A. G. Azov, I. Barnes, R. Bennett, A. Berry, J. Bhai, A. Bignell, K. Billis, S. Boddu, L. Brooks, M. Charkhchi, C. Cummins, L. Da Rin Fioretto, C. Davidson, K. Dodiya, S. Donaldson, B. El Houdaigui, T. El Naboulsi, R. Fatima, C. G. Giron, T. Genez, J. G. Martinez, C. Guijarro-Clarke, A. Gymer, M. Hardy, Z. Hollis, T. Hourlier, T. Hunt, T. Juettemann, V. Kaikala, M. Kay, I. Lavidas, T. Le, D. Lemos, J. C. Marugán, S. Mohanan, A. Mushtaq, M. Naven, D. N. Ogeh, A. Parker, A. Parton, M. Perry, I. Piližota, I. Prosovetskaia, M. P. Sakthivel, A. I. A. Salam, B. M. Schmitt, H. Schuilenburg, D. Sheppard, J. G. Pérez-Silva, W. Stark, E. Steed, K. Sutinen, R. Sukumaran, D. Sumathipala, M.-M. Suner, M. Szpak, A. Thormann, F. F. Tricomi, D. Urbina-Gómez, A. Veidenberg, T. A. Walsh, B. Walts, N. Willhoft, A. Winterbottom, E. Wass, M. Chakiachvili, B. Flint, A. Frankish, S. Giorgetti, L. Haggerty, S. E. Hunt, G. R. IIsley, J. E. Loveland, F. J. Martin, B. Moore, J. M. Mudge, M. Muffato, E. Perry, M. Ruffier, J. Tate, D. Thybert, S. J. Trevanion, S. Dyer, P. W. Harrison, K. L. Howe, A. D. Yates, D. R. Zerbino, P. Flicek, Ensembl 2022. *Nucleic Acids Res.* **50**, D988–D995 (2022).

22. V. A. Adalsteinsson, G. Ha, S. S. Freeman, A. D. Choudhury, D. G. Stover, H. A. Parsons, G. Gydush, S. C. Reed, D. Rotem, J. Rhoades, D. Loginov, D. Livitz, D. Rosebrock, I. Leshchiner, J. Kim, C. Stewart, M. Rosenberg, J. M.

Francis, C.-Z. Zhang, O. Cohen, C. Oh, H. Ding, P. Polak, M. Lloyd, S. Mahmud, K. Helvie, M. S. Merrill, R. A. Santiago, E. P. O'Connor, S. H. Jeong, R. Leeson, R. M. Barry, J. F. Kramkowski, Z. Zhang, L. Polacek, J. G. Lohr, M. Schleicher, E. Lipscomb, A. Saltzman, N. M. Oliver, L. Marini, A. G. Waks, L. C. Harshman, S. M. Tolaney, E. M. Van Allen, E. P. Winer, N. U. Lin, M. Nakabayashi, M.-E. Taplin, C. M. Johannessen, L. A. Garraway, T. R. Golub, J. S. Boehm, N. Wagle, G. Getz, J. C. Love, M. Meyerson, Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. *Nat. Commun.* **8**, 1324 (2017).

23. C. Wang, O. Wallerman, M.-L. Arendt, E. Sundström, Å. Karlsson, J. Nordin, S. Mäkeläinen, G. R. Pielberg, J. Hanson, Å. Ohlsson, S. Saellström, H. Rönnberg, I. Ljungvall, J. Häggström, T. F. Bergström, Å. Hedhammar, J. R. S. Meadows, K. Lindblad-Toh, A novel canine reference genome resolves genomic architecture and uncovers transcript complexity. *Commun Biol*. **4**, 185 (2021).

24. H. Li, R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. **25**, 1754–1760 (2009).

25. A. R. Quinlan, I. M. Hall, BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics*. **26**, 841–842 (2010).

26. Twist Bioscience, "Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System" (2021), (available at https://www.twistbioscience.com/sites/default/files/resources/2021-10/Protocol_NGS_LP2.0_EnzFrag_UniversalAdapterSystem_27SEPT21_Rev%202.0.pdf).

27. Twist Bioscience, Twist target enrichment fast hybridization protocol, (available at https://www.twistbioscience.com/resources/protocol/twist-target-enrichment-fast-hybridization-protocol).

28. Twist Bioscience, Twist target enrichment standard hybridization v2 protocol, (available at https://www.twistbioscience.com/resources/protocol/twist-target-enrichment-standard-hybridization-v2-protocol).