

# Rapid, High Throughput Genotyping Solutions for Companion Animals

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## Abstract

Cats and dogs are integral parts of our lives, and as such we want to understand as much as possible about their health. Advances in genotyping help identify deleterious alleles that can be important in breeding and veterinary care. Applied Biosystems™ recently released high-throughput genotyping solutions for feline and canine genome analysis. In this study, we demonstrate practical workflows for collecting and purifying DNA samples from companion animals. Using these samples, we introduce the SwiftArray™ Assay for genotyping and show how it provides high-quality data in 30 hours. Finally, we show how the results can be used to identify traits and breeds in dogs and cats. Together these results demonstrate that companion animal breeders, veterinary practices and direct-to-consumer genotyping companies can quickly and accurately obtain genotypic information.

## Introduction

Why genotype our companion animals?

- Identify inherited disease risks
  - Enables research into early screening, targeted monitoring, and investigating preventive care.
- Clarifies breed ancestry
  - Can inform behavior expectations, nutritional needs, and exercise recommendations
  - Can help research into drug choice or dosage where pharmacogenomic variants are known.
- Reduce the propagation of harmful alleles by informing mating decisions
  - May help improve the success of breeding programs
- Improves the power of genome-wide association studies
  - More accurate prevalence estimates for genetic conditions
  - Help accelerate the research of new diagnostics and therapeutics in veterinary medicine.
- Supports research into more effective vaccination, parasite-control, and zoonotic disease surveillance strategies by public health authorities.

## Materials and Methods

### Samples

Samples were collected from local colleagues' pets.

### Materials

- Thermo Scientific Genotubes™
- Invitrogen™ RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE
- Applied Biosystems™ Axiom™ Canine HD Array
- Applied Biosystems™ Axiom™ Feline HD Array
- Applied Biosystems™ SwiftArray™ Target Preparation kit
- Applied Biosystems™ SwiftArrayStudio™ Analyzer

### Methods

Buccal swabs were collected by individual owners from their pets. Genomic DNA was isolated from buccal swabs using the method described in the RecoverAll kit, except the protease digestion volume was increased to 400µl, and the temperature was 65C for 1 hour. 200µl of digest was used in the rest of the protocol. Targets were prepared for hybridization to the microarray using the method described in the SwiftArray reagent kit. Targets loaded on the SwiftArrayStudio for automated data collection, including 16 hr hybridization, processing, washing and imaging.

### Data Analysis

Raw data files were analyzed for QC using Applied Biosystems™ Axiom™ Analysis suite. Further analysis was performed using custom canine or feline interpretive software.

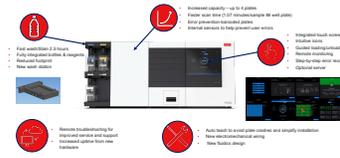


Figure 1. Features in SwiftArrayStudio that enhance usability and reliability.



Figure 2. Features in SwiftArray Assay that speed time-to-answer and reduce consumables used.

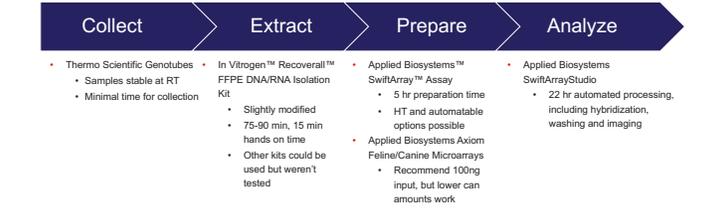


Figure 3. Workflow used in this study for collecting DNA and genotyping companion animals using SwiftArray solution

## Results



Figure 4. Description of content found on Axiom Feline Genotyping array

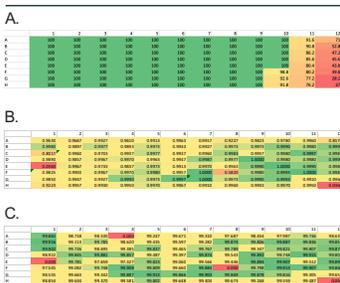


Figure 5. Quality control metrics of analysis using Feline Genotyping array. Each sample was analyzed in triplicate on three separate arrays. A. Input amount of genomic DNA analyzed (ng). B. Average dQC (dish QC, measures signal-to-noise in non-polymorphic regions of the genome) of each sample in the three replicates. C. Average total call rate for each sample in the three replicates.



Figure 6. Description of content found on Axiom Canine Genotyping array



Figure 7. Quality control metrics of analysis using Canine Genotyping array. Each sample was analyzed in triplicate on three separate arrays. A. Input amount of genomic DNA analyzed (ng). B. Average dQC (dish QC, measures signal-to-noise in non-polymorphic regions of the genome) of each sample in the three replicates. C. Average total call rate for each sample in the three replicates.

	Cat Samples	Dog Samples
Total number of markers	662,069	712,332
Number of input samples	288	287
Samples with dQC >= 0.82	93.40%	93.73%
dQC Median	0.967	0.959
Average passing QC CR	99.54%	98.96%
Average Sample CR	99.69%	99.51%
Percent of markers with high performance in dataset	97.20%	93.80%
Average sample reproducibility	99.99%	99.93%
Gender Calls		
Female	136	110
Male	118	120
Gender call rate	100%	100%
AxAS displayed values:		
AxAS PolyHighResolution	89.29%	69.83%
AxAS NoMismatches	5.59%	12.79%
AxAS MegaHighResolution	2.30%	11.15%
AxAS CallRateBelowThreshold	0.98%	2.25%
AxAS OffTargetVariant	0.05%	0.44%
AxAS Other	1.78%	3.50%
AxAS OtherNA	0.03%	0.03%

Figure 8. Summary of results obtained from cat and dog samples used in this study. Values are either sums or average values of the triplicates (given as percentage).

Dogs	
Top 10 Breeds with at least 0.1% representation (96 ThermoFisher samples)	
1	Border Collie
2	12 Day Poole
3	11 Golden Retriever
4	9 Weim
5	8 American Cocker Spaniel
6	8 East European Shepherd
7	7 American Staffordshire Terrier
8	7 Chihuahua
9	7 German Shepherd dog
10	7 Labrador Retriever
11	7 Weim

Figure 9. Most popular breeds of small samples of companion animals from Thermo Fisher Scientific colleagues

## Conclusions

- Buccal swabs collected with Thermo Scientific Genotubes can be used as a source of genomic DNA
- Applied Biosystems Axiom Canine and Feline arrays facilitate genotyping of companion animals
- Up to 96 samples can be analyzed on a single Axiom array
- Applied Biosystems SwiftArray Solution includes simplified target preparation and fully automated Axiom array processing
- Genotyping results can be obtained in approximately 30 hours when using the SwiftArray Solution

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