Microarrays

Rapid companion animal genotyping with the SwiftArrayStudio Microarray Analyzer

In this application note, we show:

- A workflow for obtaining companion animal DNA from buccal swabs
- A simplified workflow for collecting genotypes at 630,000 loci of dog and cat genomes
- High-quality genotype information can be obtained from companion animals in less than 30 hours

Introduction

There are an estimated 600 million cats and more than 700 million dogs that are kept as pets worldwide. They are as much a part of our lives as our spouses, our children, and our extended family. We therefore want to understand as much about their health as possible. Advances in genotyping help identify deleterious gene variants that can be important for understanding breeds and for veterinary care.

Genotyping cats and dogs offers significant benefits for both individual animal health and broader population management. For individual owners, genotyping can clarify breed ancestry, informing behavior expectations, nutritional needs, and exercise recommendations [1]. Genetic testing can identify inherited disease risks (e.g., cardiomyopathies, eye diseases, bleeding disorders [1-2]), enabling earlier screening, targeted monitoring, and researching preventive care plans. Genotyping can guide dose selection or drug choice where pharmacogenomic variants are known to affect response to a treatment [3]. For breeders and shelters, genotyping helps reduce the propagation of harmful alleles by informing mating decisions, supporting healthier breeding programs, and giving adopters transparent information about an animal's likely size, temperament, or health trajectory [4-5]. On a population scale, aggregating genomic and phenotype data across millions of pets supports more accurate prevalence estimates for genetic conditions, improves the power of genome-wide association studies, and accelerates the development of new diagnostics and therapeutics in veterinary research [4]. Finally, for public health author-

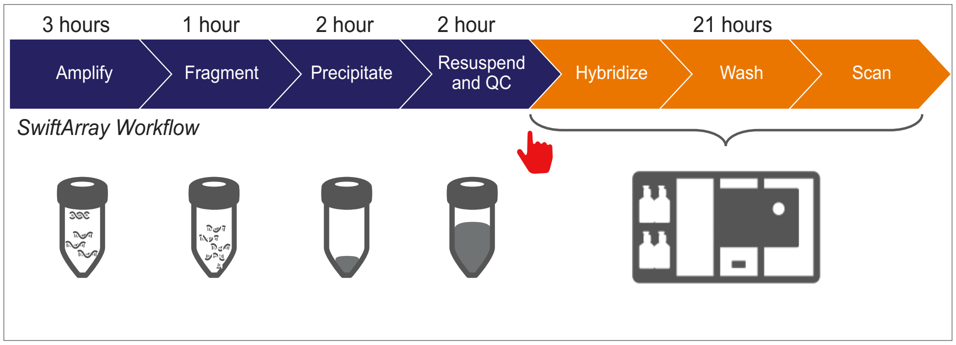


Figure 1. Introducing the SwiftArrayStudio Microarray Analysis solution. The SwiftArray target preparation steps can be completed in about eight hours in a single working day. On-instrument, autonomous array processing can generate data within 21 hours of loading. Overall, a full set of genotypes and copy number calls from an Axiom plate can be generated in about 30 hours. Different colors represent workflow steps on different days; the finger represents a touch point on the instrument, where plates and/or reagents are loaded onto the instrument.

ities, better genetic and demographic data on the companion animal population support more effective vaccination, parasite-control, zoonotic disease surveillance strategies [6-7], and therapeutics in veterinary research [4]. Finally, for public health authorities, better genetic and demographic data on the companion animal population support more effective vaccination, parasite-control, and zoonotic disease surveillance strategies [6-7].

Thermo Fisher Scientific continues to be an innovator of genetic analysis tools. To meet the growing demands for speed in microarray analyses, we recently developed the Applied Biosystems™ SwiftArrayStudio™ Microarray Analyzer and SwiftArray™ Assay. This solution consists of a DNA target preparation workflow that can be completed in a single workday, and a new instrument that automates hybridization, washing, and scanning without any in-process touch points. The SwiftArrayStudio Microarray Analyzer can provide data on an Applied Biosystems™ Axiom™ array plate after an overnight hybridization and scan (Figure 1). This assay and improved workflow reduce the sample-to-answer time from 5 days to 2 days, allowing researchers to gather and interpret genetic data to keep pace with their needs.

Design of experiment

In this application note, we show how the SwiftArrayStudio solution can be used for companion animal genotyping. Briefly, to reflect real-world sample collection conditions, we asked colleagues to obtain buccal samples from their pet cats and dogs using Thermo Scientific™ GenoTube™ Livestock Swabs. Genomic DNA was purified from the swabs using a modified protocol for the Invitrogen™ RecoverAll™ Total Nucleic Acid Isolation kit for FFPE. Up to 100 ng of DNA was processed using the manual SwiftArray Assay workflow in triplicate. Genotypes were obtained using Applied Biosystems™ Axiom™ Feline HD and Axiom™ Canine HD microarray plates. These arrays were designed with extensive consultation with subject matter experts and contain over 630,000 SNP markers that facilitate identification of variants, including those associated with traits like coat color, morphology, and disease risk. For more information, see see our **companion animal web page**. The prepared samples and plates were loaded onto the SwiftArrayStudio Microarray Analyzer for automated data collection. The data were initially analyzed using Axiom Analysis Suite, and meaningful trait information was obtained from collaborators.

A. INPUT AMOUNT (ng)

	1	2	3	4	5	6	7	8	9	10	11	12
A	>100	100	100	100	100	100	100	100	100	70	21.4	50
B	>100	100	100	100	100	100	100	100	100	62	19.28	25
C	100	100	100	100	100	100	100	100	100	44	50	32
D	100	100	100	100	100	100	100	100	96	44	50	30
E	100	100	100	100	100	100	100	100	94	44	50	29
F	100	100	100	100	100	100	100	100	86	43	50	31
G	100	100	100	100	100	100	100	100	80	42	50	32
H	100	100	100	100	100	100	100	100	78	32	50	30

B. Average DNA Amount Recovered (µg) *Median = 1067 µg*

	1	2	3	4	5	6	7	8	9	10	11	12
A	649.1	1101.0	1149.3	1030.3	962.6	1144.7	1143.3	1154.0	1119.7	1099.7	891.1	1074.1
B	812.4	1106.7	1121.0	1089.0	1087.7	1095.0	1108.0	1119.0	1115.7	1069.3	825.9	1082.7
C	921.7	1064.6	1090.1	1099.3	1034.3	1057.9	1066.2	1060.3	1042.9	1006.7	1060.7	962.2
D	1079.3	1039.5	1037.7	1046.7	1012.3	1039.7	992.3	911.5	929.9	959.8	885.0	659.9
E	1127.0	1031.5	1091.7	1078.7	1079.3	1062.7	1091.7	1082.0	1062.9	1041.0	770.3	1040.0
F	1080.7	1059.6	1110.7	943.5	1067.0	1037.6	1113.7	1083.0	1104.7	1020.0	1117.3	1026.8
G	1140.3	1089.2	1073.7	1093.7	1038.9	1055.3	1105.0	1115.7	1095.3	1099.7	1029.8	869.1
H	708.2	1016.1	1045.8	1106.4	1079.3	1064.1	1143.0	1126.7	1138.3	1120.0	978.9	855.9

C. Average DQC *Median = 0.9977*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.551	1.000	0.982	0.999	0.582	0.995	0.999	0.998	1.000	1.000	0.998	0.998
B	0.038	0.997	0.998	1.000	0.998	0.999	0.996	0.998	0.999	1.000	0.995	1.000
C	0.999	0.997	0.994	0.994	0.997	0.997	0.997	1.000	1.000	1.000	1.000	0.995
D	0.988	0.993	0.996	0.999	0.998	0.992	0.999	0.999	0.999	0.996	0.997	0.787
E	0.999	0.997	0.992	0.995	0.998	0.999	0.998	0.999	0.999	0.998	0.960	0.991
F	0.990	0.992	0.997	0.956	0.994	0.984	0.999	0.999	1.000	0.999	0.992	0.995
G	0.998	0.985	0.993	0.995	0.995	0.993	0.999	0.989	1.000	1.000	0.999	0.322
H	0.991	0.996	0.999	0.999	0.995	0.998	1.000	1.000	1.000	1.000	0.387	0.961

D. Average Total Call Rate (%) *Median = 99.18*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	99.486	98.903	99.195	0.000	98.431	98.982	99.063	99.725	99.723	99.196	98.869
B	0.000	99.174	99.321	99.182	99.030	99.667	97.988	99.117	99.559	99.532	98.876	99.653
C	99.233	99.221	98.531	98.423	99.004	99.460	99.024	99.368	99.603	99.270	99.515	98.347
D	0.000	98.574	99.074	99.553	99.157	99.138	99.382	99.227	99.209	99.203	98.684	0.000
E	99.622	99.055	98.217	99.106	99.534	99.516	98.944	98.316	98.865	99.252	0.000	0.000
F	98.346	98.333	98.757	0.000	98.225	97.505	98.950	99.512	99.443	99.354	98.903	99.433
G	99.059	0.000	99.503	98.329	97.987	98.742	99.399	98.588	99.654	99.541	99.367	0.000
H	99.660	98.593	99.519	98.942	99.022	99.341	99.709	99.202	99.110	99.341	0.000	0.000

Figure 2. Quality control metrics of analyses using Canine Genotyping array. Each sample was analyzed in triplicate on three separate arrays, and the average of the three plates is shown here. **(A)** Input amount of genomic DNA analyzed (ng). **(B)** Average amount of DNA recovered from the SwiftArray Assay target preparation workflow. **(C)** dQC (dish QC, measures signal-to-noise in non polymorphic regions of the genome) of each sample in the three replicates. **(D)** Average total call rate for each sample in the three replicates.

Recovery of genomic DNA

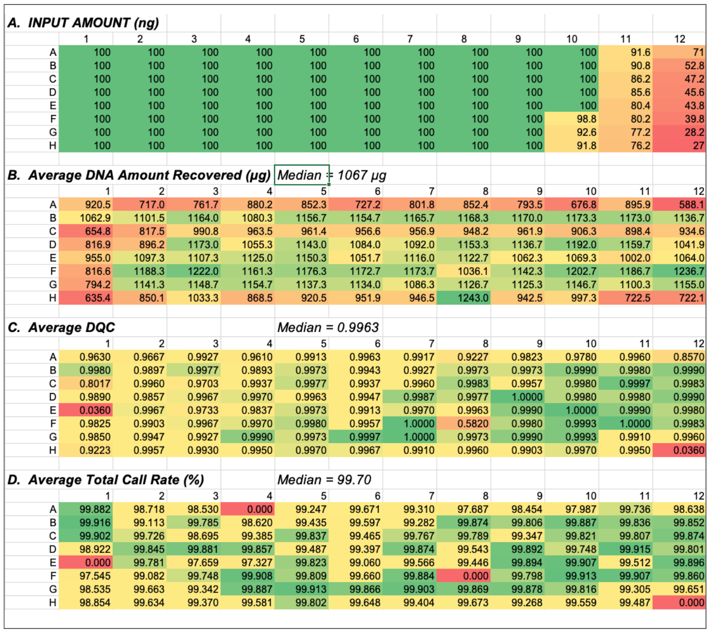
We collected a variety of representative samples from colleagues and shelters, resulting in 82 dog and 98 cat samples. Buccal swabs were collected from pets and stored at room temperature until used. DNA was recovered manually (i.e., with no automation) using the Ambion™ Recoverall™ FFPE DNA with the following adjustments: 400 µl lysis buffer/sample was used, the swabs were incubated with lysis solution at 65°C for 1 hour, and 200 µl of the resulting lysate was used according to the protocol supplied with the Recoverall kit. Final DNA concentrations were measured with the Invitrogen™ Qubit™ fluorimeter. Recovered amounts ranged from 81 ng to 9.6 µg for cats, and 57.6 ng to 5 µg for dogs.

Target preparation for SwiftArray Assay and loading of SwiftArrayStudio Microarray Analyzer

The SwiftArray assay recommends 100 ng of genomic DNA as an input amount, so samples were diluted to the necessary concentration (5 ng/µl in 20 µl). In cases where not enough DNA was recovered, we used the maximum input volume (20 µl). The DNA quantities input into the SwiftArray Assay are shown in Figures 2 and 3. Note that some of the dog samples (wells C11-H12) were replicates of other samples on the same plate but using less input DNA.

Each sample was analyzed in triplicate on different days using manual SwiftArray workflow as described in the SwiftArray Assay user guide. As described in the user guide, it is recommended that the amount of DNA produced and recovered be quantified before proceeding with the hybridization. The average amount of DNA recovered from the dog and cat SwiftArray assay triplicates is shown. Plates are recommended to have a median recovery of around 1000 µg/well; the dog plates had average three-plate median of 1067 µg and the cat plates had average three-plate median of 1066 µg. Furthermore, analysis of fragment sizes by agarose gel electrophoresis showed the samples had correct size range of DNA fragments (data not shown).

Having established that the samples met quality standards, they were prepared and loaded on the SwiftArrayStudio Microarray Analyzer for automated hybridization (16 hour hybridization time), washing and scanning. The total time on the instrument was about 21 hours.



A. INPUT AMOUNT (ng)

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100	100	100	100	100	100	100	100	100	91.6	71
B	100	100	100	100	100	100	100	100	100	100	90.8	52.8
C	100	100	100	100	100	100	100	100	100	100	86.2	47.2
D	100	100	100	100	100	100	100	100	100	100	85.6	45.6
E	100	100	100	100	100	100	100	100	100	100	80.4	43.8
F	100	100	100	100	100	100	100	100	100	98.8	80.2	39.8
G	100	100	100	100	100	100	100	100	100	92.6	77.2	28.2
H	100	100	100	100	100	100	100	100	100	91.8	76.2	27

B. Average DNA Amount Recovered (µg) *Median = 1067 µg*

	1	2	3	4	5	6	7	8	9	10	11	12
A	920.5	717.0	761.7	880.2	852.3	727.2	801.8	852.4	793.5	676.8	895.9	588.1
B	1062.9	1101.5	1164.0	1080.3	1156.7	1154.7	1165.7	1168.3	1170.0	1173.3	1173.0	1136.7
C	654.8	817.5	990.8	963.5	961.4	956.6	956.9	948.2	961.9	906.3	898.4	934.6
D	816.9	896.2	1173.0	1055.3	1143.0	1084.0	1092.0	1153.3	1136.7	1192.0	1159.7	1041.9
E	955.0	1097.3	1107.3	1125.0	1150.3	1051.7	1116.0	1122.7	1062.3	1069.3	1002.0	1064.0
F	816.6	1188.3	1222.0	1161.3	1176.3	1172.7	1173.7	1036.1	1142.3	1202.7	1186.7	1236.7
G	794.2	1141.3	1148.7	1154.7	1137.3	1134.0	1086.3	1126.7	1125.3	1146.7	1100.3	1155.0
H	635.4	850.1	1033.3	868.5	920.5	951.9	946.5	1243.0	942.5	997.3	722.5	722.1

C. Average DQC *Median = 0.9963*

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.9630	0.9667	0.9927	0.9610	0.9913	0.9963	0.9917	0.9227	0.9823	0.9780	0.9960	0.8570
B	0.9980	0.9897	0.9977	0.9893	0.9973	0.9943	0.9927	0.9973	0.9973	0.9990	0.9980	0.9990
C	0.8017	0.9960	0.9703	0.9937	0.9977	0.9937	0.9960	0.9983	0.9957	0.9980	0.9997	0.9983
D	0.9890	0.9857	0.9967	0.9970	0.9963	0.9947	0.9987	0.9977	1.0000	0.9980	0.9980	0.9990
E	0.0360	0.9967	0.9733	0.9837	0.9973	0.9913	0.9970	0.9963	0.9990	1.0000	0.9990	0.9980
F	0.9825	0.9903	0.9967	0.9970	0.9980	0.9957	1.0000	0.5820	0.9980	0.9993	1.0000	0.9983
G	0.9850	0.9947	0.9927	0.9990	0.9973	0.9997	1.0000	0.9973	0.9990	0.9993	0.9910	0.9960
H	0.9223	0.9957	0.9930	0.9950	0.9970	0.9967	0.9910	0.9960	0.9903	0.9970	0.9950	0.0360

D. Average Total Call Rate (%) *Median = 99.70*

	1	2	3	4	5	6	7	8	9	10	11	12
A	99.882	98.718	98.530	0.000	99.247	99.671	99.310	97.687	98.454	97.987	99.736	98.638
B	99.916	99.113	99.785	98.620	99.435	99.597	99.282	99.874	99.806	99.887	99.836	99.852
C	99.902	99.726	98.695	99.385	99.837	99.465	99.767	99.789	99.347	99.821	99.807	99.874
D	98.922	99.845	99.881	99.857	99.487	99.397	99.874	99.543	99.892	99.748	99.915	99.801
E	0.000	99.781	97.659	97.327	99.823	99.060	99.566	99.446	99.894	99.907	99.512	99.896
F	97.545	99.082	99.748	99.908	99.809	99.660	99.884	0.000	99.798	99.913	99.907	99.860
G	98.535	99.663	99.342	99.887	99.913	99.866	99.903	99.869	99.878	99.816	99.305	99.651
H	98.854	99.634	99.370	99.581	99.802	99.648	99.404	99.673	99.268	99.559	99.487	0.000

Figure 3. Quality control metrics of analyses using the Feline Genotyping array. Each sample was analyzed in triplicate on three separate arrays, and the average of the three plates is shown here. **(A)** Input amount of genomic DNA analyzed (ng). **(B)** Average amount of DNA recovered from the SwiftArray Assay target preparation workflow. **(C)** Average dQC (dish QC, measures signal-to-noise in non polymorphic regions of the genome) of each sample in the three replicates. **(D)** Average total call rate for each sample in the three replicates.

Scanning quality metrics

Following hybridization, washing and scanning on the SwiftArrayStudio Microarray Analyzer, we analyzed the QC metrics generated by the Applied Biosysystems™ Axiom™ Analysis Suite and default best practices parameters (see the Axiom Analysis Suite User Guide). One of these, DishQC (DQC), measures the amount of overlap between two homozygous peaks created by non polymorphic probes, with a value of 1.0 considered optimal. Among the canine samples, six failed the default DQC metric (0.82), corresponding to an approximate 93.8% pass rate. For the cat samples, four failed to meet the default DCQ metric (passing rate 95.8%).

Another quality measurement generated by Axiom Analysis Suite is the average passing QC call rate (average percentage of autosomal SNPs with a call other than NoCall, as measured at the sample QC step of passing samples within a plate). Among the dog samples that passed DQC, six more failed the default call rate threshold (97%); similarly, one additional cat sample failed to meet the threshold. However, in the other samples the data quality was high, giving median total call rates of 99.18% for passing dog samples and 99.67% for passing cat samples. Notably, these passing samples included several with substantially less than the recommended 100 ng DNA input.

Table 1. Performance of SwiftArray solution for canine and feline microarrays. Values shown are the average of three triplicate plates analyzing buccal swabs of pet DNA. Abbreviations: DQC: Dish QC; CR: call rate. For more details, see the **Axiom Analyis Suite User Guide.**

Metric	Cat Samples	Dog Samples
Total number of markers	662,069	712,332
Samples with DQC ≥ 0.82	95.8%	93.8%
DQC median	0.9963	0.9977
Median passing QC CR	99.7%	98.2%
Average number of samples with passing QC CR	95.8%	87.5%
Percent of markers with high performance in dataset	97.2%	93.8%
Average sample reproducibility	99.99%	99.93%
Gender calls (all three replicates)		
Female	136	110
Male	118	120
Axiom Analysis Suite displayed values		
PolyHighResolution	89.29%	69.83%
NoMinorHom	5.59%	12.79%
MonoHighResolution	2.30%	11.15%

Table 2. Top 20 dog breeds represented in a collection of pets. Note that most of these are due to mixtures of characteristic loci detected that were in the dogs, not necessarily pure breeds.

Breed	Number
Toy Poodle	12
Golden Retriever	11
Vizsla	9
American Cocker Spaniel	8
East European Shepherd	8
American Staffordshire Terrier	7
Chihuahua	7
German Shepherd	7
Laborador Retriever	7
Poodle	7
English Shepherd	5
Siberian Husky	5
Staffordshire Bull Terrier	5
Akbash	4
Central Asian Shepherd Dog	4
Italian Greyhound	4
Australian Cattle Dog	3
Blue Gascony Griffon	3
Bull Terrier	3
English Cocker Spaniel	3

Assessment of genotypic clustering quality showed that 91.5% of canine markers were classified as polymorphic high resolution, no minor homozygotes, or monoallelic high resolution (Table 1), while 95.4% of feline markers fell into these clustering categories. For both sets of data, the remainder fell into less informative classes of genotyping clusters (CallRateBelowThreshold, OffTargetVariant, OtherMA or Other). Thus, despite the variable quality of these samples, the SwiftArray assay workflow can generate high-quality genotyping data.

Converting genotypes to traits

The goal of obtaining genotyping data is to gain information on traits, breeds, pathologies, etc. To convert the genotype data to possible phenotypes, we relied on collaborators. For dogs, we sent the data to a collaborator who had developed a custom breed identification pipeline, matching genotypes at specific loci to over 200 dog breeds. Using the data collected from colleagues' dogs, we tallied the most common breed loci identified in these samples (Table 2). Note these do not represent the number of pure-breed dogs; rather, the list indicates which breeds are likely to have contributed to the makeup of the mixed-breed pets owned by our colleagues.

Similarly, an external collaborator provided a more detailed conversion of selected genotypes to selected cat traits (Table 3). Included in this data set were alleles for known genetic pathologies (not shown); fortunately, none of the cats were positive for these alleles. Note that for both the canine and feline arrays, more than 630,000 markers were analyzed; the results shown here are a small fraction of the total collected. For more information on the loci contained on the feline and canine microarrays, see our **companion animal web page**.

Table 3. Tally of selected cat traits in a collection of 92 cats. Some traits and genes are listed twice; this due to the two different alleles of the same gene being examined. Note that this is a subset of the more that 640,000 genotypes analyzed on the array.

Trait	Gene	# Ref homo	# Ref hetero	# Alt homo
Burmese coat color	*TRY*	90	1	1
Ticked	*DKK4*	85	4	3
Polydactyly	*LMBR1*	90	2	0
Sphynx Hairless	*KRT71*	70	19	1
Chocolate	*TYRP1*	85	7	0
Coat color, dilute	*MLPH*	35	33	24
Long hair	*FGF5*	64	25	3
Long hair	*FGF5*	49	32	11
Blotched tabby	*LVRN*	22	41	28
Coat color, non-agouti (black)	*ASIP*	8	35	49
Coat color, Salmiak	*KIT*	92	0	0
Curly coat, Devon Rex	*KRT71*	66	12	10
Curly coat, Ural Rex	*LIPH*	92	0	0
Coat color, russet	*MC1R*	92	0	0
Coat color, copal	*MC1R*	92	0	0
Tail, short	*TBXT*	92	0	0
Tail, short	*TBXT*	91	1	1
Munchkin, standard	*UGDH*	92	0	0

Summary and conclusions

In this application note, we describe a simplified method for obtaining genotypic information from dogs and cats. We showed how genomic DNA can be collected from buccal swabs using GenoTube swabs, and purified using RecoverAll kits. We also showed how the SwiftArrayStudio Microarray Analyzer and Assay can be used to generate high quality genotypic data from a variety of real-world users. Importantly, the SwiftArray Assay can generate these data in less than 30 hours. The SwiftArray-Studio Microarray Processor Solution, together with Axiom Feline and Canine microarrays, is therefore poised to provide breeders, veterinarian researchers, and providers of genetic data with tools that can generate high-quality genotyping information rapidly, accurately, and reliably.

To obtain the library files optimized for the SwiftArrayStudio Microarray Analyzer, please speak with your local FAS.

Acknowledgements

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Ordering information

Description	Cat. No
SwiftArrayStudio™ Microarray Analyzer	00-0471
Axiom™ SwiftArrayStudio Consumables Kit	952674
Axiom™ Feline HD Array	551642
Axiom™ Canine HD Array	550869
Thermo Scientific™ GenoTube™ Livestock Swabs	9062010
Invitrogen™ RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	AM1975
Invitrogen™ E-Gel™ 48 Agarose Gel with SYBR Safe stain, 4% agarose	G820804
25-bp DNA Ladder	931343

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