Automated high-throughput proteomic analysis of stored blood cells from a large cohort of non-domestic felids

Kevin L. Schauer[1], Kevin Yang[1], Amirmansoor Hakimi[1], Eugen Damoc[2] and Lily A.B. Parkinson[3]

[1] *Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA, USA, 95134,* [2] *Thermo Fisher Scientific, Hanna-Kunath Str. 11, 28199, Bremen, Germany,* [3] *Brookfield Zoo Chicago, 3300 Golf Road, Brookfield, IL, USA, 60513*

Abstract

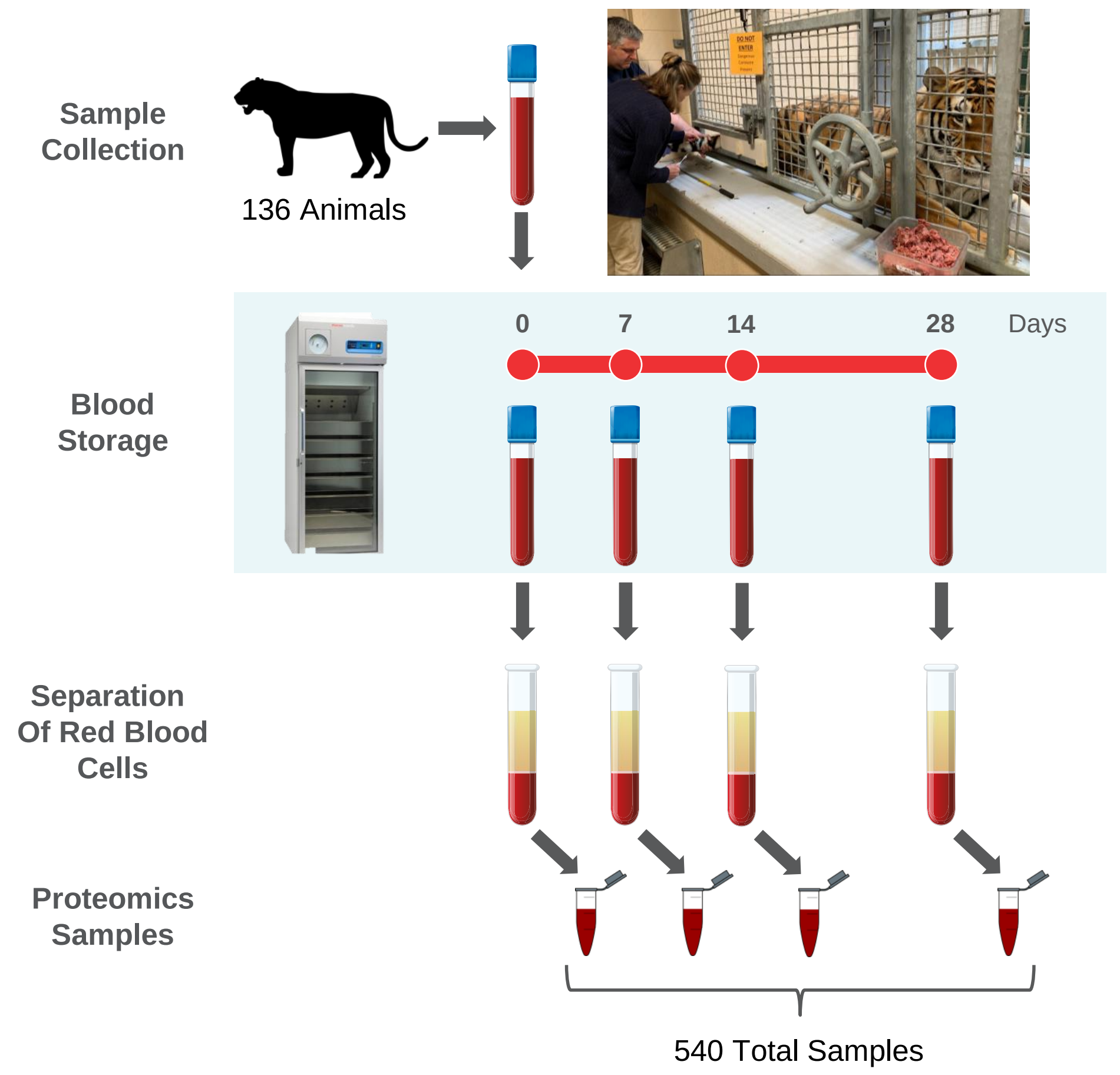
Purpose: Blood transfusions can be a life-saving treatment for animals that have become anemic due to trauma or disease. However, adverse, often life-threatening, complications can occur if the donor and recipient blood types are not appropriately matched. In non-domesticated cat species, very little is known about the different blood types found in different species, making transfusions in most situations risky. Additionally, since non-domestic cats are often found in zoos where only a few individuals of each species are housed, there is a need to be able to either transfuse blood from one species to another, or store blood for prolonged periods of times in case of future emergencies.

Methods: Blood samples were obtained from 136 non-domestic cats, consisting of 18 different species, housed at AZA (Association of Zoos and Aquariums) accredited institutions across the United States. Fresh blood samples were aliquoted and stored in a clinical blood transfusion refrigerator for 0, 7, 14, or 28 days, after which the red blood cells were pelleted and stored at -80ºC until analysis. Samples were also crossmatched to determine compatibility. Pelleted red blood cells were then prepared for bottom-up proteomic analysis using the Thermo Scientific™ AccelerOme™ automated sample preparation platform, which enables standardized, hands-off operation and provides robust workflows for label free proteomics applications.

Results: The AccelerOme automated platform for sample preparation allowed for protein lysis, DNA removal, protein reduction, alkylation, protease digestion, and sample cleanup with no user intervention. Trypsin digested red blood cell samples were then analyzed using high-throughput, capillary flow LC-MS/MS analysis on a Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer, allowing for the analysis of 100 samples per day. Data was saved directly to the Thermo Scientific™ Ardia™ platform, and processing via Thermo Scientific™ Proteome Discoverer™ software running the CHIMERYS™ search algorithm was started automatically after the completion of each run. These methods allowed for the identification of more than 53,000 peptides from over 7000 proteins across the 1070 individual runs.

Study Design

Figure 1. Sample collection and processing summary. Blood was collected from animals housed at AZA institutions during routine examinations and then stored in a clinical blood transfusion refrigerator for 0, 7, 14, or 28 days after which the red blood cells will be pelleted and frozen at -80ºC until proteomic analysis.



Sample Collection

136 Animals

Blood Storage

0 7 14 28 Days

Separation Of Red Blood Cells

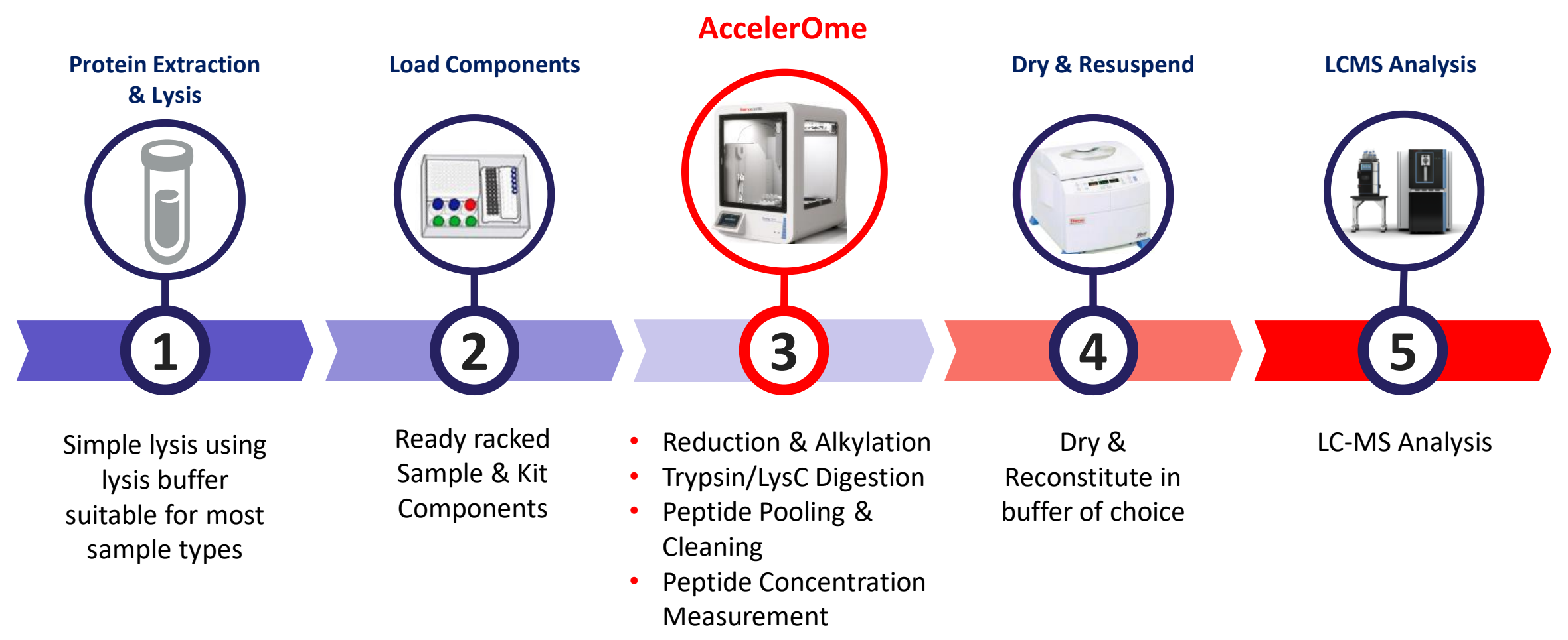
Proteomics Samples

540 Total Samples

Sample Preparation

The AccelerOme has an Experiment Designer software that guides the user through the experiment planning process to input sample names and assign study factors, values, and provides an estimate of statistical power. It also benefits from an integrated touchscreen display with user interface for instrument control and operation through a graphical wizard. The liquid handling robot has the capacity to process up to 36 label-free samples, 33 Thermo Scientific™ TMT11plex isobaric labeling reagent samples or 32 Thermo Scientific™ TMTpro™ 16plex label reagent samples per session. The AccelerOme has an automated and standardized workflow used to increase reproducibility and productivity, which is part of an integrated workflow solution, from experiment design and sample preparation, to LC-MS analysis reducing training requirements while improving data quality.

Figure 2. Automated sample preparation with the AccelerOme liquid handling robot.



AccelerOme

1. Protein Extraction & Lysis — Simple lysis using lysis buffer suitable for most sample types
2. Load Components — Ready racked Sample & Kit Components
3. AccelerOme
   - Reduction & Alkylation
   - Trypsin/LysC Digestion
   - Peptide Pooling & Cleaning
   - Peptide Concentration Measurement
4. Dry & Resuspend — Dry & Reconstitute in buffer of choice
5. LCMS Analysis — LC-MS Analysis

LC-MS Analysis

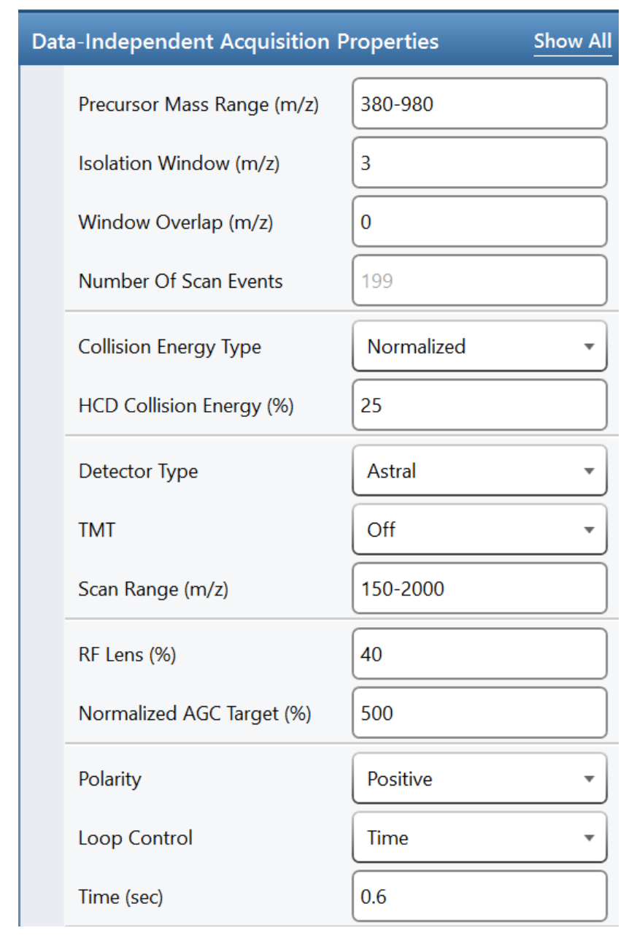
LC Method

- *Instrument:* Thermo Scientific™ Vanquish™ Neo UHPLC
- *Analytical Column:* EASY-Spray™, 2 µm C18, 150 µm X 15 cm
- *Mobile phases:* [A] 0.1% FA in H2O, [B] 0.1% FA in 80% ACN
- *Method duration:* 13 minutes + loading and equilibration (~3 minutes)
- *Pump flow rate:* 2.0 – 2.5 µL/min
- *Injection scheme:* Trap and elute using ZebraWash for trap column
- *Column temperature:* 50˚C
- *Injection volume:* 0.2 µL injections (200 ng on the column)
- *Replication:* Duplicate injections

MS Method

Here, an Orbitrap Astral mass spectrometer was run using a data-independent acquisition (DIA) scheme consisting of 199, 3 m/z windows that spanned from 380-980 m/z. MS1 scans were collected every 0.6 seconds at 240K resolution with a normalized AGC target of 500% (5e6), and 3 ms maximum injection time. Additional method parameters can be seen in Figure 3.

Figure 3. Orbitrap Astral instrument method summary.



Data-Independent Acquisition Properties — Show All

Precursor Mass Range (m/z)	380-980
Isolation Window (m/z)	3
Window Overlap (m/z)	0
Number Of Scan Events	199
Collision Energy Type	Normalized
HCD Collision Energy (%)	25
Detector Type	Astral
TMT	Off
Scan Range (m/z)	150-2000
RF Lens (%)	40
Normalized AGC Target (%)	500
Polarity	Positive
Loop Control	Time
Time (sec)	0.6

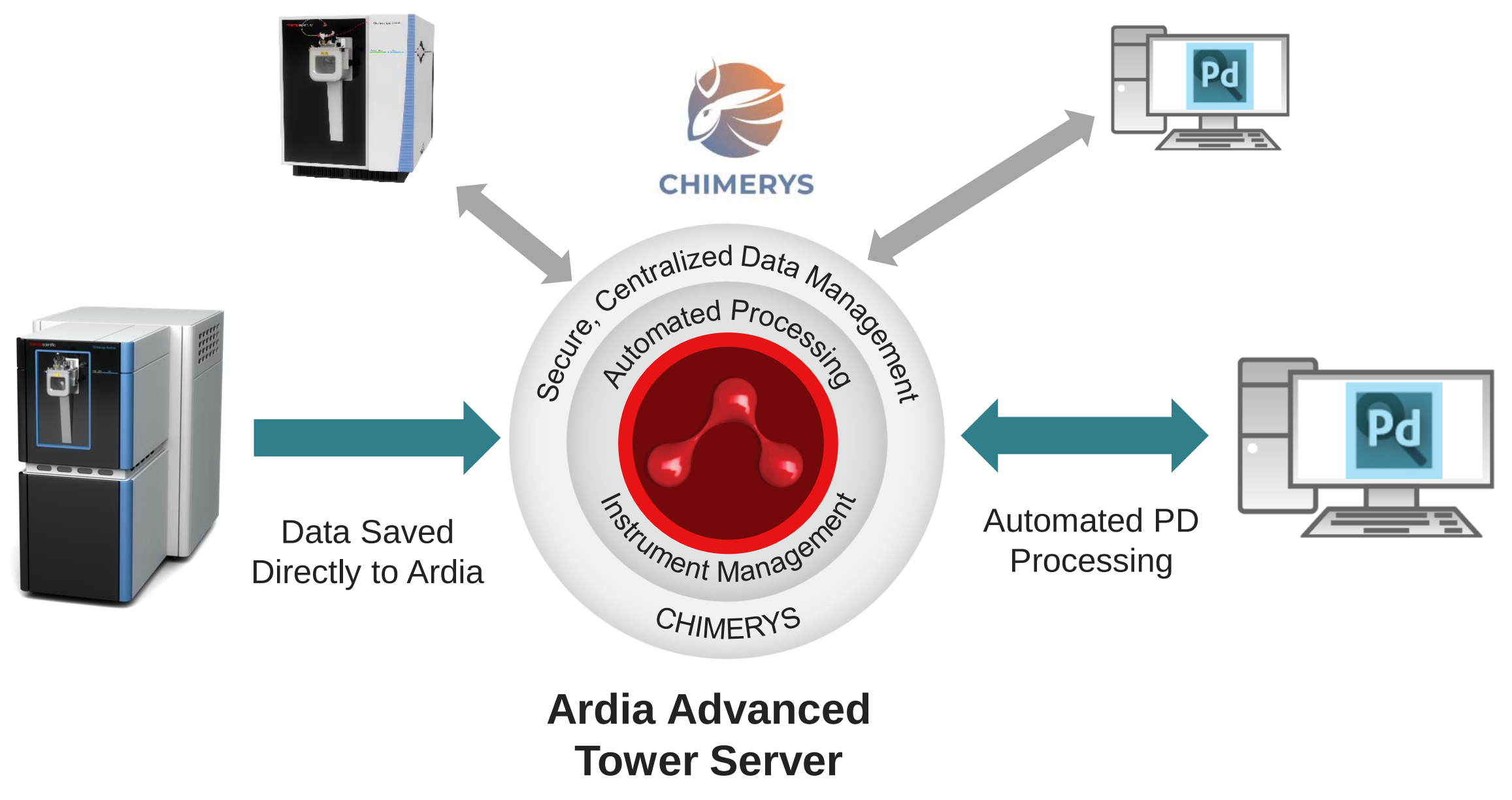
Data Analysis

Ardia Platform

Acquired LC-MS data was written directly to the Ardia server via connection of Thermo Scientific™ Xcalibur™ 4.7 software to the platform (Figure 4). The Proteome Discoverer processing method was specified in the Xcalibur sequence, allowing data processing as described below to automatically begin immediately post acquisition (Figure 5).

Connection of the LC-MS system to the Ardia platform also allowed for remote, real-time monitoring of acquisition throughout data collection. The Ardia Instruments application provided real-time estimates for the remaining acquisition time and showed real-time chromatograms as data collection proceeded.

Figure 4. Data collection, management, and analysis strategy using the Ardia platform. Acquired data was saved directly to the Ardia server and processing via Proteome Discoverer was automatically triggered as each injection completed.



Data Saved Directly to Ardia

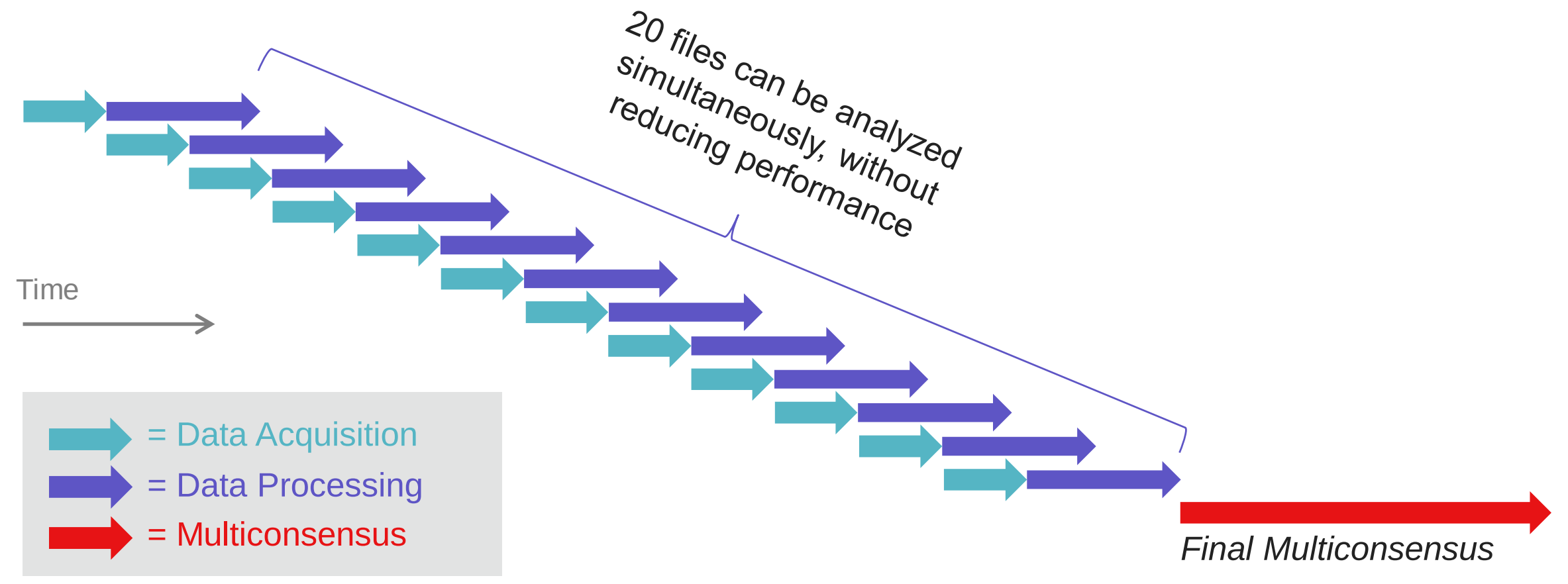
Automated PD Processing

Ardia Advanced Tower Server

Proteome Discoverer

Data was searched using the CHIMERYS 2.0 algorithm produced by MSAID, which is integrated into Proteome Discoverer 3.1 SP1. The CHIMERYS algorithm runs directly on the Ardia Advances server, so no cloud subscription is required. The Ardia server allows for up to 20 simultaneous CHIMERYS searches in parallel without a decrease in performance. A database downloaded from UniProt that contained all protein entries (including sub-taxa) from the *Feliformia* suborder was used for all searches. A small pilot study consisting of two samples from each species was analyzed prior to the start of the main searches to produce a CHIMERYS inclusion file, allowing for Match Between Runs (MBR) to be performed while analyzing data on a file-by-file basis. Upon completion of data acquisition and processing, a single multiconsensus was run to combine all analyses into a single result file.

Figure 5. Schematic illustrating the progression of data processing via Proteome Discoverer in parallel with data acquisition.

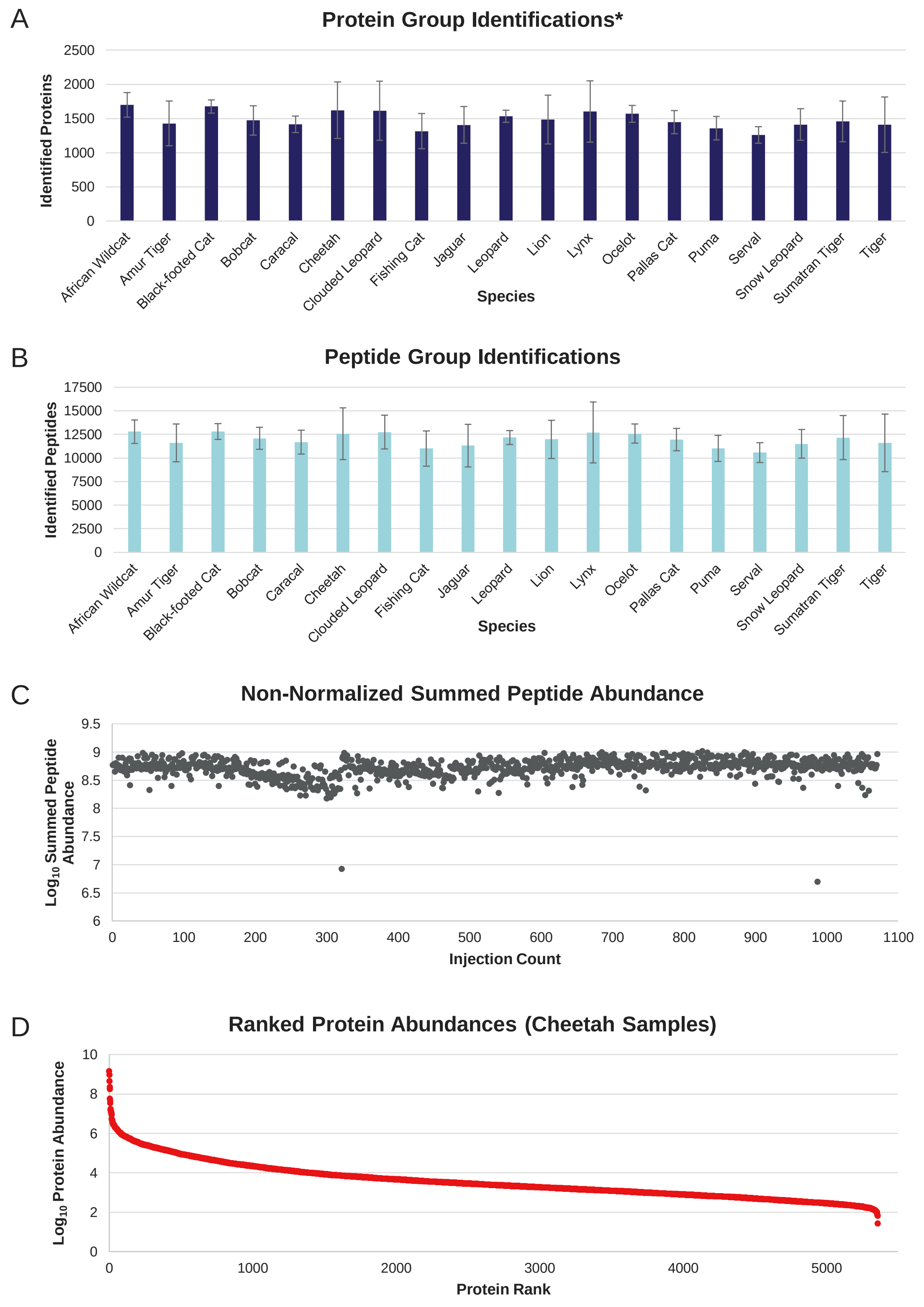


🟦 = Data Acquisition
🟪 = Data Processing
🟥 = Multiconsensus

Results

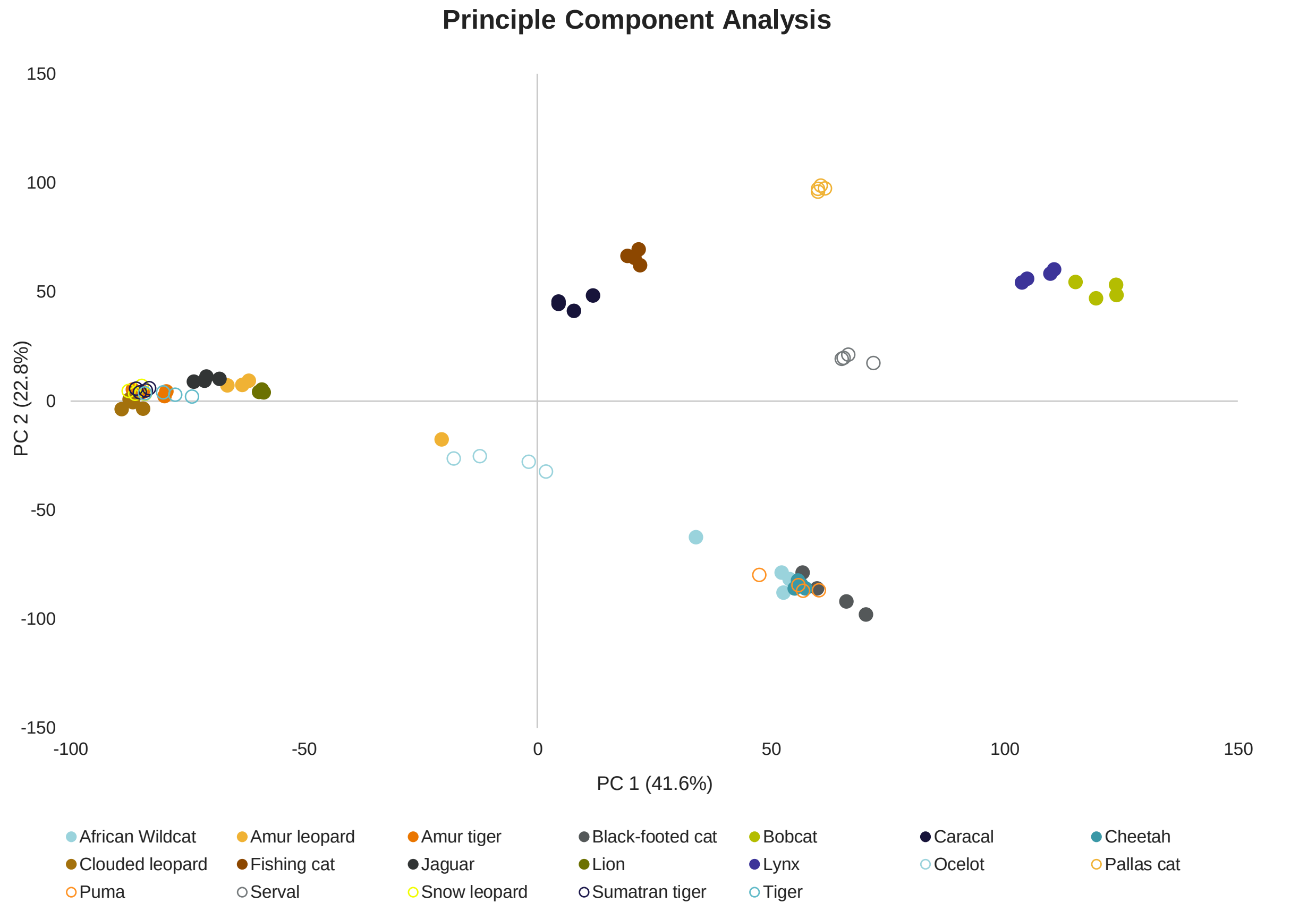
In all, a total of 1072 injections were completed in this study using samples derived from 136 individual cats. On average, more than 10,000 peptides were identified in each sample, mapping to approximately 1500 proteins (Figures 6a and 6b). However, the actual number of unique proteins is likely lower as there was significant redundancy in the database used as it contained entries from numerous species. No significant changes in sensitivity were observed across the 16 days of acquisition (Figure 6c), and protein signal intensities spanning more than 7 orders of magnitude were observed (Figure 6d). Principle component analysis (PCA) showed clear clustering of samples derived from each species, which closely mimicked the phylogenetic relationships of the species studied.

Figure 6. Average protein group (A) and peptide group (B) identifications per sample across species. Protein group identifications are likely an overestimation due to database redundancy. (C) Non-normalized sum protein abundances across all samples analyzed. (D) Ranked protein abundances of quantified proteins from the 156 cheetah samples analyzed here. Protein signal intensities spanned more than 7 orders of magnitude.



*Protein group identifications are likely an overestimate due to database redundancy

Figure 7. Principle component analysis (PCA) plot derived from grouped peptide abundances of all analyzed samples. Clear clustering within species and amongst closely related species is observed.



Conclusions

- The Vanquish Neo and Orbitrap Astral combination is an ideal platform for high-throughput proteomics studies requiring analysis of 100+ samples per day with high depth of coverage.
- The Orbitrap Astral is well suited for experiments where high dynamic range is required. Proteins with signal intensities spanning more than 7 orders of magnitude were observed here.
- Automation of acquisition via Xcalibur and processing via Proteome Discoverer and CHIMERYS with the Ardia platform simplifies data management and reduces processing time for large cohort studies.
- AccelerOme offers simplified sample preparation with minimized user involvement and improved reproducibility through instrument functionality and automation. It helped increased efficiency and productivity through pre-built and validated sample preparation methods and reagents delivered in kit format, ensuring experiment democratization.

Acknowledgements

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