# High-throughput HMW DNA animal blood extraction and sequencing on the PacBio Revio system

Deborah Moine,[1] Adam Bates[3], Michelle Kim,[2] Jacob Brandenburg[1], Nadia Sellami,[1] Jackson Mingle,[2] Jeffrey Burke,[2] Julian Rocha,[2] Aurelie Souppe,[1] Heather Ferrao,[1] Gregory Concepcion,[1] Caroline Howard,[3] and Kelvin J Liu.[2]

1. PacBio, 1305 O'Brien Drive, Menlo Park, CA 94025, 2. PacBio, 701 E. Pratt Street, Baltimore, MD 21202, 3. Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge, UK

## Introduction

The Darwin *Tree of Life* project is a large biodiversity initiative aiming to generate high-quality genomes for 70,000 species of eukaryotes across Britain and Ireland. For large-scale projects such as these, high-throughput (HT) solutions are critical; PacBio Nanobind HT DNA extraction kits combined with the Revio system address these needs by significantly increasing throughput and lowering the cost of long-read sequencing.

### Workflow

We present fully automated methods for HT high molecular weight (HMW) DNA extraction, shearing, library preparation, and PacBio HiFi sequencing of blood from animals with non-nucleated and nucleated red blood cells (nRBCs).

| Nanobind HT HMW-DNA extraction, 2h30 | SRE HT size selection, 3h30 | HT shearing + library prep, 6h |
| --- | --- | --- |

We can prepare 96 samples from DNA extraction to libraries that are ready for loading in about twelve hours.

1) **HT HMW DNA extraction** is performed using a Nanobind CBB HT kit and the ThermoFisher KingFisher APEX or the Hamilton NIMBUS Presto systems. The kit uses Nanobind disks which feature micro- and nano-structured silica wrinkles to shield bound DNA from damage during extraction. Extraction is done on a 96-well plate and can yield from **~4 to 20 µg of HMW DNA in 2.5 hours.**

2) **HT size selection** is automated on a Hamilton NGS STAR (protocol under development**) to remove DNA <10 kb using the *Short Read Eliminator* (SRE) kit** on unsheared DNA.

3) **HT shearing + library preparation** is also automated by using an automated liquid handler to pipette **shear HMW DNA to 15–20 kb** (protocol under development). HMW DNA can also be sheared using the Diagenode Megaruptor 3 system. **HT library preparation** is performed using the SMRTbell prep kit 3.0 (SPK 3.0) on the Hamilton NGS STAR. Manual library preparation can also be performed using the SPK 3.0 kit.
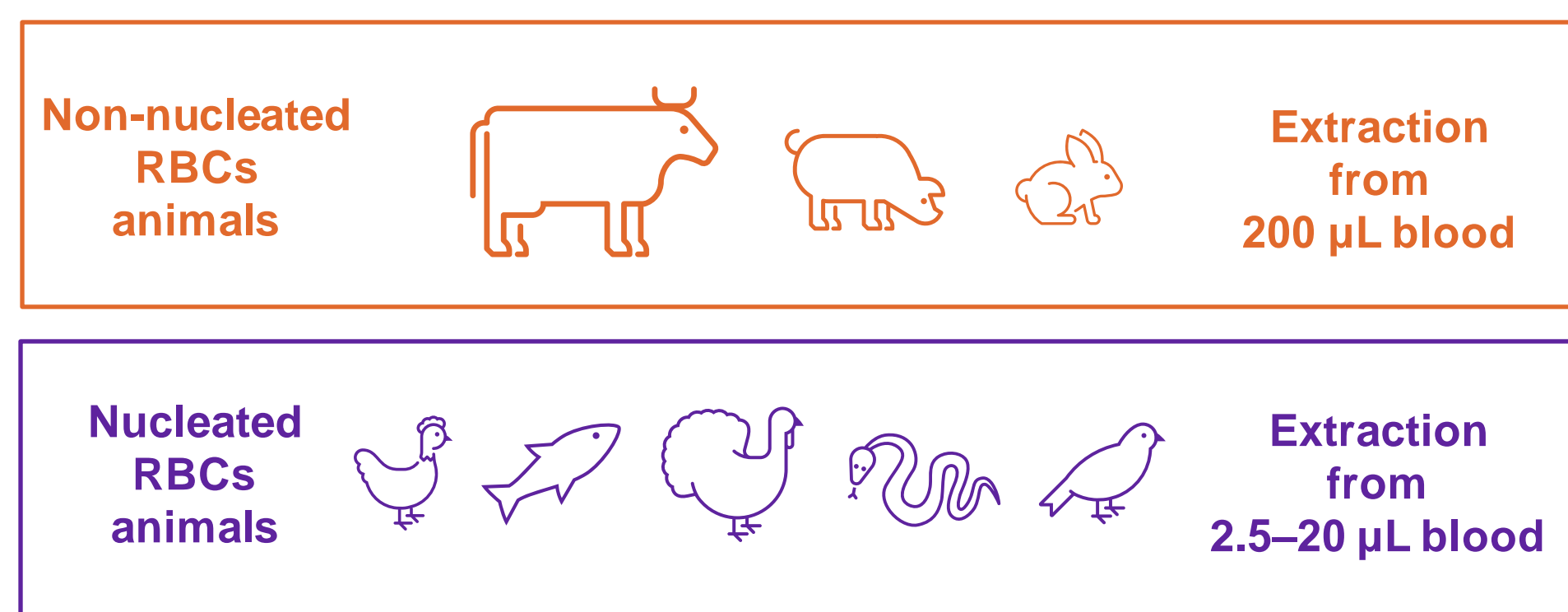
**Figure 1.** Nanobind HT CBB kit and Kingfisher Apex automation system (Thermo Fisher)

## Samples analyzed

DNA was extracted from the **whole blood** of multiple animals including mammalian and non-mammalian species. For the mammalian animals (bovine, rabbit, porcine), DNA was extracted from 200 µL blood, whereas for the non-mammalian animals (fish, bird, and reptile), DNA was extracted from 2.5 to 20 µL.

This difference in input volume is because the red blood cells (RBCs) of mammals do not contain nuclei. In contrast, the RBCs of most other vertebrates are nucleated, thus requiring 10–100× lower blood input volumes in DNA extractions for these animals.

| Non-nucleated RBCs animals | Extraction from 200 µL blood |
| --- | --- |

| Nucleated RBCs animals | Extraction from 2.5–20 µL blood |
| --- | --- |

## Extraction results

Blood samples were extracted in a 96-well plate on Thermo Fisher KingFisher Apex or Hamilton Nimbus Presto systems. Total run time was 2 h 30 min.

| Sample name | Taxon | Blood volume | DNA yield | DNA mode size |
| --- | --- | --- | --- | --- |
| Bovine 1 | *Bos taurus* | 200 µL | **15.0** µg | **119** kb |
| Bovine 2 | *Bos taurus* | 200 µL | **12.2** µg | **50** kb |
| Minipig | *Sus scrofa* | 200 µL | **6.8** µg | **111** kb |
| Rabbit | *Oryctolagus cuniculus* | 200 µL | **3.9** µg | **188** kb |
| Chicken 1 | *Gallus domesticus* | 2.5 µL | **11.5** µg | **137** kb |
| Chicken 2 | *Gallus domesticus* | 2.5 µL | **10.7** µg | **87** kb |
| Tuna | *Thunnus orientalis* | 5 µL | **12.5** µg | **125** kb |
| Turkey | *Cathartes aura* | 10 µL | **21.0** µg | **165** kb |
| Viper | *Squamata viperidae* | 10 µL | **12.0** µg | **126** kb |
| Kestrel | *Falconiforms falconidae* | 20 µL | **13.0** µg | **102** kb |

DNA mode size as measured by Femto Pulse is >80 kb for majority of samples — example electropherograms are shown below.

Bovine 2 – mode size 50 kb
Minipig – mode size 111 kb
Rabbit – mode size 188 kb
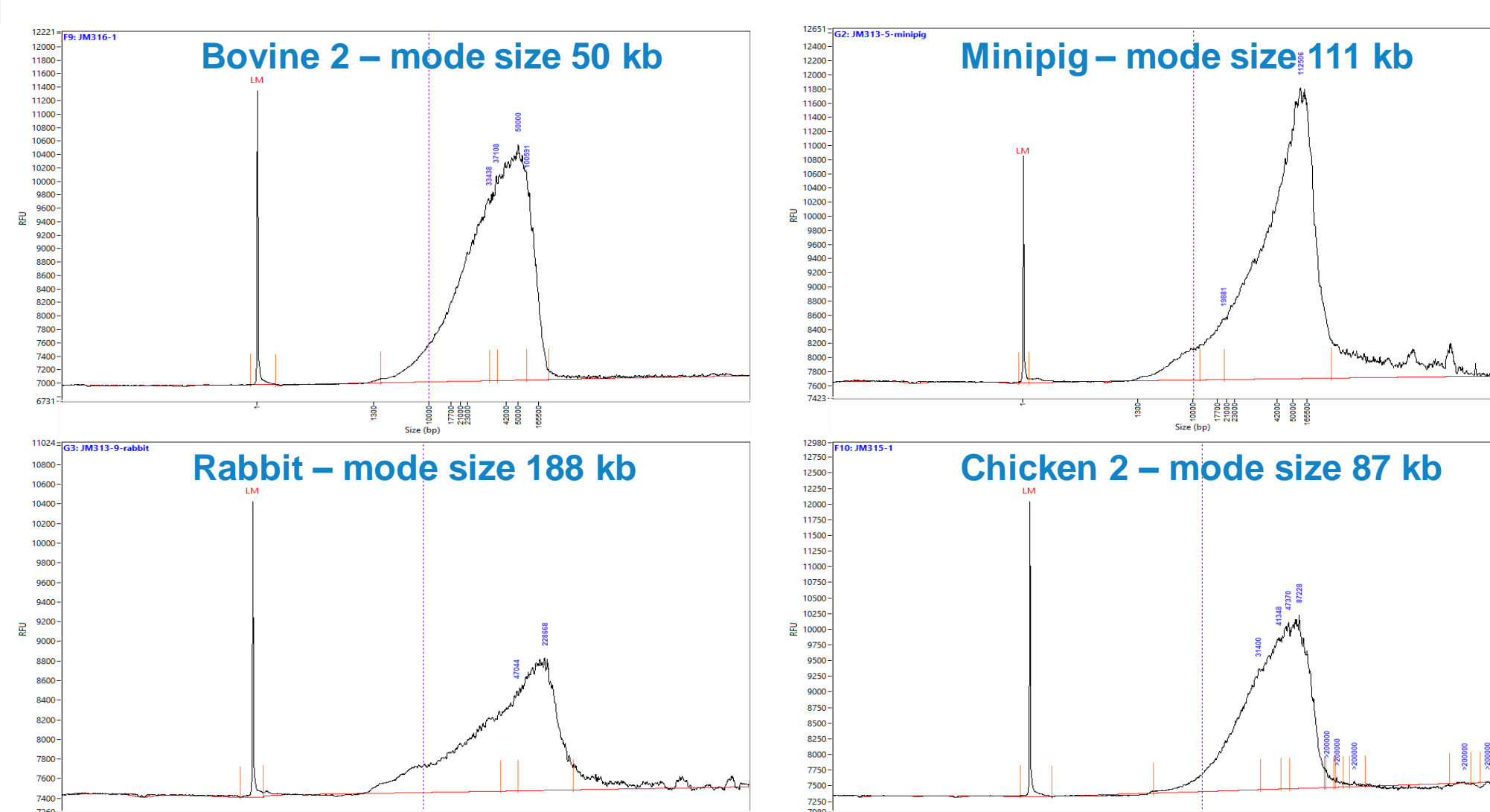Chicken 2 – mode size 87 kb

**Figure 2.** HMW DNA size distribution on Femto Pulse system (Agilent Technologies)

## Library result

Libraries were prepared following SPK 3.0 workflow automated on Hamilton NGS star or manually.

Final libraries DNA mode size measured by Femto Pulse were between 15 and 18 kb.

## Sequencing on PacBio Revio system

Samples were sequenced on the Revio system at 225 pM to achieve approx. 30× HiFi coverage.

| Sample | HiFi yield | Mean HiFi RL | Median QV | Coverage |
| --- | --- | --- | --- | --- |
| Bovine 1 | **82 Gb** | 14,504 bp | Q35 | **28×** |
| Bovine 2 | **106 Gb** | 15,988 bp | Q34 | **37×** |
| Minipig | **97 Gb** | 15,750 bp | Q35 | **36×** |
| Rabbit | **93 Gb** | 15,041 bp | Q35 | **27×** |
| Chicken 1 | **98 Gb** | 16,384 bp | Q34 | **82×** |
| Chicken 2 | **91 Gb** | 16,182 bp | Q34 | **76×** |
| Tuna | **101 Gb** | 16,759 bp | Q34 | **128×** |
| Turkey | **87 Gb** | 16,081 bp | Q35 | **79×** |
| Viper | **92 Gb** | 15,414 bp | Q34 | **65×** |
| Kestrel | **83 Gb** | 16,672 bp | Q34 | **65×** |

Bovine 2 Mean HiFi 16 Kb
Minipig Mean HiFi 15.7 Kb
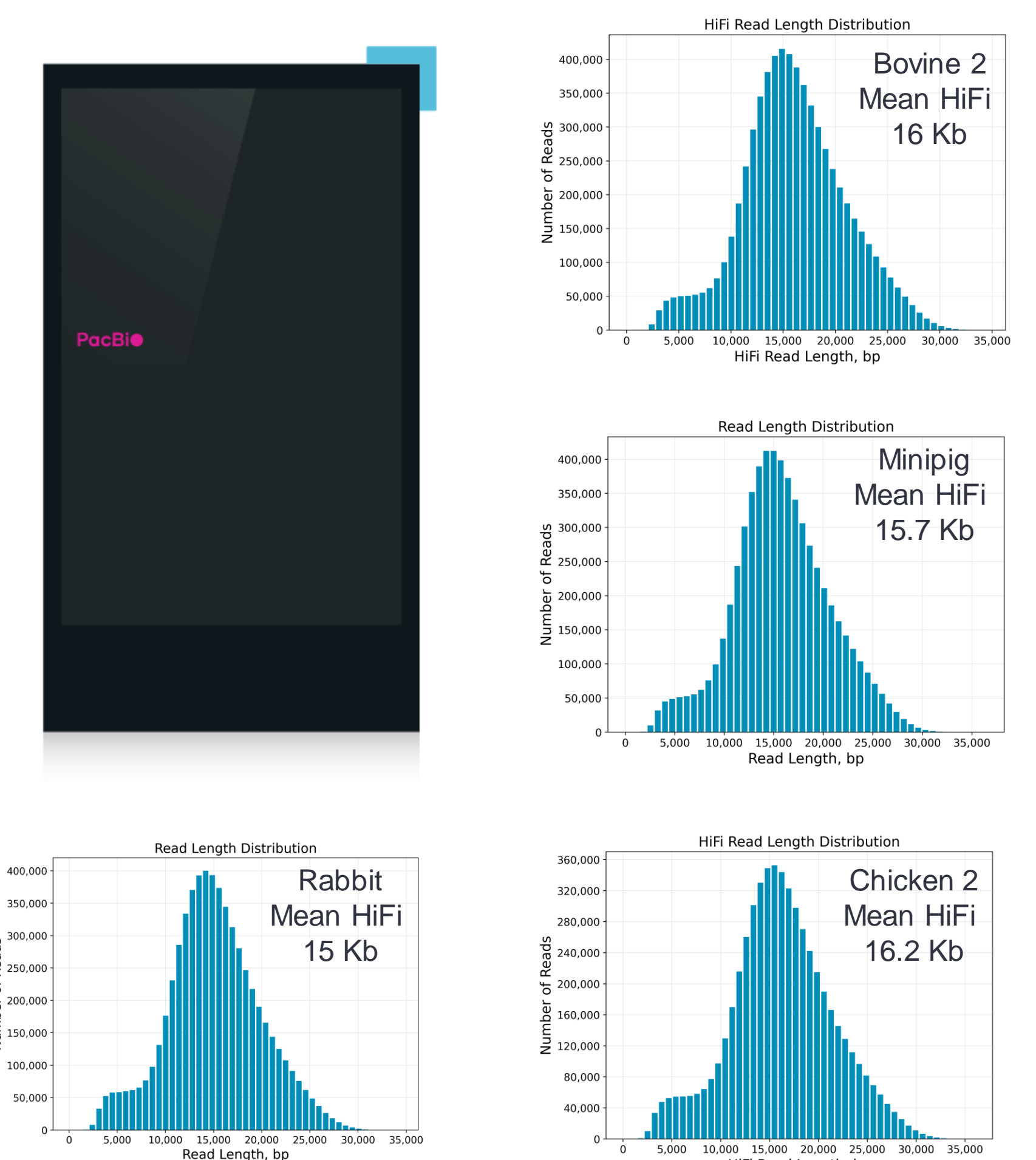Rabbit Mean HiFi 15 Kb
Chicken 2 Mean HiFi 16.2 Kb

**Figure 3.** Revio system and HiFi read length distribution plot for 3 samples

### *De novo* assembly

For bovine 1 sample, *de novo* assembly was run with 28× coverage obtained with one Revio SMRT Cell using *hifiasm* with default parameter. **This bovine assembly shows high contiguity with contig N50 >23 Mb and high quality with accuracy >QV 56. Each haplotype is phased.**

| | Hap 1 | Hap 2 |
| --- | --- | --- |
| **Consensus accuracy** | QV 56.4 | QV 56.0 |
| **N50 contig length** | 25.4 Mb | 23.0 Mb |
| **Max contig length** | 111.7 Mb | 105.5 Mb |
| **Total size** | 3.04 Gb | 2.94 Gb |
| **Number of contigs** | 727 | 710 |

## Conclusion

**We demonstrated a high-throughput, automated workflow for processing animal blood samples from extraction using Nanobind HT CBB kit through HiFi sequencing on the Revio system.**

**All sequenced samples generated ~30× coverage of HiFi data sufficient for *de novo* assembly.**

## Acknowledgements