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## Volume reduction and automation of DNAPrep libraries for low pass sequencing

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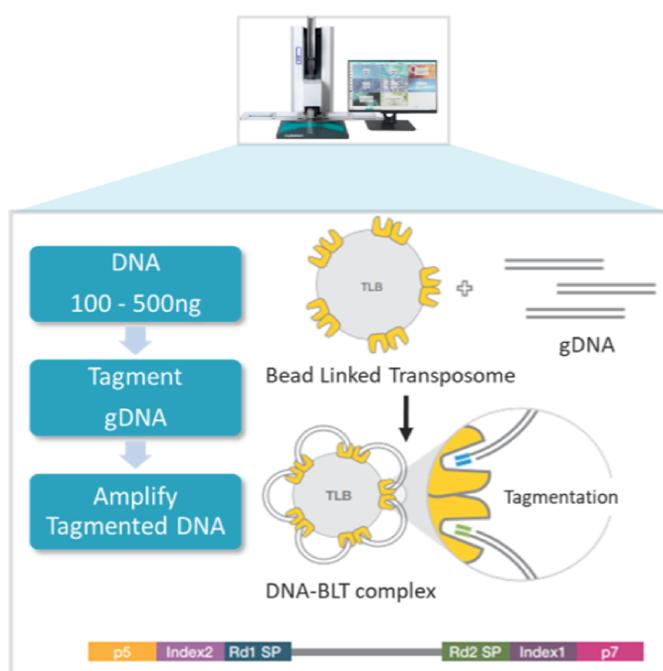
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## Background and objectives

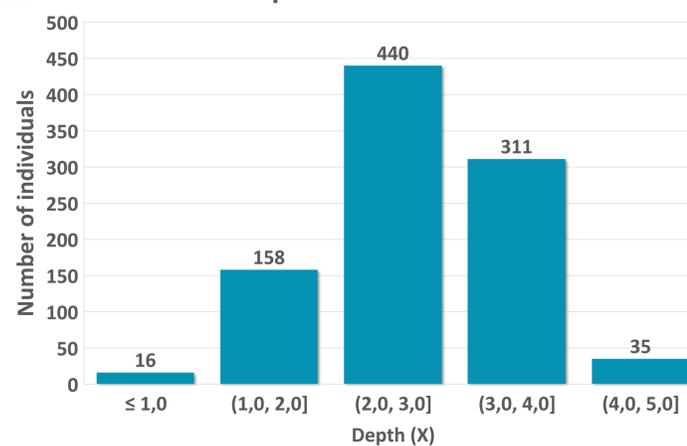
To characterize a population's haplotypic variability, **low pass sequencing** represents an interesting alternative to genotyping DNA-microarrays. Although this new technology is also costly, it offers greater precision of genetic information. We have therefore reduced reagent and DNA volumes by automating our Illumina **DNA Prep library** preparation protocol with the **Mosquito HV** (SPT LabTech, UK). For this purpose, we intend to evaluate this strategy for the **porcine species**. We used a family F2 protocol based on crossbreeds between Large White (LW) and Meishan (MS) individuals. It comprises around **1000 pigs**, including 12 F0 (6 LW and 6 MS), 30 F1 (6 males and 24 females) and 1000 F2. In addition to the evaluation of bioinformatic phasing methods for the **imputation** and reconstruction of **haplotypes**. The exploitation of these data will enable the precise characterization of **recombination sites**. This will provide an understanding of the reasons why a large region of the X chromosome recombines or not. The SeqOccIn **porcine pilot project** gave us the opportunity to (i) **automate the DNA prep protocol** to significantly reduce volumes with very good results and (ii) generate **good quality low pass sequencing data** for genotype imputation (validated by Mendelian compatibility).

## Protocol optimisation: automation with volume reduction

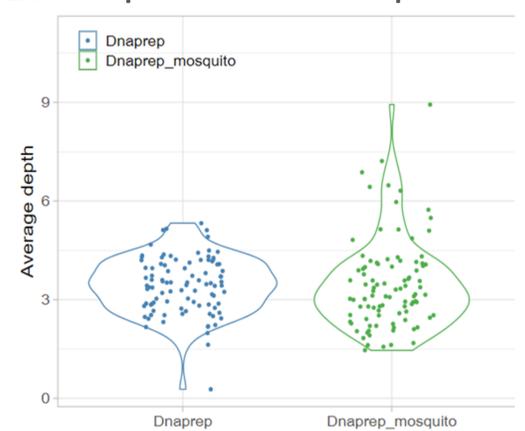
### 1A DNA Prep library workflow on Mosquito HV



### 1B Depth distribution



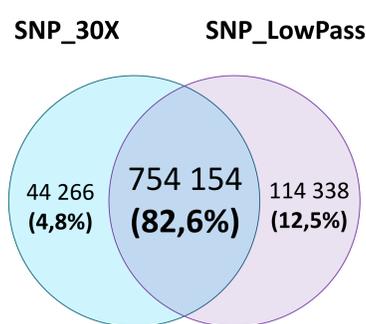
### 1C Depth distribution comparison



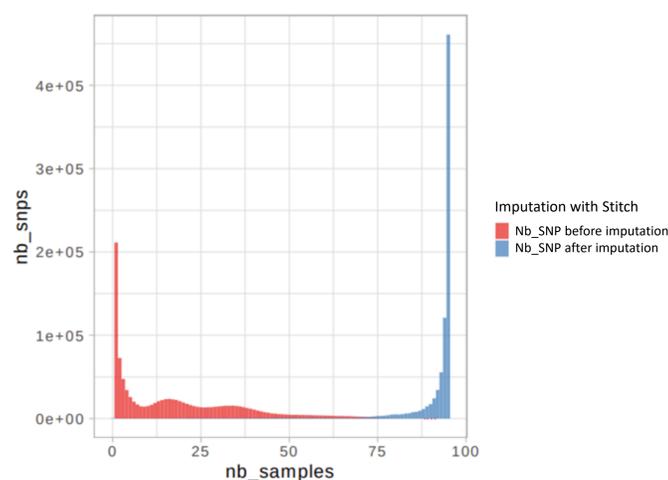
**Figure 1.** **1A.** DNA Prep library workflow. This kit uses **tagmentation chemistry**, which combines the steps of DNA fragmentation and adapter ligation. It also eliminates the need to quantify libraries prior to pooling for sequencing. This protocol has been **automated on the Mosquito HV**, with the volumes being **reduced by a factor of 5** for this project. **1B.** Distribution of coverage obtained on the cohort of 960 pigs using the DNA Prep library protocol. The mean coverage is **2.7X**. Heterogeneity is expected since balancing is based entirely on initial DNA quantification. **1C.** Graphic representation of **the average depth across the genome** (equivalent to the size of the library). This graph confirms that there is the **same variation in depth** with (green) or without Mosquito HV (blue).

## Protocol validation

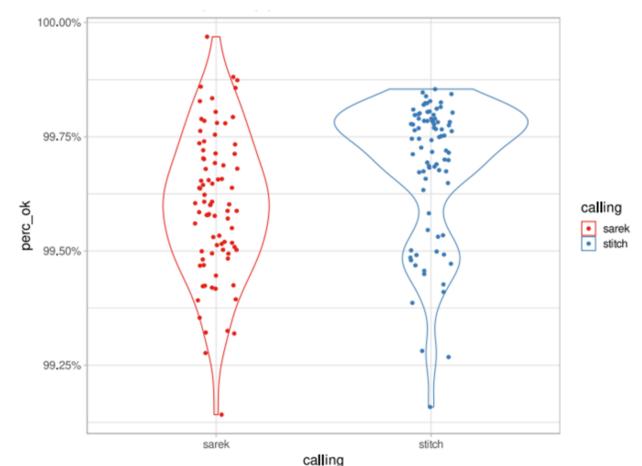
### 2A



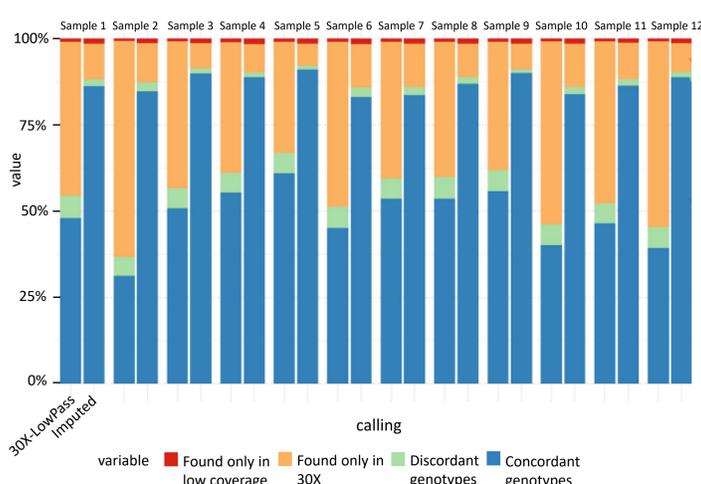
### 2C Number of genotyped samples distribution



### 2D Mendelian compatibility per trio



### 2B Concordance between low pass data and 30X reference



**Figure 2.** The parents (F0) were sequenced in 30X and the descendants (F1-F2) at 1X low pass. The tools used for **genotype imputation** are **nf-core/sarek** and **Stitch**. nf-core/sarek is a workflow designed to **detect variants** across the entire genome. The Stitch algorithm runs **without reference panels**. **2A.** Sparse allows to detect a similar set of variants compared to **high-coverage sequencing**. The phasing method on the trio detects **82.6% of variants**. **2B.** Graph of the **imputation effect** on genotyping of individuals sequenced at 30X and low pass. Imputation increases the percentage of identical genotypes between low pass and 30X (blue), corrects genotypes (green) and reduces false positives (red/orange). **2C.** The distribution of the number of variants in the same sample shows the imputation effect on the number of samples genotyped (blue). The **quality of the genotyping** is visible through the Mendelian plot. **2D.** There is **no degradation of symmetry after imputation** (blue) and genotyping is better corrected.

## Conclusion & perspectives

- Low-pass sequencing makes it possible to generate high-quality whole-genome genotyping data (pilot project results) at a cost close to that of a DNA-microarrays.
- We will develop volume reduction by automating other protocols on the Mosquito HV.