

Sam Ho¹, Tim Mosbruger², Amalia Dinou², Dimitri Monos^{2,3}

¹ Histocompatibility and Immunogenetics Laboratory, Gift of Hope Organ and Tissue Donor Network, Itasca, IL

² Immunogenetics/Clinical Laboratories, The Children's Hospital of Philadelphia, Philadelphia, PA

³ Department of Pathology and Lab Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Introduction:

Xenotransplantation with genetically modified pig organs offers a promising solution to the shortage of donor organs for transplantation. The swine major histocompatibility complex (MHC) contains numerous immune-related genes, including xenoreactive swine leukocyte antigens (SLA), which may play a key role in xenograft rejection. Comprehensive and accurate characterization of this region is essential for identifying novel xenoantigens and guiding genetic modifications to reduce immunogenicity, enhance xenocompatibility, and promote long-term graft survival. Here, we present a method for complete haplotypic characterization of the swine MHC using Oxford Nanopore Technologies' (ONT) adaptive sampling (AS) for sequencing and a custom assembly pipeline.

Methods:

Ultra-long DNA was extracted from the blood of three miniature pig breeds (Hanford, Sinclair and Yucatan) with known, blinded SLA genotyping. ONT AS was used to enrich the MHC (2.5 Mb) by targeting three reported MHC haplotypes extracted from published whole genome assemblies. The highly repetitive centromeric sequence separating MHC class I-III from class II was avoided. MHC-specific reads were extracted, assembled, SLA typed and annotated with a pipeline leveraging custom and existing tools.

RNA was extracted with the QIAmp Blood kit and sequenced on the Illumina platform. RNA-seq reads were aligned using STAR to a custom reference containing the assembled haplotypes and non-MHC reference sequence. Gene expression was estimated genome-wide with RSEM and reported as Transcripts per Million (TPM).

Results:

Sequencing generated long reads (N50 66-81 kb) that were >5x enriched for the MHC, resulting in 15-20x depth of coverage per haplotype. Assemblies produced complete contigs across each of class I-III (1,618-1,733 kb) and class II (502-594 kb) (Figure 1). The Sinclair pig was completely homozygous across the MHC. The other two animals were completely phased across class I-III and class II, but phase was lost at the centromere.

Assembly SLA typing was compared against the known typing at the SLA-1, 2, 3, DRB1, DQA and DRB1 loci and was 100% concordant with SBT at the first-field level (Figure 2). The assembled haplotypes were complex, with duplicate SLA-1 genes in some haplotypes.

Allele-specific SLA expression was determined for each animal (Figure 2), revealing strong expression for SLA-1 and SLA-2, low expression of the SLA-3 null and variable expression for SLA-1 duplicate genes.

Both SLA class I genes and pseudogenes were highly demethylated, even though the expression of pseudogenes is low (Figure 3).

Expression of class I and class II genes are similar between human and swine, even though the class I genes are not direct orthologs (Figure 4).

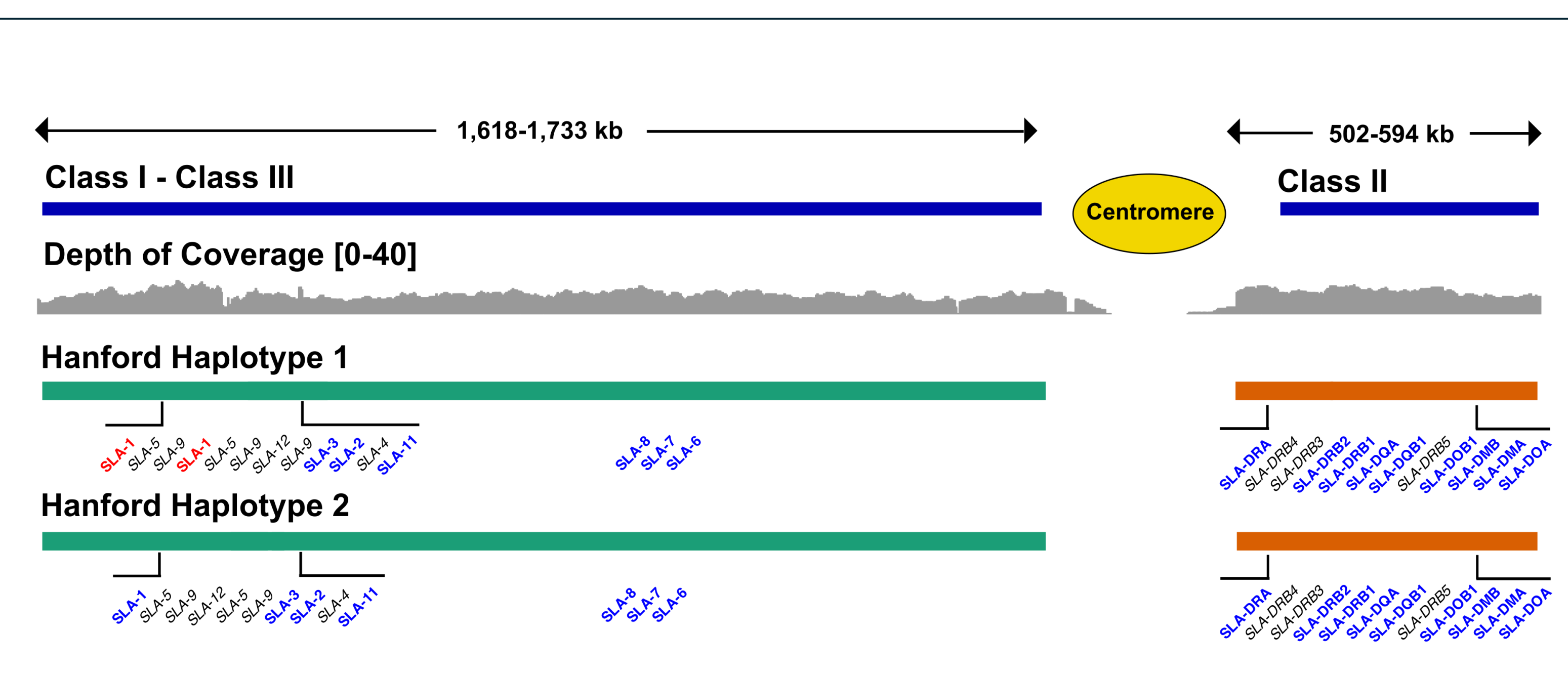


Figure 1. Representative assembly and typing. The assembly pipeline produced complete contigs across the class I-III region (green bar) and class II region (orange bar). Phase is lost at the centromere. SLA gene relative locations on the haplotype are shown, including protein coding genes (blue) and pseudogenes (black). Haplotypes can contain multiple copies of the same protein coding gene (red).

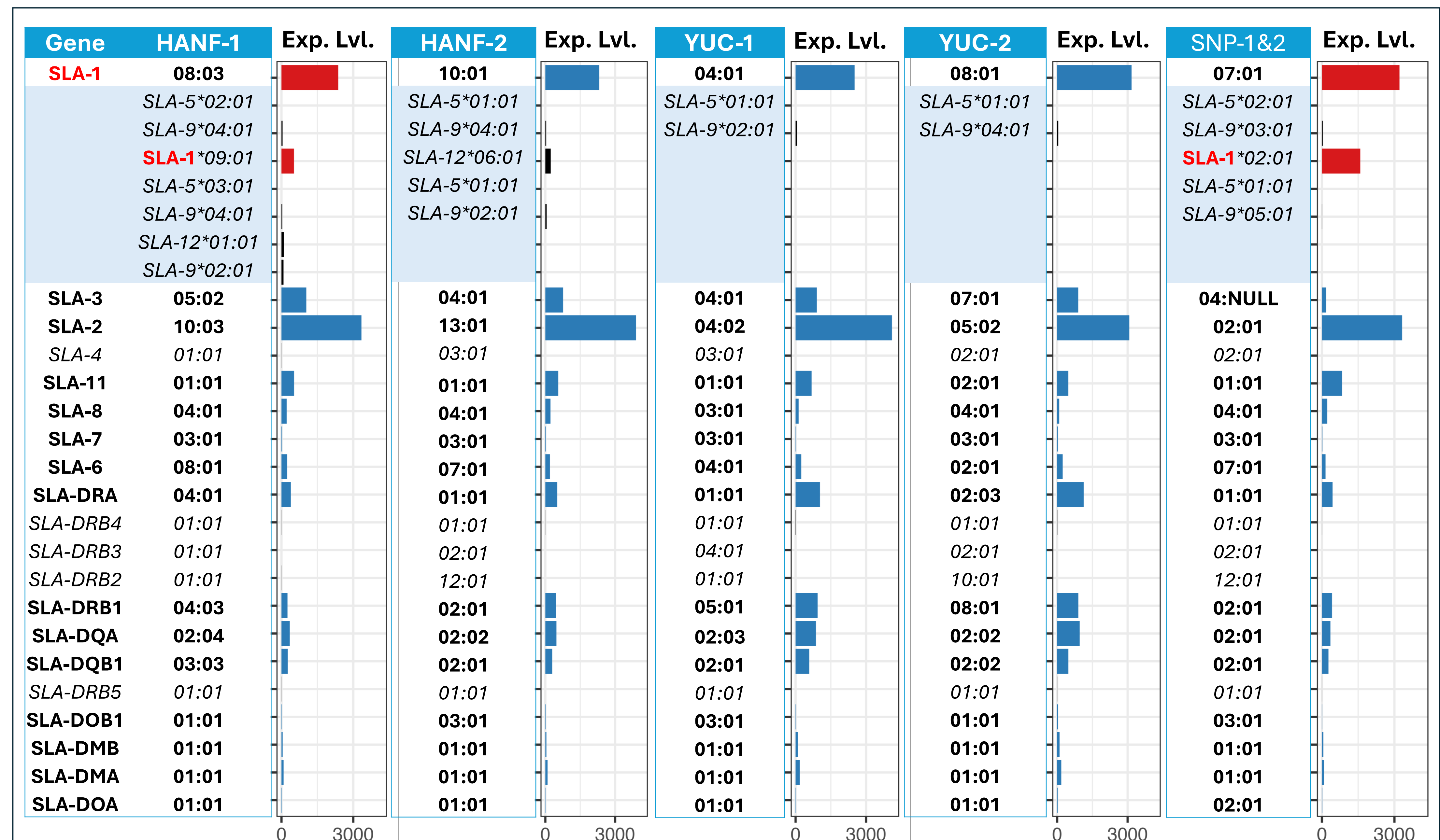


Figure 2. SLA Typing and Expression. Haplotypes were annotated with the best matching IMGT allele at each protein coding gene (bold) and pseudogene (italics). SLA typing at loci SLA-1, 2, 3, DRB1, DQA and DQB1 were 100% concordant with SBT at the first-field level. Allele-specific expression from blood was estimated for each pig and shown adjacent to the haplotype. The SLA-1 and SLA-2 genes were the highest expressing genes across the haplotypes. Duplicate copies of SLA-1 have different expression levels (red).

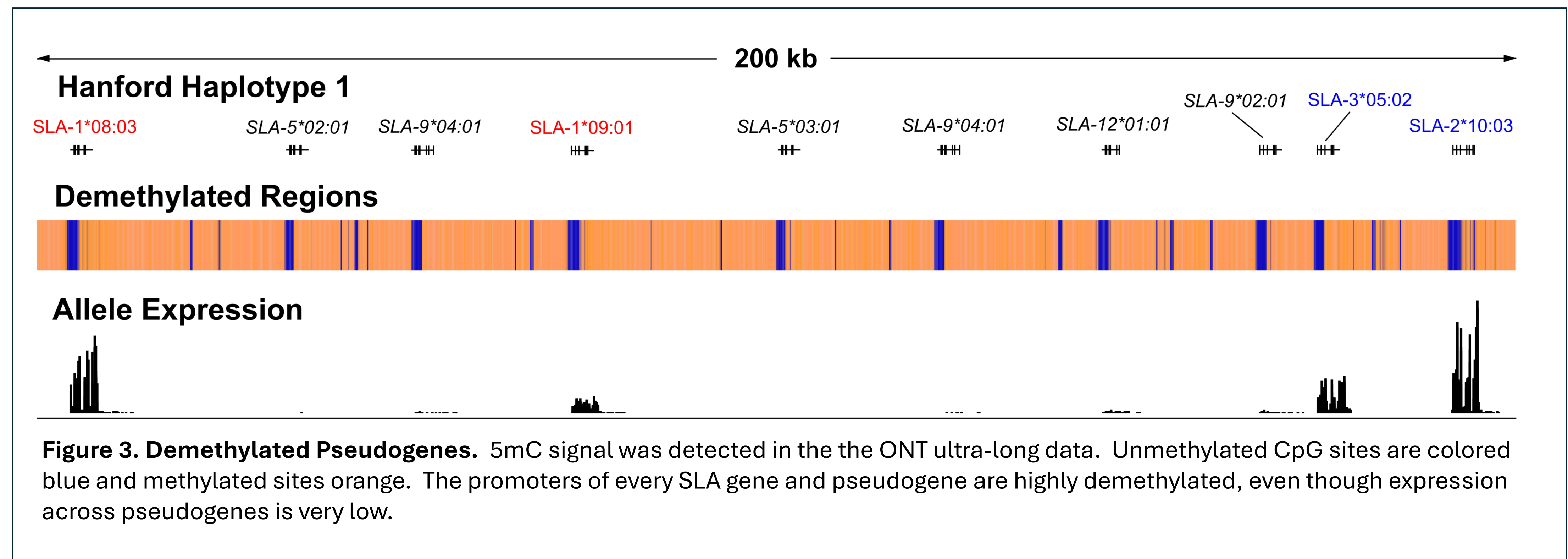


Figure 3. Demethylated Pseudogenes. 5mC signal was detected in the the ONT ultra-long data. Unmethylated CpG sites are colored blue and methylated sites orange. The promoters of every SLA gene and pseudogene are highly demethylated, even though expression across pseudogenes is very low.

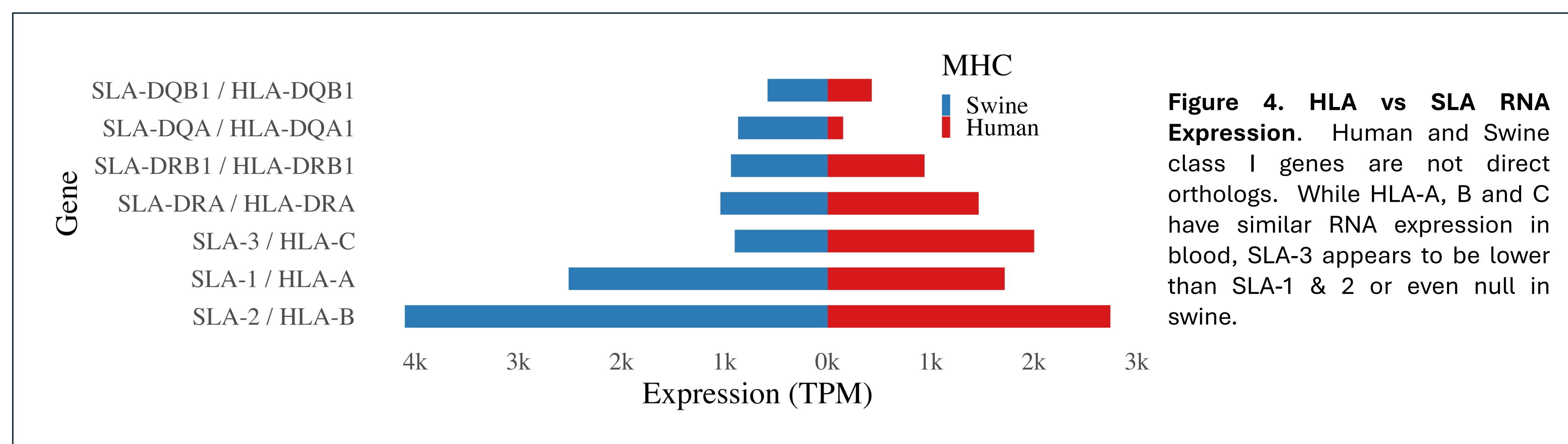


Figure 4. HLA vs SLA RNA Expression. Human and Swine class I genes are not direct orthologs. While HLA-A, B and C have similar RNA expression in blood, SLA-3 appears to be lower than SLA-1 & 2 or even null in swine.

Conclusions:

We have demonstrated that ONT AS paired to our analysis pipeline can generate credible haplotype-resolved MHC assemblies for three pigs representing diverse haplotypes. The accuracy, low cost and fast turnaround time of this method will hopefully facilitate future xenotransplantation studies.