



MHC Genotyping via Deep Sequencing

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Introduction

Since 2009, the O'Connor laboratory has provided high-throughput MHC genotyping for investigators utilizing nonhuman primates to study cellular immunity. Our sequence-based approach was initially designed using the Roche/454 Life Sciences Genome Sequencer Junior instrument to perform massively parallel pyrosequencing of an MHC class I genotyping amplicon; this method was also adapted for MHC class II DRB locus genotyping. As alternative next-generation sequencing platforms like the Illumina MiSeq and Pacific Biosciences Sequel instruments have become available, we have explored how best to use these technologies to improve our sequence-based MHC genotyping methods. We currently use the MiSeq to perform short-amplicon genotyping focused on the most polymorphic segments of MHC genes. We have established a Fluidigm Access Array primer panel for MHC genotyping spanning both the class I (A, B, I, and E) and class II (DRB, DQA, DQB, DPA, and DPB) regions. We are also currently developing full-length MHC class I and class II high-resolution genotyping using single molecule real-time (SMRT) sequencing on the PacBio Sequel instrument. This assay is ideal for novel allele discovery and provides the highest possible resolution MHC genotyping, since the entire coding region is analyzed. We are also exploring the utility of the PacBio method for other immune- important loci beyond the MHC, such as the killer immunoglobulin-like receptor and Fc receptor loci.

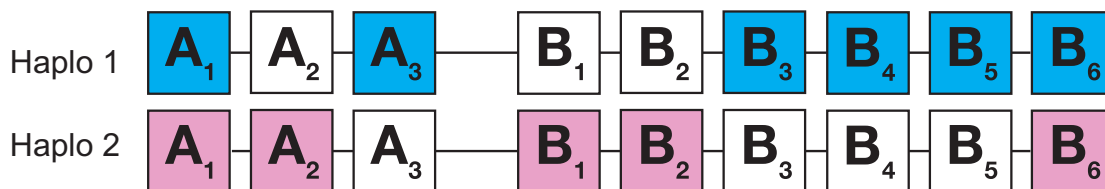
Difficulties Inherent in MHC Class I Genotyping of Macaques

It has been said that the MHC region of macaques has 'unparalleled complexity' when compared to the MHC regions of other species. This creates unique challenges for MHC genotyping.

Every animal has a variable number of MHC class I alleles

MHC-A and *MHC-B* loci in macaques are genetically unstable and have undergone a complex series of duplications. Unlike HLA class I haplotypes in humans, where three loci (*HLA-A*, *HLA-B*, *HLA-C*) each encode a single allele (e.g., the *HLA-B* locus on the maternal haplotype encodes *HLA-B*27:01* and the *HLA-B* locus on the paternal haplotype encodes *HLA-B*41:01*), a single macaque haplotype may have more than 20 MHC class I genes. Not all of these genes encode molecules that are stably expressed on the cell surface. This means that an animal might have one haplotype with 10 functional MHC class I alleles and a second haplotype with 6 functional MHC class I alleles, for a total of 16 expressed MHC class I molecules.

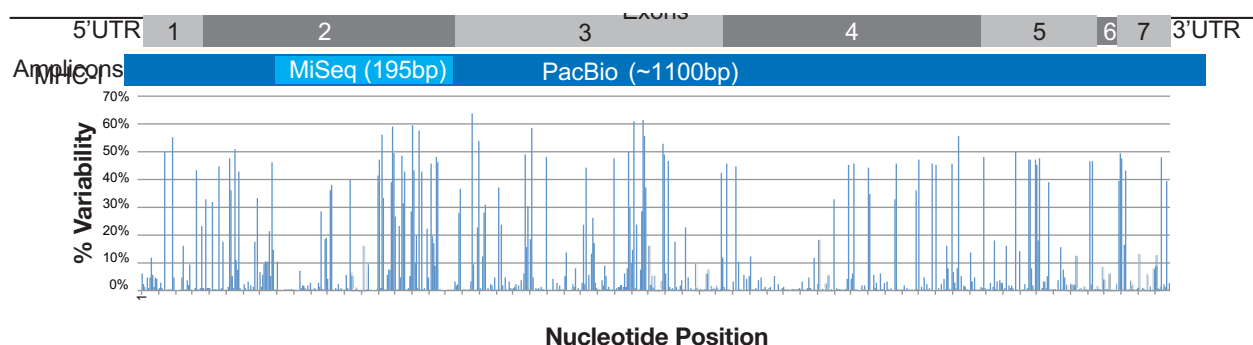
A simplified cartoon of the macaque MHC class I region is shown here (there are actually more *MHC-A* and *MHC-B* loci on each haplotype than in the cartoon). The blue shaded boxes represent those genes expressed on the paternal haplotype while the pink shaded boxes indicate genes expressed on the maternal haplotype.



In this example, the macaque would express 11 distinct MHC class I transcripts (4 *MHC-A* alleles and 7 *MHC-B* alleles). These genes are inherited as haplotypes, or defined groups of alleles, from the parents. We generally consider the *MHC-A* and *MHC-B* haplotypes independent from each other, since the MHC class I A and I B regions are separated by ~1 Mb which allows for relatively frequent recombination to occur on a population basis.

Macaque MHC class I alleles are exceptionally polymorphic

The MHC class I genes are among the most polymorphic in the genome, undoubtedly reflecting their centrality to host immunity. This exceptional polymorphism greatly complicates genotyping assays. Many other high-throughput genotyping tools rely on discriminating single-nucleotide polymorphisms that uniquely identify individual alleles. For example, the allele-specific PCR primers used to detect *Mamu-A1*001:01* anneal specifically to a sequence that is present in *Mamu-A1*001:01* but no other Indian rhesus macaque MHC class I alleles. **The problem? We do not know all other rhesus macaque MHC class I alleles - there may be hundreds of alleles that have not yet been described. The situation is even more extreme in cynomolgus macaques from certain origins and pig-tailed macaques, for which many fewer alleles have been identified to date.** To overcome this issue, we developed primers located within conserved regions of MHC class I alleles, so that each pair of primers has the ability to amplify all *MHC-A* and *MHC-B* alleles that are encoded by an animal. A schematic of our MHC class I primer design is shown in the diagram below.



The amplicons generated for MiSeq genotyping span the highly polymorphic exon 2 portions that encode the peptide binding region; the PacBio sequencing amplicons span the full coding region, with primers located in the UTRs.

MHC class I allele variants are largely population-specific, but allele lineages are shared

The specific MHC class I allelic variants observed in Indian rhesus macaques are seldom found in Chinese rhesus macaques (and vice versa). However, it is currently unknown if or how much these often subtle allelic variants influence peptide presentation and overall infectious disease course; therefore, to simplify haplotype designations we use allele lineage (for instance, *Mamu-A1*001* or *Mamu-B*017*) as the base unit for data analysis. Additionally, we define unique haplotypes as different configurations of 'major' MHC class I lineages only. The variable number of MHC class I lineages expressed by each macaque are detected over a wide range of transcript abundance when sequencing from RNA templates. 'Major' alleles account for the preponderance of total transcripts detected and we assume the bulk of immune responses would be dictated by the complement of 'major' alleles expressed by each macaque. A schematic of the reduction of a set of specific alleles down to the final haplotype designation for an example *Mamu-A* haplotype is shown on the next page.

Names are assigned to each haplotype based on the presence of a diagnostic major lineage, typically the most transcriptionally abundant allele contained on a haplotype but occasionally selected based on known biological significance (i.e., the B017 haplotype). Related versions of a base haplotype, denoted with a lowercase letter following the haplotype name (for instance, B001a and B001b), result when there are differences in the complement of major lineages accompanying the diagnostic allele.

Alleles	A1*007:04	A2*05:02:01	A6*01:nov:02
Lineages	A1*007	A2*05	A6*01
‘Major’	A1*007	A2*05	A6*01
Haplotype	A007		

Moving beyond rhesus macaques, there is evidence that some lineage-level haplotypes may be shared between rhesus, cynomolgus, and pig-tailed macaques. However, cynomolgus and pig-tailed macaques likely also express many haplotypes not observed in rhesus macaques. Our genotyping methods allow us to assign new haplotypes in these species as they are observed.

For instances where full resolution of the specific MHC class I allelic variant is important, like transplantation protocols and analysis of nonhuman primates from under-explored populations with less complete MHC allele libraries where short amplicon genotyping is minimally informative, PacBio SMRT sequencing of full-length MHC amplicons will produce the necessary resolution.

Difficulties Inherent in MHC Class II Genotyping of Macaques

To a lesser extent, the difficulties for MHC class I genotyping also apply to MHC class II DRB. This locus is the most polymorphic of the MHC class II loci, but each macaque only expresses 2 to 4 alleles per haplotype (4-8 per animal). The remaining five MHC class II loci (DQA, DQB, DPA, DPB, and DRA) only express one allele per haplotype (2 per animal) and are thus comparatively easy to genotype. However, each MHC class II locus is distinct enough that different primers are required for amplification of each locus (unlike MHC class I, where a single pair of exon 2 primers can amplify all A, B, I, and E loci). The DRA locus is essentially monomorphic in macaques, with most variation described to date being either synonymous nucleotide changes that do not change the protein, or intronic variation. We do not include the DRA locus in our Fluidigm/MiSeq primer panel, but do include it in the PacBio MHC class II full-length sequencing assay.

Just as with MHC class I, there have been relationships identified between specific MHC class II alleles and control of SIV in Indian rhesus macaques; however, these associations which almost certainly exist in other species of macaques have not yet been shown outside of that specific geographic population. Though inter-species allele sharing is more common for the MHC class II loci, previous studies have shown that there are still numerous alleles which have only been described to date in specific populations of macaques. Thus there is also a great need to expand the current population- specific MHC class II allele databases.

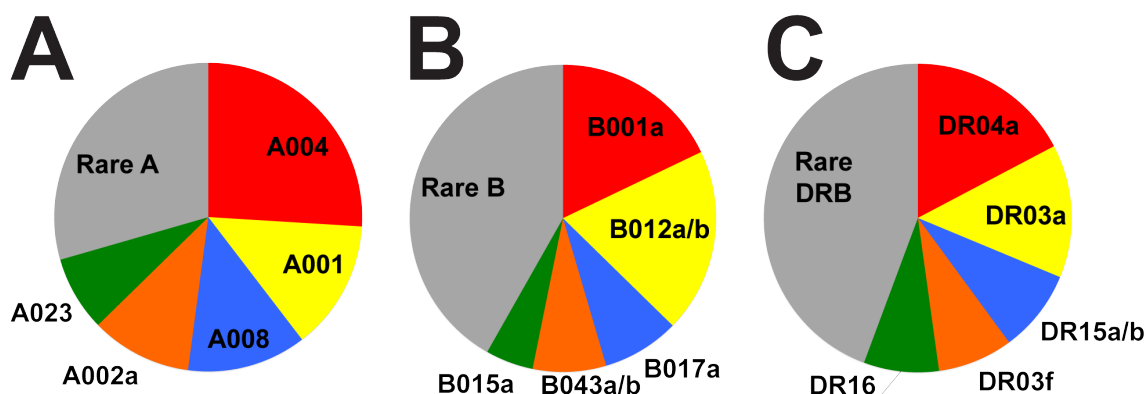
MHC Genotyping of Mauritian Cynomolgus Macaques

The MHC complement of the entire population of Mauritian cynomolgus macaques is limited to seven haplotypes, referred to as M1-M7. This restricted MHC diversity greatly simplifies genotyping analysis. We have developed a MiSeq sequence-based genotyping assay for Mauritian cynomolgus macaques using a Fluidigm panel of exon 2 primers for the MHC class I and class II loci and an automated pipeline for calling haplotypes based on the detection of diagnostic alleles for the M1-M7 haplotypes for each region (A, B, DRB, DQA/B, DPA/B). This makes genotyping of Mauritian cynomolgus macaques very straightforward.

MHC Genotyping of Indian Rhesus Macaques

Indian rhesus macaques from various breeding centers also exhibit restricted MHC diversity, though not as drastic as the Mauritian cynomolgus macaques. We have genotyped Indian rhesus macaques from over six different breeding centers

throughout the country, and identified that a very limited number of *Mamu-A*, *Mamu-B*, and *Mamu-DRB* haplotypes account for over half of the MHC diversity of Indian rhesus macaques available for research protocols, as shown here.



Though frequencies of each haplotype can vary between facilities, genotyping analysis of Indian rhesus macaques is more straightforward than other outbred populations of nonhuman primates because a large number of animals contain these most abundant haplotypes. We have validated our Fluidigm panel of exon 2 primers for MHC class I and class II for use in MiSeq genotyping of Indian rhesus macaques.

MHC Genotyping of Other Nonhuman Primate Populations

We have performed MiSeq MHC class I and class II genotyping on many other populations of nonhuman primates to date, such as Chinese rhesus macaques, Indonesian cynomolgus macaques, cynomolgus macaques from Chinese breeding facilities, and pig-tailed macaques. Because these populations are overall more diverse than Indian rhesus and Mauritian cynomolgus macaques, there is an increased chance we will encounter novel haplotypes during routine MiSeq genotyping. In populations with particularly small databases of known MHC alleles, the MiSeq assay may be only minimally informative since results are scored through comparison to the allele database. Alternatively, the PacBio assay can be used to unambiguously resolve novel alleles in these populations.

MHC Genotyping Process

We currently offer two assays for nonhuman primate MHC genotyping; the assay performed for a particular cohort is determined by the species and geographic origin of primate to genotype and the required level of allelic resolution. For **Indian-origin rhesus** and **Mauritian-origin cynomolgus** macaques, our standard assay includes Fluidigm Access Array exon 2 amplification from genomic DNA templates, followed by Illumina MiSeq sequencing. This assay produces lineage-level haplotypes for the MHC-A, MHC-B, MHC-DRB, MHC-DQ, and MHC-DP regions. For other nonhuman primate species, Fluidigm exon 2 amplification from total RNA templates followed by MiSeq sequencing can be performed. However, this assay may be only minimally informative, particularly for primates with small databases of known MHC alleles, since haplotypes may not be resolved for animals containing previously uncharacterized novel alleles.

For **other nonhuman primate populations**, or samples where **unambiguous, allele-level resolution is required**, our PacBio assay is highly recommended. This assay includes MHC class I and/or class II full-length amplification from total RNA templates, followed by PacBio Sequel SMRT sequencing. This assay produces high-resolution haplotypes for the MHC-A and MHC-B regions for class I typing, and MHC-DRA/B, MHC-DQA/B, and MHC-DPA/B regions for class II typing.

Starting material for genotyping

For MHC genotyping of **Indian rhesus** or **Mauritian cynomolgus** macaques, we require one of the following:

- 25 µl of genomic DNA at a concentration of ≥ 60 ng/µl
- 2 ml of fresh or frozen whole blood in EDTA tubes
- 5×10^6 cryopreserved PBMC

Genomic DNA samples may be shipped overnight at room temperature. Blood samples can be shipped either fresh or frozen; fresh blood samples should be shipped overnight with cold packs, and frozen blood samples should be shipped overnight on dry ice. Cell samples should be shipped overnight on dry ice. Genomic DNA isolations will be performed on whole blood or cells using a Promega Maxwell instrument.

For MHC genotyping of **other nonhuman primate species**, or **any samples for PacBio sequencing**, we require one of the following:

- 25 µl of total RNA at a concentration of ≥ 10 ng/µl
- 20 µl of cDNA synthesized using the Superscript III First-Strand Synthesis System for RT-PCR from Invitrogen
- 2 ml of fresh (not frozen) whole blood in EDTA tubes
- 5×10^6 cryopreserved PBMC

cDNA samples may be shipped overnight at room temperature. RNA and cell samples should avoid freeze-thaw cycles and should be shipped overnight on dry ice. Whole blood samples should be shipped overnight with cold packs. These conditions must be followed to minimize degradation of RNA from the starting material during shipping. Total RNA isolations will be performed on whole blood or cells using a Promega Maxwell instrument.

Synthesis of cDNA from RNA template

For our RNA-based assays, we use 50 ng of total RNA as a template for first-strand cDNA synthesis using the Superscript III First-Strand Synthesis System for RT-PCR from Invitrogen. This cDNA is then utilized to generate cDNA-PCR amplicons.

PCR amplification

For **MiSeq short-amplicon genotyping**, a panel of primers amplifying exon 2 of MHC class I, class II DRB, class II DQA/B, and class II DPA/B loci are amplified in a Fluidigm Access Array microfluidics chip. This chip facilitates

simultaneous amplification of 48 samples by up to 48 different primer pairs, adds MiSeq sequencing adapters and sample-specific barcodes to each amplicon, and pools the different amplicons for each sample upon harvest from the chip. Pooled amplicons for each sample are then SPRI-bead purified, quantitated, and normalized.

For **PacBio high-resolution genotyping**, the amplicons span the entire full-length coding region of MHC class I or MHC class II alleles. Since these amplicons include multiple exons, these primers must be used with a cDNA template. PacBio barcode identifier sequence tags are incorporated via the PCR primers, and amplicons are SPRI-bead purified, quantitated, and normalized.

Pooling and sequencing: MiSeq genotyping

We generally pool barcode-tagged amplicons for approximately 192 samples into a single pool for MiSeq sequencing. Cluster formation is performed on-instrument, greatly simplifying sample preparation. Additional details about the MiSeq instrument system and sequencing workflow can be found on the Illumina website (<http://www.illumina.com/systems/miseq.ilmn>).

Pooling and sequencing: PacBio genotyping

We typically pool barcode-tagged amplicons for 32 MHC class I or 32 MHC class II samples (all six loci) together into a single pool for PacBio Sequel SMRT sequencing. PCR products are circularized prior to long-read sequencing on a Sequel instrument. Additional details about the Sequel instrument system and sequencing workflow can be found on the PacBio website (<http://www.pacb.com/products-and-services/pacbio-systems/>).

Analysis of data

After images are processed and nucleotides are called on the MiSeq instrument, sequence reads are binned by barcode tag and compared against a species-specific curated in-house database of primate MHC sequences available in IPD/GenBank to map each animal's sequences against all known MHC alleles for that particular species. Any sequences that show 100% identity to a known species-specific MHC allele over the entire exon 2 read are enumerated as that allele. For **Mauritian cynomolgus** macaques, the results of comparison to the allele database are passed through an auto-calling script that predicts most likely A, B, DRB, DQ, and DP haplotypes for each animal based on the presence of diagnostic alleles for each of the seven haplotypes (M1-M7) in each region. The results of the auto-calling script are manually verified and final M1-M7 haplotype results for each region are reported for each animal. For **other nonhuman primates**, the 'major' alleles are manually examined and assigned to most likely haplotype using species-specific lists of previously described haplotypes, and by looking for shared groups of 'major' alleles across the cohort submitted for genotyping. Lineage-level A, B, DRB, DQ, and DP haplotypes are assigned and reported for each animal.

For **PacBio data analysis**, sequence reads are binned by barcode tag, then filtered to remove insertion/deletion base calling artifacts, short reads, and likely PCR chimeras. Reads that pass filtering are compared to a species-specific database of MHC alleles. Any 100% matches to full-length database alleles are enumerated as that allele and are not passed through any additional processing. Any 100% matches to short database alleles and any sequences that do not perfectly match a database allele are further processed to cluster identical sequences together. Clusters supported by 3 or more independent reads are retained, compared again to the database of known alleles, and categorized as either extensions of previously identified alleles or putative novel alleles. These sequences are manually curated and added to the species-specific database, as well as submitted to GenBank/IPD for official nomenclature. All raw PacBio sequence reads are compared against the updated species-specific database of alleles, containing the new extension and putative novel alleles, generating a table of number of reads per allele per animal in the PacBio cohort. These results are examined to assign most likely haplotype as described above for the MiSeq genotyping analysis. Results are reported as a list of full-resolution A, B, DR, DQ, and DP alleles, with a haplotype summary per region available for each animal.

Typical Next-Generation Genotyping Results

Depth of coverage

The following table illustrates the average yields of sequence reads for an MHC genotyping next-generation sequencer run.

Sequencer	Avg Total Pass Filter Reads	Avg MHC Class I Reads Identified/Sample	Avg MHC Class II Reads Identified/Sample
MiSeq	~16,000,000	~28,000 (pool of 192 samples)	~14,000 (pool of 192 samples)
PacBio	~30,000/SMRT cell	~1,000 (pool of 32, 1 SMRT cell)	~1,000 (pool of 32, 1 SMRT cell)

The averages listed here reflect a typical genotyping run; not all samples are equally successful, so this is not a guarantee of level of genotyping coverage. Since we anticipate identifying fewer total MHC class II sequences per animal, we generate fewer of those amplicons in the primary PCR for MiSeq runs.

Example genotyping reports

Figures 1-4 on pages 10-13 of this document show examples of the genotyping reports that we generate. Upon completion of data analysis, we will provide a file summarizing the data obtained for each cohort. Most MiSeq reports will include a simple summary of the haplotypes observed in each sample, as well as the definitions table of all alleles associated with each haplotype (Figure 1). We also include a sequencing results summary (Figure 2), including a simple list of alleles identified in all samples and total reads identified for each sequence in each animal (as shown here for the MHC class I region of three trio sets of Indian rhesus macaques). Comparable reports are created for Mauritian cynomolgus macaque MiSeq genotyping (Figure 3), which includes the simple haplotype summary shown at the top of the figure, and additional sequencing result details. The example shown here includes a list of alleles and total reads identified for each *Mafa-A* and *Mafa-B* allele observed in each animal, and a phase-inferred chromosome report for each animal sorted into simple heterozygotes, recombinant animals lacking a *Mafa-A1*063* allele, and recombinants with that allele. Genotyping reports for PacBio data will look very similar to MiSeq reports (Figure 4), but with full-length allele resolution (as shown here for a group of related pig-tailed macaques). Inclusion of any reports or graphical representations beyond the simple allele summary will be based on the needs of each individual client.

Unless previously specified otherwise, we also reserve the right to use any samples submitted for genotyping assays for future genotyping development projects. This may include analysis of novel MHC alleles, expanding genotyping efforts to other immune-important loci, using cohort haplotype information and frequencies in aggregated lists of MHC haplotypes observed in a species or population, and the like. Since these analyses are performed subsequent to, not at the time of production of an independent genotyping report, the results of any such analyses will not be reported to the individual client (but are absolutely essential to improving future genotyping runs for all clients).

Contact Information

For more information or to discuss the specifics of your project, please contact the UW-Madison WNPRC Genomics Services unit at uwgenomicservices@primate.wisc.edu. Before starting any genotyping, we require execution of a service agreement (example appended to the end of this document) and generation of a purchase order. An [on-line service request form](#) must be submitted prior to starting any service. For additional information, please see our website (<https://primate.wisc.edu/research-services/genomics-services/>).

Costs for MHC Genotyping - MiSeq Assays

Genotyping costs per animal are shown in the tables below. **These rates are effective from May 1, 2024 through April 30, 2025.** Rates are subject to change annually. Direct cost recovery is used to set the base rate for University of Wisconsin clients (Tier 1). For clients outside of the UW, we must also recover indirect costs (facilities & administrative overhead, etc.). The current rates for these indirects are 38.5% for other US federal and non-profit entities (Tier 2), 55.5% for foreign universities and non-profits (such as foreign NIH equivalents, e.g. the UK's NHS) and US for-profit entities using federal funds, and 72.5% for for-profit entities (Tier 3). A more detailed breakdown of costs is available upon request.

Mauritian cynomolgus macaque MiSeq MHC genotyping

	Whole Blood	gDNA
Reagents Total/Animal	\$52	\$39
Labor Total/Animal	\$66	\$57
Total Costs	\$118	\$96
Tier 1 - UW-Madison	\$118.00	\$96.00
Tier 2 - Federal & Non-Profit	\$163.43	\$132.96
Tier 2a - Specialty Non-Profit	\$183.49	\$149.28
Tier 3 - For-Profit	\$203.55	\$165.60

Indian rhesus and other nonhuman primate species MiSeq MHC genotyping

	Whole Blood	gDNA
Reagents Total/Animal	\$52	\$39
Labor Total/Animal	\$91	\$82
Total Costs	\$143	\$121
Tier 1 - UW-Madison	\$143.00	\$121.00
Tier 2 - Federal & Non-Profit	\$198.06	\$167.59
Tier 2a - Specialty Non-Profit	\$222.37	\$188.16
Tier 3 - For-Profit	\$246.68	\$208.73

Standard turnaround time for all MiSeq genotyping assays is 8-12 weeks from receipt of samples. These assays can also be expedited at a 50% premium on top of the Tier 1 rates (plus any applicable overhead; expedited Indian rhesus macaque MiSeq genotyping from client-provided whole blood, for instance, would be available for \$214.50 to Tier 1 clients, \$297.08 for Tier 2 clients, etc.). The extra charge is to cover the rush sample processing and running of a MiSeq run with less than the optimal number of samples, and reduces turnaround to 4-6 weeks.

Costs for MHC Genotyping - PacBio Assays

Genotyping costs per animal are shown in the table below. **These rates are effective from May 1, 2024 through April 30, 2025.** Rates are subject to change annually. Direct cost recovery is used to set the base rate for University of Wisconsin clients (Tier 1). For clients outside of the UW, we must also recover indirect costs (facilities & administrative overhead, etc.). The current rates for these indirects are 38.5% for other US federal and non-profit entities (Tier 2), 55.5% for foreign universities and non-profits (such as foreign NIH equivalents, e.g. the UK's NHS) and US for-profit entities using federal funds, and 72.5% for for-profit entities (Tier 3). A more detailed breakdown of costs is available upon request.

PacBio high-resolution MHC genotyping

	MHC Class I	MHC Class II	MHC Class I + II
Reagents Total/Animal	\$56	\$108	\$142
Labor Total/Animal	\$133	\$162	\$256
Total Costs	\$189	\$270	\$398
Tier 1 - UW-Madison	\$189.00	\$270.00	\$398.00
Tier 2 - Federal & Non-Profit	\$261.77	\$373.95	\$551.23
Tier 2a - Specialty Non-Profit	\$293.90	\$419.85	\$618.89
Tier 3 - For-Profit	\$326.03	\$465.75	\$686.55

Standard turnaround time for all PacBio genotyping assays is 10-14 weeks from receipt of samples. We are unable to offer an expedited turnaround for these assays. These assays must be performed from total RNA templates; samples for which high-quality RNA cannot be obtained will not be processed for PacBio genotyping.

Figure 1. MHC Haplotype Summary and Definitions

Summary of haplotypes observed in each sample

Animal ID	Relation	# Class I reads identified	Mamu-A Haplo 1	Mamu-A Haplo 2	Mamu-B Haplo 1	Mamu-B Haplo 2	# DRB reads identified	Mamu-DRB Haplo 1	Mamu-DRB Haplo 2
r89033	Sire1	2195	A002a	A002a	B043a	B001a	741	DR03f	DR02
r04035	Offspring1	2173	A002a	A002a	B043a	B055	641	DR03f	DR04a
r89056	Dam1	2615	A004	A002a	B048	B055	259	DR15a	DR04a
rh2071	Sire2	849	A001	A023	B048	B024a	323	DR15a	DR04a
r04024	Offspring2	1875	A001	A007	B048	B008	548	DR15a	DR03f
r97061	Dam2	2258	A001	A007	B001a	B008	314	DR16	DR03f
r98068	Sire3	1723	A008	A011	B001a	B017a	231	DR09a	DR11a
r05084	Offspring3	913	A008	A004	B001a	B001a	386	DR09a	DR03a
r87110	Dam3	2456	A004	A004	B043a	B001a	664	DR06	DR03a

Alleles associated with each haplotype

Mamu-A Haplotype Name	Diagnostic Major	Minor 1	Minor 2	Minor 3	Minor 4
A001	A1*001	A2*05	-	-	-
A002a	A1*002	-	A3*13	A4*14	-
A004	A1*004	A2*05	-	A4*14	-
A007	A1*007	A2*05	-	-	A6*01
A008	A1*008	A2*05	A3*13	-	-
A011	A1*011	A2*05	-	A4*14	A6*01
A023	A1*023	-	-	A4*14	-

Mamu-B Haplotype Name	Diagnostic Major	Major 2	Major 3	Minor 1	Minor 2	Minor 3	Minor 4	Minor 5	Minor 6	Minor 7
B001a	B*001	B*007	B*030	B*045	B*046	B*057	B*060	B*072	B*082	-
B008	B*008	-	-	B*006	B*072	B*079	B*082	-	-	-
B017a	B*017	B*029	B*068	B*060	B*061	B*072	-	-	-	-
B024a	B*024	B*019	-	B*046	B*051	B*057	B*072	B*082	B*109	-
B043a	B*043	B*027	B*030	B*057	B*060	B*072	B*092	B*098	-	-
B048	B*048	B*041	-	B*046	B*064	B*072	B*079	B*109	B*116	B*134
B055	B*055	B*052	B*058	B*072	-	-	-	-	-	-

Mamu-DRB Haplotype Name	Allele 1	Allele 2	Allele 3	Allele 4
DR02	DRB3*04:03	DRB*W3:05	-	-
DR03a	DRB1*03:03	DRB1*10:07	-	-
DR03f	DRB1*03:06	DRB1*10:03	-	-
DR04a	DRB1*03:09	DRB*W2:01	-	-
DR06	DRB*W3:03/16	DRB*W4:01	-	-
DR09a	DRB1*04:04	DRB*W3:07	DRB*W7:02	-
DR11a	DRB*W20:02	DRB*W25:01	-	-
DR15a	DRB*W6:06	DRB*W21:04	DRB*W26:03	-
DR16	DRB1*03:10	DRB*W1:01	DRB*W6:02	DRB*W6:09

*haplotype names are still undergoing refinement

Figure 2. MiSeq MHC Class I Sequencing Summary

Animal ID	r89033	r04035	r89056	rh2071	r04024	r97061	r98068	r05084	r87110
Relationship	Sire 1	Progeny 1	Dam 1	Sire 2	Progeny 2	Dam 2	Sire 3	Progeny 3	Dam 3
# Reads Identified	529	5,300	5,070	1,651	2,587	3,315	3,636	2,405	5,263
Mamu-A Haplotype 1	A002a	A002a	A002a	A023	A007	A007	A011	A004	A004
Mamu-B Haplotype 1	B001a	B055	B055	B024a	B008	B008	B017a	B001a	B001a
Mamu-A Haplotype 2	A002a	A002a	A004	A001	A001	A001	A008	A008	A004
Mamu-B Haplotype 2	B043a	B043a	B048	B048	B048	B001a	B001a	B001a	B043a
Mamu-A Major									
A1*001				78	105	132			
A1*002	39	434	193						
A1*004			148					77	354
A1*007					174	229			
A1*008							213	113	
A1*011							185		
A1*023				75					
Mamu-A Minor									
A2*05				78	297	374	439	150	
A3*13							116	52	
A3*13,A4*14	28	284	135						
A4*14			258	90				105	458
A6*01					131	166	165		
Mamu-B									
B*001	22					117	106	143	164
B*006					48	87			
B*007	10					116	122	186	158
B*008					164	165			
B*017							219		
B*019				67					
B*024				60					
B*027	21	155							173
B*029							197		
B*030	84	273				184	215	177	557
B*035,B*049	35	604	270				223	338	605
B*041			87	27	46				
B*043	14	147							134
B*046				141					
B*048			253	64	135				
B*051				67	154	189			
B*052		81	55						
B*054		202	189						
B*055		197	167						
B*057	5	81		27					86
B*058		194	148						
B*060							150		268
B*061							57		
B*063	39	179	430	76	147	134	178	238	259
B*064			306	102	155				
B*072				69	134	285			
B*074	17	386	199				140	152	368
B*078					90	133	109		
B*079					140	171			
B*082				53					
B*092	17	163							154
B*097			224				131		
B*098	40	631	282				184	237	579
B*109			295	160	141				
B11L*01					8	17			
B16*01	55	441	474	156	99	147	292	207	397
B17*01	69	560	694	189	288	439	195	230	232
B20*01	34	288	263	72	131	230			317

Figure 3. Mauritian Cynomolgus Macaque Full MHC Genotyping Report

OC#ID	AB001	AB002	AB003	AB004	AB005	AB006	AB007	AB008
##Reads#Mapped	58,505	54,500	57,876	29,288	19,156	15,548	34,986	33,975
Mafa/A#Haplotype#1	M3	M4	M4	M3	M2	M2	M1	M1
Mafa/A#Haplotype#2	M4	M4	M4	M4	M4	M4	M1	M3
Mafa/B#Haplotype#1	M2	M4	M1	M3	M2	M3	M1	M1
Mafa/B#Haplotype#2	M4	M4	M3	M4	M4	M4	M2	M3
Mafa/DRB#Haplotype#1	M2	M1	M1	M3	M2	M3	M1	M1
Mafa/DRB#Haplotype#2	M4	M4	M3	M3	M4	M4	M2	M3
Mafa/DQA/DQB#Haplotype#1	M2	M1	M1	M3	M2	M3	M1	M1
Mafa/DQA/DQB#Haplotype#2	M4	M4	M3	M3	M4	M4	M2	M3
Mafa/DPA/DPB#Haplotype#1	M3	M1	M1	M3	M2	M3	M1	M1
Mafa/DPA/DPB#Haplotype#2	M4	M4	M3	M3	M4	M3	M2	M3
Mafa/A1								
05_M1M2M3_A1_063g	209			158	136	109	349	417
05_M4_A1_031_01	519	1036	946	323	312	303		
Mafa/A2/4/5								
06_M1M2M3M4_A2_05g	866	804	682	489	438	286	679	726
06_M1M2M3M6_A4_01g	137			114	127	157	259	335
06_M4M7_A5_30_01g	386	956	822	331	382	364		
Mafa/B								
12_M1M2M3M4M5M7_B17_01g	3,106	3,250	2,321	2,007	1,752	1,735	1,705	2,009
12_M1M4M7_B_060g1	293	678	256	208	273	241	265	374
12_M1_B_046_01_01			110				92	111
12_M1_B_057_02			82				31	60
12_M1_B_064_01			137				209	358
12_M1_B_104_01_01			10				8	
12_M1_B_131_02_01							9	
12_M1_B_134_02			301				387	470
12_M1_B_144_02			275				241	392
12_M1_B_152_01N			233				310	337
12_M2M3_B_079g	89		125	96	85	103	160	199
12_M2M5_B_098g B_098_01_B_098_04	135			6	138	9	158	9
12_M2_B11L_01g2ex	163				160		150	
12_M2_B_019_03	301				216		150	
12_M2_B_109_04	310				326		351	
12_M2_B_109_06	248				370		383	
12_M2_B_148_01_01	70				357		280	
12_M2_B_150_01_01	81				33		32	
12_M2_B_162	106				46		99	
12_M3M4_B20_01_02	237	677	38	345	242	267		41
12_M3M6M7_I_01g I_01_10_I_01_11_I_01_22			156	116		103		118
12_M3_B_075_01			353	314		329		398
12_M3_B_098_05			190	178		96		210
12_M3_B_165_01			458	372		426		578
12_M4M6_B16_01_04	17	95		76	89	85		
12_M4_B_027_02	56	173		73	116	125		
12_M4_B_051_03	227	669		217	210	148		
12_M4_B_088_01	338	838		227	191	170		
12_M4_B_127_nov_01	186	455		169	156	90		
12_M4_B_147_01	109	242		82	141	112		
12_M4_I_03_01_01	86	235		112	147	171		
12_M5_B_051_04				7	5			

Total#	Mafa2A#	Mafa2B#	Mafa2DRB#	Mafa#DQ#	Mafa2DP#	Mafa2A#	Mafa2B#	Mafa2DRB#	Mafa#DQ#	Mafa2DP#
OC#ID	Reads#	Haplotype#	Haplotype#	Haplotype#	Haplotype#	Haplotype#	Haplotype#	Haplotype#	Haplotype#	Haplotype#
Mapped		1	1	1	1	1	2	2	2	2
Simple Heterozygous Animals										
AB008	33975	M1	M1	M1	M1	M1	M3	M3	M3	M3
AB005	19156	M2	M2	M2	M2	M2	M4	M4	M4	M4
Recombinant Animals (A1*063-)										
AB002	54500	M4	M4	M4	M4	M4	M4	M4	M1	M1
AB003	57876	M4	M1	M1	M1	M1	M4	M3	M3	M3
Recombinant Animals										

AB007	34986	M1	M1	M1	M1	M1	M1	M2	M2	M2	M2	M2
AB004	29288	M3	M3	M3	M3	M3	M3	M4	M4	M3	M3	M3
AB001	58505	M4	M4	M4	M4	M4	M4	M3	M2	M2	M2	M3
AB006	15548	M2	M3	M3	M3	M3	M3	M4	M4	M4	M4	M3

Figure 4. PacBio Full-Length MHC Class I Genotyping Report

Animal ID			PT190	PT136	PT129	PT135	PT172		
Relationship			Dam 1	Progeny 1	Sire	Progeny 2	Dam 2		
Mane-A Haplotype 1			A084	A004	A004	A052:A063	A052:A063		
Mane-A Haplotype 2			A010	A010	A019	A019	A082		
Mane-B Haplotype 1			B104	B025	B025	B150	B150		
Mane-B Haplotype 2			B015a	B015a	B069a	B069a	B016a		
# Reads Evaluated	Totals	Ave	20,972	4,194	3410	4476	4155	3999	4932
# Identical Full-length Reads			6,158	1,232	842	1350	1350	1158	1458
% Identical Full-length Reads			29.17		24.7	30.2	32.5	29.0	29.6
# Full-length Alleles			13		13	13	11	12	14
Mane-A1									
A1*004:01	e	404	2		208	196			
A1*010:SP	n	363	2	142	221				
A1*019:nov:01	n	447	2			239	207		
A1*052:01	p	420	2				167	252	
A1*063:nov:03	n	316	2				122	194	
A1*082:03	p	262	1						262
A1*084:01:01	p	150	1	150					
Mane-A minors									
A2*05:03:01	p	6	2	3					3
A2*05:07	n	5	2	2	3				
A3*13:01:01	p	48	1						48
A4*01:01:01	e	24	2			17	7		
A6*01:nov:03	n	6	3		2	2	2		
Mane-B									
B*015:01	e	453	2	154	299				
B*016:01	e	89	1						89
B*017:02	p	243	2				122	121	
B*025:SP	n	1,088	2		482	606			
B*027:nov:04	n	17	2		5	12			
B*046:01:01	e	2	1	2					
B*064:01	p	3	2	2	1				
B*068:02:01	e	158	2	47	111				
B*068:06	e	94	1						94
B*068:nov:03	n	161	2				84	77	
B*069:01	p	351	2			208	143		
B*072:02:02	e	7	1	7					
B*088:01	p	19	2	11	8				
B*104:02	p	309	1	309					
B*107:02	p	52	2			33	19		
B*112:02	e	13	1						13
B*115:nov:02	n	7	1			7			
B*116:01	p	40	2			24	16		
B*119:01	p	12	1	12					
B*150:nov:02	n	558	2				267	291	
Mane-I									
I*01:02	e	5	2				2	3	
I*01:06	e	6	1						6
I*01:nov:03	n	9	2		3	6			
I*01:nov:06	n	5	2	1	4				
I*01:nov:07	n	3	1		3				
I*01:nov:08	n	5	1						5
Novel full length sequence									
Novel full length sequence	n	14							
Extension of partial sequence	e	11							
Identical to known sequence	p	13							

Wisconsin National Primate Research Center Service Agreement

This Service Agreement ("Agreement") is entered into as of the date of last signature below ("Effective Date"), between the Board of Regents of the University of Wisconsin System on behalf of the Wisconsin National Primate Research Center at the University of Wisconsin-Madison, a public educational institution of the State of Wisconsin, with an address at 21 N. Park Street, Suite 6401, Madison, WI 53715 ("University" or "WNPRC"), and The Trustees of the **University of Noname**, a company having its principal place of business at Neverland, AK 99999 ("Service Recipient").

Whereas the service contemplated by this agreement is of mutual interest and benefit to the University and to the Service Recipient, and will further the University's instructional, research or outreach objectives in a manner consistent with its status as a non-profit, tax-exempt, educational institution.

Now therefore, the parties hereto agree as follows:

1. Statement of Work

The Service Recipient agrees to have the WNPRC provide the following service ("Service"):

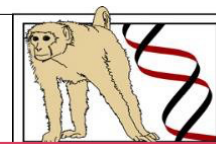
Indian rhesus macaque MiSeq MHC Class I + Class II genotyping (blood samples)

\$143.00/sample (base rate) x 1.385 (cost recovery of 38.5%) x 10 samples = \$1,980.55

The WNPRC agrees to use reasonable efforts to perform the Service. The Service Recipient acknowledges that the WNPRC makes no express or implied warranties for results of this Service unless specifically identified in this agreement. The Service is limited to the scope of work as provided in this Agreement, and does not include any consultation that the WNPRC may provide to Service Recipient before or after the performance of Service. The terms and conditions of this Agreement do not govern any rights or duties that may be associated with any such consultation.

2. Term of Agreement

This Agreement shall begin on the Effective Date, and continue until **4/30/2025**, and may be extended by mutual agreement of the parties. Terms which by their nature are intended to survive shall not be affected by the expiration or termination of this Agreement.



3. Payment Terms

Service Recipient shall pay WNPRC in U.S. dollars the total amount of **\$198.06 per sample as noted in the statement of work.**

Per National Institutes of Health (NIH)/Office of Research Infrastructure Programs (ORIP) guidelines and as determined by the WNPRC in coordination with the Service Recipient, this agreement is considered a Tier 2 agreement and cost recovery will be in accordance with the applicable Tier rate structure. **The 38.5% rate will be applied as a separate line item on the invoice.**

While it is estimated that this amount is sufficient to conduct the Service outlined in the scope of work, the WNPRC will submit to Service Recipient a revised budget requesting additional funds in the event the Service Recipient requests additional services.

4. Payment Schedule

Email Purchase Orders (each a "PO") to:

Katie Frisch
Grants Manager
kolson@primate.wisc.edu

If electronic submission is not possible, fax the PO to: (608) 263-4031.

WNPRC will invoice the Service Recipient and such invoices shall be due within 30 days of receipt.

Checks shall be made payable to: Wisconsin National Primate Research Center

And sent to:

Debra L. Jurmu
Associate Director, Operational Services
Wisconsin National Primate Research Center
1220 Capitol Court
Madison, WI 53715

5. Notices

Notices and communications hereunder shall be deemed made if given by registered or certified mail, postage prepaid, and addressed to the party to receive such notice or communication at the address given below, or such other addresses as may hereafter be designated in writing.

If to Service Recipient:

Service Recipient Scientist:

Polly Pi
Department of Surgery
University of Noname
1000 Campus Drive
Neverland, AK 99999
Tel: (000) 000-9000
polly.pi@noname.edu

Service Recipient Administrative Contact:

Alison Alpha
Department of Surgery
University of Noname
2000 Campus Drive
Neverland, AK 99999
Tel: (000) 000-1800
alison.alpha@noname.edu

If to WNPRC:

WNPRC Scientist:

David O'Connor
Genetics Services Unit Head
Wisconsin National Primate Research Center
585 Science Drive
Madison, WI 53711
Tel: (608) 890-0845
dhocconno@wisc.edu

WNPRC Administrative Contact:

Matt Raymond
Grants Coordinator
Wisconsin National Primate Research Center
1220 Capitol Court, Room 210
Madison, WI 53715
Tel: (608) 263-3542
Fax: (608) 262-8946
mdraymond@wisc.edu

6. Entire Agreement

Unless otherwise explicitly agreed by WNPRC in writing, this Agreement, with the Wisconsin National Primate Research Center Standard Terms and Conditions attached hereto as Exhibit A, embodies the entire understanding between the WNPRC and the Service Recipient for this Service, and any prior or contemporaneous representations, either oral or written, are hereby superseded. No amendments or changes to this Agreement, including without limitation, changes in the scope of work, total estimated cost, and period of performance, shall be effective unless made in writing and signed by authorized representatives of each party.

IN WITNESS WHEREOF,

The parties hereto have executed this Agreement by proper persons thereunto duly authorized.

The Trustees of the University of Noname

**Board of Regents of the
University of Wisconsin System**

By: _____

By: _____

Name: _____

Name: _____

Title: _____

Title: _____

Date: _____

Date: _____

Example

EXHIBIT A
Wisconsin National Primate Research Center
Standard Terms and Conditions

The Wisconsin National Primate Research Center (“WNPRC”), a center of the University of Wisconsin-Madison, offers service in support of research. The WNPRC reserves the right to decline to perform any services that it determines not to be in the best interests of the University of Wisconsin-Madison, the University of Wisconsin System, or the State of Wisconsin.

All Service Agreements entered into by WNPRC are subject to these Standard Terms and Conditions (“Terms”) unless specifically agreed to otherwise in writing by the WNPRC.

1. Pricing and Payment Terms and Payment Schedule

The WNPRC shall be paid the amount specified in the Agreement.

2. Acceptance of Proposal

WNPRC’s receipt of a signed Service Agreement or other form of a signed written contractual agreement constitutes Service Recipient and WNPRC’s acceptance of the proposed scope of work, price, and terms and conditions.

The Agreement may be executed in any number of counterparts, each of which is deemed to be an original, but which together shall constitute but one instrument. This Agreement shall be considered accepted once it has been executed by both parties. A signature delivered by facsimile or electronic means will be considered binding for each party.

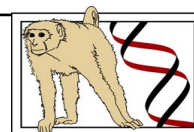
3. Termination

The Service Recipient upon sixty (60) days written notice may terminate performance under this Agreement; performance may be terminated by the WNPRC if circumstances beyond its control preclude continuation of the Service. Upon termination by the Service Recipient, WNPRC will be reimbursed for all costs and non-cancelable commitments incurred in the performance of the Service and not yet paid for, such reimbursements together with other payments not to exceed the total estimated project cost specified in the service agreement.

In the event that either party hereto shall commit any breach of or default in any of the terms or conditions of the Agreement, and also fail to remedy such default or breach within thirty (30) days after written notice thereof from the other party hereto, the party giving notice may, at its option and in addition to any other remedies which it may have at law or in equity, terminate this Agreement by sending notice of termination in writing to the other party to such effect, and such termination shall be effective as of the date of the receipt of such notice.

4. Publicity

Neither party shall use the name of the other party (including staff members of such other party), in connection with any products, press releases, promotions, or advertising without the prior written approval of the other party. This shall not include internal documents available to the public that identify the existence of the Agreement.



Notwithstanding the restrictions above, and in recognition of the support provided to WNPRC by the National Institutes of Health, which allows WNPRC to provide such Services, any publication resulting from the Services provided by WNPRC under this Agreement shall contain the following acknowledgement: "Research in this publication was supported in part by the Office Of The Director, National Institutes of Health under Award Number P51OD011106 to the Wisconsin National Primate Research Center, University of Wisconsin-Madison. This research was conducted/conducted in part (as applicable) at a facility constructed with support from Research Facilities Improvement Program grant numbers RR15459-01 and RR020141-01. The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health."

5. Confidentiality

Unless otherwise required by law, governmental proceeding, or court order, WNPRC will exercise reasonable efforts to maintain in confidence all Confidential Information (as defined herein).

Confidential Information shall mean any information, documentation and materials specifically developed or prepared for Service Recipient by the WNPRC in performance of the Services, and any proprietary or confidential information disclosed or submitted to WNPRC by the Service Recipient that is designated in writing as confidential at the time of disclosure. Confidential Information does not include information which:

- a) Is generally available in the public domain or becomes available to the public through no act of the WNPRC;
- b) Is independently known prior to receipt thereof or is discovered independently by an employee of WNPRC who had no access to the Confidential Information; or
- c) Is made available to the WNPRC as a matter of lawful right by a third party.

The WNPRC retains the right to refuse to accept any Confidential Information that is not considered to be essential to the completion of the Service. The obligations of the WNPRC under this paragraph shall survive and continue for one (1) year after this Agreement ends.

6. Liability

The Service Recipient agrees to hold the WNPRC harmless from any loss, claim, damage, or liability of any kind arising out of or in connection with this Agreement, except to the extent that it is founded upon or grows out of the acts or omissions of any of the officers, employees or agents of the University of Wisconsin System while acting within the scope of their employment where protection is afforded by ss.893.82 and 895.46(1), Wis. Stats.

7. Warranties

THE UNIVERSITY AND WNPRC MAKE NO WARRANTIES, EXPRESSED OR IMPLIED, AS TO ANY MATTER WHATSOEVER, INCLUDING, WITHOUT LIMITATION, THE CONDITION OF THE SERVICE OR DELIVERABLES, WHETHER TANGIBLE OR INTANGIBLE, OR DEVELOPED UNDER THIS AGREEMENT; OR THE OWNERSHIP, MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT OF THE SERVICE OR DELIVERABLES. THE UNIVERSITY AND WNPRC SHALL NOT BE LIABLE FOR ANY DIRECT, INDIRECT, CONSEQUENTIAL, SPECIAL OR OTHER DAMAGES SUFFERED BY ANY PERSON RESULTING FROM THIS SERVICE OR THEIR USE OF THE DELIVERABLES.

8. Equipment

Equipment, supplies, and materials purchased or produced under this Agreement shall be owned by WNPRC, unless expressly stated otherwise herein.

9. Assignment

Neither party shall assign this Agreement to any third party without the prior written consent of the other party; however, the Service Recipient may assign this Agreement to a successor in ownership of all or substantially all its business assets, provided that such successor shall expressly assume in writing the obligation to perform in accordance with the terms and conditions of this Agreement. Any other purported assignment shall be void.

10. Independent Contractor

In the performances of all services hereunder:

- a) The WNPRC shall be deemed to be and shall be an independent contractor and, as such, shall not be entitled to any benefits applicable to employees of the Service Recipient; and
- b) Neither party is authorized or empowered to act as agent for the other for any purpose and shall not on behalf of the other enter into any contract, warranty, or representation as to any matter. Neither party shall be bound by the acts or conduct of the other.

11. Independent Inquiry

Nothing in this Agreement shall be construed to limit the freedom of University researchers who are participants in this Agreement, whether paid under this Agreement or not, from engaging in similar projects made independently under other grants, contracts, or agreements with parties other than the Service Recipient.

12. Insurance

The University warrants and represents that it has adequate liability protection, such protection being applicable to officers, employees, and agents while acting within the scope of their employment by the University. The University has no liability insurance policy as such that can extend protection to any other person.

13. Governing Law

This Agreement shall be governed by the laws of the State of Wisconsin, excluding its conflict of laws provisions.

14. Export Control

Each party agrees that it shall comply with any applicable import and export control laws, rules, and regulations relating to the import and export of technical information, materials, or products in connection with the Service conducted under this Agreement. Service Recipient shall provide WNPRC in writing prior to providing WNPRC with access to any export regulated information or materials. Such notification shall include all associated classification numbers. WNPRC reserves the right to refuse receipt of any information or materials that are subject to export controls.

15. Compliance with Laws

Each party shall comply fully with all applicable federal, state, and local laws and regulations, including treaties such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora. Further, each party shall comply with all applicable regulations in the event that they share or transfer non-human primate biological materials with or to any other individual or organization.
