Major histocompatibility complex haplotyping and long-amplicon allele discovery in cynomolgus macaques from Chinese breeding facilities

Julie A. Karl¹, Michael E. Graham¹, Roger W. Wiseman¹, Katelyn E. Heimbruch², Samantha M. Gieger², Gaby G. M. Doxiadis³, Ronald E. Bontrop³, and David H. O'Connor^{1,2*}

¹ Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, Wisconsin 53705

² Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, Wisconsin 53715

³ Department of Comparative Genetics and Refinement, Biomedical Primate Research Centre, Rijswijk, The Netherlands

* Corresponding Author: David H. O'Connor, Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, 585 Science Drive, Madison, WI, 53711

Phone: 608-890-0845

Fax: 608-265-8084

Email: doconnor@primate.wisc.edu

Key words: *Macaca fascicularis*; MHC class I; PacBio long-amplicon sequencing; RNA transcript-based haplotypes

ABSTRACT

Very little is currently known about the major histocompatibility complex (MHC) region of cynomolgus macaques (*Macaca fascicularis*; *Mafa*) from Chinese breeding centers. We performed comprehensive MHC class I haplotype analysis of 100 cynomolgus macaques from two different centers, with animals from different reported original geographic origins (Vietnamese, Cambodian, and Cambodian/Indonesian mixed-origin). Many of the samples were of known relation to each other (sire, dam, and progeny sets), making it possible to characterize lineage-level haplotypes in these animals. We identified 52 *Mafa-A* and 74 *Mafa-B* haplotypes in this cohort, many of which were restricted to specific sample origins. We also characterized full-length MHC class I transcripts using Pacific Biosciences (PacBio) RS II single-molecule real-time (SMRT) sequencing. This technology allows for complete read-through of unfragmented MHC class I transcripts (~1,100 bp in length), so no assembly is required to unambiguously resolve novel full-length sequences. Overall, we identified 313 total full-length transcripts in a subset of 72 cynomolgus macaques from these Chinese breeding facilities; 131 of these sequences were novel and an additional 116 extended existing short database sequences to span the complete open reading frame. This significantly expands the number of *Mafa-A*, *Mafa-B*, and *Mafa-I* full-length alleles in the official cynomolgus macaque MHC class I database. The PacBio technique described here represents a general method for full-length allele discovery and genotyping that can be extended to other complex immune loci such as MHC class II, killer immunoglobulin-like receptors, and Fc gamma receptors.

INTRODUCTION

Cynomolgus macaques (*Macaca fascicularis*; *Mafa*) are native to much of continental and insular Southeast Asia, but there are no feral cynomolgus macaques living in China. However, Chinese breeding facilities are one of the largest exporters of cynomolgus macaques for use in biomedical research. According to the Centers for Disease Control and Prevention, China provided over 70% of the total cynomolgus macaques imported into the United States in fiscal year 2014. These animals are derived from other geographic populations of cynomolgus macaques, most likely from Thailand, Laos, Vietnam, Cambodia, and Malaysia, but also possibly including animals from Indonesia and the Philippines. Cynomolgus macaques from Chinese breeding facilities therefore are genetically heterogeneous, and this heterogeneity may be particularly biologically relevant in highly diverse genomic regions like the major histocompatibility complex (MHC).

The MHC is both highly complex and important – the gene products encoded by the MHC present peptides to T-cells, playing an essential role in immune response to pathogens and other non-self peptides (Bontrop 2006). In macaques, the MHC class I region is highly polymorphic and extensively duplicated (Daza-Vamenta et al. 2004; Otting et al. 2005), with different chromosomes carrying different numbers of functional *Mafa-A* and *Mafa-B* genes. Many investigators have examined the MHC class I region of cynomolgus macaques from various origins (Budde et al. 2010; Campbell et al. 2008; Kita et al. 2009; Lawrence et al. 2012; Ling et al. 2012; Otting et al. 2007, 2009, 2012; Pendley et al. 2008; Saito et al. 2012; Shiina et al. 2015; Uda et al. 2004, 2005; Zhang et al. 2012; Zhuo et al. 2011), but only a limited number of studies have looked at cynomolgus macaques from Chinese breeding facilities (Krebs et al. 2005; Westbrook et al. 2015).

Here we describe a comprehensive study of 100 samples collected from two different breeding facilities in China that include individuals reported to be of Vietnamese, Cambodian, and mixed Cambodian/Indonesian origins. Many of the samples were related to at least one other animal in the study, allowing us to characterize lineage-level haplotypes based on shared Illumina short-read transcript sequences. We then performed full-length MHC class I transcript analysis on a subset of the samples using the PacBio long-read single-molecule real-time (SMRT) sequencing system. This analysis improved on a previous MHC class I study using PacBio circular consensus SMRT sequencing (Westbrook et al. 2015) by using more advanced sequencing chemistry, and a data analysis pipeline focused on the highest quality reads that did not need to account for sequencing reads with insertion/deletion errors.

MATERIALS AND METHODS

Animals

A total of 100 whole blood samples were collected from two different breeding facilities in China. Thirty samples from reported Vietnamese-origin animals were obtained from Yulin Hongfeng Experimental Animal's Domesticating and Breeding Center (Guangxi, China). A total of 70 samples were obtained from Hainan Jingang Laboratory Animal Co., Ltd. (Hainan, China) - 57 samples were obtained from reported Cambodian-origin animals, and 13 samples were from mixed Cambodian/Indonesian-origin animals. Wherever possible, sire/dam/progeny trio sets or parent/progeny duos were collected to obtain samples that share MHC haplotypes that are identical by descent; a total of 71 samples across both facilities were directly related to at least one other animal in the cohort. Samples were collected into TRIzol Reagent (ThermoFisher Scientific, Waltham, MA, USA) to stabilize the total RNA for shipment from China.

RNA isolation, cDNA synthesis, and PCR preparation

Total RNA was isolated from the blood samples and cDNA was produced from the isolated RNA using the RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific) following manufacturer's protocols. For Illumina MiSeq genotyping, a 195 bp amplicon spanning the most polymorphic region of MHC class I molecules was generated using primers designed in conserved regions of exon 2, as shown in Supplemental Fig. 1. Amplification was performed using a 4-primer system to add unique barcodes to each sample, using consensus sequence adapters and barcodes designed by the Fluidigm Corporation (San Francisco, CA, USA), as detailed in Supplemental File 1.

For PacBio RS II full-length MHC class I transcript sequencing of 72 samples from the full cohort, an ~1,150 bp amplicon with primers located in the 5'UTR and 3'UTR of MHC class I sequences was generated as shown in Supplemental Fig. 2. A cocktail of two forward primers and three reverse primers was used to maximize homology with the majority of MHC class I sequences. A unique set of barcoded primers was used for each sample, with the barcodes integrated onto the ends of the sequence-specific oligos at synthesis. Barcodes are available from Pacific Biosciences (Menlo Park, CA, USA). Primer sequences are shown in Supplemental Fig. 2, and PCR conditions are detailed in Supplemental File 1.

All PCR products were purified using the AMPure XP PCR purification kit (Agencourt Bioscience

Corporation, Beverly, MA, USA) and quantified using the Quant-iT dsDNA HS Assay kit and a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA), following the manufacturer's protocols.

Sequencing

The MHC class I exon 2 genotyping amplicons were sequenced on an Illumina MiSeq instrument (San Diego, CA, USA), and the full-length MHC class I transcripts were sequenced on a PacBio RS II instrument (Menlo Park, CA, USA) following the respective manufacturer's protocols. Additional sequencing details are provided in Supplemental File 1, and an overview of the PacBio sequencing process is shown in Supplemental Fig. 3.

Data analysis

Processing of the MHC class I exon 2 genotyping amplicon was performed as described previously for MHC class II amplicons (Karl et al. 2014) using a custom-built command line pipeline to merge reads, trim primers, and compare MiSeq reads against a database of known cynomolgus macaque transcripts. *Mafa-A* and *Mafa-B* lineage-level haplotypes were determined for each of the 100 samples essentially as previously described (Heimbruch et al. 2015; Karl et al. 2013), first examining the sire/dam/progeny trio sets, then the parent/offspring duos, to look for sets of co-inherited sequences. Haplotypes for the unrelated animals in this cohort were inferred based on similarity to those observed in the related sets of animals.

A custom semi-automated pipeline was also developed for analysis of the PacBio MHC class I full-length transcripts; an overview of this pipeline is shown in Supplemental Fig. 4. Full details of the pipeline are available in Supplemental File 1, and the scripts and tools used are publicly available (<https://bitbucket.org/dholab/mhc-long-amplicon-allele-discovery-in-mafa-from-china>). In brief, the PacBio reads of insert (the consensus sequence generated from all passes around the circular SMRTbell template) were processed to eliminate indel sequences, short reads, and chimeric sequences using the SMRT Analysis v2.3 Reads of Insert protocol. Processed reads perfectly matching known full-length cynomolgus macaque transcripts were removed prior to clustering by using bbmap (version 34) to map reads to known full-length allele sequences. Reads not identical to known allele sequences were clustered using USEARCH -cluster_fast (version v8.0.1517_i86osx32) and requiring 100% identity within clusters. Clusters supported by three or more identical full-length reads were then binned as either extensions of known partial cynomolgus macaque sequences or putative novel transcripts, and a genotyping table with read

counts per sample was generated. All extension and putative novel sequences were manually validated using Geneious Pro v9.0 (Biomatters Limited, Auckland, New Zealand) to perform BLAST alignments and create BAM alignment files of the reads of insert against the validated transcripts. Validated sequences were submitted to GenBank and the Immuno Polymorphism Database for the Major Histocompatibility Complex genes of Non-Human Primates (IPD-MHC NHP) (<http://www.ebi.ac.uk/ipd/mhc/nhp/index.html>) (de Groot et al. 2012; Robinson et al. 2013) for official nomenclature.

Data availability

Primer sequences are listed in Supplemental Fig. 1 and 2. Lineage-level *Mafa-A* and *Mafa-B* haplotypes assigned to each animal from the MiSeq exon 2 data are available in Supplemental Table 1, and full MiSeq read counts per transcript per animal are available in Supplemental Fig. 5. Full resolution *Mafa-A* and *Mafa-B* haplotypes assigned to each of the 72 animals examined by PacBio sequencing are available in Supplemental Table 2, and full PacBio read counts per transcript per animal are available in Supplemental Fig. 6. Novel and extension full-length sequences as listed in Table 1 are available from GenBank (LN851916-LN852021, LN994391-LN994530, LN998188, KY047492).

RESULTS AND DISCUSSION

Lineage-level haplotype analysis

In human and macaque MHC class I nomenclature, closely related alleles originating from the same gene are assigned to the same lineage group, signified by the 2-3 digits between the asterisk and the first colon of MHC class I sequence names. Since the Illumina MiSeq exon 2 genotyping amplicon is focused on a small segment of the MHC class I molecule, it is generally only sufficient for determining the lineage groups of any detected MHC class I sequences (considered ‘three-digit’ resolution in human HLA genotyping and referred to as ‘lineage-level’ resolution here). However, it provides a rapid, high-throughput methodology for haplotype analysis, especially within related animals. Using the parent/progeny trio and duo sets within these two cohorts, we described haplotypes of *Mafa-A* and *Mafa-B* transcripts inherited together from parents to offspring. Two representative trio sets with parents and their progeny are illustrated in Fig. 1a. Any transcripts observed as shared between parent and offspring were assigned to the inherited haplotype; any remaining transcripts observed within a parent, or in an offspring with just one representative parent, were assigned to the alternate haplotype. For animals without a direct relative in the cohort, haplotypes were inferred based on identity to the defined inherited haplotypes. We focused on the ‘major’ transcripts with high levels of steady state RNA for haplotype definitions. Abbreviated haplotype names were assigned based on presence of a major ‘diagnostic’ sequence that was typically the most abundant transcript on the haplotype, i.e., B013 haplotypes express the diagnostic major transcript *Mafa-B*013*. Different combinations of major allele lineages inherited with each diagnostic transcript were denoted with lowercase letters following the haplotype name, e.g., *Mafa-B*013* inherited with *Mafa-B*007* was designated B013a and *Mafa-B*013* inherited with *Mafa-B*137* was designated B013d. The full list of *Mafa-A* and *Mafa-B* haplotypes defined in these cohorts of cynomolgus macaques from Chinese breeding facilities is given in Supplemental Table 1.

A total of 52 distinct *Mafa-A* haplotypes and 74 unique *Mafa-B* haplotypes were identified in the 100 animals (200 chromosomes) evaluated in this cohort (Supplemental Table 1). A summary of the lineage-level haplotypes observed in each animal is shown in Fig. 2, with haplotypes directly inherited from parent to offspring denoted with solid outlined boxes. Full MiSeq haplotyping results, including reads supporting each transcript call per sample, are also given in Supplemental Fig. 5. While overall the large number of related animals in the cohort skews haplotype frequencies, it should be noted that four *Mafa-A* haplotypes (A006, A022a, A027, and A065) and three *Mafa-B* haplotypes (B013d, B028d, and B039e) are each observed in ten or more individuals. There are 1-2

major alleles expressed on each *Mafa-A* haplotype, accompanied by 0-5 minor alleles. Each *Mafa-B* haplotype is associated with 1-7 expressed major transcripts and between 0-15 minor transcripts.

Though many studies have examined the lists of MHC class I transcripts expressed in cynomolgus macaques of multiple geographic origins, few have studied the haplotype structures in those animals (Budde et al. 2010; Campbell et al. 2008; Otting et al. 2009, 2012; Saito et al. 2012; Shiina et al. 2015). The best-characterized population of cynomolgus macaques is from the island of Mauritius, where a limited number of founding animals were deposited on the island in the 1500s. Since the entire current population arose from a small number of ancestors, the diversity of their MHC region (and their entire genome) is limited to five lineage-level *Mafa-A* haplotypes (A031, A032a, A033, A060, A063) and seven lineage-level *Mafa-B* haplotypes (B019a, B045a, B075a, B104a, B147, B151a, B164a) (Budde et al. 2010; Wiseman et al. 2013). The diversity observed in these cohorts of 100 individuals from two Chinese breeding facilities represents a ten-fold increase in diversity at each locus compared to Mauritian cynomolgus macaques. While both populations of cynomolgus macaques are popular for biomedical research, this difference in overall diversity suggests that investigators should carefully consider their objectives when choosing a source for their animals. For protocols where MHC may be a confounding factor, like SIV and transplantation research, the restricted diversity of the Mauritian cynomolgus macaque population is particularly attractive. When testing vaccines or other drug therapies for potential negative side effects however, an extremely diverse source like cynomolgus macaques from Chinese breeding facilities may be advantageous. The overall diversity observed in this cohort also suggests that researchers should have their animals haplotyped if the MHC is a possible modifying factor, since this study revealed a large number of haplotypes despite the fact that multiple related animals were evaluated.

This study likely represents a snapshot of the total MHC diversity available within cynomolgus macaques from Chinese facilities, largely dependent on the country of origin of their breeding animals. Of the 52 total *Mafa-A* haplotypes observed here, nine (17%) were observed exclusively in animals of reported Vietnamese heritage, 17 (33%) were of reported Cambodian-origin only, and 4 (8%) were observed in the small set of reported hybrid Cambodian/Indonesian-origin animals (Supplemental Table 1). Only 22 (42%) *Mafa-A* haplotypes were observed in samples from multiple origins. The results for the 74 *Mafa-B* haplotypes are even more striking with only 21 (28%) observed in samples from multiple origins. Among the remaining *Mafa-B* haplotypes, 14 (19%) were exclusive to Vietnamese-origin animals, 26 (35%) exclusive to Cambodian-origin, and 13 (18%) were observed only in the

mixed Cambodian/Indonesian-origin individuals. No previous MHC haplotype studies have been performed in either Vietnamese- or Cambodian-origin cynomolgus macaques, but limited studies have characterized haplotypes in Filipino, Indonesian, and Malaysian cynomolgus macaques (Campbell et al. 2008; Otting et al. 2009, 2012; Saito et al. 2012; Shiina et al. 2015). The cohorts studied here appear more MHC diverse than those sampled from the previously described insular and extremely southern continental Asian populations (it is unclear from precisely where the Malaysian cohort was initially derived).

Full-length allele discovery

In this study we employed a novel method for next-generation sequencing of full-length MHC class I alleles, using the PacBio RS II SMRT long read sequencing instrument. In our previous study with P4-C2 PacBio sequencing (Westbrook et al. 2015), data analysis relied on creation of contigs at 99% identity (largely to account for insertion/deletion artifacts) to define transcripts. Since this initial study, advances in PacBio sequencing with P6-C4 reagents and analysis software have eliminated the need to include less than 100% identical reads for identification of novel sequences. From the full 100 animal cohort, we selected 72 total samples across both breeding centers for PacBio sequencing based on lineage-level haplotype diversity. Table 1 presents a complete list of all 313 full-length MHC class I transcripts that we detected in these 72 individuals; these include 126 *Mafa-A*, 174 *Mafa-B*, and 13 *Mafa-I* transcripts. The complete PacBio sequencing results for each animal including numbers of identical sequencing reads supporting each transcript call are shown in Supplemental Fig. 6. Overall, nearly 80% of the transcripts identified in this study added new information to the existing *Mafa* MHC class I allele databases – 131 (42%) of the sequences were completely novel to the database, and 116 (37%) extended short but previously named database sequences to cover full-length open reading frames (Fig. 3).

This study improved the extant cynomolgus macaque allele databases in both total number of sequences and, more importantly, in the percentage of known transcripts that have been unambiguously described over the full coding region. The November 2015 update of IPD-MHC NHP contained a total of 899 *Mafa-A*, *Mafa-B*, and *Mafa-I* sequences. Of these, only 386 (43%) were full-length from start codon to stop codon. This study adds an additional 131 novel transcripts to the database, bringing the total to 1,030 named MHC class I sequences in the cynomolgus macaque database. With the additional 116 extant sequences extended to full length, the database of *Mafa-A*, *Mafa-B*, and *Mafa-I* transcripts following this study now contains 633 full-length sequences (61% of the 1,030 total

transcripts).

We also examined distinct, full resolution haplotypes in the samples that were PacBio sequenced to compare to the previously determined lineage-level haplotypes from the full cohort. Fig. 1b shows the PacBio haplotype results for two representative trio sets of samples, and Supplemental Table 2 provides the complete list of full resolution haplotypes observed in the cohort. Haplotype names were retained from the lineage-level haplotype results, but sub-haplotypes (indicated with Roman numerals in the “Sub-Haplo” column of Supplemental Table 2) were assigned for any distinctions in specific transcripts. For instance, the lineage-level haplotype A001 has four (i-iv) distinct sub-haplotypes in the PacBio data, as they all vary from each other at the transcript level (A001i – A001iv carry *Mafa-A1*001:01:02*, *Mafa-A1*001:01:03*, *Mafa-A1*001:02:01*, and *Mafa-A1*001:05*, respectively). For the PacBio haplotypes, both major and minor specific transcripts were considered when assigning sub-haplotypes. As expected, results were concurrent with the lineage-level haplotypes overall, but the full-length allelic resolution provided by PacBio sequencing resulted in a significant expansion of the number of both *Mafa-A* and *Mafa-B* haplotypes. In total we identified 92 and 101 distinct full-resolution *Mafa-A* and *Mafa-B* haplotypes, respectively, in the 72 PacBio-sequenced samples. As with the lineage-level haplotypes, more than 90% of the *Mafa-A* and *Mafa-B* PacBio haplotypes were only observed in individuals from a single origin - 26 *Mafa-A* and 29 *Mafa-B* haplotypes were distinct to Vietnamese-origin samples, 43 *Mafa-A* and 46 *Mafa-B* haplotypes were only observed in Cambodian-origin samples, and 15 *Mafa-A* and 15 *Mafa-B* haplotypes were distinct to the hybrid Cambodian/Indonesian-origin samples (Supplemental Table 2).

It is unclear how many of these allelic distinctions make a functional biological difference in regards to immune responses. Although such confirmatory studies are beyond the scope of this report, we predict that specific nonsynonymous nucleotide variants between two closely related alleles may result in significant biological differences, particularly when the nucleotide differences alter the resulting amino acid within the peptide binding pocket. Therefore, full resolution genotyping provided by PacBio sequencing is particularly attractive for researchers looking for linkage between a specific immune response and specific MHC class I genotypes.

Overall, this study describes some of the first MHC class I *Mafa-A* and *Mafa-B* haplotypes in cynomolgus macaques from Chinese breeding facilities, including samples of reported Vietnamese, Cambodian, and mixed Cambodian/Indonesian origins. It also describes an improved PacBio long read sequencing approach to characterize full-length MHC class I transcripts without contig assembly, using only the highest quality reads. The transcripts

identified here both increase the total number of known *Mafa-A*, *Mafa-B*, and *Mafa-I* sequences, and significantly improve the percentage of full-length transcripts in the nonhuman primate IPD database. Finally, the general PacBio-based method described here for long-amplicon sequencing can also be applied to other complex immune loci of macaques such as MHC class II, killer immunoglobulin-like receptors, and Fc gamma receptors, as well as those of other model organisms.

ACKNOWLEDGMENTS

The authors thank Catherine Westbrook, Suzanne Mate, and Galina Koroleva at the U.S. Army Medical Research Institute of Infectious Diseases, and Katherine Munson and the staff of the University of Washington PacBio Sequencing Services core for performing PacBio RS II sequencing. We also thank Annemiek J.M. de Vos-Rouweler for RNA isolations and preparation of cDNAs used in this study. Finally, we thank Nel Otting, Natasja de Groot and the IMGT Non-human Primate Nomenclature Committee for obtaining accession numbers and providing official *Mafa* transcript nomenclature designations for the sequences described here.

This research was supported by contracts HHSN272201600007C and HHSN272201100013C from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, and was conducted at a facility constructed with support from the Research Facilities Improvement Program (RR15459-01, RR020141-01).

REFERENCES

- Bontrop RE (2006) Comparative genetics of MHC polymorphisms in different primate species: duplications and deletions. *Hum Immunol* 67(6):388-397
- Budde ML, Wiseman RW, Karl JA, Hanczaruk B, Simen BB, O'Connor DH (2010) Characterization of Mauritian cynomolgus macaque major histocompatibility complex class I haplotypes by high-resolution pyrosequencing. *Immunogenetics* 62(11-12):773-780
- Campbell KJ, Detmer AM, Karl JA, Wiseman RW, Blasky AJ, Hughes AL, Bimber BN, O'Connor SL, O'Connor DH (2008) Characterization of 47 MHC class I sequences in Filipino cynomolgus macaques. *Immunogenetics* 61(3):177-187
- Daza-Vamenta R, Glusman G, Rowen L, Guthrie B, Geraghty DE (2004) Genetic divergence of the rhesus macaque major histocompatibility complex. *Genome Res* 14(8):1501-1515
- de Groot NG, Otting N, Robinson J, Blancher A, Lafont BA, Marsh SG, O'Connor DH, Shiina T, Walter L, Watkins DI, Bontrop RE (2012) Nomenclature report on the major histocompatibility complex genes and alleles of Great Ape, Old and New World monkey species. *Immunogenetics* 64(8):615-631
- Heimbruch KE, Karl JA, Wiseman RW, Dudley DM, Johnson Z, Kaur A, O'Connor DH (2015) Novel MHC class I full-length allele and haplotype characterization in sooty mangabeys. *Immunogenetics* 67(8):437-445
- Karl JA, Bohn PS, Wiseman RW, Nimityongsukul FA, Lank SM, Starrett GJ, O'Connor DH (2013) Major histocompatibility complex class I haplotype diversity in Chinese rhesus macaques. *G3 (Bethesda)* 3(7):1195-1201
- Karl JA, Heimbruch KE, Vriezen CE, Mironczuk CJ, Dudley DM, Wiseman RW, O'Connor DH (2014) Survey of major histocompatibility complex class II diversity in pig-tailed macaques. *Immunogenetics* 66(11):613-623
- Kita YF, Hosomichi K, Kohara S, Itoh Y, Ogasawara K, Tsuchiya H, Torii R, Inoko H, Blancher A, Kulski JK, Shiina T (2009) MHC class I A loci polymorphism and diversity in three Southeast Asian populations of cynomolgus macaque. *Immunogenetics* 61(9):635-648
- Krebs KC, Jin Z, Rudersdorf R, Hughes AL, O'Connor DH (2005) Unusually high frequency MHC class I alleles in Mauritian origin cynomolgus macaques. *J Immunol* 175(8):5230-5239
- Lawrence J, Orysiuk D, Prashar T, Pilon R, Fournier J, Rud E, Sandstrom P, Plummer FA, Luo M (2012) Identification of 23 novel MHC class I alleles in cynomolgus macaques of Philippine and

- Philippine/Mauritius origins. *Tissue Antigens* 79(4):306-307
- Ling F, Zhuo M, Ni C, Zhang GQ, Wang T, Li W, Wei LQ, Du HL, Wang JF, Wang XN (2012) Comprehensive identification of high-frequency and co-occurring Mafa-B, Mafa-DQB1, and Mafa-DRB alleles in cynomolgus macaques of Vietnamese origin. *Hum Immunol* 73(5):547-553
- Otting N, de Groot N, de Vos-Rouweler AJ, Louwerse A, Doxiadis GG, Bontrop RE (2012) Multilocus definition of MHC haplotypes in pedigreed cynomolgus macaques (*Macaca fascicularis*). *Immunogenetics* 64(10):755-765
- Otting N, de Vos-Rouweler AJ, Heijmans CM, de Groot NG, Doxiadis GG, Bontrop RE (2007) MHC class I A region diversity and polymorphism in macaque species. *Immunogenetics* 59(5):367-375
- Otting N, Doxiadis GG, Bontrop RE (2009) Definition of Mafa-A and -B haplotypes in pedigreed cynomolgus macaques (*Macaca fascicularis*). *Immunogenetics* 61(11-12):745-753
- Otting N, Heijmans CM, Noort RC, de Groot NG, Doxiadis GG, van Rood JJ, Watkins DI, Bontrop RE (2005) Unparalleled complexity of the MHC class I region in rhesus macaques. *Proc Natl Acad Sci U S A* 102(5):1626-1631
- Pendley CJ, Becker EA, Karl JA, Blasky AJ, Wiseman RW, Hughes AL, O'Connor SL, O'Connor DH (2008) MHC class I characterization of Indonesian cynomolgus macaques. *Immunogenetics* 60(7):339-351
- Robinson J, Halliwell JA, McWilliam H, Lopez R, Parham P, Marsh SG (2013) The IMGT/HLA database. *Nucleic Acids Res* 41(Database issue):D1222-D1227
- Saito Y, Naruse TK, Akari H, Matano T, Kimura A (2012) Diversity of MHC class I haplotypes in cynomolgus macaques. *Immunogenetics* 64(2):131-141
- Shiina T, Yamada Y, Aarnink A, Suzuki S, Masuya A, Ito S, Ido D, Yamanaka H, Iwatani C, Tsuchiya H, Ishigaki H, Itoh Y, Ogasawara K, Kulski JK, Blancher A (2015) Discovery of novel MHC-class I alleles and haplotypes in Filipino cynomolgus macaques (*Macaca fascicularis*) by pyrosequencing and Sanger sequencing : Mafa-class I polymorphism. *Immunogenetics* 67(10):563-578
- Uda A, Tanabayashi K, Fujita O, Hotta A, Terao K, Yamada A (2005) Identification of the MHC class I B locus in cynomolgus monkeys. *Immunogenetics* 57(3-4):189-197
- Uda A, Tanabayashi K, Yamada YK, Akari H, Lee YJ, Mukai R, Terao K, Yamada A (2004) Detection of 14 alleles derived from the MHC class I A locus in cynomolgus monkeys. *Immunogenetics* 56(3):155-163
- Westbrook CJ, Karl JA, Wiseman RW, Mate S, Koroleva G, Garcia K, Sanchez-Lockhart M, O'Connor DH,

- Palacios G (2015) No assembly required: Full-length MHC class I allele discovery by PacBio circular consensus sequencing. *Hum Immunol* 76(12):891-896
- Wiseman RW, Karl JA, Bohn PS, Nimityongskul FA, Starrett GJ, O'Connor DH (2013) Haplessly hoping: macaque major histocompatibility complex made easy. *ILAR J* 54(2):196-210
- Zhang GQ, Ni CH, Ling F, Qiu W, Wang HB, Xiao Y, Guo XJ, Huang JY, Du HL, Wang JF, Zhao SJ, Zhuo M, Wang XN (2012) Characterization of the major histocompatibility complex class I A alleles in cynomolgus macaques of Vietnamese origin. *Tissue Antigens* 80(6):494-501
- Zhuo M, Wang HB, Ling F, Wang JF, Wang XN (2011) Eighteen novel MHC class I A alleles identified in Vietnamese-origin cynomolgus macaques. *Tissue Antigens* 78(2):139-142

FIGURE LEGENDS

Fig. 1 Representative trio set results by a) MiSeq genotyping and b) PacBio full-length sequencing methods. The major transcripts for two representative trio sets are shown here, with progeny located between the sire and dam, showing direct transcript inheritance from parents to offspring. Inherited haplotypes are outlined in solid boxes. Groups of *Mafa-A* and *Mafa-B* transcripts co-inherited together as a haplotype are colored correspondingly. Transcripts shown in bold font are diagnostic major sequences used to name the haplotypes. Transcripts shown in grey font were only observed by MiSeq. Values indicate the number of reads for each sequence per sample

Fig. 2 Summary of MHC class I *Mafa-A* and *Mafa-B* haplotypes observed in each macaque. Samples are organized by geographic origin into family groups, and directly inherited haplotypes are outlined with solid boxes. The full list of transcripts associated with each haplotype are available in Supplemental Table 1

Fig. 3 Evaluation of PacBio full-length transcripts. A breakdown of the 313 total identified *Mafa-A*, *Mafa-B*, and *Mafa-I* full-length transcripts

Table 1: Full-length transcripts observed by PacBio sequencing

Allele Name	Full-Length Accession Number	# Animals	# Reads	ORF Length (bp)	Category	Longest Previous Length (bp)	Previous Accession Number(s)
<i>Mafa-A1*001:01:02</i>	LN851916	3	123	1098	extension	1062	FR682846
<i>Mafa-A1*001:01:03</i>	LN994391	1	31	1098	novel	-	-
<i>Mafa-A1*001:02:01</i>	LN998188	1	54	1098	extension	1039	AY958101
<i>Mafa-A1*001:05</i>	LN851917	2	51	1098	novel	-	-
<i>Mafa-A1*002:02</i>	LN994392	1	35	1098	extension	1003	AB447609
<i>Mafa-A1*003:01</i>	LN994393	1	48	1098	extension	1065	AM295829
<i>Mafa-A1*003:03</i>	LN994394	1	78	1098	extension	1062	FR682835, HQ113982, HQ131743
<i>Mafa-A1*003:05</i>	LN994395	1	73	1098	extension	544	HQ131758
<i>Mafa-A1*003:06</i>	LN851918	3	337	1098	novel	-	-
<i>Mafa-A1*003:07</i>	LN994396	1	53	1098	novel	-	-
<i>Mafa-A1*004:02</i>	LN994397	1	60	1098	extension	1003	AB448751, HQ113954, HQ131752
<i>Mafa-A1*006:01:01</i>	LN851919	7	528	1098	extension	1062	AY958098, FR682819
<i>Mafa-A1*007:01</i>	LN851920	2	627	1098	extension	1042	AY958089, HQ113975
<i>Mafa-A1*007:06</i>	LN994398	1	163	1098	extension	1065	FR691532, KP307886
<i>Mafa-A1*008:01</i>	KP307887	1	178	1098	extant	1098	AY958104, HQ113960, HQ131766, KP307887
<i>Mafa-A1*010:04</i>	EU203706	1	117	1098	extant	1098	EU203706
<i>Mafa-A1*010:05</i>	EU203707	1	115	1098	extant	1098	EU203707, FR686840
<i>Mafa-A1*010:07</i>	LN851921	1	50	1098	novel	-	-
<i>Mafa-A1*015:01</i>	LN851922	3	309	1098	extension	1039	AY958087
<i>Mafa-A1*018:01</i>	LN851923	2	337	1098	extension	1062	AY958090, FR682839
<i>Mafa-A1*018:06</i>	FM246489	2	279	1098	extant	1098	FM246489
<i>Mafa-A1*018:08</i>	LN994399	1	36	1098	novel	-	-
<i>Mafa-A1*022:01</i>	LN994400	1	17	1098	extension	1042	AY958096, LN994401
<i>Mafa-A1*022:04</i>	LN851924	5	634	1098	extension	822	HM236273, HQ113976, HQ131745
<i>Mafa-A1*022:05</i>	LN851925	1	141	1098	extension	1062	FR682826, HQ113977, HQ131770
<i>Mafa-A1*022:06</i>	KY047492	1	293	1098	extension	1062	FR682828, HQ131736
<i>Mafa-A1*022:09:01</i>	KP307910	1	58	1098	extant	1098	KP307910
<i>Mafa-A1*022:09:02</i>	LN851926	1	47	1098	novel	-	-
<i>Mafa-A1*026:01</i>	KP307888	3	306	1098	extant	1098	AY958119, HQ131773, KP307888
<i>Mafa-A1*027:01</i>	LN851927	6	705	1098	extension	1065	AY958103, KP307889
<i>Mafa-A1*027:02</i>	LN994402	1	41	1098	extension	1042	AY958113
<i>Mafa-A1*027:03</i>	LN994403	1	102	1098	extension	1003	AB447615
<i>Mafa-A1*028:01</i>	LN851928	1	57	1098	extension	1062	FR682824, HQ113969, HQ131769
<i>Mafa-A1*028:03</i>	LN994404	1	225	1098	novel	-	-
<i>Mafa-A1*028:04</i>	LN994405	1	65	1098	novel	-	-
<i>Mafa-A1*028:05</i>	LN994406	1	22	1098	novel	-	-
<i>Mafa-A1*032:03:02</i>	LN851929	2	195	1098	extension	1062	FR682840
<i>Mafa-A1*033:02</i>	LN851930	2	157	1098	novel	-	-
<i>Mafa-A1*038:01</i>	LN851931	8	894	1098	extension	1065	AM295825, AY958114, FR682847, HQ131737
<i>Mafa-A1*040:01:01</i>	LN851932	3	275	1089	extension	1062	AY958115, FR682842
<i>Mafa-A1*040:03</i>	LN994407	1	70	1089	extension	1042	AY958095
<i>Mafa-A1*041:01</i>	LN851933	3	350	1098	extension	1042	AY958116

<i>Mafa-A1*042:01</i>	LN994408	1	109	1098	extension	1039	AY958117
<i>Mafa-A1*042:02</i>	LN851934	1	85	1098	extension	1062	FR682858
<i>Mafa-A1*043:01</i>	LN994409	1	144	1098	extension	1062	AY958118, FR686841
<i>Mafa-A1*043:10</i>	KP307853	2	127	1098	extant	1098	KP307853, KP307884
<i>Mafa-A1*043:11</i>	LN994410	1	43	1098	novel	-	-
<i>Mafa-A1*045:01</i>	LN851935	1	107	1098	extension	1062	AY958088, AY958120, FR682843
<i>Mafa-A1*045:03</i>	LN994411	1	114	1098	novel	-	-
<i>Mafa-A1*053:01</i>	LN994412	1	57	1098	extension	1065	AB447562, AM295839, HQ113973, HQ131754
<i>Mafa-A1*055:01</i>	LN994413	1	108	1098	extension	1065	AM295840
<i>Mafa-A1*055:02</i>	LN994414	1	214	1098	novel	-	-
<i>Mafa-A1*056:03</i>	KP307890	5	428	1098	extant	1098	KP307890
<i>Mafa-A1*057:02</i>	LN994415	1	85	1098	extension	1065	KP307880
<i>Mafa-A1*058:02</i>	LN851936	2	194	1098	extension	1062	FR682836
<i>Mafa-A1*060:04</i>	LN851937	1	51	1098	extension	1003	AB447574
<i>Mafa-A1*063:03:01</i>	LN851938	2	162	1098	extension	1062	AY958099, FR682832
<i>Mafa-A1*064:03</i>	LN994416	1	287	1098	extension	1003	AB447608
<i>Mafa-A1*065:02</i>	LN851939	1	36	1098	extension	1003	AB447611
<i>Mafa-A1*065:03</i>	LN851940	3	144	1098	extension	1062	AB447614, FR682853
<i>Mafa-A1*065:04:01</i>	LN851941	1	71	1098	extension	1062	AB448748, FR682825, FR682850
<i>Mafa-A1*065:05</i>	LN994417	1	59	1098	novel	-	-
<i>Mafa-A1*066:06</i>	LN851942	1	102	1098	extension	721	HQ113962, HQ131765
<i>Mafa-A1*067:01</i>	KP307908	4	584	1098	extant	1098	AM295855, FR682827, KP307908
<i>Mafa-A1*070:01</i>	EU203708	2	74	1098	extant	1098	AM295858, EU203708, FR682831
<i>Mafa-A1*074:01:01</i>	LN851943	2	220	1098	extension	1003	AB447596
<i>Mafa-A1*077:01:02</i>	LN994418	1	81	1098	extension	1003	AB447613
<i>Mafa-A1*077:02:01</i>	LN994419	1	101	1098	novel	-	-
<i>Mafa-A1*077:02:02</i>	LN994420	1	275	1098	novel	-	-
<i>Mafa-A1*078:01:01</i>	AB154767	1	111	1098	extant	1098	AB154767
<i>Mafa-A1*085:02</i>	LN851944	2	198	1098	extension	1062	FR682820
<i>Mafa-A1*086:01:02</i>	LN851945	2	12	981	novel	-	-
<i>Mafa-A1*086:03</i>	LN994421	1	5	981	extension	822	GU130454
<i>Mafa-A1*086:05</i>	LN994422	1	5	981	novel	-	-
<i>Mafa-A1*089:06</i>	LN851946	1	68	1098	extension	1003	AB447610
<i>Mafa-A1*090:02:02</i>	LN994423	1	10	1098	novel	-	-
<i>Mafa-A1*090:03</i>	LN851947	4	19	1098	novel	-	-
<i>Mafa-A1*092:01</i>	LN994424	1	41	1098	extension	1062	AB447594, FR682823, FR682833, HQ113961, HQ131768
<i>Mafa-A1*092:03</i>	LN994425	1	123	1098	extension	1065	FM246488
<i>Mafa-A1*092:04</i>	KP307869	1	103	1098	extant	1098	KP307869
<i>Mafa-A1*095:02</i>	LN994426	1	99	1098	novel	-	-
<i>Mafa-A1*096:02</i>	LN994427	2	129	1098	novel	-	-
<i>Mafa-A1*097:02</i>	LN994428	1	119	1098	novel	-	-
<i>Mafa-A1*101:02:02</i>	LN851948	1	67	1098	novel	-	-
<i>Mafa-A1*102:03:01</i>	LN851949	3	256	1098	novel	-	-
<i>Mafa-A1*102:03:02</i>	LN994429	1	146	1098	novel	-	-
<i>Mafa-A1*124:01</i>	LN994430	1	63	1098	extension	641	AB583237
<i>Mafa-A1*130:01</i>	LN851950	1	66	1098	extension	1080	KP307844
<i>Mafa-A2*01:01</i>	LN994431	1	62	1098	extension	1062	FR682848, HQ113963, HQ131750

<i>Mafa-A2*05:06:02</i>	LN994432	4	6	1098	extension	822	EF550522
<i>Mafa-A2*05:06:04</i>	LN994438	2	14	1098	novel	-	-
<i>Mafa-A2*05:22</i>	LN851952	2	14	1101	extension	822	EF589357
<i>Mafa-A2*05:25:01</i>	LN994433	5	16	1098	extension	822	EF589360, HP823075
<i>Mafa-A2*05:31</i>	LN851953	2	18	1101	extension	822	EF550520
<i>Mafa-A2*05:40</i>	LN994434	2	5	1098	extension	822	GQ131777
<i>Mafa-A2*05:42</i>	LN994435	1	20	1083	extension	822	GQ131775
<i>Mafa-A2*05:45</i>	LN994436	1	4	1098	extension	822	GQ131771
<i>Mafa-A2*05:46</i>	LN851954	2	9	1101	extension	1065	GQ131770, HQ230581
<i>Mafa-A2*05:50</i>	GU063713	1	1	1098	extant	1098	GU063713, LC053847
<i>Mafa-A2*05:51</i>	LN851955	1	3	1101	extension	822	GU130445
<i>Mafa-A2*05:55</i>	LN851956	5	48	1101	extension	1080	KP307855
<i>Mafa-A2*05:57:01</i>	LN851951	6	34	1098	extension	822	HQ992789
<i>Mafa-A2*05:58</i>	LN994437	1	5	1098	novel	-	-
<i>Mafa-A2*05:59</i>	LN994439	2	6	1098	novel	-	-
<i>Mafa-A2*24:07</i>	LN851957	1	49	1098	novel	-	-
<i>Mafa-A2*24:08</i>	LN994440	1	211	1098	novel	-	-
<i>Mafa-A3*13:02</i>	LN851958	2	27	1098	extension	1042	AY958111
<i>Mafa-A3*13:07</i>	LN851959	4	55	1098	extension	1062	FR682834, GQ131768, HM161349, HQ113968, HQ131739
<i>Mafa-A3*13:14N</i>	LN851960	2	5	1011	extension	822	GU130464
<i>Mafa-A3*13:15</i>	LN994441	1	6	1098	extension	641	AB583238
<i>Mafa-A3*13:17:02</i>	LN851961	1	5	1098	novel	-	-
<i>Mafa-A3*13:20</i>	LN851962	1	15	1098	novel	-	-
<i>Mafa-A3*13:21</i>	LN994442	1	6	1098	novel	-	-
<i>Mafa-A3*13:22</i>	LN994443	1	14	1098	novel	-	-
<i>Mafa-A4*01:02</i>	LN851963	2	9	1098	extension	1042	AY958121
<i>Mafa-A4*01:08</i>	LN994444	6	11	1011	extension	822	GQ131766
<i>Mafa-A4*01:12</i>	LN994445	1	6	1011	novel	-	-
<i>Mafa-A4*14:01</i>	LN851964	9	31	1011	extension	1065	AM295880
<i>Mafa-A4*14:03</i>	LN851965	21	110	1101	extension	1065	FR682829, GQ131781, HQ113965, HQ131747, LC043320
<i>Mafa-A4*14:04</i>	LN851967	1	3	1101	extension	822	GQ131767
<i>Mafa-A4*14:13</i>	KP307856	1	22	1092	extant	1092	KP307856
<i>Mafa-A4*14:15</i>	LN851968	1	6	1107	novel	-	-
<i>Mafa-A4*14:16</i>	LN851966	1	3	1101	novel	-	-
<i>Mafa-A4*14:18</i>	LN994446	1	4	1107	novel	-	-
<i>Mafa-A4*14:19</i>	LN994447	1	7	1101	novel	-	-
<i>Mafa-A5*30:06</i>	KP307871	1	1	1089	extant	1089	KP307871
<i>Mafa-B*001:01:01</i>	KP307893	7	239	1089	extant	1089	AY958126, KP307893
<i>Mafa-B*001:01:02</i>	LN994449	1	54	1089	novel	-	-
<i>Mafa-B*001:02</i>	LN994448	1	19	1089	novel	-	-
<i>Mafa-B*002:04</i>	LN994450	3	472	1089	extension	822	HM161352, HQ114001, HQ131729, HQ131749
<i>Mafa-B*006:01:02</i>	LN994451	1	12	1089	novel	-	-
<i>Mafa-B*007:01:01</i>	GU063753	17	606	1089	extant	1089	AY958137, GU063753, HM161384, HM236279
<i>Mafa-B*007:01:05</i>	LN851969	6	155	1089	extension	822	HM161391, HQ114005, HQ131716
<i>Mafa-B*007:05</i>	LN851970	4	98	1089	extension	544	HQ131707
<i>Mafa-B*007:06:02</i>	LN994453	1	48	1089	novel	-	-

<i>Mafa-B*007:07</i>	LN851971	3	53	1089	novel	-	-
<i>Mafa-B*007:08</i>	LN851972	7	235	1089	novel	-	-
<i>Mafa-B*007:10</i>	LN994452	1	27	1089	novel	-	-
<i>Mafa-B*008:01</i>	LN994454	1	56	1089	extension	544	HQ131700
<i>Mafa-B*010:01</i>	AB195452	2	112	1089	extant	1089	AB195452
<i>Mafa-B*011:01</i>	AY958143	1	42	1089	extant	1089	AY958143, EU203717, GU063737
<i>Mafa-B*013:03</i>	LN994455	2	45	1089	extension	822	FJ178813
<i>Mafa-B*013:06</i>	LN851973	1	32	1089	extension	822	GQ131788, HQ113998, HQ131703
<i>Mafa-B*013:09</i>	KP307894	3	269	1089	extant	1089	FJ178814, HQ131706, KP307894
<i>Mafa-B*013:10</i>	LN851974	2	42	1089	extension	1056	HM161366, HQ131722, KP307895
<i>Mafa-B*013:11</i>	LN994456	1	45	1089	extension	1056	FM212794
<i>Mafa-B*013:13</i>	LN851975	1	27	1089	novel	-	-
<i>Mafa-B*013:14</i>	LN851976	2	104	1089	novel	-	-
<i>Mafa-B*013:15</i>	LN994457	1	44	1089	novel	-	-
<i>Mafa-B*013:16</i>	LN994458	1	118	1089	novel	-	-
<i>Mafa-B*013:17</i>	LN994459	1	18	1089	novel	-	-
<i>Mafa-B*013:18</i>	LN994460	1	4	1089	novel	-	-
<i>Mafa-B*014:01</i>	LN851977	2	14	1089	extension	1056	FM212831
<i>Mafa-B*014:02</i>	LN851978	1	8	1089	novel	-	-
<i>Mafa-B*015:01</i>	LN851979	2	49	1089	extension	1042	AY958132, HM235701
<i>Mafa-B*015:05</i>	LN994461	1	42	1089	novel	-	-
<i>Mafa-B*015:06</i>	LN994462	1	106	1089	novel	-	-
<i>Mafa-B*016:01</i>	EU203701	1	118	1092	extant	1092	EU203701
<i>Mafa-B*016:02</i>	LN994463	1	61	1092	novel	-	-
<i>Mafa-B*017:02</i>	KP307896	2	43	1080	extant	1080	HQ131701, KP307896
<i>Mafa-B*018:01:01</i>	KP307897	3	178	1089	extant	1089	AY958138, KP307897
<i>Mafa-B*018:03</i>	LN994464	1	13	1089	novel	-	-
<i>Mafa-B*019:03</i>	DQ979879	1	7	1086	extant	1086	DQ979879, GU063743
<i>Mafa-B*019:04</i>	LN994465	1	33	1086	novel	-	-
<i>Mafa-B*021:02</i>	KP307859	8	127	1077	extant	1077	KP307859, KP307845
<i>Mafa-B*021:04</i>	LN994466	1	10	1077	novel	-	-
<i>Mafa-B*021:05</i>	LN994467	2	37	1077	novel	-	-
<i>Mafa-B*025:02</i>	LN851980	1	110	1086	novel	-	-
<i>Mafa-B*027:03:01</i>	LN851982	1	3	1096+	extension	659	HG977466
<i>Mafa-B*027:03:02</i>	LN851981	2	19	1096+	novel	-	-
<i>Mafa-B*028:02</i>	KP307898	6	18	1089	extant	1089	AY958130, KP307898
<i>Mafa-B*028:03</i>	LN994468	1	6	1089	extension	1042	AY958128, LC043325
<i>Mafa-B*028:04</i>	EU046324	2	20	1089	extant	1089	EU046324, HM235716, HQ131708
<i>Mafa-B*030:01:01</i>	LN851983	7	277	1089	extension	1042	AY958133
<i>Mafa-B*030:01:05</i>	LN994472	2	22	1089	novel	-	-
<i>Mafa-B*030:02</i>	LN994469	1	16	1089	extension	1042	AY958134
<i>Mafa-B*030:03:01</i>	KP307900	2	10	1089	extant	1089	AY958135, HQ131687, KP307900
<i>Mafa-B*030:03:02</i>	LN994470	5	18	1089	extension	822	FJ719167, HQ131726
<i>Mafa-B*030:04</i>	LN994471	1	48	1089	extension	822	FJ719169
<i>Mafa-B*030:15</i>	KP307857	2	34	1089	extant	1089	KP307857
<i>Mafa-B*030:16</i>	LN851984	2	22	1089	novel	-	-
<i>Mafa-B*030:17</i>	KP307899	5	28	1089	extant	1089	FJ178822, KP307899

<i>Mafa-B*030:18</i>	LN994473	2	5	1089	novel	-	-
<i>Mafa-B*030:19</i>	LN994474	1	4	1089	novel	-	-
<i>Mafa-B*031:03</i>	LN994475	2	21	1095	novel	-	-
<i>Mafa-B*034:01</i>	LN851985	1	62	1089	extension	1042	AY958127
<i>Mafa-B*034:03</i>	LN851986	2	131	1089	extension	819	HQ113997, HQ131704
<i>Mafa-B*034:04</i>	LN994476	1	34	1089	extension	1056	KP307879
<i>Mafa-B*036:01:02</i>	EU606046	2	39	1080	extant	1080	EU606046, FM212796
<i>Mafa-B*037:01</i>	AY958149	2	22	1089	extant	1089	AY958149, HQ114004, HQ131697
<i>Mafa-B*038:01</i>	KP307860	2	31	1089	extant	1089	KP307860
<i>Mafa-B*039:01</i>	KP307902	11	674	1089	extant	1089	HM161344, HQ113949, HQ113992, HQ114010, HQ131713, HQ131762, KP307902
<i>Mafa-B*039:02</i>	LN994477	2	87	1089	novel	-	-
<i>Mafa-B*041:02</i>	KP307858	4	28	1089	extant	1089	KP307858, KP307874, KP307882, LC053850
<i>Mafa-B*041:03</i>	LN851987	1	9	1089	extension	1071	KP307846
<i>Mafa-B*043:01</i>	LN994478	2	33	1089	extension	646	AB569230
<i>Mafa-B*043:02</i>	LN994479	1	15	1089	novel	-	-
<i>Mafa-B*044:01:01</i>	KP307903	10	113	1080	extant	1080	FM212817, HM235712, KP307903
<i>Mafa-B*044:01:03</i>	LN994480	1	43	1080	novel	-	-
<i>Mafa-B*044:04</i>	HM581964	1	47	1080	extant	1080	HM581964
<i>Mafa-B*045:07</i>	LN994481	2	21	1089	novel	-	-
<i>Mafa-B*047:03</i>	LN994482	1	53	1089	novel	-	-
<i>Mafa-B*048:06</i>	LN851988	1	54	1089	novel	-	-
<i>Mafa-B*050:02:02</i>	LN994483	1	15	1080	novel	-	-
<i>Mafa-B*050:05</i>	LN851989	5	55	1080	extension	546	HM161343, HQ131714
<i>Mafa-B*050:08</i>	LN851990	2	10	1080	extension	1003	LC043337
<i>Mafa-B*056:01</i>	KP307904	10	717	1089	extant	1089	AY958131, HQ131699, KP307904
<i>Mafa-B*056:04</i>	KP307848	1	29	1089	extant	1089	KP307848
<i>Mafa-B*056:05:01</i>	LN994484	1	52	1089	extension	1056	HM235703, HQ131733, KP307905
<i>Mafa-B*056:05:02</i>	LN994485	1	54	1089	novel	-	-
<i>Mafa-B*060:01</i>	EU203692	4	6	1080	extant	1080	EU203692
<i>Mafa-B*060:04</i>	HM581968	5	12	1080	extant	1080	AB569226, HM581968, HQ131693
<i>Mafa-B*060:12</i>	LN851991	1	3	1080	novel	-	-
<i>Mafa-B*060:13</i>	LN851992	12	57	1080	novel	-	-
<i>Mafa-B*060:14</i>	LN994487	1	5	1080	novel	-	-
<i>Mafa-B*060:16</i>	LN994486	1	8	1080	novel	-	-
<i>Mafa-B*061:03:01</i>	KP307851	2	48	1080	extant	1080	KP307851
<i>Mafa-B*061:03:02</i>	LN851993	1	11	1080	novel	-	-
<i>Mafa-B*061:04:01</i>	KP307861	6	270	1080	extant	1080	KP307861, KP307843
<i>Mafa-B*061:04:02</i>	LN851994	2	77	1080	novel	-	-
<i>Mafa-B*061:05</i>	LN994488	2	5	1089	novel	-	-
<i>Mafa-B*064:05</i>	LN994489	1	4	1077	novel	-	-
<i>Mafa-B*065:02</i>	LN851995	3	28	1089	extension	822	HM161351, HQ113986, HQ131727
<i>Mafa-B*065:04</i>	LN851996	5	70	1089	novel	-	-
<i>Mafa-B*065:05</i>	LN994490	1	40	1089	novel	-	-
<i>Mafa-B*066:01</i>	KP307849	1	16	1089	extant	1089	KP307849
<i>Mafa-B*067:01</i>	LN994491	1	47	1089	novel	-	-
<i>Mafa-B*067:02</i>	LN994492	1	31	1089	novel	-	-

<i>Mafa-B*068:02</i>	FM212839	4	79	1089	extant	1089	FM212839
<i>Mafa-B*068:04</i>	LN851997	7	110	1089	extension	641, 1056	AB569236, KP307906
<i>Mafa-B*068:06:01</i>	KP307865	6	120	1089	extant	1089	KP307865
<i>Mafa-B*068:06:02</i>	LN994493	1	23	1089	novel	-	-
<i>Mafa-B*068:07:01</i>	KP307850	1	20	1089	extant	1089	KP307850
<i>Mafa-B*068:07:02</i>	LN994494	2	57	1089	novel	-	-
<i>Mafa-B*068:10</i>	LN851998	1	5	1089	novel	-	-
<i>Mafa-B*069:03</i>	LN852001	3	144	1089	extension	544	HQ131732
<i>Mafa-B*069:04</i>	LN851999	2	113	1089	extension	822	HM161434, HQ114002, HQ131710
<i>Mafa-B*069:05</i>	LN852000	2	152	1089	novel	-	-
<i>Mafa-B*069:06</i>	LN994495	1	90	1089	novel	-	-
<i>Mafa-B*069:07</i>	LN994496	1	28	1089	extension	822	HM161424
<i>Mafa-B*070:04</i>	LN852002	2	10	1080	extension	1070	KP307842
<i>Mafa-B*072:02</i>	HM581961	1	3	1089	extant	1089	HM581961
<i>Mafa-B*072:04</i>	LN852003	2	5	1089	novel	-	-
<i>Mafa-B*073:02</i>	KP307876	2	82	1089	extant	1089	HM161413, KP307876
<i>Mafa-B*073:03</i>	LN994497	1	18	1089	novel	-	-
<i>Mafa-B*074:02</i>	LN852004	7	132	1080	extension	646	AB569228
<i>Mafa-B*075:01</i>	EU203718	1	73	1080	extant	1080	AY958150, EU203718, GU063741
<i>Mafa-B*077:01</i>	FM212843	1	88	1089	extant	1089	FM212843
<i>Mafa-B*077:02</i>	LN852005	1	59	1089	novel	-	-
<i>Mafa-B*079:01:01</i>	AM943362	1	1	1080	extant	1080	AM943362
<i>Mafa-B*079:02:01</i>	LN994499	5	9	1080	extension	822	HM236276
<i>Mafa-B*081:01</i>	LN852006	2	31	1086	extension	822	AB569225, HM161374, HQ131721
<i>Mafa-B*082:03</i>	LN994500	6	20	1116	novel	-	-
<i>Mafa-B*083:02</i>	KP307907	1	151	1089	extant	1089	HM236289, KP307907
<i>Mafa-B*083:04</i>	LN994501	1	16	1089	novel	-	-
<i>Mafa-B*085:01</i>	KP307852	7	214	1089	extant	1089	AY958124, HM161375, HQ113995, KP307852, LC043363
<i>Mafa-B*088:04</i>	LN852007	8	13	1089	novel	-	-
<i>Mafa-B*088:05</i>	LN994502	6	12	1089	novel	-	-
<i>Mafa-B*089:01:02</i>	EU392125	6	18	1116	extant	1116	EU392125, FJ178818, HM161360, HQ113989, JF729417, LC043349
<i>Mafa-B*091:03</i>	LN994503	1	70	1089	extension	822	HM161370
<i>Mafa-B*093:02</i>	LN852008	5	242	1089	extension	822	HM161359, HQ113988, HQ114006, HQ131734
<i>Mafa-B*095:03</i>	LN994504	1	12	1089	novel	-	-
<i>Mafa-B*095:04</i>	LN994505	1	173	1089	novel	-	-
<i>Mafa-B*098:03:02</i>	LN994508	3	5	1089	novel	-	-
<i>Mafa-B*098:04</i>	LN994506	3	6	1089	extension	981	HM581969
<i>Mafa-B*098:12</i>	LN994507	6	15	1089	novel	-	-
<i>Mafa-B*101:02</i>	LN994509	2	164	1089	extension	819	HQ113999, HQ131702
<i>Mafa-B*103:01:02</i>	LN994510	1	6	1080	novel	-	-
<i>Mafa-B*104:01:02</i>	FM212841	6	698	1089	extant	1089	FM212841, HQ131684, HQ131774
<i>Mafa-B*105:01</i>	LN852009	2	80	1089	extension	819	HQ114013, HQ131692
<i>Mafa-B*110:01:01</i>	LN852010	4	162	1089	extension	822	HM161339, HM161378, HQ131725
<i>Mafa-B*110:01:02</i>	LN994511	1	13	1089	novel	-	-
<i>Mafa-B*117:03</i>	LN852011	2	26	1080	novel	-	-
<i>Mafa-B*117:04</i>	LN994512	1	18	1080	novel	-	-

<i>Mafa-B*124:01:01</i>	EU203714	1	27	1080	extant	1080	EU203714
<i>Mafa-B*124:01:03</i>	KP307862	6	105	1080	extant	1080	KP307862, KP307847
<i>Mafa-B*124:03</i>	LN852012	2	9	1080	novel	-	-
<i>Mafa-B*124:04</i>	LN994513	1	6	1080	novel	-	-
<i>Mafa-B*137:03</i>	EU203723	6	138	1089	extant	1089	EU203723, EU392117, HM235700, HQ131688, HQ131740, JF729416, LC043364
<i>Mafa-B*137:05</i>	LN852013	1	12	1089	novel	-	-
<i>Mafa-B*137:06</i>	LN994515	2	32	1089	novel	-	-
<i>Mafa-B*137:07</i>	LN994514	1	4	1089	novel	-	-
<i>Mafa-B*137:08</i>	LN994516	1	11	1089	novel	-	-
<i>Mafa-B*139:02</i>	FM212840	2	19	1086	extant	1086	FM212840
<i>Mafa-B*140:01:02</i>	LN994517	1	27	1089	novel	-	-
<i>Mafa-B*144:01</i>	LN994518	2	38	1080	extension	1042	AY958122, HQ113983
<i>Mafa-B*145:01</i>	LN852014	3	198	1089	extension	1042	AY958123, HM235702, HQ113984, HQ131728
<i>Mafa-B*146:01:01</i>	LN852015	1	24	1089	extension	1042	AY958125
<i>Mafa-B*148:01:01</i>	LN994519	1	22	1089	novel	-	-
<i>Mafa-B*149:01</i>	EU606047	2	4	1089	extant	1089	EU606047
<i>Mafa-B*149:02</i>	LN852016	2	10	1089	extension	822	HG977501, HQ110973
<i>Mafa-B*150:02</i>	LN994520	1	96	1089	extension	1056	FM212814
<i>Mafa-B*155:01</i>	EU203684	1	45	1080	extant	1080	EU203684, HM235713
<i>Mafa-B*156:01</i>	EU203694	2	57	1089	extant	1089	EU203694, FJ178816
<i>Mafa-B*161:05</i>	LN852017	2	57	1089	novel	-	-
<i>Mafa-B*164:02</i>	GQ153333	1	49	1089	extant	1089	GQ153333, LC043371
<i>Mafa-B*181:03</i>	LN994521	1	41	1089	novel	-	-
<i>Mafa-B*184:01:01</i>	LN994522	2	162	1089	extension	822	JN032108
<i>Mafa-B*184:01:02</i>	LN994523	2	100	1089	novel	-	-
<i>Mafa-B*203:01</i>	LN994498	1	81	1089	novel	-	-
<i>Mafa-I*01:01:02</i>	LN994530	1	9	1089	novel	-	-
<i>Mafa-I*01:09</i>	AB195465	1	2	1089	extant	1089	AB195465
<i>Mafa-I*01:11:02</i>	LN852020	14	46	1089	novel	-	-
<i>Mafa-I*01:14:02</i>	LN852021	4	5	1089	novel	-	-
<i>Mafa-I*01:18:01</i>	LN994524	8	34	1089	extension	1056	FM246497
<i>Mafa-I*01:22</i>	HM581970	1	2	1089	extant	1089	AF161864, HM581970
<i>Mafa-I*01:29</i>	LN852018	2	6	1089	novel	-	-
<i>Mafa-I*01:30</i>	LN852019	4	4	1089	novel	-	-
<i>Mafa-I*01:31</i>	LN994525	4	6	1089	novel	-	-
<i>Mafa-I*01:32</i>	LN994526	3	6	1089	novel	-	-
<i>Mafa-I*01:33</i>	LN994527	4	8	1089	novel	-	-
<i>Mafa-I*01:34</i>	LN994528	1	6	1089	novel	-	-
<i>Mafa-I*01:35</i>	LN994529	1	6	1089	novel	-	-

Summary of 313 full-length MHC class I transcripts identified in 72 cynomolgus macaques from Chinese breeding facilities

a

MiSeq Genotyping Results						
Animal ID	GC015	GC074	GC016	GC017	GC075	GC018
Relationship	sire	prog	dam	sire	prog	dam
# Reads Mapped	19,320	16,573	21,992	20,732	20,179	21,227
<i>Mafa-A</i> Haplotype 1	A063	A063	A026	A067	A067	A028
<i>Mafa-A</i> Haplotype 2	A065	A041	A041	A067	A096	A096
<i>Mafa-B</i> Haplotype 1	B085	B085	B145	B045a	B045a	B069d
<i>Mafa-B</i> Haplotype 2	B034a	B013a	B013a	B013d	B039e	B039e
<i>Mafa-A</i> Major Alleles						
A1*026			4581			
A1*028						4331
A1*041		3067	3688			
A1*063	4391	3035				
A1*065	3857					
A1*067				11656	5194	
A1*096					6292	6075
<i>Mafa-B</i> Major Alleles						
B*007		1693	1778		1341	2487
B*013		1843	1792	1076		
B*034	2415					
B*036				303	420	
B*037				1908	1389	
B*039					926	849
B*044	678	541				
B*045				2107	1433	
B*068			306			
B*069						2720
B*074						784
B*083						1106
B*085	921	706				
B*137				1930		
B*144			3129			
B*145			2749			
B*146	2494					

b

PacBio Full-Length Sequencing Results						
Animal ID	GC015	GC074	GC016	GC017	GC075	GC018
Relationship	sire	prog	dam	sire	prog	dam
# Reads Mapped	339	384	329	402	151	275
<i>Mafa-A</i> Haplotype 1	A063	A063	A026	A067	A067	A028
<i>Mafa-A</i> Haplotype 2	A065	A041	A041	A067	A096	A096
<i>Mafa-B</i> Haplotype 1	B085	B085	B145	B045a	B045a	B069d
<i>Mafa-B</i> Haplotype 2	B034a	B013a	B013a	B013d	B039e	B039e
<i>Mafa-A</i> Major Alleles						
A1*026:01			80			
A1*028:04						65
A1*041:01		118	101			
A1*063:03:01	95	67				
A1*065:04:01	71					
A1*067:01				316	45	
A1*096:02					46	83
<i>Mafa-B</i> Major Alleles						
B*007:01:01					17	18
B*007:08		47	47			
B*007:10						27
B*013:14		53	51			
B*013:17				18		
B*034:01	62					
B*036:01:02				27	12	
B*037:01				14	8	
B*039:01					12	36
B*044:01:01	27	22				
B*045:07				16	5	
B*068:06:01			7			
B*069:07						28
B*074						nd
B*083:04						16
B*085:01	37	34				
B*137:03				8		
B*144			nd			
B*145:01			34			
B*146:01:01	24					

Center - Origin	Animal ID	Relation	Mafa-A Haplo 1	Mafa-A Haplo 2	Mafa-B Haplo 1	Mafa-B Haplo 2	PacBio Typed	Center - Origin	Animal ID	Relation	Mafa-A Haplo 1	Mafa-A Haplo 2	Mafa-B Haplo 1	Mafa-B Haplo 2	PacBio Typed
Yulin Hongfeng - Vietnamese	GC001	sire	A001	A056a	B155b	B028a	yes	Hainan Jingang - Cambodian	GC013	sire	A028	A224a	B015d	B028d	yes
	GC067	prog	A001	A043	B155b	B039e			GC073	prog	A028	A040	B015d	B039e	
	GC002	dam	A015	A043	B069e	B039e	yes		GC014	dam	A096	A040	B056a	B039e	
	GC003	sire	A045	A042	B065a	B025a	yes		GC015	sire	A063	A065	B085	B034a	yes
	GC068	prog	A045	A007	B065a	B039e			GC074	prog	A063	A041	B085	B013a	yes
	GC004	dam	A007	A007	B039e	B039e	yes		GC016	dam	A026	A041	B145	B013a	yes
	GC005	sire	A038	A064	B077	B001b	yes		GC017	sire	A067	A067	B045a	B013d	yes
	GC069	prog	A038	A130	B077	B013f			GC075	prog	A067	A096	B045a	B039e	yes
	GC006	dam	A065	A130	B161	B013f	yes		GC018	dam	A028	A096	B069d	B039e	yes
	GC007	sire	A074a	A070	B093b	B028b	yes		GC019	sire	A006	A092a	B104d	B017a	yes
	GC070	prog	A074a	A066	B093b	B013d			GC076	prog	A006	A027	B104d	B001b	yes
	GC008	dam	A022a	A066	B048b	B013d	yes		GC020	dam	A038	A027	B091	B001b	yes
	GC009	sire	A060	A070	B056a	B028a	yes		GC021	sire	A018a	A003	B093a	B013b	yes
	GC071	prog	A060	A043	B056a	B145			GC077	prog	A018a	A057	B093a	B028d	
	GC010	dam	A043	A043	B013h	B145	yes		GC022	dam	A053	A057	B039e	B028d	yes
	GC011	sire	A038	A074a	B013d	B013d	yes		GC045	sire	A065	A027	B028b	B056a	yes
	GC072	prog	A038	A033	B013d	B083c	yes		GC090	prog	A065	A130	B028b	B039e	
	GC012	dam	A089	A033	B056f	B083c	yes		GC046	dam	A065	A130	B015a	B039e	
	GC026	dam	A065	A102	B101a	B008	yes		GC033	sire	A040	A032b	B028d	B069f	yes
	GC079	prog	A043	A102	B039e	B008			GC083	prog	A040	A015	B028d	B013d	
	GC028	dam	A027	A027	B034b	B056e	yes		GC084	prog	A040	A065	B028d	B018b	yes
	GC080	prog	A040	A027	B083b	B056e	yes		GC085	prog	A040	A007	B028d	B045a	
	GC030	dam	A022a	A022a	B093a	B093a	yes		GC038	dam	A026	A067	B043b	B083d	yes
	GC081	prog	A085	A022a	B013f	B093a	yes		GC086	prog	A124	A067	B015b	B083d	yes
	GC032	dam	A018b	A006	B002b	B002b	yes		GC040	dam	A027	A007	B069a	B028b	
	GC082	prog	A027	A006	B104d	B002b	yes		GC087	prog	A010a	A007	B013d	B028b	
	GC025	unrelat	A018b	A102	B002a	B077			GC047	sire	A006	A102	B056a	B077	yes
	GC027	unrelat	A018a	A022a	B093a	B137	yes		GC091	prog	A006	A056b	B056a	B039g	yes
	GC029	unrelat	A003	A004	B013d	B039d	yes		GC053	sire	A022a	A028	B039e	B013a	yes
	GC031	unrelat	A065	A070	B025a	B104d			GC094	prog	A022a	A022a	B039e	B083b	
Hainan Jingang - Cambodian/Indonesian	GC088	prog (s)	A022a	A078	B001a	B019b			GC055	sire	A022b	A045	B069a	B001a	yes
	GC089	prog (s)	A022a	A003	B001a	B043c	yes		GC095	prog	A022b	A067	B069a	B093a	
	GC023	sire	A022a	A022a	B015c	B001a	yes		GC057	sire	A006	A085	B028a	B010a	yes
	GC078	prog	A022a	A077	B015c	B184			GC096	prog	A006	A022a	B028a	B013a	
	GC024	dam	A001	A077	B047b	B184	yes		GC060	dam	A015	A056b	B017a	B104d	yes
	GC049	sire	A001	A010b	B069a	B104d	yes		GC097	prog	A065	A056b	B039e	B104d	yes
	GC092	prog	A001	A077	B069a	B016	yes		GC034	unrelat	A042	A056b	B013d	B039d	yes
	GC051	sire	A001	A058	B156	B069g	yes		GC035	unrelat	A007	A041	B028d	B043a	yes
	GC093	prog	A001	A018a	B156	B019a	yes		GC036	unrelat	A026	A077	B039d	B083a	yes
	GC041	unrelat	A018c	A038	B065b	B181	yes		GC037	unrelat	A003	A058	B069h	B069h	yes
	GC042	unrelat	A055	A078	B075a	B019b	yes		GC039	unrelat	A022a	A022a	B028d	B028d	
	GC050	unrelat	A038	A092a	B015a	B028e	yes		GC043	unrelat	A006	A015	B013d	B039e	
	GC052	unrelat	A010a	A055	B056g	B164a	yes		GC044	unrelat	A006	A015	B013d	B039e	
									GC048	unrelat	A022a	A224a	B095a	B145	yes
									GC054	unrelat	A022a	A074a	B065a	B093a	
									GC056	unrelat	A027	A027	B013i	B013i	yes
									GC058	unrelat	A018a	A022a	B093a	B145	
									GC059	unrelat	A007	A008	B028d	B056a	yes
									GC061	unrelat	A015	A032b	B039f	B056a	yes
									GC062	unrelat	A028	A097	B001a	B101a	yes
									GC063	unrelat	A102	A102	B010a	B013j	yes
									GC064	unrelat	A038	A043	B056h	B184	yes
									GC065	unrelat	A010a	A040	B013d	B069a	yes
									GC066	unrelat	A002	A003	B001f	B002a	yes
									GC098	unrelat	A022a	A065	B028e	B104d	
									GC099	unrelat	A003	A092b	B001a	B056a	yes
									GC100	unrelat	A001	A038	B161	B039d	yes

Figure 3

bioRxiv preprint doi: <https://doi.org/10.1101/084947>; this version posted November 2, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-ND 4.0 International license.