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Review Article

Enhancers in Proboscis Monkey: A Primer

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ABSTRACT

Enhancers are indispensable elements in various developmental stages, orchestrating numerous biological processes via the elevation of gene expression with the aid of transcription factors. Enhancer variations have been linked to various onset of genetic diseases, highlighting their equal importance as the coding regions in the genome. Despite the first enhancer, SV40 been discovered four decades ago, the progress in enhancer identification and characterization is still in its infancy. As more genome sequences are made available, especially from that of the non-human primates, we can finally study the enhancer landscape of these primates that differs evolutionarily from that of human. One interesting genome to investigate is that of the proboscis monkey as it is deemed as one of the most ancient primates alive to date with unique morphological and dietary characteristics; it is also one of the IUCN endangered species with the strong demands for immediate conservation. In this review, we provide some justifications and considerations of selecting the proboscis monkey as a model for enhancer landscape discovery. It is hoped that more conservation research and protective measures can come in time to prevent this species from extinction.

Keywords: Conservation, enhancer, gene regulation, primate, proboscis monkey

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INTRODUCTION

Enhancer, as its name suggests, is an essential regulatory DNA element capable of enhancing and elevating gene transcription and all other processes that

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occur at post-transcription as described in the central dogma of molecular biology (Pennacchio et al., 2015). Enhancers are vitally indispensable as it plays major roles in orchestrating evolutionarily important phenotypes as well as biological processes at numerous developmental stages (Pennacchio et al., 2015). The magic the enhancers have that differs them from gene promoters is that they can regulate adjacent and distal genes in the bidirectional orientation and locality-unrestricted manner (Melo et al., 2013; Natoli & Andrau, 2012).

Despite the fact that the first enhancer, SV40 was discovered over four decades ago by Banerji et al. (1981), it was only recently that enhancers were once again being promoted into the limelight of the molecular biology field for its significance in disease-related genetics as the first disease-related enhancer was found in Hirschsprung disease (Grice et al., 2005). Another crucial driving force for this phenomenon is no other than the emergence of next generation sequencing which has unravelled genome sequences of various species (Baker, 2012). The completion of genome sequencing of human and other famous model organisms had revealed more than just previously undiscovered evolutionarily conserved non-coding regions but also functionally conserved (but not necessarily sequence-wise conserved) regions believed to function as enhancers (Melton et al., 2015). On the side note, among the emerging genome sequencing projects initiated in the late 20th century (Gordon et al., 2016; Prüfer et al., 2012; The Marmoset Genome Sequencing and Analysis Consortium, 2014), the non-human primates are one of the major highlights as they represent the closest evolution counterparts to human and the high similarities they share with human in terms of coding and non-coding regions are very valuable for biomedical genetic studies especially (Harding, 2013).

The proboscis monkey, *Nasalis larvatus*, which is endemic to Borneo Island is one of the interesting non-human primate subjects to study. This species is deemed to be the most primitive colobine based on its morphological characteristics as well as exceptional diploid number of 2n=2x=48 (Chiarelli, 1966; Soma et al., 1974; Stanyon et al., 1992). These ancient adaptive traits above that are possessed by none other than the proboscis monkey, are very beneficial for the investigation of the enhancer landscape in primates, especially in its most ancient form to study on how evolutionary divergence of these enhancers would lead to phenotypic variations and speciation. As enhancers are known for their rapid evolution especially across mammals and recently evolved enhancers are found to be the dominators in mammalian regulatory landscapes (Villar et al., 2015), thus it is interesting to discover the effects of gain-of-function or loss-of-function of these enhancers on the emergence of genetic disease throughout the divergence process in primates. The variations in enhancer sequences are also known to be associated with the onset of various developmental and genetic diseases known to date in human (Kim et al., 2011; Kleftogiannis

et al., 2015). Furthermore, this monkey species is currently listed as endangered by IUCN (Meijaard et al., 2008). Therefore, by exploring the enhancer landscape in its most ancient form in the most primitive primate like the proboscis monkey, we can understand how evolution had changed the enhancer landscape in primates and further aid in conservation research like antibody synthesis against elephant endotheliotropic herpesvirus (EEHV) (Kochagul et al., 2018), pathogen combatting in white-nose syndrome in bats (Palmer et al., 2018) as well as the toxicology gene expression studies on endemic *Rasbora* fish (Lim et al., 2018b). Besides, the proboscis monkey enhancer landscape is useful in the understanding of the adaptive phenotypic traits that occur in the environment for displaced wildlife (Luo & Lin, 2016; Vogt, 2017) for more effective conservation measures and strategies in future. In this review, we provide some justifications and considerations of selecting the proboscis monkey as a model for enhancer landscape discovery and conservation.

Gene Regulation

The expression of gene in cell and tissue is governed by DNA components termed the regulatory elements, they control the amount of gene products produced spatially and temporally at different developmental stages (Laybourn, 2001; Scott, 2000). The regulation of gene expression is conducted in various ways and forms ranging from chromatin remodelling, transcription initiation, transcription, transcript modifications, mRNA degeneration, translation initiation, translation, posttranslational modifications to protein transport and protein degradation. Each of these stages are tightly monitored to ensure the survivability and adaptability of the host organism towards diverse environmental stimuli (Laybourn, 2001; Scott, 2000). In eukaryotic organisms, the gene regulation mechanism is much more complex as compared to their prokaryote counterparts as it involves multifaceted networks and numerous cross-acting regulatory elements (Scott, 2000; Watson et al., 2014).

The regulatory modules such as the promoter and enhancers determine the expression level of the gene via transcription factor binding. The core promoter is present in all eukaryotic genes and the TATA box (TATAAAAAA) is the most abundantly found example (Watson et al., 2014). The strong conservation of the core promoter across all protein-coding genes can be observed from its structure and binding factor whereas other upstream promoters varies in terms of binding factors and structures (Wray et al., 2003). Enhancers, on the other hand, are generally located in non-coding genomic regions where they are either sequence- or functionally conserved (or both) across different species (Levine & Tjian, 2003). The RNA polymerase II requires the interactions between the enhancers and promoters along with the recruitment of general transcription factors (TFIIA, -B, -D, -E, -F and -H) and chromatin remodelling complexes (RSF, PBAF, SWI/SNF and ACF) in order to initiate gene transcription (Watson et al., 2014).

Cis-regulatory modules like the enhancers play major roles in shaping the phenotypes of species evolutionarily as well as enabling numerous important biological processes such as morphogenesis and anatomy development to occur in an ordered manner without causing chaos like the cancer progression (Watson et al., 2014). The association between protein-coding regions and the onset of genetic diseases has been well studied throughout the decades, however it was not until recently that researchers start placing focus on the non-coding genomic regions and discover the effects of their variants on disease-related phenotypic differences (Kim et al., 2011; Visel et al., 2009).

Enhancer

The term 'enhancer' was first introduced by De Villiers and Schaffner (1981) to define a 72 bp DNA sequence repeat that can significantly activate the β-globin gene from rabbit. The proposed action of enhancer was described as element that could alter the superhelical density of DNA, facilitated the accessibility of RNA polymerase II and allowed for nuclear matrix binding (De Villiers & Schaffner, 1981).

Enhancers are short DNA elements with lengths ranging from 50 to 1500 base pairs, capable to serve as binding platforms for transcriptional activators such as transcription factors (Blackwood & Kadonaga, 1998). Upon binding of these activators, enhancer becomes functional and can elevate the transcription of gene it regulates to a much higher level. Enhancers are mostly cis-acting, they can be found up to 1 Mbp away either upstream or downstream from the gene (Blackwood & Kadonaga, 1998). The enhancer can function in both forward and backward directions of the DNA reads, making them more versatile than promoters in terms of their mode of activations. Some enhancers can even function in the form of enhancer-originating RNAs (eRNAs) where RNA polymerase II is recruited by enhancer itself and together with general transcription factors, eRNAs are transcribed (Melo et al., 2013; Natoli & Andrau, 2012). The eRNAs can significantly improve the efficiency of enhancers (Melo et al., 2013; Natoli & Andrau, 2012).

The first enhancer identified was the SV40 enhancer (Banerji et al., 1981) and this enhancer was found to be highly efficient in enhancing the expression of beta-globin gene in HeLa cell line. In their study, they first cloned the rabbit hemoglobin beta 1 gene isolated from rabbit and insert the gene into a recombinant expression plasmid containing SV40 enhancer. The recombinant plasmid containing SV40 enhancer had successfully produced 200-fold more gene transcripts as compared to plasmid without SV40 enhancer (Banerji et al., 1981). Banerji et al. (1981) concluded that this enhancer can improve gene transcription in both orientations and at any positions (1400 bp upstream or 3300 bp downstream from transcription start site) from the rabbit beta-globin gene. Since then, many enhancers in the human genome such as HACNS1, sensory vibrissae enhancer, forebrain subventricular zone enhancer and

penile spine enhancer have been discovered, characterized and deposited in various databases like the VISTA and FANTOM5 (Andersson et al., 2014; McLean et al., 2011; Visel et al., 2007).

The identification of enhancers can be conducted via two main approaches, namely the experimental and the computational approach. The experimental wet lab approach involves reporter assays (e.g. undirected integration of enhancer-reporter vector, enhancer trap and transient transgenesis assay) and high-throughput assays (e.g. STARR-seq and RNA-seq) in search for candidate enhancers that can activate the reporter gene (Arnold et al., 2013; Cao & Yip, 2016; Kvon, 2015; Mello et al., 1991; Patwardhan et al., 2009; Pennacchio et al., 2006; Schwarzer & Spitz, 2014; Visel et al., 2009). The disadvantage of the experimental wet lab approach is that the enhancer is usually tested on a context (in terms of disease states, developmental stages and chromatin states) different from the original context, making it hardly reflected on the 'actual' context in the living system (Cao & Yip, 2016). The computational approach on the other hand enables for a wider scope of enhancer identification on a genome-wide scale in a much shorter period and lower cost. There are various enhancer predictor tools such as GMFR-CNN, CSI-ANN, LS-GKM, DeepBind and iEnhancer-2L (Alipanahi et al., 2015; Firpi et al., 2010; Ghandi et al., 2014; Liu et al., 2015; Wong et al., 2016) that utilizes various gold standards of enhancer (namely histone modifications, transcription binding motifs, evolutionary conservation, DNA methylation and chromatin accessibility) to predict them from the genome (intergenic regions and non-coding regions) with various accuracies achieved (Cao & Yip, 2016; Lim et al., 2018a).

The link between coding regions and onset of numerous genetic diseases had been long established and that of the non-coding regions are picking up their paces. Throughout centuries, the search for the ultimate cure of genetic diseases in human via the genetic approaches such as gene therapy and gene editing faced various challenges and obstacles (Mubiru et al., 2008, 2011; Phillips et al., 2014). One of the major concerns is that human testing is restricted by ethical issues and human rights, therefore model organisms are normally used as test subjects beforehand before administration is to be done onto humans. There are many vertebrate model organisms such as the *Danio rerio* (zebrafish), *Mus musculus* (mouse), *Mesocricetus auratus* (golden hamster) and *Oryzias latipes* (medaka) being well studied to aid in the understanding of vital biological pathways and mechanisms leading to genetic diseases (Dooley & Zon, 2000; Fan et al., 2014; Lin et al., 2016; Perlman, 2016; Wittbrodt et al., 2002).

Recently, biomedical researches have been focusing on the potential of non-human primates as model organisms for disease studies and gene therapies. The advantage of using non-human primates as model organisms is that they share higher similarities with human in terms of genetic

contents, dietary factors, responses to environmental stimuli and even epigenomics (Huang et al., 2015). Besides, they also share physiological resemblances such as cognitive aging, reproduction, cognition, development and neuroanatomy with human (Phillips et al., 2014). Moreover, the primates are important disease models for primate-specific diseases such as AIDS as well as prostate diseases, lung malfunction syndrome and androgen receptor related diseases (Mubiru et al., 2008, 2011; Phillips et al., 2014). On the side note, non-human primates can be divided into a few categories, namely New World, Old World, prosimians, hominoids and Great Apes. Among them, the New World and Old World monkeys are more widely used as primate models (Phillips et al., 2014).

At earlier stages in primate epigenomic field, the functional epigenomic comparison studies among primates are restricted to lymphoblastoid cell lines only (Cain et al., 2011; Shibata et al., 2012; Zhou et al., 2014) and across 20 selected mammals this was conducted on the whole organ (liver) context (Villar et al., 2015). The histone modification H3K27ac (one of the enhancer mark) from different developmental stages of limb was compared across human, mouse and rhesus macaque (Cotney et al., 2013). Then, the FZD8 enhancer in the developing neocortex was examined for human and chimpanzee (Boyd et al., 2015). The iPSC of chimpanzee was also used as a model for the comparisons of neural crest cell enhancers in chimpanzee and human (Prescott et al., 2015). The abovementioned studies had proven that how enhancer variants across primates as well as absence or inactivity of enhancer strongly affected the speciation and divergence process during evolution. The milestones established by these previous studies are the stepping stones for the discovery of more functionally significant enhancers that are primate-specific and evolutionary significant.

A group of researchers had started to work on annotating the proboscis monkey genome and further predicted enhancers from the chromosome 18 of the genome using five different enhancer predictor tools (Omar et al., 2017). In their study, they utilized five different enhancer predictor tools (namely GMFR-CNN, CSI-ANN, LS-GKM, DeepBind and iEnhancer-2L) that use different epigenetic features (such as CTCF, EP300, HSK4me1, H3K4me3 and H3K27ac marks) as benchmark in identifying enhancers from the proboscis monkey genome (Alipanahi et al., 2015; Firpi et al., 2010; Ghandi et al., 2014; Liu et al., 2015; Omar et al., 2017; Wong et al., 2016). Omar et al. (2017) had consolidated the outcomes from the five enhancer predictor tools and found that the utilization of various epigenetic features in enhancer prediction had indeed improved the prediction power in general. Nevertheless, they leave the window open with the statement saying that some other epigenetic marks such as DNase I hypersensitivity, GATA1 and TAL1 are not included in their study, and enhancers in other chromosomes of proboscis monkey are yet to be explored in the future for evolutionary and medical studies contributing to larger scientific discoveries in future.

An interesting study on liver specific enhancers in human across various ethnics by Kim et al. (2011) had shown that the enhancer variants that were found across different ethnics might contribute to differing drug responses and thus this might provide the ultimate solution to the adverse drug events that had caused high mortality in this modern era. It has been widely known that enhancer variants can lead to several genetic diseases (Kleftogiannis et al., 2015), discovering these enhancer variants in endangered ancient primates like the proboscis monkey would greatly drive future conservation research.

In this review, the proboscis monkey was chosen as one of the potential primate candidates because of several unique aspects it possesses that are clearly distinctive from the other non-human primates known to date (which will be discussed in detail in the following sections) and the strong needs to protect it from the brink of extinction.

Proboscis Monkey

The proboscis monkey (*Nasalis larvatus*), also known as the long-nosed monkey, is one of the Asia's largest native monkey species. It is an Old World Monkey belonging to the Cercopithecidae family and it is exclusively endemic to Southeast Asian Island of Borneo. This monkey species is currently listed as endangered by IUCN (Meijaard et al., 2008). This reddish-brown skin-coated monkey can be easily distinguished from other monkeys via their unique morphology and appearance: their large and fleshy nose with growth capacity up to 7 inches in length (Harding, 2013). The proboscis monkey has grey limbs and large pot-shaped bellies. The sexual dimorphism is very distinctive where the size of the male is twice as big as the female in terms of head-body length and weight (Harding, 2013). The proboscis monkey possesses unique external nasal cartilages to support its huge nose (Maier, 2000) and it is the only member in the colobine genus that owns a narrow, cercopithecine-like interorbital pillar (Delson, 1994).

The proboscis monkey commonly survives in groups of females and one dominant male together with their young (Bennett & Gombek, 1993; Boonratna, 1993, 2002). Groups of some males and the all-males group had also been reported (Boonratna, 1999; Murai, 2004). The social interactions in the group of around 9-60 individuals are mostly peaceful with minor aggressions (Bennett & Gombek, 1993; Boonratna, 1999; Yeager, 1992). The natural habitats of the proboscis monkeys are mainly riverine, dipterocarp and swamp forests (Bennett & Gombek, 1993). This monkey is one of the best swimmers among non-human primates despite the fact that they live most of their lives on trees foraging for flowers, insects, leaves and fruits (Boonratna, 1993; Sebastian, 2000). On average, they could live up to fifteen to twenty years (Harding, 2013).

Significance of Proboscis Monkey in Enhancer Studies

The booming effects coming from the rise of the next generation sequencing had pathed the way for the completion of genome

sequencing in various primates (e.g. rhesus monkey, marmoset, bonobo, gorilla and orangutan) (Gordon et al., 2016; Prüfer et al., 2012; The Marmoset Genome Sequencing and Analysis Consortium, 2014). Likewise, there are also some on-going primate genome sequencing projects on drill, mouse lemur, sooty mangabey, gibbon, baboon, white and black colobus, sifaka lemur and owl monkey (Baylor College of Medicine-Human Genome Sequence Center [BCM – HGSC], 2016). Above all, the recently sequenced genome of the proboscis monkey is the one primate genome that is stepping new into the limelight (Abdullah et al., 2014). This primate genome is essential for the opening of a new window for the genome-wide discovery of enhancers for several reasons.

The unique morphological traits of the proboscis monkey are one of the reasons it is viewed as the most primitive primate. From the Pleistocene to the Holocene, the Asian colobines are exposed to extensive adaptive radiation in which they are subjected to a vast range of adaptations and selection pressures in numerous environments with differing altitudes, climates and vegetations (Davies, 1994). These adaptations have interestingly introduced a myriad of diversity in terms of differentiation in the structures of the body and social behaviours, the speciation process that is still progressing till today (Davies, 1994). Peng et al. (1993) had conducted classification of Asian colobines (on 123 skulls) based on 14 characteristics and further deduced the morphology-based evolutionary phylogeny. The traits of colobine monkeys (namely *Nasalis*, *Pygathrix*, *Prebytis*, *Rhinopithecus* and *Presbytiscus* (*Rhinopithecus avunculus*)) are subjected to discriminant, one-way and cluster analyses (Peng et al., 1993). In most of the cases, the dentition, cranial skeleton, cranial morphology as well as the general anatomy are the major discriminant factors among the colobine genera. Moreover, Peng et al. (1993) proposed the possibility of the proboscis monkey of being a primitive based on features it shared with the *Rhinopithecus*: skull structure, highly-distinctive sexual dimorphism, terrestrial movement and proportions of the extremities. Thus, these morphological characteristics of the proboscis monkey may suggest that it may belong to one of the long-isolated genera within the colobines (Giusto & Margulis, 1981; Groves, 1989; Peng et al., 1993). Morphological characteristics such as brain, skull and facial structure are governed greatly by enhancers, and essentially it is believed that these enhancers are vital contributors towards primate evolution. For instance, the human HARE5 (human-accelerated regulatory enhancer) displayed dramatic performance as compared to that of chimpanzee in corticogenesis and neural progenitor cell cycle in developing neocortex; the brain size of transgenic mice is also much bigger with the presence of this enhancer from human in contrast to that of chimpanzee (Boyd et al., 2015). Besides, genes associated with enhancer divergence in both neural crest of human and chimpanzee are enriched with species-biased enhancers, indicating the potential

of enhancers in orchestrating the facial morphological variations between both primates (Prescott et al., 2015). Therefore, the ancient morphological characteristics such as the nose and skull structure of the proboscis monkey is an indication of a unique enhancer landscape that is yet to be explored.

The proboscis monkey is unique among other primates in terms of their digestive physiology. Like other colobine monkeys, the proboscis monkey is a foregut fermenter and it possesses overdeveloped salivary glands that are capable of high saliva production (Bigoni et al., 2003). Their large stomachs are four-chambered and are responsible for cellulose digestion especially in the forestomach where symbiotic microorganisms are abundant in amount. The first two stomach chambers (*presaccus* and *saccus gastricus*) allow for actions of symbiotic microbiota to disintegrate cellulose with saliva as pH buffer whereas the other two stomach chambers (*tubus gastricus* and *pars pilorica*) digest the bacteria using numerous digestive enzymes (Oates et al., 1994). Interestingly, adaptive convergence was found to occur between the lysozyme of colobine monkey and that of the ruminant, thus marking the striking differences between the lysozyme of colobine monkeys and that of the mammalian (including that of human) lysozyme (Stewart et al., 1987). The dietary habits of a host organism have substantial effects on its epigenetic regulations as well as developmental origins of health and diseases (Mochizuki et al., 2017). Generally, the methylation of histones and DNA (the key player in epigenetics) are affected by any bioactive elements or conditions that can influence the AdoHcy (S-adenosylhomocysteine) and AdoMet (methyl donor of methylations) levels in the host (Choi & Friso, 2010). A total of 738 species-specific genes were discovered from the whole genome of the proboscis monkey where genes such as the expanded SusE outer membrane protein (PF14292) and glycogen synthase I (*GYSI*) gene are associated with starch utilization (Tamrin, 2016). In addition, the sweet taste receptor *Tas1r2* gene of the proboscis monkey was found to be greatly diverged from all its other anthropoid primate counterparts, which explains for the dietary shift in this species (Tamrin, 2016). Besides, the gut microbiome also plays part in orchestrating the epigenome (in terms of chromatin modelling and DNA alterations) via the synthesis of low molecular weight byproducts that eventually contribute to the DNA methylation process (Lewis & Tollefsbol, 2017). The unique gut microbiome and dietary of proboscis monkey are another two key reasons for the need for enhancer studies in this species because it is interesting to discover how the dietary habits of ruminant in primates affect the enhancer landscape as whole. This is one of the adaptive traits we wish to explore in endangered wildlife like the proboscis monkey and further improve our comprehension on how this unique epigenome of proboscis monkey is associated with the enhancer landscape they possess (Luo & Lin, 2016; Vogt, 2017).

The karyotypes of mammals and primates were extensively investigated by Müller (2006) in search for the ancestral primate karyotype. In his study, he discovered that the differences between the inferred ancestral mammalian karyotype (2n=2x=46) and ancestral primate karyotype (2n=2x=50) were small with fusions and fissions involving chromosome 4, 8, 10, 12 and 22 (Müller, 2006). Of all prosimians studied, the primitive karyotype is not present, and their karyotypes are highly derived. The karyotype diversity of the New World monkeys is greatly varied with a wide range of chromosome numbers from 2n=2x=16 to 2n=2x=62, the inferred ancestral karyotype for this group is 2n=2x=54. Among the New World monkeys included in the study, only the common marmoset (*Callithrix jacchus*) and Pygmy marmoset (*Callithrix pygmaea*) have chromosome number strongly conserved to that of human, which is 2n=2x=46 and 2n=2x=44 respectively. The hominoids studied depicted diverse karyotype ranging from 2n=2x=38 to 2n=2x=52 with *Hylobates* (2n=2x=44) having the closest karyotype to that of human. All the Old World monkeys investigated generally have strong conserved karyotypes with the exception of African green monkey (*Chlorocebus aethiops*) (2n=2x=60) and *Cercopithecus wolfi* (2n=2x=72). The baboons and macaques share the same chromosome number (2n=2x=42) whereas leaf-eating monkeys like the white and black colobus (*Colobus guereza*) possess chromosomal number of 2n=2x=48. The proboscis monkey was reported to possess chromosome number of 2n=2x=48 which was considered fairly conserved compared to human (Chiarelli, 1966; Soma et al., 1974; Stanyon et al., 1992). In short, in the selection of suitable primate model organism that have conserved karyotype to that of human, the common marmoset and Pygmy marmoset has the highest potential among the New World monkeys whereas the proboscis monkey and the white and black colobus are among the most feasible Old World monkey candidates. Comparing the chromosome of the proboscis monkey with that of human, a reciprocal translocation followed by pericentric inversion had led to the events of fragmentation and association of human chromosome 1 and 19 onto chromosome 5 and 6 of proboscis monkey (Bigoni et al., 2003). Chromosomal translocation can sometimes change gene expression and enhancer functioning that favours the overexpression of oncogene when oncogene is proximity to strong enhancer of other gene, thus causing cancer (McNeil et al., 2003). The chromosomal dissimilarities between human and proboscis monkey, when added with the knowledge on the enhancer landscape of this non-human primate, would be a big advantage for us to discover the adaptive traits of this wildlife. Moreover, this is also essential for future decisions on the best effective medical treatments and optimal drug dosage for species conservation.

FUTURE PERSPECTIVES

The genome-wide enhancer identification in proboscis monkey is a trend we foresee happening in the near future as we discussed in the previous sections. From its various morphological characteristics that suggest that it is one of the most primitive primates belonging to a long isolated genera, to its one-of-its-kind cytogenetics as well as its dietary habits, these had spiked our interest in understanding the epigenetic and enhancer landscape of this species. -

The importance of enhancer landscape discovery in the proboscis monkey can be seen in terms of how they can be used to aid in conservation measures and disease treatment in future. Now that the genome of the proboscis monkey had been sequenced, we can finally conduct an epigenome comparison between this species and that of human to discover the uniquely ancient and unevolved enhancer landscape. This serves as an important milestone to identify the enhancer variants in proboscis monkey that are disease-causing and further plan on the strategies to conserve this species via conservation research like antibody synthesis against elephant endotheliotropic herpesvirus (EEHV) (Kochagul et al., 2018) and pathogen combatting in white-nose syndrome in bats (Palmer et al., 2018). Besides, the proboscis monkey enhancer landscape is useful in the understanding of the adaptive phenotypic traits that occur in the environment for displaced wildlife (Luo & Lin, 2016; Vogt, 2017) for more effective conservation measures and strategies in future.

In a nutshell, the potential of the proboscis monkey in the role of providing us with the most primitive enhancer landscape is undeniably huge in the near future. It is now essential for consolidation of efforts in the discovery of enhancers from the genome of the proboscis monkey and further characterize them functionally to enhance our understanding on the onset and treatment of some genetic diseases accounted by enhancer variations across the proboscis monkey and that of human.

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