# XXX

## Introduction

XXX

## Material and Method

The elephant sampled in this study were in the Asian … elephant (Elephas maximus ~~maximus, Linnaeus, …~~), which are actually present in the … part of Thailand. In this study, 33 adult and healthy individuals are selected for sample collection. Among them X are male and X are female. As the studied aims to investigate the SNPs ~~responsible~~ of the development of Ivory, the presence of tusk are … visually and then recorded (Table SX). With a collaboration with the … farm, …, Thailand, the blood samples were collected by … X ml of blood for each individual. All … of the animals were conducted with respect the guidelines provided by the Institutional Animals Ethics Committee (IAEC). Once the biological samples were collected, they are stored at -X° C until genomic DNA extraction.

The DNA extraction were isolated using the salting out method (Miller et al. …). Polymerase chain reaction were performed according to the following parameter : … . The amplified DNA were … at a concentration of … . Gel electrophoresis were then performed to check … of the obtained DNA using a … % agarose gel. The ddRAD sequencing library was prepared to selectively target a … target site on the genome by using the … and the … enzyme and the … adapter. The DNA sequencing were performed following the … instructions which provided fastq sequence file that will be used in the next step.

Sequence quality parameters were performed using FastQC (ref), and only the reads having length greather than … bp were selected using the … software (ref). The sequences were trimmed using the Trimmomatic version … (ref), and then aligned to a reference genome i.e. *Elephas maximums* (Linnaeus …) (ref) available in NCBI (AN …, accessed on …) and alignment index were built using BWA (ref). The obtained map file were in the S… (SAM) (ref) format, which were converted to … (BAM) (ref) format using Samtools version … (ref), and ultimatelly sorted.

The obtained map file were used to call SNPs using the STACKS pipeline version 1.9 (ref). The ref\_map.pl utility was used to create the … of SNP loci. ~~First, sequences aligned to the same genomic location were stacked together and merged to form loci. Loci with a sequencing depth of three or more reads per individual were retained and catalogues have been created. SNPs at each locus were selected using a maximum likelihood frame- work.~~ Then the populations program was called from the “…” toolkit using the following parameter : 0.70 for the minimum samples per population, 0.05 for the minimum m… allele frequencies, 0.7 for the max observed heterozygosity. The STACKS pipeline returned a variant calling file (VCF) as an output and further SNP ~~statistical~~ analysis were done with those files. The “gen” utility from Plink version … (ref) were used to generate the SNP … summary data from the VCF file. By editing the “…” file provided by the “gen” utility, the trait data of each individual were … to the PLINK pipeline. The … test were performed using the “fisher” … test from the PLINK toolkit. The fisher … summary data were used to plot the Manhattan plot highlighting the trait-associated loci.

By using the VCF Tools version … (ref), all SNPS present on the X, Y chromosomes and mitochondrial DNA were removed and were not studied in the further step. Minor allele frequency (MAF < 0.05), missing genotypes (0.8) and HWE deviation (P < 0.001) filtering was done using PLINK version … to only … high-quality SNPs for … .

~~Genome annotation were performed using the … software version …~~

~~The distribution of those SNPs were studied comparatively on the exonic, intronic or expressed region.~~

~~Phylogenetic relationship between individuals were studied by using the Neighbor joining (NJ) algorihm by choosing a bootstrap value of X.~~

~~Genotyping,~~

~~Map nucleotide marker~~

~~Aligned sequence~~

~~On a reference genome~~

~~At the … Institute of … , Bangkok, Thailand,~~

~~Genetic improvement was carried out ???~~

~~Individual animals were selected from unrelated pedigree and … for their trait, …~~

~~Tusk, ivory quality,~~

~~Density, size~~

~~The trait under studied can be observed visually, and recorded in a …~~

~~Both male and female were sampled, as the tusk only develop in adult elephant, all individuals have enough maturity to have their tusk be developed.~~

~~We selected genotypes for animals (~~*~~n~~* ~~= 33)~~

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## Results

The … data includes the sex, the hair type (long or short) and the presence of tusk or not (Table SX).

In this study a total of … SNP were … from 33 individuals of elephant from the ddRAD sequences.

The total number of haplotypes were …

… SNPs were distributed among the elephant sequenced …, withit, … were found in X individuals having developed tusk and X were found in tuskless specimen.

Maximum number of SNPs were on chromosome 1 (as it is the largest chromosome in the *Elephas maximus* genome), and the chromosome X have the less number of SNPs.

The SNPs have an average relative density of X loci/Mbp were found (Fig X; Table X).

Among all X chromosomes, no significant accumulation of SNPs were found on any particular chromosome.