

# 披毛犀线粒体基因组的获取和分析

Acquisition and analysis of mitochondrial genome of Woolly rhinoceros

汇报人：刘卿梅

组员：刘氏河 孙媛媛 周正

Background

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Future Plan

## Background



Charles R. Knight, 'Men of the Old Stone Age'



Guérin, 1989; Prothero et al., 1989

披毛犀（*Coelodonta antiquitatis*）又名 *Woolly Rhinoceros*。是一种已天绝的犀牛，从更新世早期出现，在亚洲地区进化，并于更新世末期天绝。[1]

披毛犀鼻端有两只由角蛋白构成的角，前端角大约长24英寸，用于推开雪吃草。毛长、耳细、脚短厚，身体敦实。[2]

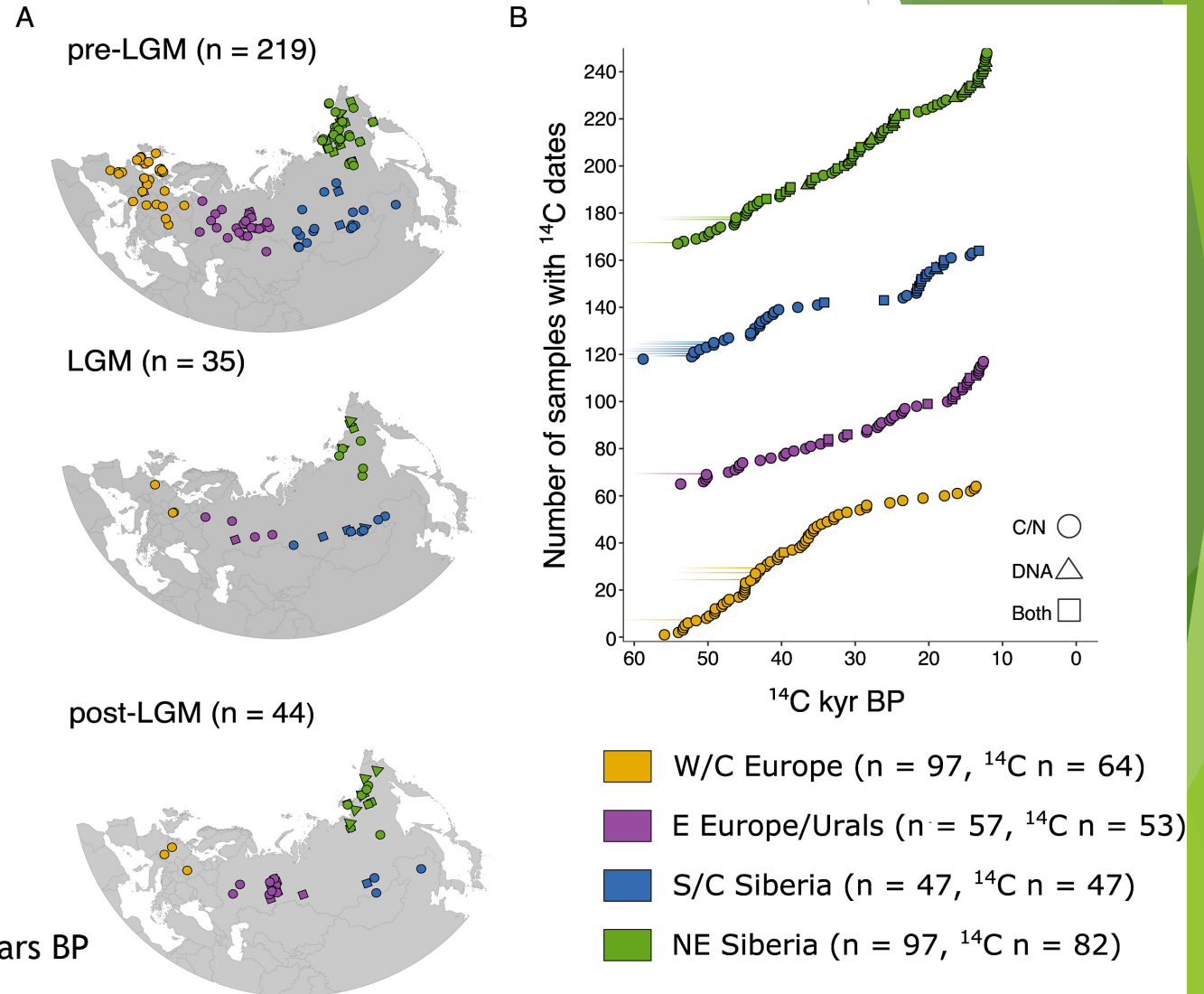
[1] AlbaRey-Iglesia etc, Quaternary Science Reviews, 2021

[2] R. Dale Guthrie, 'Frozen Fauna of the Mammoth Steppe'

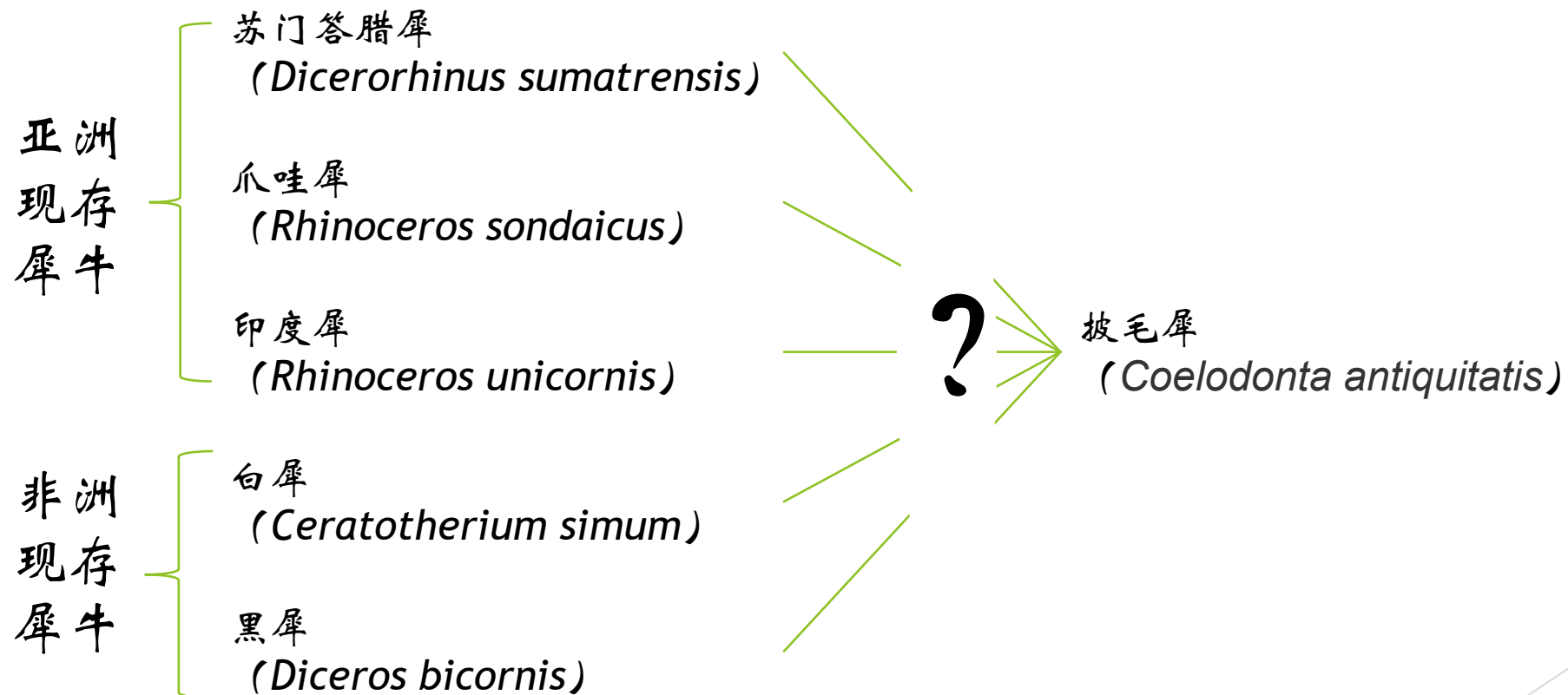
# Background

化石证据表明，披毛犀主要分布在欧洲和西伯利亚地区。直到公元前16000年之前，长毛犀仍然存在于欧亚大陆的大部分地区。从公元前14600年开始，该物种的范围已经缩小，因此仅在乌拉尔山脉、西伯利亚南部和西伯利亚东北部发现了披毛犀。

pre-LGM: >24,600 <sup>14</sup>C years BP/28,660 cal years BP  
LGM: 24,600-17,000 <sup>14</sup>C years BP/28,660-20,520 cal years BP  
post-LGM: <17,000 <sup>14</sup>C years BP/< 20,520 cal years BP



## Background



# Background

Cell



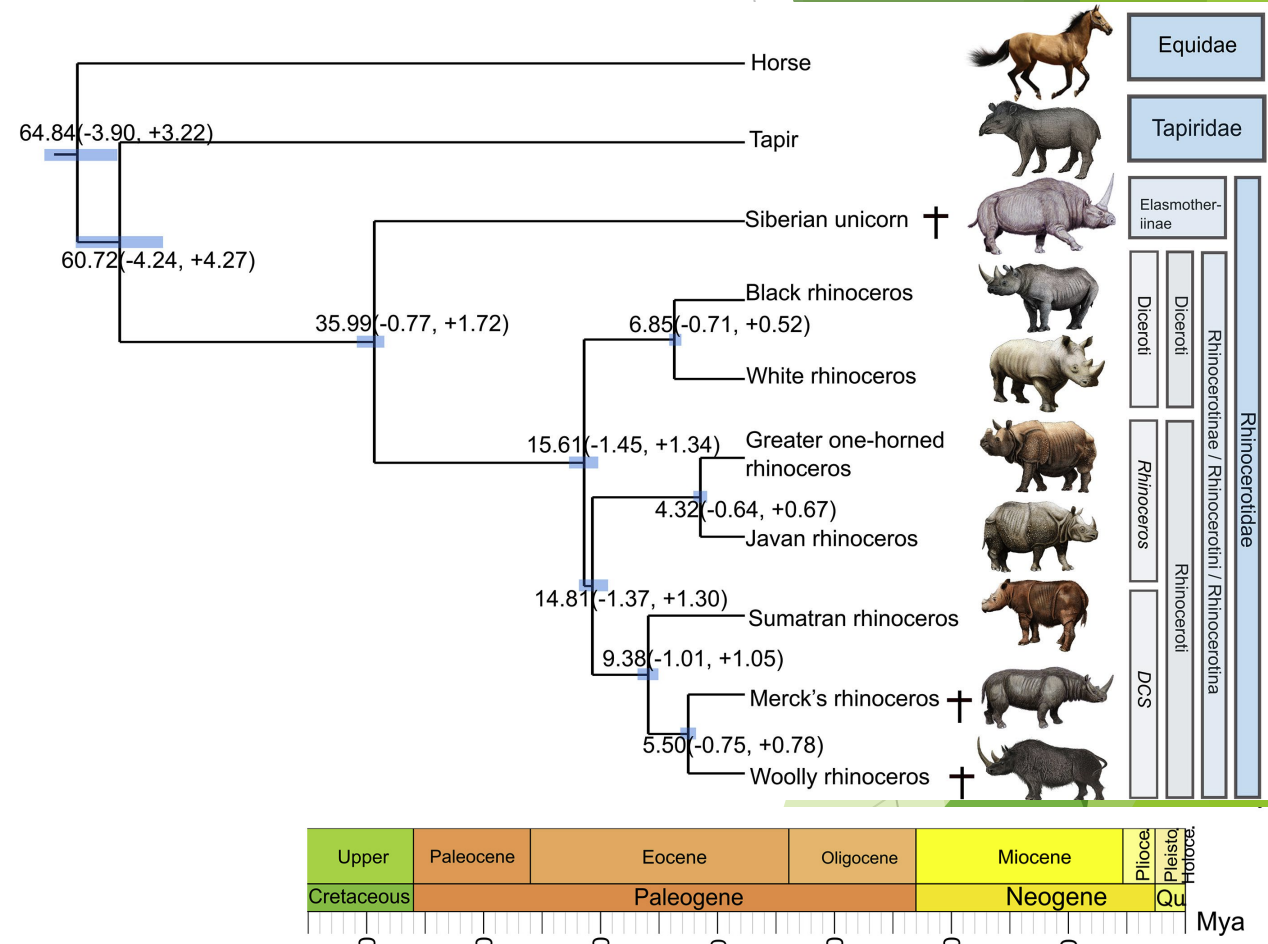
Volume 184, Issue 19, 16 September 2021, Pages 4874-4885.e16

Article

## Ancient and modern genomes unravel the evolutionary history of the rhinoceros family

Shanlin Liu<sup>1, 2, 37</sup>, Michael V. Westbury<sup>2</sup>, Nicolas Dussex<sup>3, 4, 5</sup>, Kieren J. Mitchell<sup>6</sup>, Mikkel-Holger S. Sinding<sup>2</sup>, Peter D. Heintzman<sup>7</sup>, David A. Duchêne<sup>2</sup>, Joshua D. Kapp<sup>8</sup>, Johannavon Seth<sup>3, 4, 5</sup>, Holly Heiniger<sup>6</sup>, Fátima Sánchez-Barreiro<sup>2</sup>, Ashot Margaryan<sup>2</sup>, Remi André-Olsen<sup>9</sup>, Binia De Cahsan<sup>2</sup>, Guanliang Meng<sup>10</sup>, Chentao Yang<sup>10</sup>, Lei Chen<sup>11</sup>, Tom van der Valk<sup>12</sup> ... M. Thomas P. Gilbert<sup>2, 35, 36</sup>

A



# 披毛犀是如何灭绝的？

- 人类捕猎？
- 气候变化？

## Background

› [Curr Biol. 2020 Oct 5;30\(19\):3871-3879.e7. doi: 10.1016/j.cub.2020.07.046. Epub 2020 Aug 13.](#)

### Pre-extinction Demographic Stability and Genomic Signatures of Adaptation in the Woolly Rhinoceros

Edana Lord <sup>1</sup>, Nicolas Dussex <sup>2</sup>, Marcin Kierczak <sup>3</sup>, David Díez-Del-Molino <sup>4</sup>, Oliver A Ryder <sup>5</sup>, David W G Stanton <sup>6</sup>, M Thomas P Gilbert <sup>7</sup>, Fátima Sánchez-Barreiro <sup>8</sup>, Guojie Zhang <sup>9</sup>, Mikkel-Holger S Sinding <sup>10</sup>, Eline D Lorenzen <sup>8</sup>, Eske Willerslev <sup>8</sup>, Albert Protopopov <sup>11</sup>, Fedor Shidlovskiy <sup>12</sup>, Sergey Fedorov <sup>13</sup>, Hervé Bocherens <sup>14</sup>, Senthilvel K S S Nathan <sup>15</sup>, Benoit Goossens <sup>16</sup>, Johannes van der Plicht <sup>17</sup>, Yvonne L Chan <sup>2</sup>, Stefan Prost <sup>18</sup>, Olga Potapova <sup>19</sup>, Irina Kirillova <sup>12</sup>, Adrian M Lister <sup>20</sup>, Peter D Heintzman <sup>21</sup>, Joshua D Kapp <sup>22</sup>, Beth Shapiro <sup>23</sup>, Sergey Vartanyan <sup>24</sup>, Anders Götherström <sup>25</sup>, Love Dalén <sup>26</sup>

Affiliations + expand

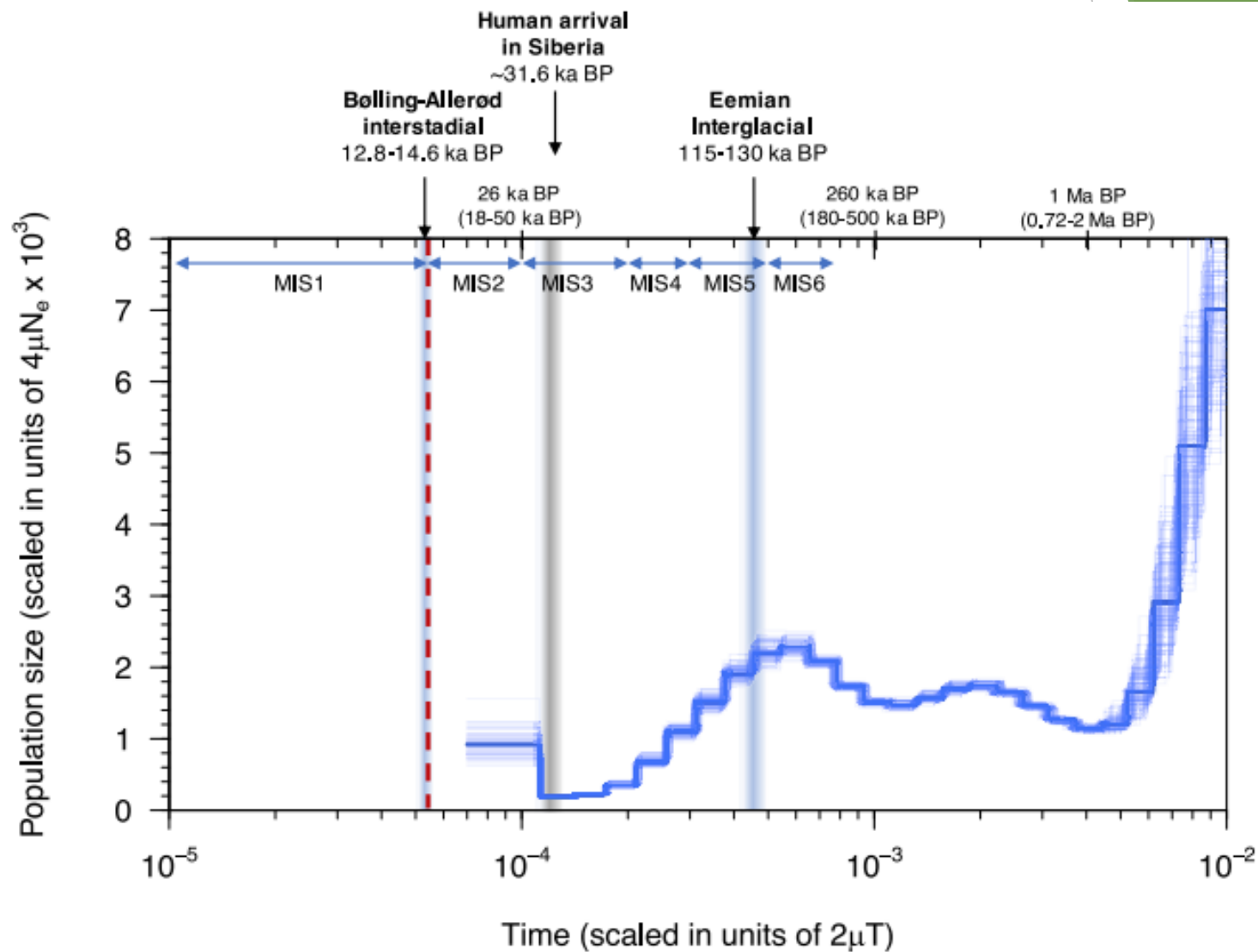
PMID: 32795436 DOI: [10.1016/j.cub.2020.07.046](#)

获得了第一个完整的披毛犀核基因组和14个线粒体基因组。  
使用PSMC分析对披毛犀的有效种群大小变化进行推测。



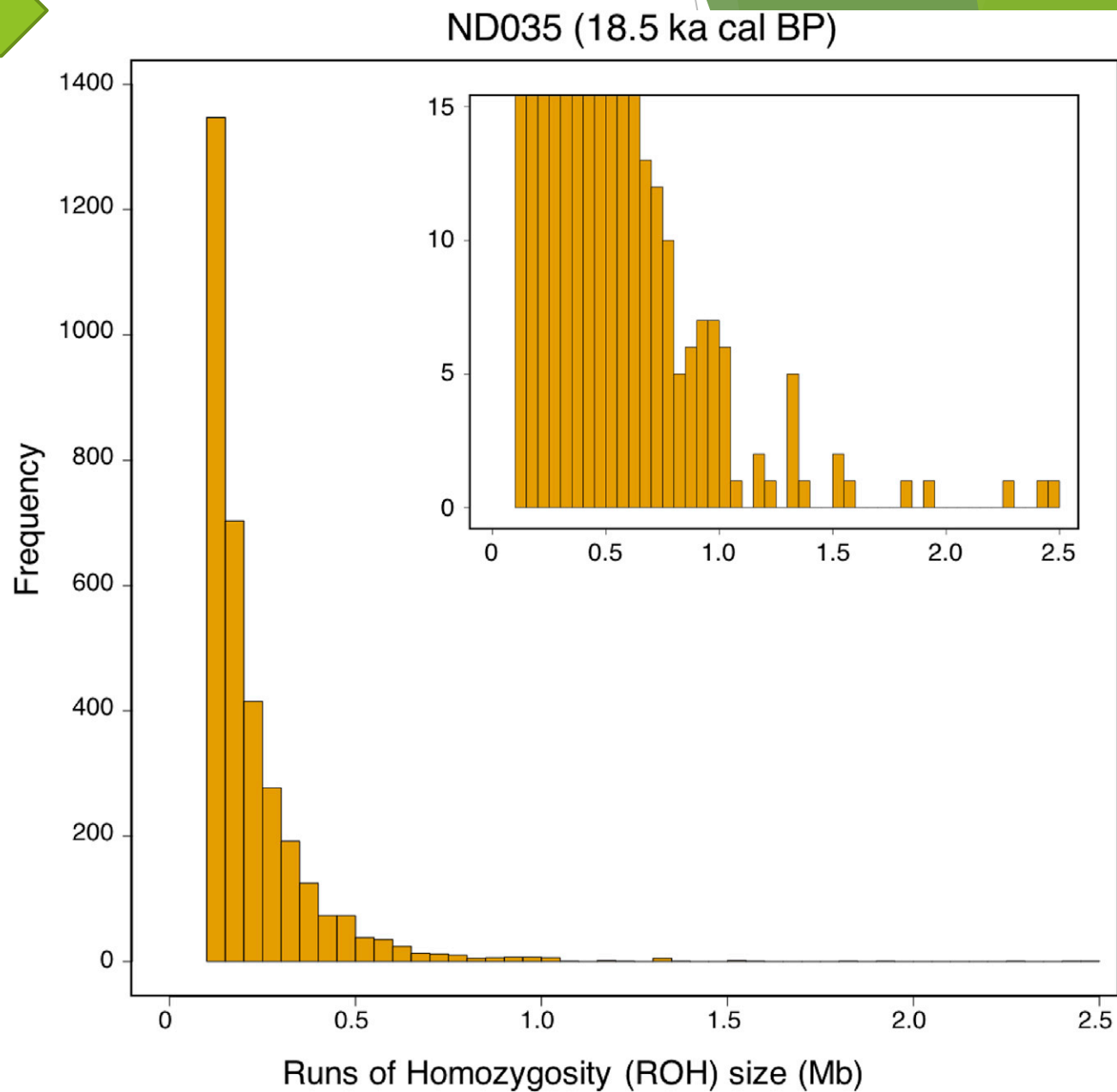
# Background

使用PSMC方法推导披毛犀随时间的有效种群大小的变化



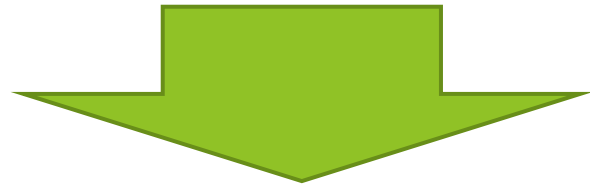
# Background

披毛犀纯合片段的频率分布



## Background

PSMC 没有显示种群规模下降  
没有小种群典型的近亲繁殖水平升高现象



人类来到西伯利亚与披毛犀种群数量下降没有关联；  
至少18.5kya前披毛犀的种群规模是稳定的；  
灭绝是迅速的；  
披毛犀的灭绝主要是由气候和植被的变化导致；

为了更好地了解灭绝的时间和速度，需要对更接近灭绝事件的个体进行额外测序！

## Methods

获得化石样品，根据  
形态学进行初步鉴定



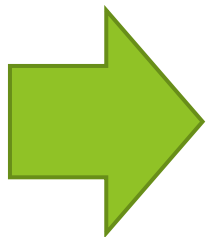
打磨样品，暴露内层的骨  
质结构，并砸碎成小块



加入proteinase K和EDTA等  
消化样品，释放DNA



使用柱吸附纯化法  
纯化获得DNA



Partial UDG 处理

NGS 建库

不处理

## Methods

NGS (Next-Generation Sequencing) 即二代测序技术。与传统的一代DNA测序技术 (Sanger Sequencing) 相比, 拥有高通量的优点。

NGS通过携带荧光基团的dNTPs表征碱基种类, 通过桥式PCR反应边合成边测序。

但由于在测序长度较长时, 荧光的读取和PCR合成不可避免地会产生位移造成测序错误, 因此NGS的测序长度一般为几百bp。近年来开发出第三代测序技术, 测序长度更长 (超过100kbp) 速度更快, 但测序精确度较低。

由于古代DNA样品在几万年的时间中大部分降解, 一般为30-60bp, 因此采用二代测序技术的PE150, 即双端各测150bp。

## Methods

获得测序数据后对测序结果进行质控、处理、分析

使用fastqc进行质控



使用AdapterRemoval去接头



对参考基因组进行环化



获得披毛犀线粒体基因组序列



使用Schmutzi计算污染



使用bwa比对到参考基因组

## Results

### NERH002.2.1

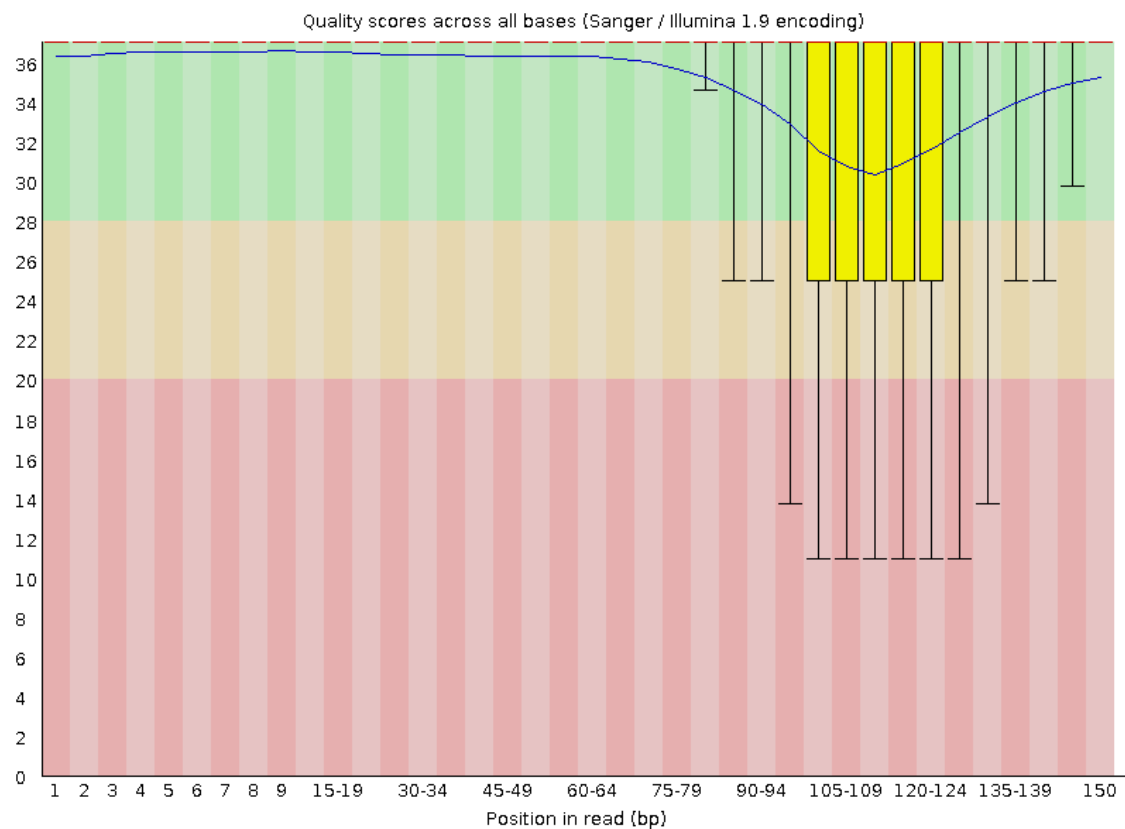
- 时间：约更新世
- 地址：黑龙江河边，无法确定位置
- Total Reads: 136873423
- Mapped Reads: 28375010
- Endogenous DNA: 22.04%
- Coverage: 0.29X
- Mean coverage: 0.42X
- Total Reads: 136873423
- Mapped Reads: 31370775
- Endogenous DNA: 23.31%
- Coverage: —
- Mean coverage: 0.4X

使用samtools flagstat、depth功能计算

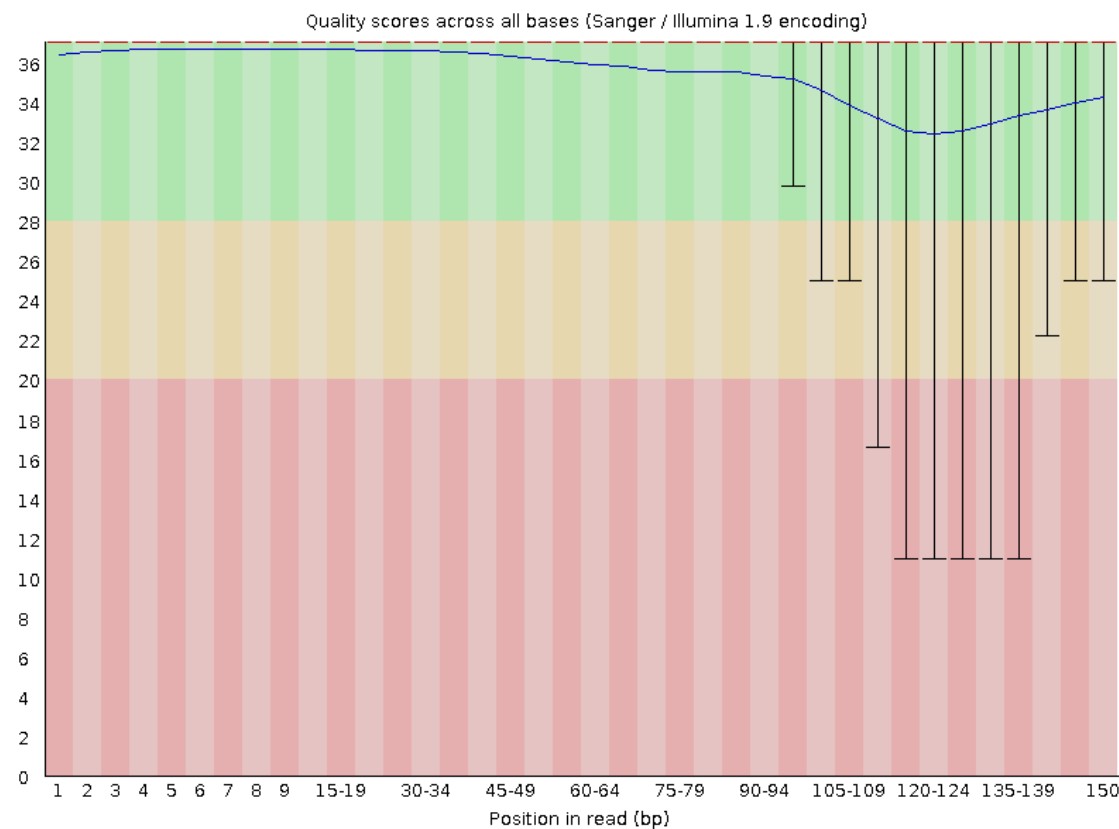
使用eager pipeline

# Results

## NERH002.2.1\_1



## NERH002.2.1\_2

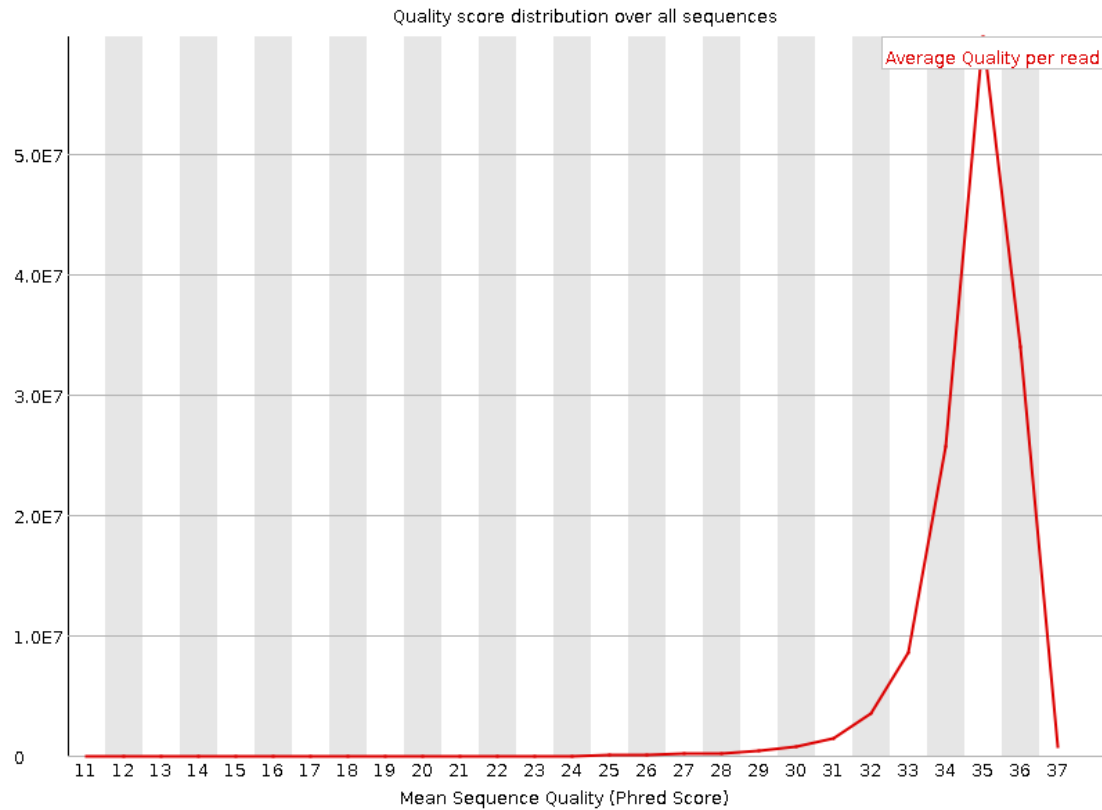


Per base sequence quality

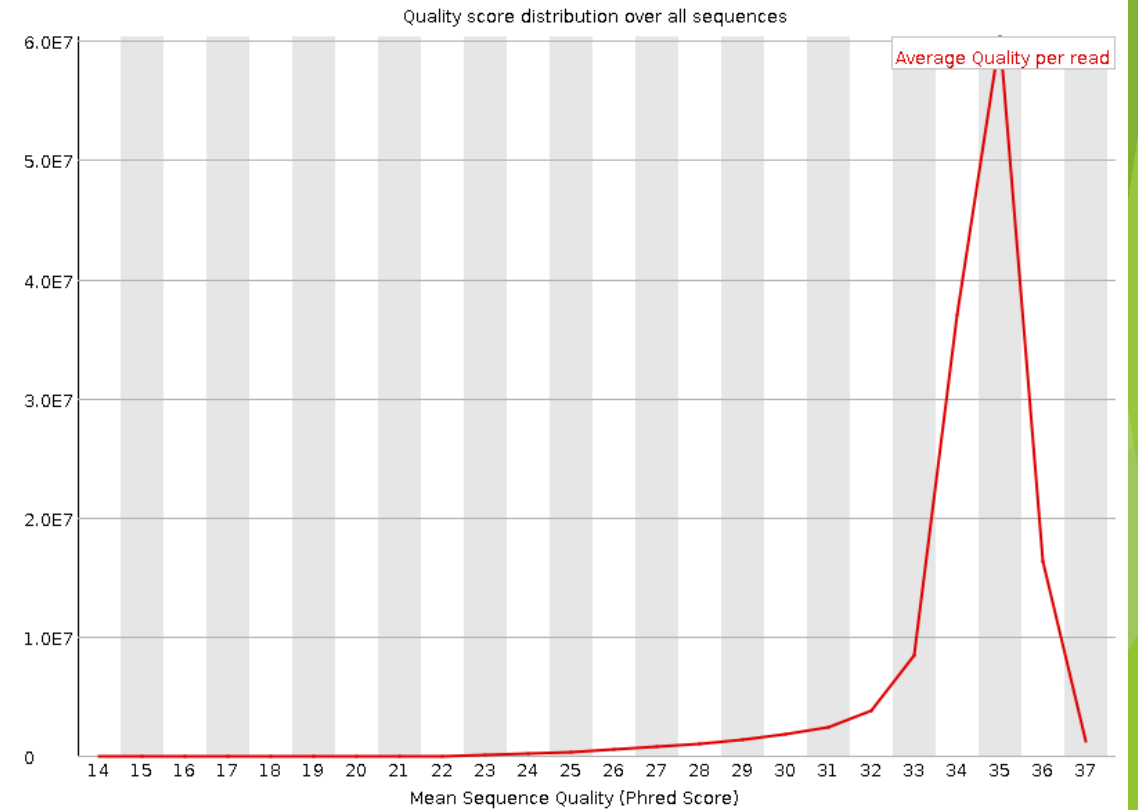


# Results

## NERH002.2.1\_1



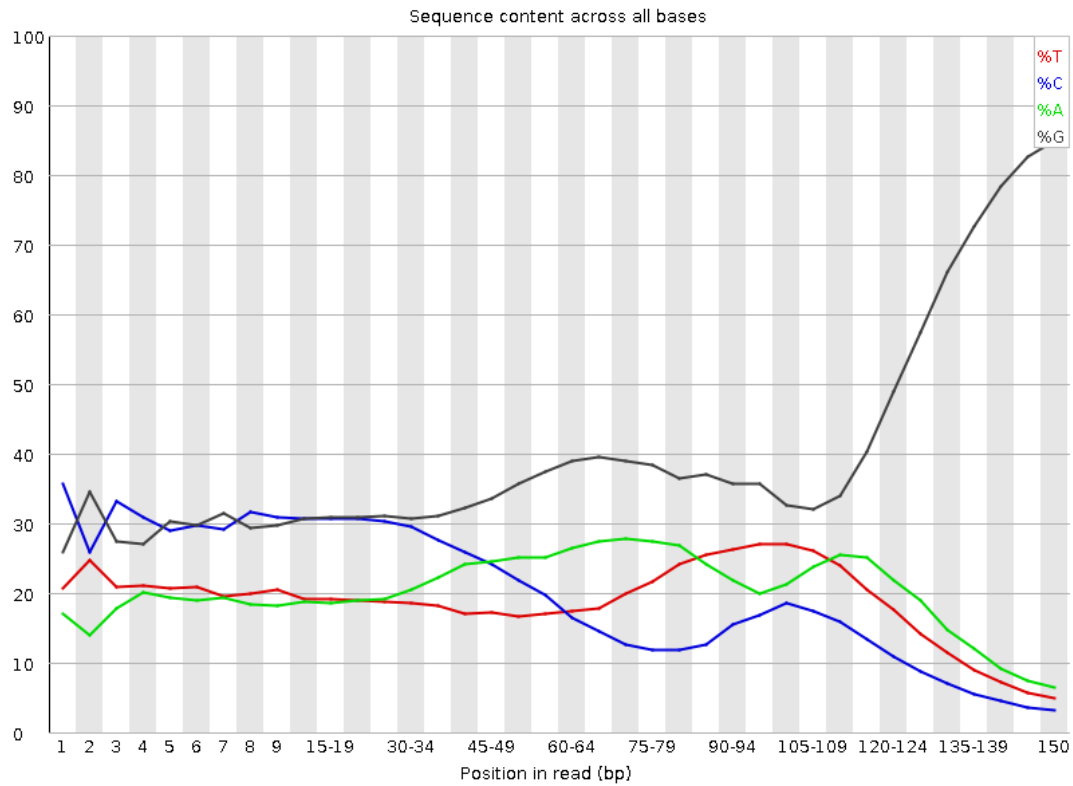
## NERH002.2.1\_2



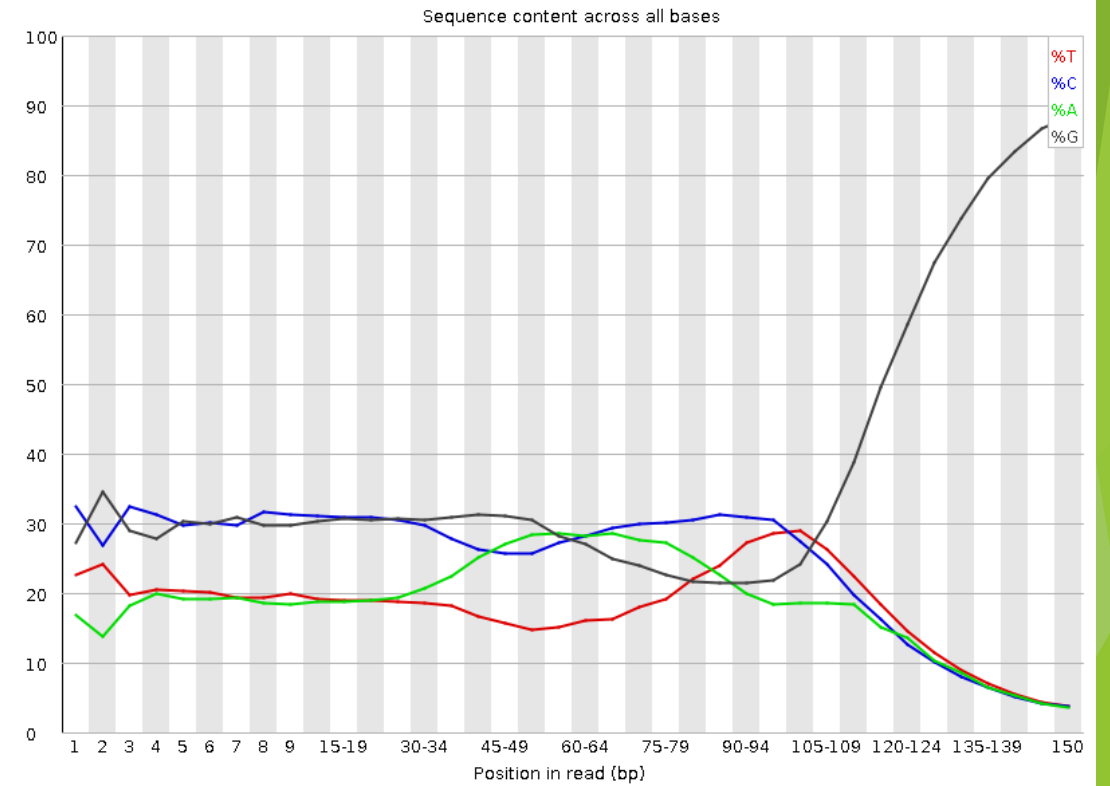
Per sequence quality scores

# Results

## NERH002.2.1\_1



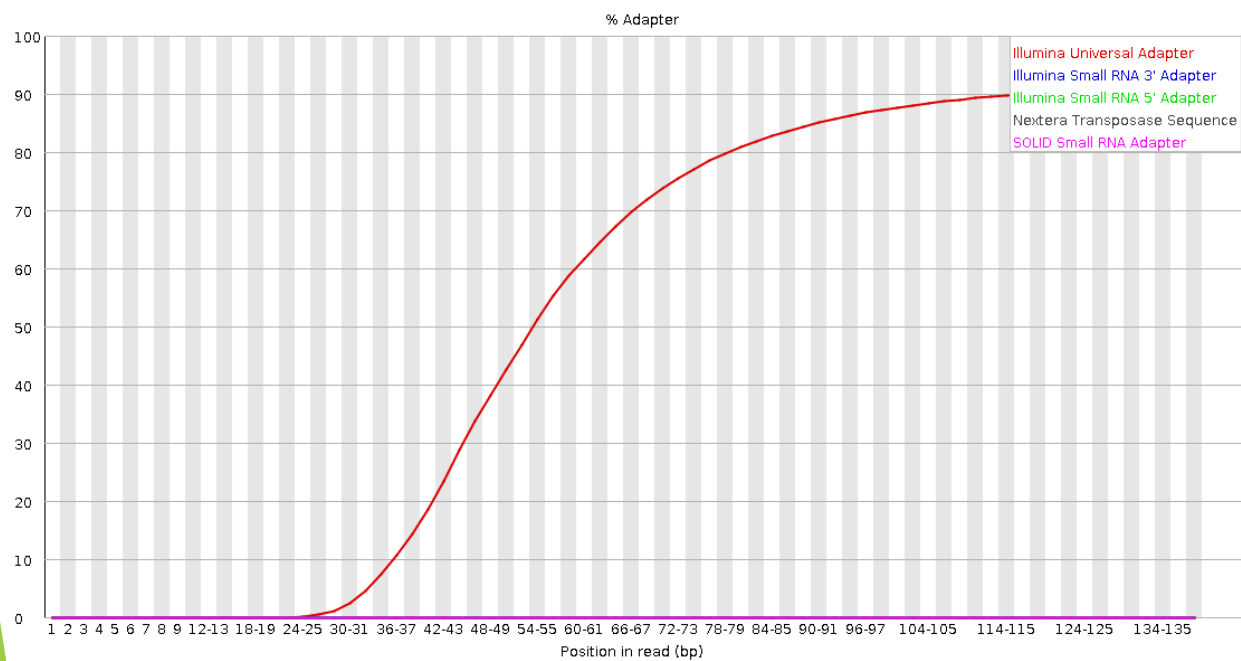
## NERH002.2.1\_2



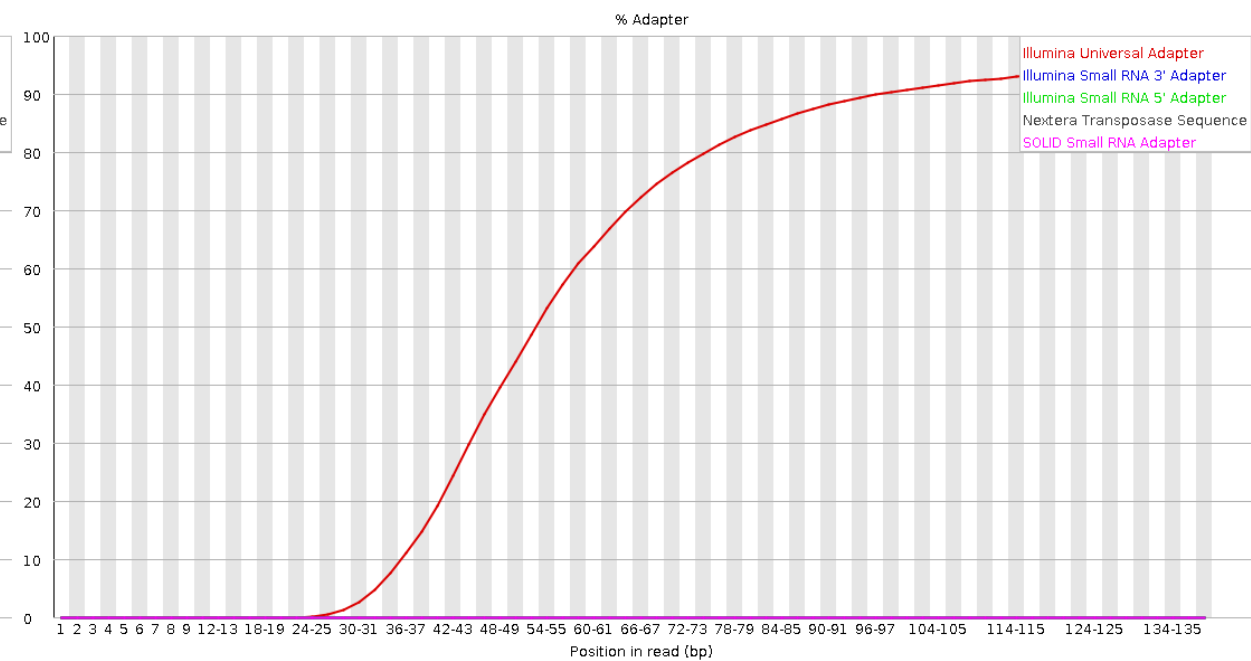
Per base sequence content

# Results

## NERH002.2.1\_1

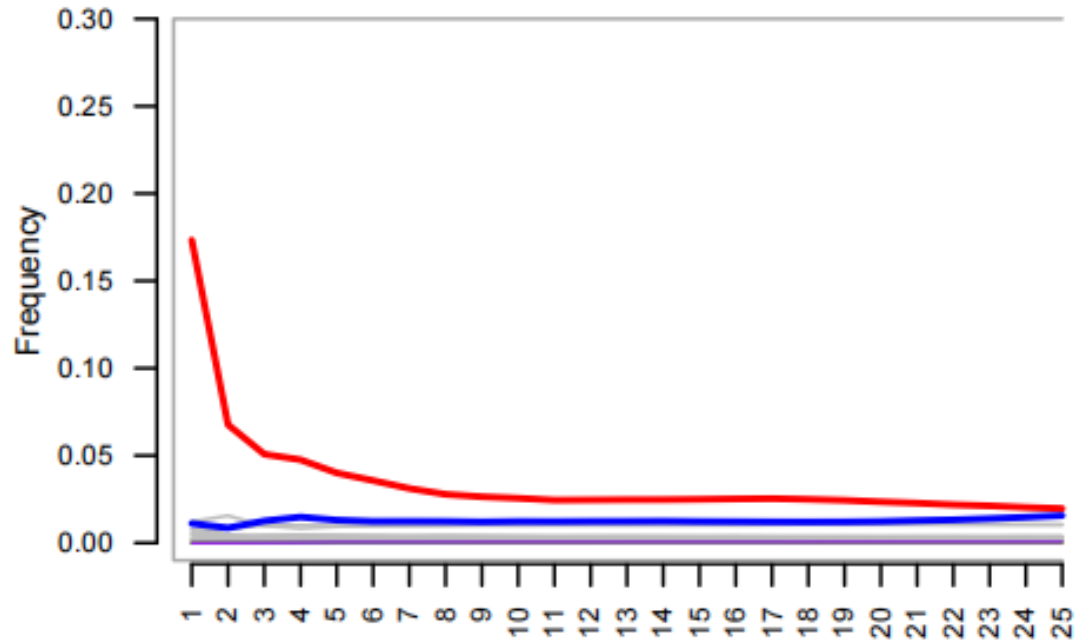


## NERH002.2.1\_2

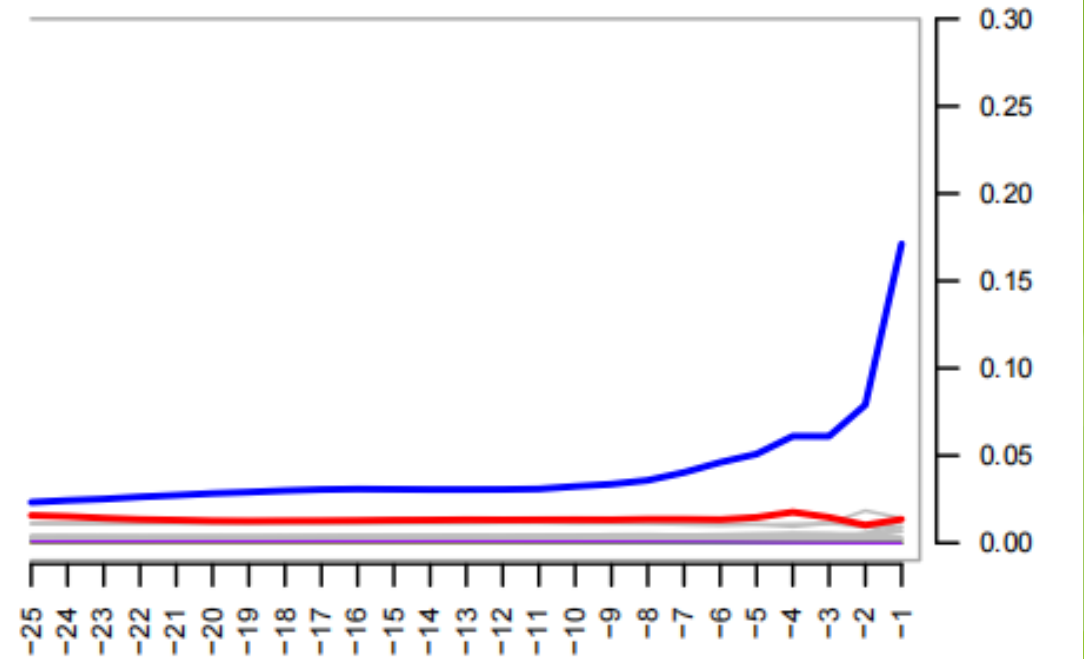


## Adapter Content

# Results



5' C to T misincorporation



3' A to G misincorporation

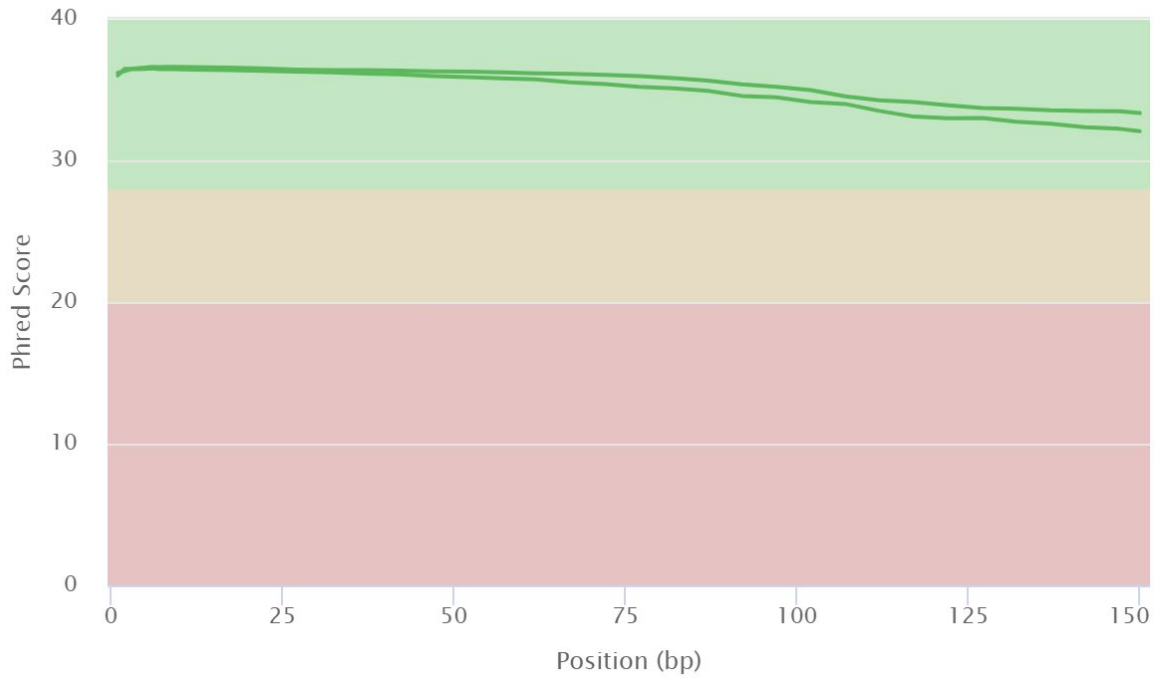
## Results

### NERH002.3.1

Sample Name	Nr. Reads Into Mapping	Nr. Mapped Reads	Endogenous DNA (%)	% Dup. Mapped Reads	5 Prime C>T 1st base	5 Prime C>T 2nd base	Mean Length Mapped Reads	Nr. Dedup. Mapped Reads	Mean cov	Median cov	% GC Dedup. Mapped Reads
NERH002.3.1	13,635,584	30,672	0.22	15.4%	6.9%	2.9%	64.21bp	25,941	0.0X	0.0X	48%

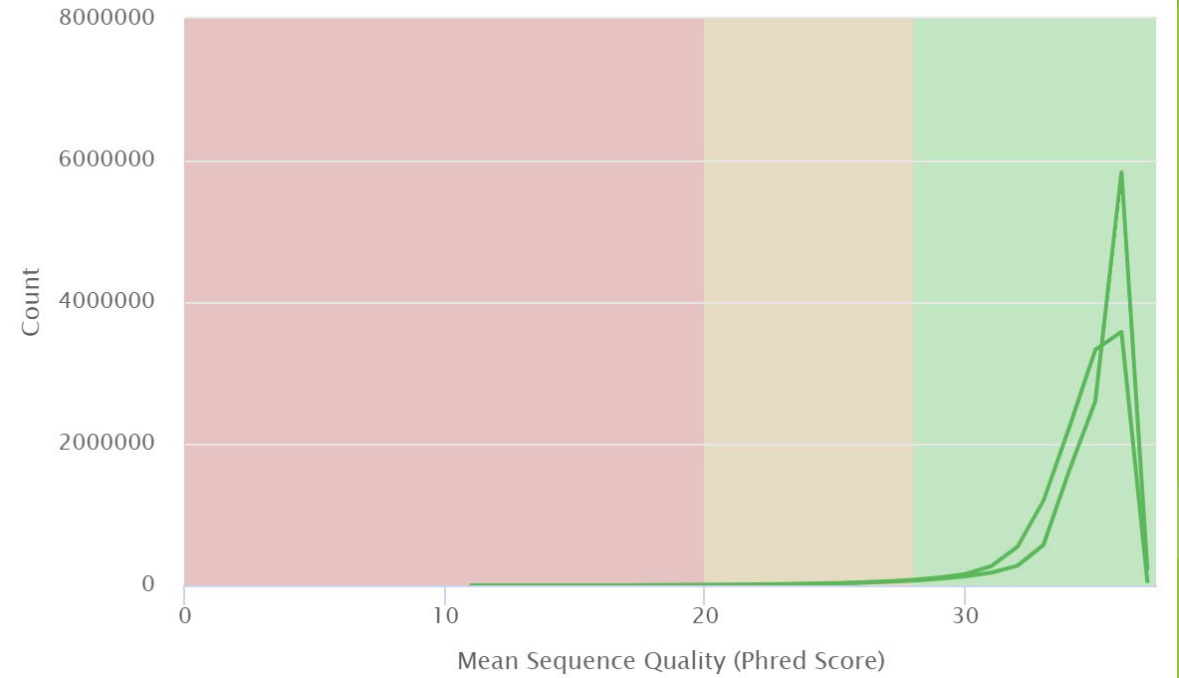
# Results

FastQC: Mean Quality Scores



Created with MultiQC

FastQC: Per Sequence Quality Scores

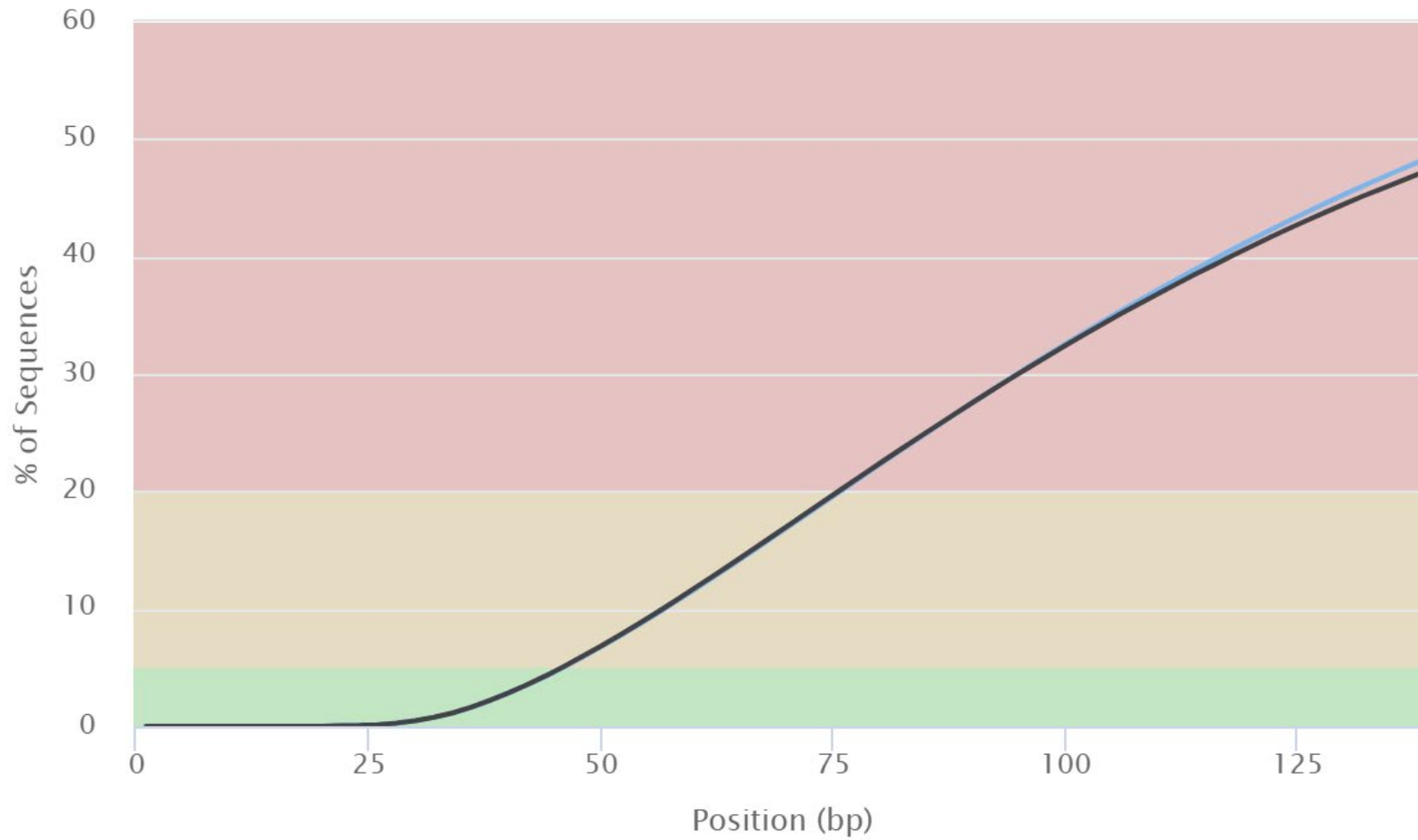


Created with MultiQC

Quality: ✓

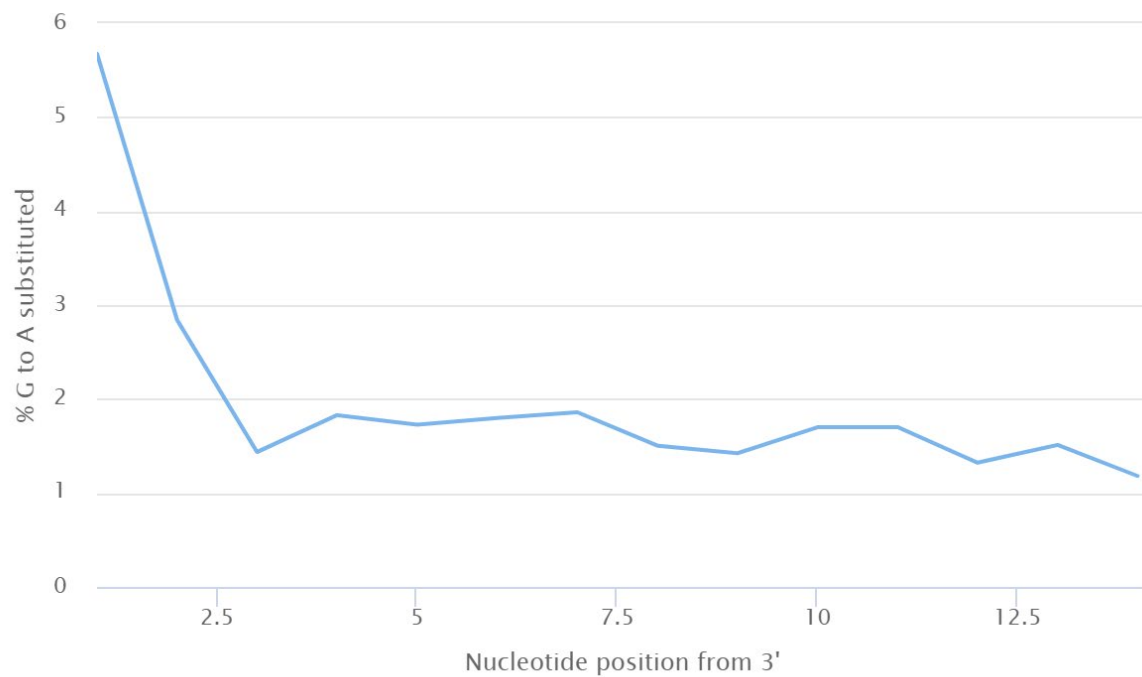
# Results

## FastQC: Adapter Content



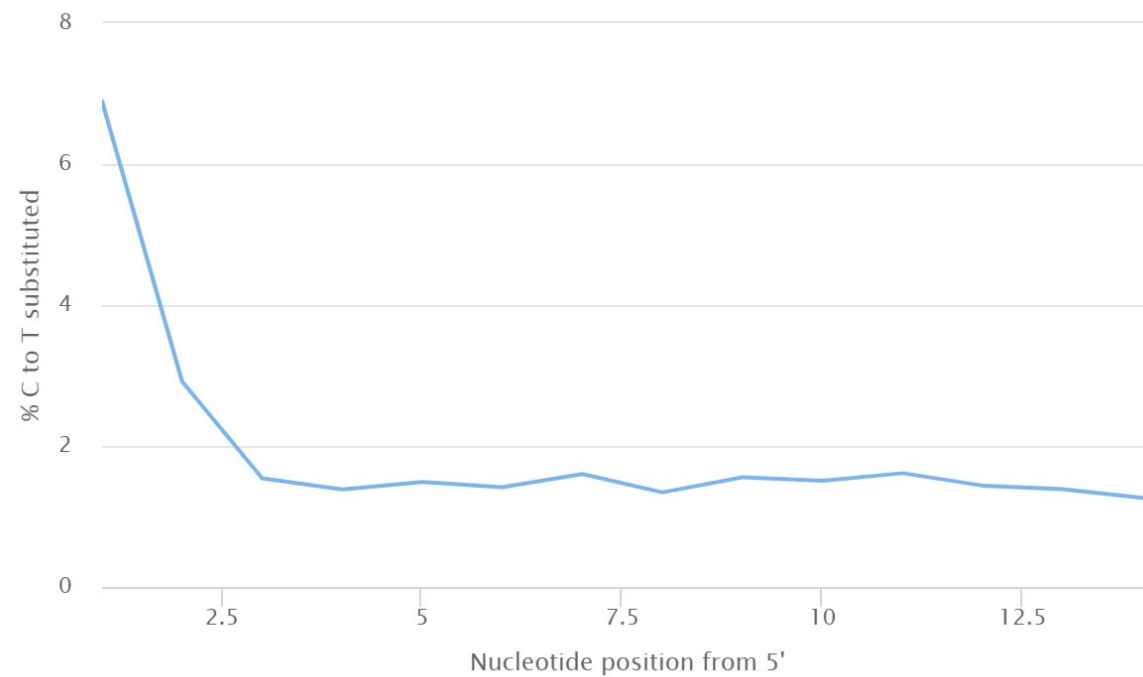
# Results

DamageProfiler: 3' G>A misincorporation plot



Created with MultiQC

DamageProfiler: 5' C>T misincorporation plot



Created with MultiQC



## Results Summary

- ◆ 成功获得了古代样品的DNA
- ◆ 建库并获得了测序结果
- ◆ 获得了样品的线粒体基因组，鉴定为披毛犀

## Future Plan

- 使用BEAST对NERH002.2.1数据与Lord文章中的线粒体基因组进行建树
- 对NERH002.2.1数据与Lord文章中的线粒体基因组构建单倍型网络

THANKS  
FOR LISTENING