**Supplementary Experiment**

**Process of Experiment** A dataset contains 600 homologous sequences selected randomly from mt genome (Tanaka *et al.*, 2004) dataset is used to execute 600 center-star MSA, which use every sequence in the set as center. Then the results of these experiments are quantitatively assessed as raw score, here, SP score (Zou *et al.*, 2009) is used.

**Transformation of SP score to Z score** SP score was normalized and transformed into a z-score. The formula is as following:

Given,

[Sample SP score mean]

[Sample SP score std. dev]

Then,

The distribution of these normalized SP scores is shown in Supplementary Figure 1.

Distribution%20of%20SP.pdf

**Supplementary Figure 1** The distribution of normalized SP score

When a MSA is carried out with a random selected center sequence, the probability of Z-score less than 4, which means the SP score is less than 17063, is 0.9983. The SP of the result of MAFFT and KAlign is between 16500 and 17000.

**Bibliography**

Tanaka,M. *et al.* (2004) Mitochondrial Genome Variation in Eastern Asia and the Peopling of Japan Mitochondrial Genome Variation in Eastern Asia and the Peopling of Japan. *Genome Res.*, 1832–1850.

Zou,Q. *et al.* (2009) An Algorithm for DNA Multiple Sequence Alignment Based on Center Star Method and Keyword Tree. *Acta Electron. Sin.*, **38**, 1746–1750.