## Simulating GBS data for pooled samples

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Nei and Li's (1979) measure of nucleotide diversity,  $\theta_{\pi}$ , is calculated using the following equation:

$$\theta_{\pi} = \sum_{ij} x_i x_j \pi_{ij} \tag{1}$$

Here,  $x_i$  and  $x_j$  represent the frequencies of the *i*th and *j*th unique sequences respectively and  $\pi_{ij}$  represents the proportion of divergent sequence between the *i*th and *j*th unique sequences.

If I assume that all lines will be unique sequences as af assumption if whole genomes are considered then the above equation can be expressed as follows:

$$\theta_{\pi} = \frac{1}{n^2} \sum_{ij} \pi_{ij} \tag{2}$$

Then, since the number of total pairwise combinations between n sequences is  $\binom{n}{2}$ , we can calculate  $\bar{\pi}$ , the mean proportional sequence divergence between any two sequences, as such:

$$\bar{\pi} = \frac{\sum_{ij} \pi_{ij}}{\binom{n}{2}} \tag{3}$$

Some simple arithmetic gives us...

$$\sum_{ij} \pi_{ij} = \binom{n}{2} \bar{\pi} \tag{4}$$

Now I insert this into equation 2:

$$\theta_{\pi} = \frac{1}{n^2} \binom{n}{2} \bar{\pi} \tag{5}$$

Solving for  $\bar{\pi}$  yields the following:

$$\bar{\pi} = \frac{\theta_{\pi} n^2}{\binom{n}{2}} \tag{6}$$