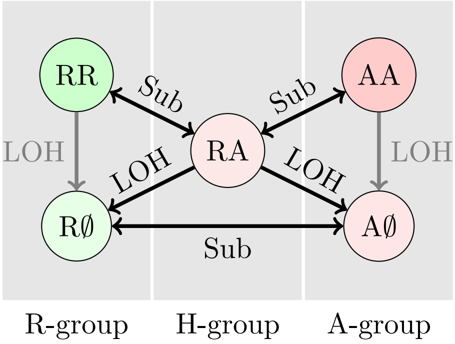
Lab rotation: theories (draft)

June 5, 2022

Mutation detection

The loci are treated as independent. The genotype of a cell at any locus is assigned to one of three groups: R (reference only), A (alternative only) and H (heterozygous). This grouping is dependent on which alleles are present in the cell and does not take copy numbers into account. More specifically, it is assumed that each locus has exactly one or two alleles in any cell. This means, while an H-group cell is guaranteed to have one reference and one alternative allele, an R- or A-group cell can possess either one or two of the same allele.



R: reference allele; A: alternative allele; ∅: lost allele;
Sub: substitution; LOH: loss of heterozygosity

Given a locus, a cell and the corresponding observation, the likelihood of the number of reference reads given a genotype G follows a beta-binomial distribution. Let $D = \{n_R, n_A\}$ be the numbers of reference and alternative reads, respectively. Then the likelihood is

$$P(D \mid G) = \mathrm{BetaBin}(n_R, n_A + n_R, f\omega, \omega - f\omega)$$

where $f = \frac{\alpha}{\alpha+\beta}$ and $\omega = \alpha + \beta$ are specific for each G. Let N be the number of cells, then the joint likelihood of all observations at this locus is

$$P(\mathbf{D} \mid \vec{G}) = \prod_{i=1}^{N} P(D_i \mid G_i)$$

For any locus, we want to find the probability that a mutation exists. Specifically, we only consider mutations that move the cell from one genotype group to another, namely LOH in heterozygous cells and substitutions. Other mutations, especially copy number changes (e.g. LOH in homozygous cells), are harder to detect using the data available and are therefore ignored for now.

Given the observations $\mathbf{D}$ at a locus, the probability that a mutation eixsts here is equal to one minus the probability that no mutation exists, i.e. that all cells are of the same genotype group. Since we assumed that all cells are independent, we have

$$\begin{aligned} P(\text{mutation} \mid \mathbf{D}) &= 1 - P(\forall i : G_i = \text{R} \mid \mathbf{D}) - P(\forall i : G_i = \text{H} \mid \mathbf{D}) - P(\forall i : G_i = \text{A} \mid \mathbf{D}) \\ &= 1 - \sum_{G_0 \in \{\text{R, H, A}\}} \frac{P(\mathbf{D} \mid \forall i : G_i = G_0) P(\forall i : G_i = G_0)}{P(\mathbf{D})} \\ &= 1 - \sum_{G_0 \in \{\text{R, H, A}\}} \frac{P(\forall i : G_i = G_0)}{P(\mathbf{D})} \prod_{i=1}^{N} P(D_i \mid G_i = G_0) \end{aligned}$$

While we already have a model for $P(D_i \mid G_i = G_0)$, it remains a question how we can estimate the values of $P(\forall i : G_i = G_0)$ and $P(\mathbf{D})$.

If we assume that genotypes of the cells are independent given the observations, we get

$$\begin{aligned} P(\text{mutation} \mid \mathbf{D}) &= \sum_{G_0 \in \{\text{R, H, A}\}} \prod_{i=1}^{N} P(G_i = G_0 \mid D_i) \\ &= \sum_{G_0 \in \{\text{R, H, A}\}} \prod_{i=1}^{N} \frac{P(D_i \mid G_i = G_0) P(G_i = G_0)}{P(D_i)} \end{aligned}$$

In this case, $P(G_i = G_0)$ is easier to estimate than $P(\forall i : G_i = G_0)$, and once it is known for all G_0 values, the normalizing factor $P(D_i)$ is also easy to obtain.
However, the assumption that the genotypes are independent might not be plausible.

Bypassing the underflow problem

When a large number of probabilities are multiplied together, the result can be so small that the floating point number underflows to zero. This problem is bypassed by using the log-scale notation, i.e. denoting the numbers by their logarithms. Then the calculation of the product becomes addition of logarithms of individual probabilities.

When marginalizing over all possible genotype profiles, the joint probabilities of each genotype profile need to be added up. Let p_1 and p_2 be two very small probabilities. To avoid underflow, p_1 and p_2 are represented by their logarithms $q_1 = \log p_1$ and $q_2 = \log p_2$, respectively. Then the logarithm of the sum of p_1 and p_2 can be calculated as follows

$$\begin{aligned}\log(p_1 + p_2) &= \log(e^{q_1} + e^{q_2}) \\ &= \log(e^{q_1}(1 + e^{q_2 - q_1})) \\ &= q_1 + \log(1 + e^{q_2 - q_1})\end{aligned}$$

This method avoids directly calculating p_1 and p_2 and reduces the risk of underflow. Since q_1 and q_2 tend to be similar, $e^{q_1 - q_2}$ will usually not underflow. Even if $e^{q_1 - q_2}$ underflows, it will have little effect on the final result because in this case p_2 is much smaller than p_1 and hence we have $p_1 + p_2 \approx p_1$. The only problem comes when $e^{q_1 - q_2}$ is too large that it overflows. It remains to be assessed how often and under which conditions this situation may occur.