1. **Reverse-engineering the genetic regulatory network**

The first part of the method consists of inferring (or reverse-engineering) the genetic regulatory network. The method is based on the GENIE3[1] method. It uses a random forest regressor to define an importance factor to each possible edge within the network. Then for each gene, the importance factors of this gene to all other genes are summed. The genes are then sorted and the gene with highest importance is seen as the most upstream gene. All importance factors going downstream to upstream are set to 0 (removed). Then an importance factor threshold needs to be selected. Every edge with an importance below this threshold is considered non-existing. Every edge with an importance above this threshold is considered as existing.

1. **Recalculate gene expressions of unperturbed cells after introducing a knockout**

Once the genetic regulatory network has been inferred, it is possible to make predictions on the effects of genetic knockouts. The predictions are done by taking the genetic profiles of the unperturbed cells and introducing a knockout (setting the expression to zero) and recalculating the genetic expressions following the genetic network. The order of which to recalculate the gene expressions is determined by iterating over a list containing the effected genes, and appending the genes that the current iterated gene effects until done iterating. And leaving only the final occurrence of each gene in this list.

1. **Calculating cell state proportions from genetic expressions**

Then, in the order determined above, the genetic expressions of the unperturbed cells are recalculated by training a regression forest that has as a target a single gene expression, and as an input only the incoming edges towards that gene. This results in a new genetic profile, for which the state is determined by a nearest neighbor method in principal component space.

[1] https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0012776&type=printable