

Automated mutagenesis can be summarised as follows; each round starts with a dilution from the previous plate:

1. Previous plate is diluted 1/400 and initial OD₆₀₀ measured to baseline. After an incubation of 2 h, OD₆₀₀ is measured for the first time.

Note: Spectrometer readings are labelled Abs0*, where * represents the 1st, 2nd, 3rd, 4th or 5th reading after dilution.

2. VB_1 then identifies the most recent spectrometer reading and assesses which cells, if any, exceed the target OD₆₀₀, and creates a count. If three or more wells are hits, the next dilution is initiated and a new mutagenesis round begins (this is initiated in the automation method).
3. VB_2 identifies the three highest OD₆₀₀ values from the current reading and the previous reading, and records an average OD₆₀₀ of these three wells. Assuming exponential growth following $N_{\text{final}} = N_{\text{initial}}(2^{kt})$, k is evaluated:

$$k = \frac{\log_2(\text{AverageTop3OD}) - \log_2(\text{PrevAverageTop3OD})}{\text{prevDuration}}$$

where prevDuration is the previous incubation length. The ideal subsequent incubation time to reach the desired OD₆₀₀ is then calculated:

$$\text{EstToTarget} = \frac{\log_2(\text{TargetOD}) - \log_2(\text{AverageTop3OD})}{k}$$

The calculated ‘EstToTarget’ is capped at 9 h; after this period of incubation a new spectrometer reading is taken and the method cycles back to step 2. This incubation is unnecessary if a new dilution was initiated after step 2.

4. If after two capped incubation times of 9 h (20 h in total or three OD₆₀₀ measurements), a 12 h incubation is applied to grow out all wells. A final spectrometer reading is taken, and the plate is diluted for the beginning of a new round.