Measurement:

Assumptions:

The density of all the cells is the same.

On centrifuging at a particular value of centrifugal force (equivalent to rpm for a particular centrifuge) for a short amount of time, the cells sedimenting are only those in a cluster with cluster size beyond a particular cutoff cluster size value.

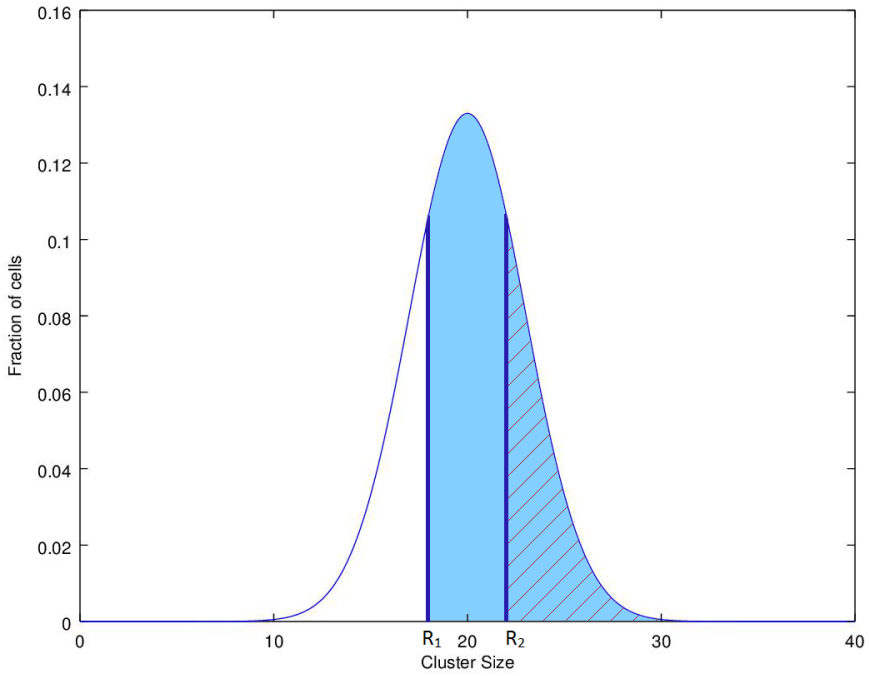
OD600 is proportional to number of cells in the culture.

The significance of short centrifugation time is that all the cells will settle down irrespective of the cluster size if centrifuged for long enough (as there is a net force in the downward direction, the magnitude of the force and settling time will depend on the viscous force which depends non-linearly on cluster size, the other forces will scale linearly with cluster size and it is viscous force that leads to different cluster sizes settling at different rates).

The motivation for the centrifugal force cutoff assumption was that the viscous force depends on the surface area, for a cluster, the reduction in surface area relative to the total surface area of all the cells in a cluster depends on the orientation of the cells in the cluster and cluster size. If you do not understand this, think of 50S and 30S ribosomal subunits attaching to each other and the total surface area of the ribosome is less than the sum of the surface areas of the individual subunits, leading to the ribosome being 70S (<80S).

Assuming that orientation doesn't significantly change the sedimentation rate significantly for a given cluster size (i.e, variance in sedimentation rate due to the different orientation of cells in a cluster for a given cluster size is much less than the difference in sedimentation rate per unit change in cluster size), thus the sedimentation rate is determined only by the cluster size.

Graphically, the effect of centrifugation is displayed below:



Blue – fraction of cells settled at a higher rpm; red lines – fraction of cells settled at a lower rpm. The cutoff cluster sizes are indicated (R_1 and R_2 respectively).

Model:

As indicated, the fraction that settles is the area under the curve beyond the cutoff cluster size corresponding to the rpm used during centrifugation.

Mathematically, the settling fraction f is:

$$f = \int_{R}^{\infty} p(n)dn$$

Where p(n) is the cluster size distribution (discrete distribution of fraction of cells versus cluster size approximated as a continuous distribution) and R is the cluster size cutoff corresponding to the rpm used.

To obtain the cluster size distribution, we must calculate df/dn. To get df/dn, we centrifuge the cultures at different rpm values close to each other. Let us assume we centrifuged at c_1 rpm, c_2 rpm (c_1 close to c_2) and the corresponding cluster size cutoffs and fraction sedimented was R_1, R_2 and f_1, f_2 respectively.

$$f_1 = \int_{R_1}^{R_2} p(n)dn + \int_{R_2}^{\infty} p(n)dn \approx p(\bar{R})(R_2 - R_1) + \int_{R_2}^{\infty} p(n)dn = p(\bar{R})(R_2 - R_1) + f_2$$

If the cluster size cutoff is assumed to decrease as the inverse square of rpm (as centrifugal force goes as square of angular frequency), the cluster size distribution is determined as follows:

$$\frac{f_1 - f_2}{R_2 - R_1} = p(\bar{R}); R = \left(\frac{d}{c^2}\right) + e; \bar{R} = \frac{R_1 + R_2}{2}$$

$$\frac{f_1 - f_2}{R_2 - R_1} = \frac{f_1 - f_2}{\frac{d}{{c_2}^2} - \frac{d}{{c_1}^2}} = \frac{(f_1 - f_2)(c_1^2 c_2^2)}{d(c_1^2 - c_2^2)} = p(\bar{R})$$

Thus, we get a scaled cluster "size" distribution with cluster size in rpm cutoff by plotting $\frac{(f_2 - f_1)(c_1^2 c_2^2)}{(c_1^2 - c_2^2)} = p(\bar{R})d$ versus (c_1+c_2)/2.

Settled fraction f was estimated as 1-(OD600 (supernatant)/OD600 (culture)) (as total cells = cells settled + cells in supernatant).

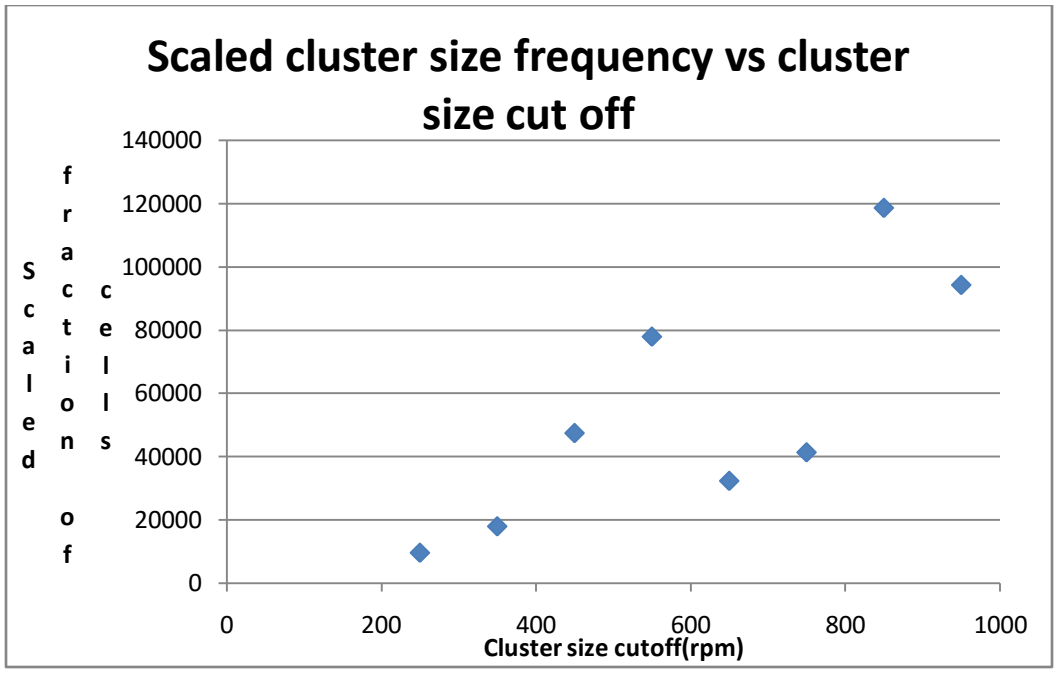
Brief Protocol:

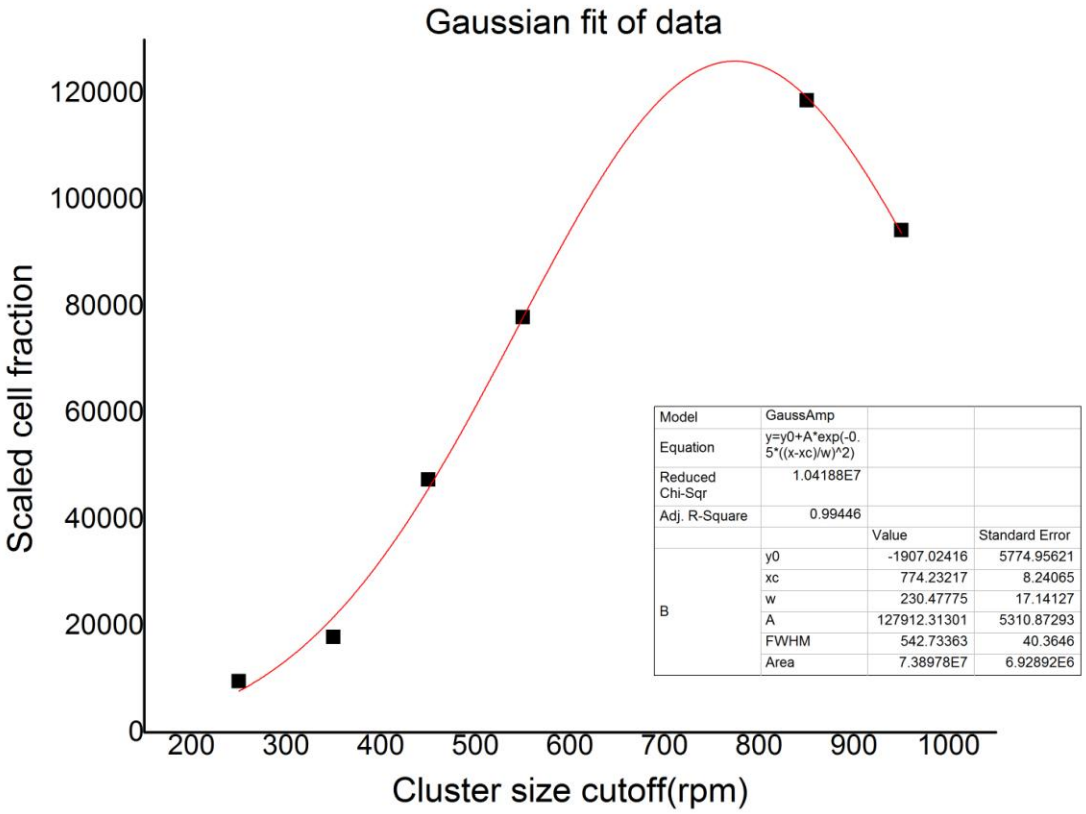
1. Overnight primary culture (of BBa_K1352000 transformants) were inoculated into a secondary culture (2% inoculation), allowed to grow to 0.6 OD600 and induced then with 0.2% arabinose.
2. Every 5 hours, 10 x 1.5mL aliquots of the culture were taken in microfuge tubes. OD600 of the culture was recorded. Each tube was spun at an rpm in the range 100-1000rpm, the supernatant was carefully removed and its OD600 measured.

Preliminary Results:

Only the dataset at 5 hours was analyzed as outlined above, at 10 hours, the culture had almost completely settled and our model only works for a well mixed suspension.

Given our limited dataset and only 2 technical replicates of the experiment, the results cannot be completely trusted due to lack of data. However, the results look promising, the plot of scaled fraction of cells versus cluster size shows a distribution that peaks at a particular size, we expected that this would be Poisson distributed, which can be approximated as Gaussian. Other than 2 points, the data seemed to show the expected trend, it appears to have a single peak and fits a Gaussian well.


Scaled cluster size frequency vs cluster size cut off
Scaled fraction of cells
140000
120000
100000
80000
60000
40000
20000
0
0
200
400
600
800
1000
Cluster size cutoff(rpm)


Gaussian fit of data
Scaled cell fraction
120000
100000
80000
60000
40000
20000
0
200
300
400
500
600
700
800
900
1000
Cluster size cutoff(rpm)
Model GaussAmp
Equation y=y0+A*exp(-0.5*((x-xc)/w)^2)
Reduced Chi-Sqr 1.04188E7
Adj. R-Square 0.99446
Value Standard Error
B y0 -1907.02416 5774.95621
xc 774.23217 8.24065
w 230.47775 17.14127
A 127912.31301 5310.87293
FWHM 542.73363 40.3646
Area 7.38978E7 6.92892E6

The further experiments we would like to do is show that the cluster size at which the distribution peaks increases with time (as number of Ag43 copies per cell is expected to increase with time) and see how the standard deviation of the distribution changes with time.