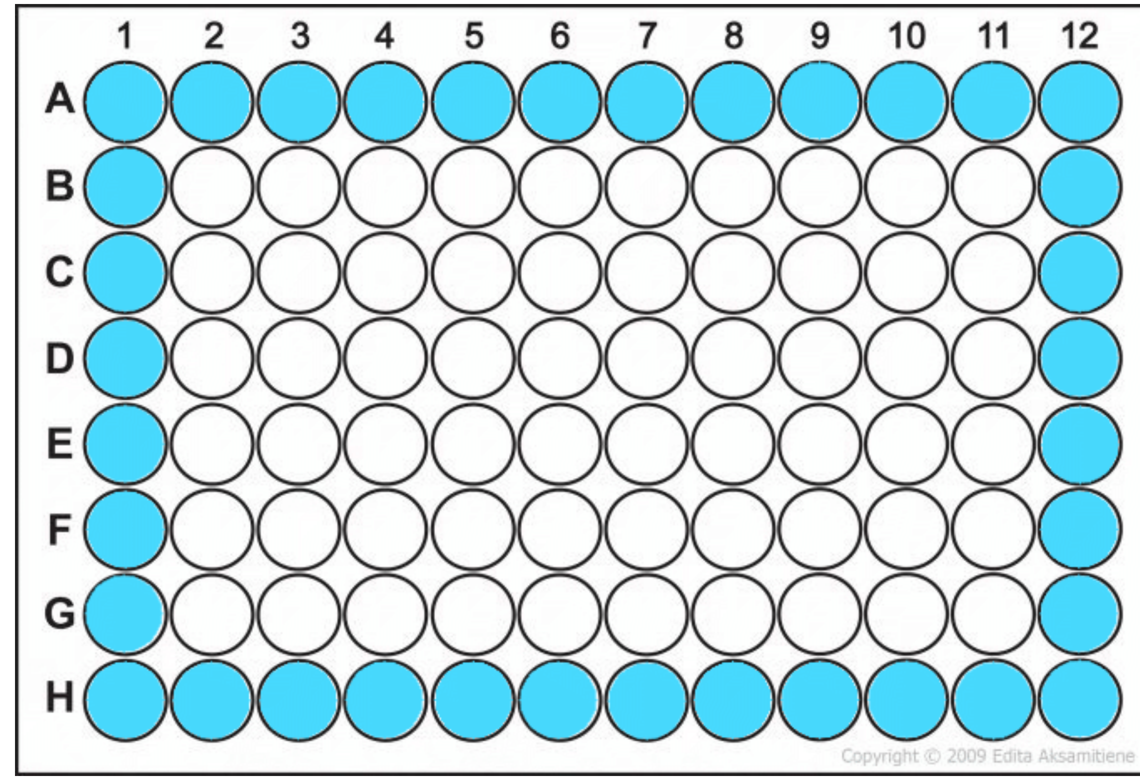
STANDARD GROWTH ASSAY — SHAKY SPEC

SHAKY SPEC GROWTH ASSAY



DAY 1

- Set up 5ml O/N in appropriate Hv-broth (YPC or Ca)

DAY 2

- Dilute O/N in fresh Hv-broth, typically 1/500 dilution

DAY 3

- Dilute O/N 1/10, to OD ~0.05, grow for ~2-3 hours
- Pipette Hv-broth (YPC or Ca) into outer wells of 48-well (250µl) or 96-well (150µl) plate as blanks
  - See shaded wells above, helps prevent evaporation of samples
- Dilute cultures and pipette in triplicate into microtitre plate, using 250µl (48-well) or 150µl (96-well)
  - Typically use 1/100 and 1/1000 dilutions, in pre-warmed Hv-broth
- Seal lid to base with 1cm microporous tape, ensuring tape doesn't protrude above the edge
  - Important to avoid tape adhesive being deposited on spectrophotometer
- Incubate at 45°C for 72 hours with double-orbital shaking at 425rpm (48-well) or 807rpm (96-well)
- Take readings every 15 minutes and export data to Excel

Wardell et al (2017); Lever et al (2023)

ANALYSING SHAKY SPEC DATA, PART 1

- Export the data on the shaky spec to an Excel file
  - Alternatively, export to a text file, then copy and paste the data into an Excel file afterwards
- Shaky spec will calculate the blank-zeroed, Spectronic-equivalent values but these may be inaccurate due to crystal formation in the blank samples. If necessary, calculate manually:

$$\frac{\text{(sample reading - mean of blank readings)}}{0.14} = \text{spectronic}$$

  - Dividing by 0.14 accounts for path length in shaky spec, vs 1 cm path length in the Spectronic
- Cultures (e.g. with different inoculation volume, or under different conditions) must be aligned so that they reach a specific OD at the same time point
  - This must be done first, so that the mean and standard error calculated next are accurate
- Manually shift cells in the Excel spreadsheet until they align at a specific OD near the beginning of log phase (e.g. ~OD 0.2) as shown in spreadsheet:

H26								
Time	0mM	0.05mM	0.1mM	0.15mM	0.25mM	Mean	STD	STE
				0.0754				
				0.0754				
	0.08254			0.0754				
	0.08968	0.09683		0.08254	0.10397			
	0.08968	0.09683		0.08968	0.10397			
	0.1254	0.10397		0.09683	0.11111			
00:14:16	0.11111	0.10397	0.14683	0.10397	0.1254	0.11825	0.01821	0.00814
00:29:16	0.11825	0.13254	0.14683	0.11825	0.1254	0.12825	0.01195	0.00535
00:44:16	0.1254	0.14683	0.15397	0.13254	0.13254	0.13825	0.01174	0.00525
00:59:16	0.13254	0.13254	0.16111	0.13968	0.14683	0.14254	0.01195	0.00535
01:14:16	0.14683	0.14683	0.16825	0.14683	0.16111	0.15397	0.0101	0.00452
01:29:16	0.16111	0.15397	0.18254	0.16111	0.16825	0.1654	0.01083	0.00484
01:44:16	0.16825	0.16825	0.18254	0.1754	0.18254	0.1754	0.00714	0.00319
01:59:16	0.18254	0.18254	0.19683	0.18968	0.21825	0.19397	0.01481	0.00662
02:14:16	0.19683	0.19683	0.20397	0.20397	0.20397	0.20111	0.00391	0.00175
02:29:16	0.21825	0.21111	0.21825	0.21825	0.2254	0.21825	0.00505	0.00226
02:44:16	0.3254	0.23254	0.25397	0.23968	0.23968	0.25825	0.03833	0.01714

ANALYSING SHAKY SPEC DATA, PART 2

- Calculate the mean, standard deviation, and standard error of the aligned repeats

$$\text{Standard error} = \frac{\text{Standard deviation}}{\sqrt{\text{Number of repeats}}}$$

- Plot the mean OD values (and standard errors) vs time on graph with $\log_2$ scale on Y axis
- Calculate the generation times using the following equations:

$$n = \frac{\log_{10} D_i - \log_{10} D_f}{\log_{10} 2}$$

n = number of generations

D_f = end OD

D_i = start OD

$$G = \frac{t}{n}$$

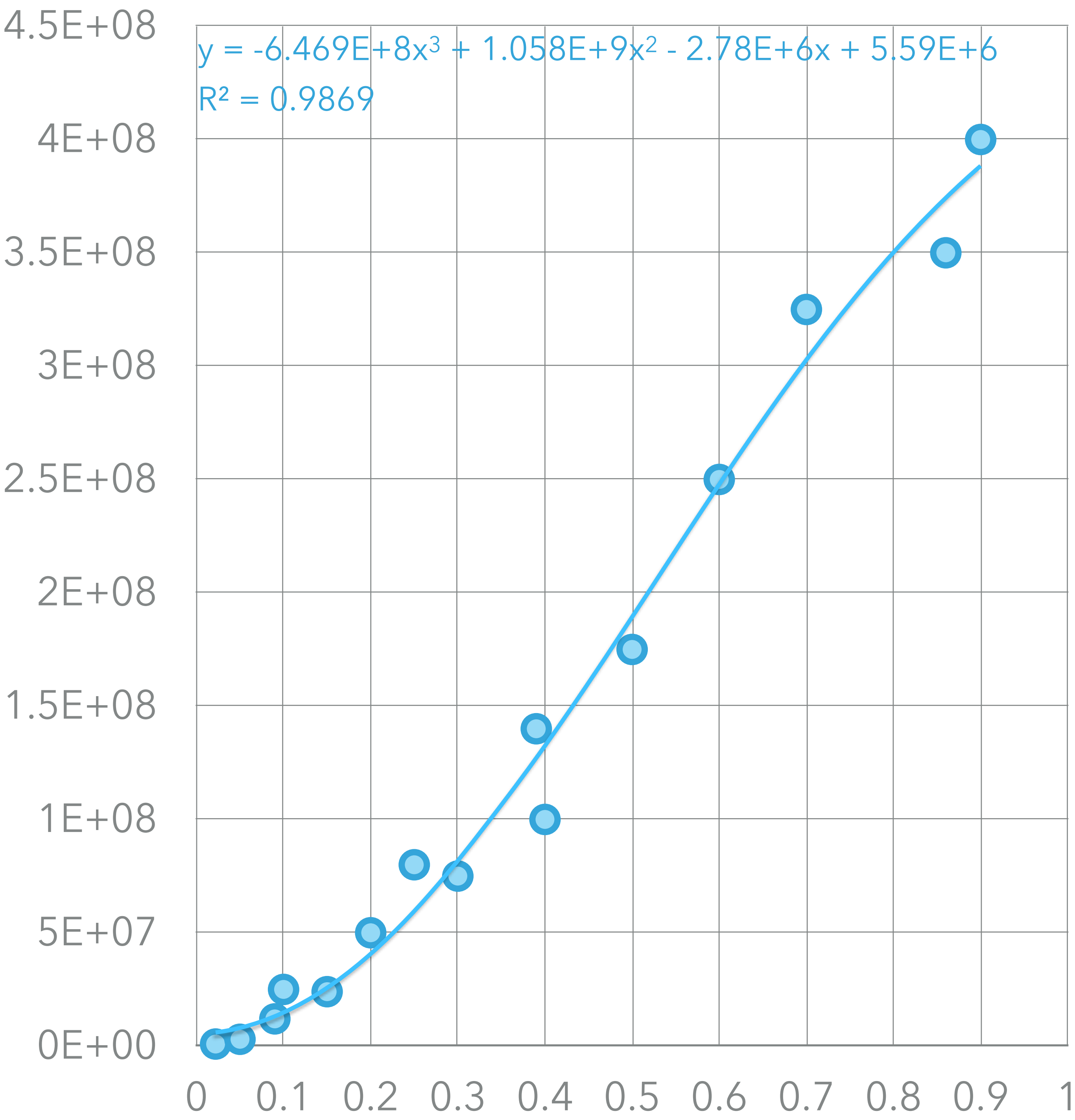
G = generation time

t = time

- Alternatively, fit a line to exponential growth portion of graph and generate an equation
- Use equation to find the time at which sample reaches a specific OD (e.g. 0.2) and then the time at which it reaches double the first OD (e.g. 0.4)
- The difference between these times is the generation time of the sample

CELLS PER ML @ OD650 VALUE

OD650	Cells/ml
0.022	9.00E+05
0.05	3.00E+06
0.09	1.20E+07
0.15	2.40E+07
0.25	8.00E+07
0.39	1.40E+08
0.86	3.50E+08
0.1	2.50E+07
0.2	5.00E+07
0.3	7.50E+07
0.4	1.00E+08
0.5	1.75E+08
0.6	2.50E+08
0.7	3.25E+08
0.9	4.00E+08



CALCULATING CULTURE DILUTIONS

- Spec the culture

$$\frac{\text{Desired OD}}{\text{Actual OD}} = x$$

$$\frac{\text{Time till needed}}{\text{generation time of strain}} = y$$

$$2\text{\textasciicircum}y = \text{dilution factor}$$

$$\frac{\text{dilution factor}}{x} = \text{Actual dilution}$$