

# growth curve

## Contents

1. Pilot . . . . .	2
2. Growth curve of 19 strains . . . . .	3

**1. Pilot**

Six strains used in the plant experiment. Eight replicates each.

## 2. Growth curve of 19 strains

- Prepare the Time

ExpID	ID	Family	Genus
H1M1R1	1	Rhizobiaceae	Rhizobium
H2M3R1	9	Rhizobiaceae	Ensifer
H2M3R2	10	Rhizobiaceae	Ensifer
H3M1R1	12	Rhizobiaceae	Ensifer
H3M3R2	15	Rhizobiaceae	Ensifer
H3M4R1	17	Rhizobiaceae	Ensifer
H3M4R2	18	Rhizobiaceae	Rhizobium
H4M5R1	23	Rhizobiaceae	Ensifer
L1M2R2	29	Rhizobiaceae	Ensifer
L2M2R1	37	Rhizobiaceae	Ensifer
L2M4R1	40	Rhizobiaceae	Ensifer
L3M1R1	44	Rhizobiaceae	Ensifer
L3M4R1	48	Rhizobiaceae	Rhizobium
L3M5R1	51	Rhizobiaceae	Ensifer
L3M6R2	54	Rhizobiaceae	Rhizobium
L4M2R2	57	Rhizobiaceae	Ensifer
L4M3R3	58	Rhizobiaceae	Ensifer
L4M4R1	59	Rhizobiaceae	Ensifer
L4M4R3	60	Rhizobiaceae	Rhizobium
L4M7R1	65	Rhizobiaceae	Ensifer

- revive glycerol stock of rhizobia on TY agar
  - prepare labelled plates
  - use a sterilized inoculation loop to streak the glycerol stock on TY agar
  - incubate the petri plates at 30C for 2-3 days until the colony is identifiably large
- prepare rhizobium inoculum
  - prepare a DW96 filled with 300uL of PBS
  - pick colonies from the petri plate and dissolve the colonies in the PBS. Use wells in column 1, 5, and 9
  - dispense 100 uL of the dissolved colony to a NUNC96 and measure od at 600 nm
  - standardize the OD600 to 0.1. For target od = 0.1 `od_target` and target inoculum volume = 300 uL `volume_target`, and given the measured od of dissolved colonies `od_dissolved`, use the code below to calculate the needed volume of the dissolved colonies `volume_dissolved` and the pbs volume `volume_pbs`
  - prepare another DW96, follow the table to add required volume of dissolved colonies and pbs to make 300 uL of standardized inoculum
  - measure the od of the standardized inoculum as `od_inoculum`
- prepare 100 uL of TY medium in NUNC96
  - inoculate 2 uL of the standardized inoculum to each well

	1	2	3	4	5	6	7	8	9	10	11	12
A	H1M1R1	H1M1R1	H1M1R1	H1M1R1	L1M2R2	L1M2R2	L1M2R2	L1M2R2	L4M3R3	L4M3R3	L4M3R3	L4M3R3
B	H2M3R1	H2M3R1	H2M3R1	H2M3R1	L2M2R1	L2M2R1	L2M2R1	L2M2R1	L4M4R1	L4M4R1	L4M4R1	L4M4R1
C	H2M3R2	H2M3R2	H2M3R2	H2M3R2	L2M4R1	L2M4R1	L2M4R1	L2M4R1	L4M4R3	L4M4R3	L4M4R3	L4M4R3
D	H3M1R1	H3M1R1	H3M1R1	H3M1R1	L3M1R1	L3M1R1	L3M1R1	L3M1R1	blank	blank	blank	blank
E	H3M3R2	H3M3R2	H3M3R2	H3M3R2	L3M4R1	L3M4R1	L3M4R1	L3M4R1	blank	blank	blank	blank
F	H3M4R1	H3M4R1	H3M4R1	H3M4R1	L3M5R1	L3M5R1	L3M5R1	L3M5R1	blank	blank	blank	blank
G	H3M4R2	H3M4R2	H3M4R2	H3M4R2	L3M6R2	L3M6R2	L3M6R2	L3M6R2	blank	blank	blank	blank
H	H4M5R1	H4M5R1	H4M5R1	H4M5R1	L4M2R2	L4M2R2	L4M2R2	L4M2R2	blank	blank	blank	blank

Use the code below to standardize OD

strain	well	od_dissolved	od_target	volume_target	volume_dissolved	volume_pbs
H1M1R1	A01	0.6202	0.1	100	16.12	83.88
H2M3R1	B01	0.5002	0.1	100	19.99	80.01
H2M3R2	C01	0.2902	0.1	100	34.46	65.54
H3M1R1	D01	0.2252	0.1	100	44.40	55.60
H3M3R2	E01	0.2322	0.1	100	43.07	56.93
H3M4R1	F01	0.2512	0.1	100	39.81	60.19
H3M4R2	G01	0.5892	0.1	100	16.97	83.03
H4M5R1	H01	0.2312	0.1	100	43.25	56.75
L1M2R2	A05	0.2282	0.1	100	43.82	56.18
L2M2R1	B05	0.1462	0.1	100	68.40	31.60
L2M4R1	C05	0.3682	0.1	100	27.16	72.84
L3M1R1	D05	0.3342	0.1	100	29.92	70.08
L3M4R1	E05	0.1482	0.1	100	67.48	32.52
L3M5R1	F05	0.6312	0.1	100	15.84	84.16
L3M6R2	G05	0.3672	0.1	100	27.23	72.77
L4M2R2	H05	0.3362	0.1	100	29.74	70.26
L4M3R3	A09	0.2422	0.1	100	41.29	58.71
L4M4R1	B09	0.3572	0.1	100	28.00	72.00
L4M4R3	C09	0.2412	0.1	100	41.46	58.54