

- *# Import some helpful packages for loading and plotting data*
- using CSV , Dates , DataFrames , Gadfly , GLM , Statistics

	Time	Replicate	OD
1	11:06:00	"H"	0.013
2	11:06:00	"S"	0.027
3	11:11:00	"1"	0.031
4	11:11:00	"2"	0.031
5	11:16:00	"3"	0.034
6	11:16:00	"4"	0.032
7	11:21:00	"5"	0.032
8	11:21:00	"6"	0.031
9	11:28:00	"H"	0.009
10	11:28:00	"S"	0.039
	more		
96	15:04:00	"6"	5.1

- begin
- *# Load CSV into a DataFrame*
- csvfile = "Growth Curve Data.csv"
- df = DataFrame(CSV.File(csvfile))
- end

	Time	Replicate	OD
1	0	"H"	0.013
2	0	"S"	0.027
3	5	"1"	0.031
4	5	"2"	0.031
5	10	"3"	0.034
6	10	"4"	0.032
7	15	"5"	0.032
8	15	"6"	0.031
9	22	"H"	0.009
10	22	"S"	0.039
more			
96	238	"6"	5.1

```

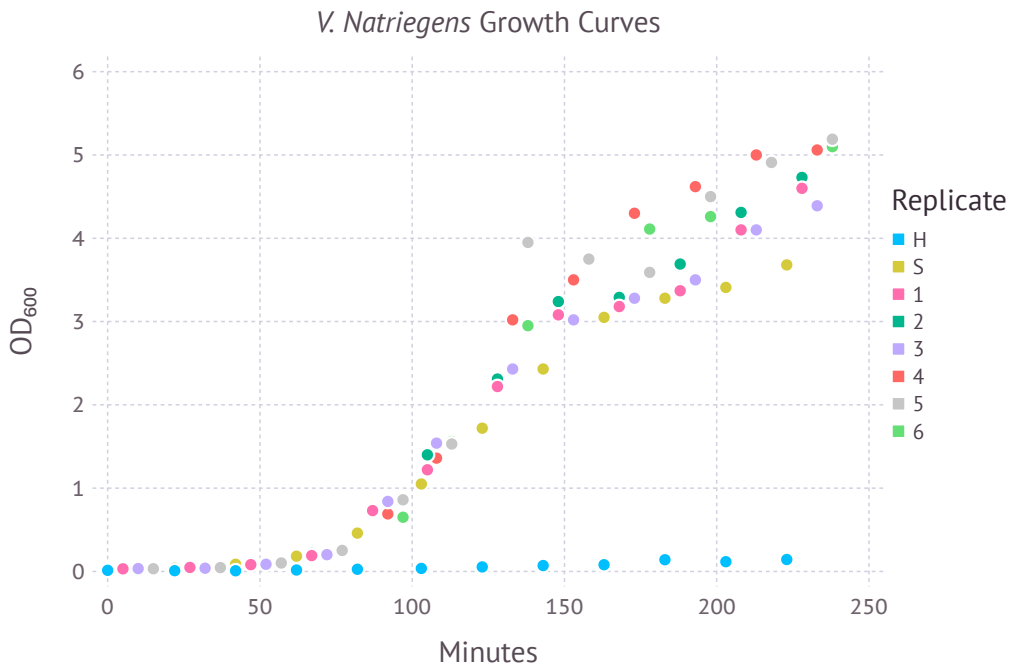
• begin
•   # Normalise times and convert to minutes
•   start = df[1, :Time]
•   pdf = transform(df, :Time => ByRow(t -> Dates.value(Minute(t - start))) => :Time)
• end

```

## Growth Curve Data

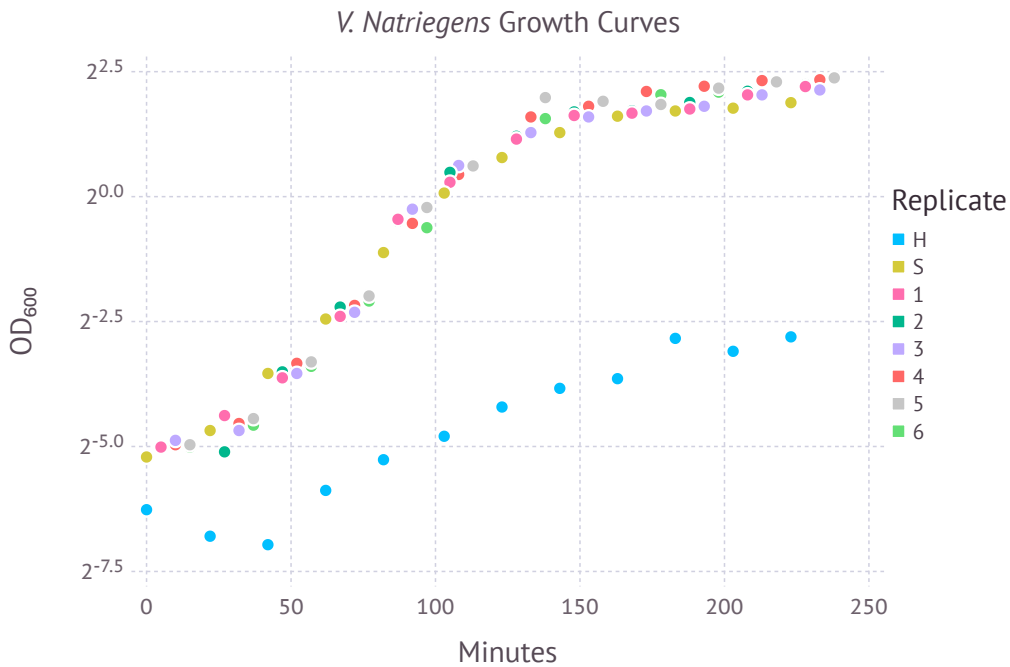
Given that OD is proportional to cell count (when properly diluted so that readings don't exceed 0.6), it can be used to track the growth of cells.

On a linear scale, this growth curve is an exponential, but be later made linear by applying a logarithmic transformation.



- *# Construct a line-scatter plot, grouping by biological replicate*
- `plot(pdf, x=:Time, y=:OD, color=:Replicate, Scale.color_discrete_hue,`
- `Guide.xlabel("Minutes"), Guide.ylabel("OD600"),`
- `Guide.title("<i>V. Natriegens</i> Growth Curves"))`

A log transformation reveals that the region between 60 and 120 minutes can be safely said to be linear



```

• # Replot, but on a log-scale so that we can pick out the exponential growth region
• plot(pdf, x=:Time, y=:OD, color=:Replicate,
•     Scale.color_discrete_hue, Scale.y_log2,
•     Guide.xlabel("Minutes"), Guide.ylabel("OD600"),
•     Guide.title("<i>V. Natriegens</i> Growth Curves"))

```

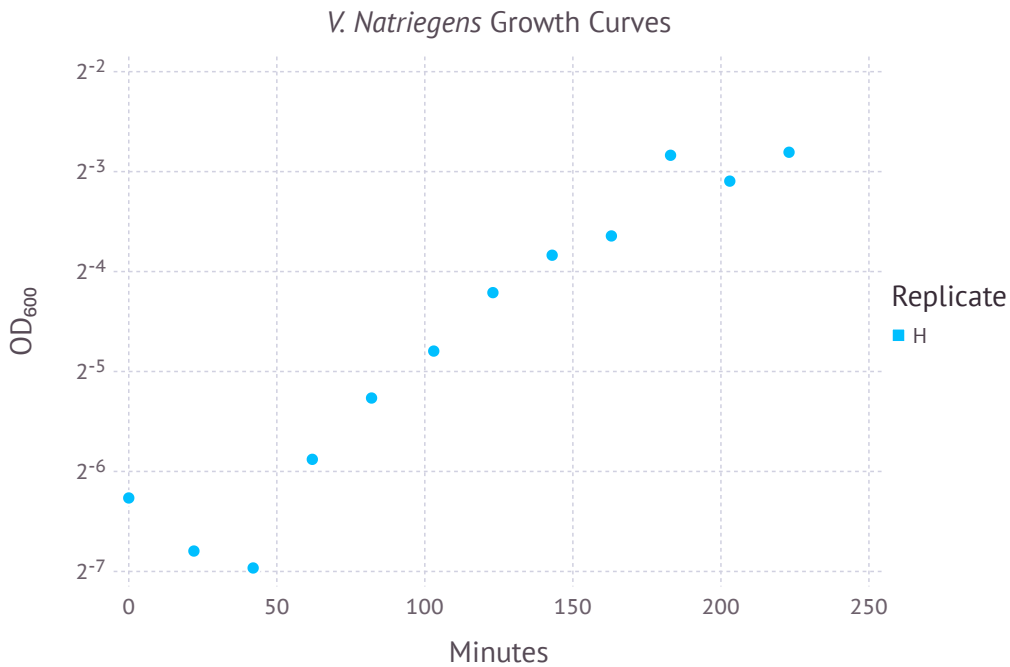
hlogdf =

	Time	Replicate	OD
1	0	"H"	0.013
2	22	"H"	0.009
3	42	"H"	0.008
4	62	"H"	0.017
5	82	"H"	0.026
6	103	"H"	0.036
7	123	"H"	0.054
8	143	"H"	0.07
9	163	"H"	0.08
10	183	"H"	0.14
11	203	"H"	0.117
12	223	"H"	0.143

```

• # Isolate the H media
• hlogdf = filter(:Replicate => r -> r == "H", pdf)

```



- *# Replot, but the isolated values so that we can pick out the exponential growth region*
- `plot(hlogdf, x=:Time, y=:OD, color=:Replicate,`
- `Scale.color_discrete_hue, Scale.y_log2,`
- `Guide.xlabel("Minutes"), Guide.ylabel("OD600"),`
- `Guide.title("<i>V. Natriegens</i> Growth Curves"))`

thlogdf =

	Time	Replicate	OD
1	62	"H"	0.017
2	82	"H"	0.026
3	103	"H"	0.036
4	123	"H"	0.054

- *# Trim the data to take a closer look at log-phase*
- `thlogdf = filter(:Time => t -> 60 <= t <= 123, hlogdf)`

	Time	Replicate	OD
1	62	"H"	-5.87832
2	82	"H"	-5.26534
3	103	"H"	-4.79586
4	123	"H"	-4.2109

- *# Log-transform the OD data*
- `transform!(thlogdf, :OD => ByRow(log2) => :OD)`

```
hols =
StatsModels.TableRegressionModel{LinearModel{GLM.LmResp{Vector{Float64}}, GLM.DensePredCho
```

```
OD ~ 1 + Time
```

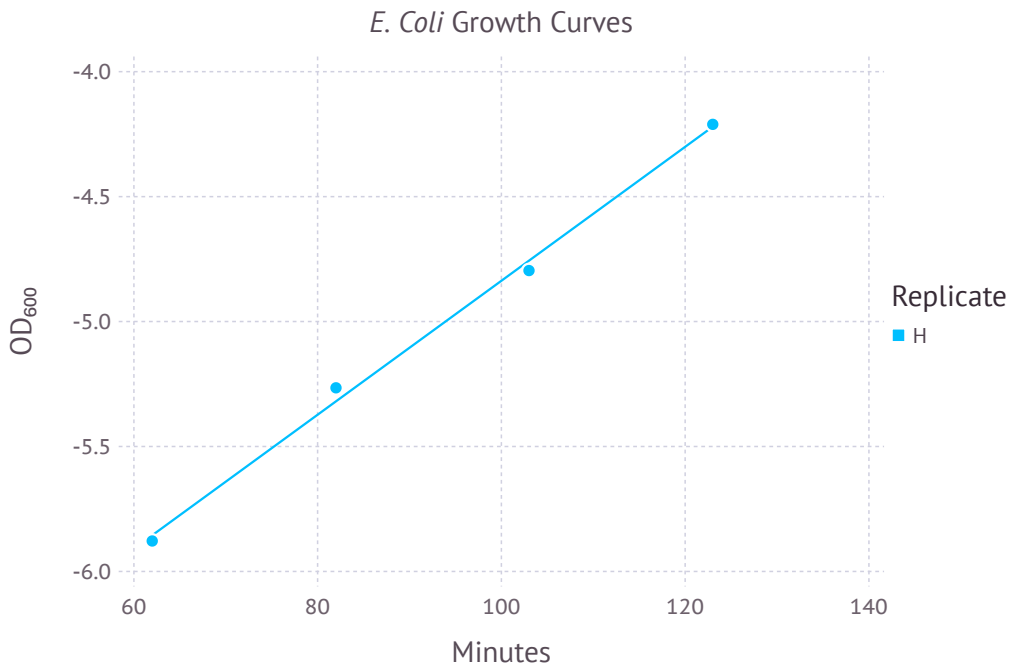
```
Coefficients:
```

	Coef.	Std. Error	t	Pr(> t )	Lower 95%	Upper 95%
(Intercept)	-7.51728	0.105276	-71.41	0.0002	-7.97025	-7.06432
Time	0.0268073	0.00110502	24.26	0.0017	0.0220528	0.0315618

- *# Perform and ordinary least-squares regression for a linear model*
- `hols = lm(@formula(OD ~ Time), thlogdf)`

```
[-5.85523, -5.31908, -4.75613, -4.21998]
```

- *# Insert a new column into our dataframe representing the model predictions*
- `thlogdf[:, :Model] = predict(hols)`



- *# And then plot it on a log-scale*
- `plot(thlogdf, x=:Time, y=:OD, color=:Replicate,`
- `Scale.color_discrete_hue, Geom.point,`
- `Guide.xlabel("Minutes"), Guide.ylabel("OD600"),`
- `Guide.title("<i>E. Coli</i> Growth Curves"),`
- *# With the line-of-best-fit superimposed*
- `layer(x=:Time, y=:Model, Geom.line))`

# Calculating Doubling-Time From Our Model

We can start with a fundamental equation that models the growth of microbes undergoing binary fission:

$$N = N_0 2^{\frac{t}{g}}$$

Where  $N$  is the current number of cells,  $N_0$  is the initial number of cells,  $t$  is time, and  $g$  is generation or doubling-time. We want to rearrange this equation to fit the model  $OD \sim \text{Time}$  after calculating the  $\log_2$  of all ODs.

Let's start by applying the  $\log_2$  to both sides of the equation:

$$\log_2 N = \log_2 N_0 + \frac{t}{g}$$

Ignoring the intercept and separating terms, we get an expression that matches our model:

$$\log_2 N = \frac{1}{g}t$$

Therefore we can conclude that  $g$  is equal to the reciprocal of our regression gradient.

The doubling time for H was ~37.3 minutes, with a growth rate of 0.0268

```
• begin
•   # Calculate doubling-time
•   g = 1/coef(hols)[2]
•   # Format it into a nice string
•   md"The doubling time for H was ~$(round(g, sigdigits=3)) minutes, with a growth
      rate of $(round(coef(hols)[2], sigdigits=3))"
• end
```



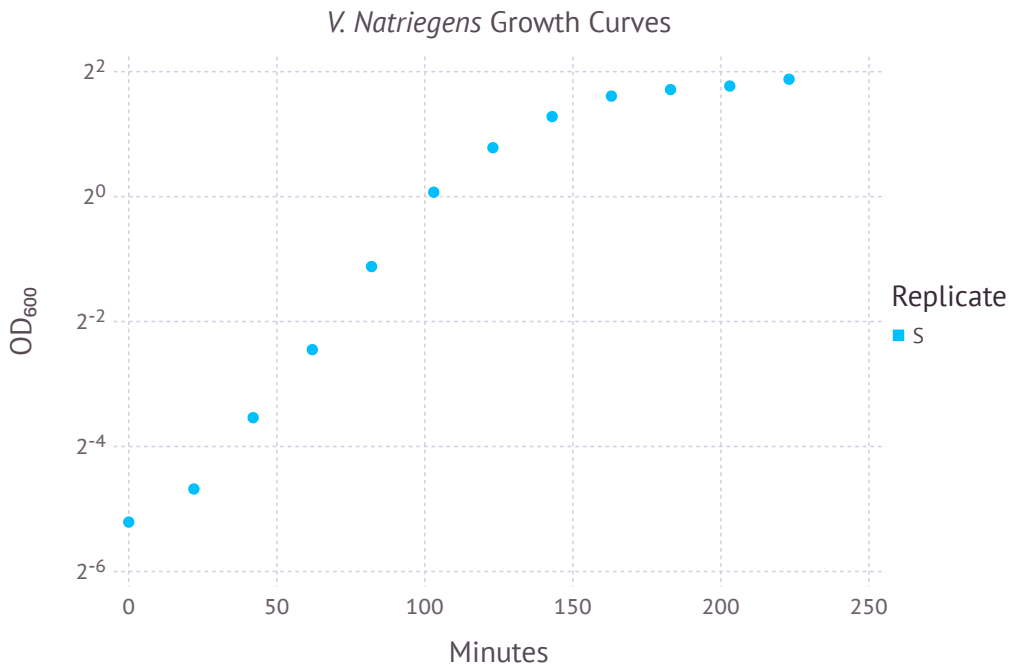
slogdf =

---

	Time	Replicate	OD
<b>1</b>	0	"S"	0.027
<b>2</b>	22	"S"	0.039
<b>3</b>	42	"S"	0.086
<b>4</b>	62	"S"	0.183
<b>5</b>	82	"S"	0.46
<b>6</b>	103	"S"	1.05
<b>7</b>	123	"S"	1.72
<b>8</b>	143	"S"	2.43
<b>9</b>	163	"S"	3.05
<b>10</b>	183	"S"	3.28
<b>11</b>	203	"S"	3.41
<b>12</b>	223	"S"	3.68

---

- *# Isolate the S media*
- `slogdf = filter(:Replicate => r -> r == "S", pdf)`



- *# Replot, but the isolated values so that we can pick out the exponential growth region*
- `plot(slogdf, x=:Time, y=:OD, color=:Replicate,`
- `Scale.color_discrete_hue, Scale.y_log2,`
- `Guide.xlabel("Minutes"), Guide.ylabel("OD600"),`
- `Guide.title("<i>V. Natriegens</i> Growth Curves"))`

tslogdf =

	Time	Replicate	OD
1	22	"S"	0.039
2	42	"S"	0.086
3	62	"S"	0.183
4	82	"S"	0.46

- *# Trim the data to take a closer look at log-phase*
- `tslogdf = filter(:Time => t -> 20 <= t <= 82, slogdf)`

	Time	Replicate	OD
1	22	"S"	-4.68038
2	42	"S"	-3.53952
3	62	"S"	-2.45008
4	82	"S"	-1.12029

- *# Log-transform the OD data*
- `transform!(tslogdf, :OD => ByRow(log2) => :OD)`

```
sols =
StatsModels.TableRegressionModel{LinearModel{GLM.LmResp{Vector{Float64}}, GLM.DensePredCho
```

```
OD ~ 1 + Time
```

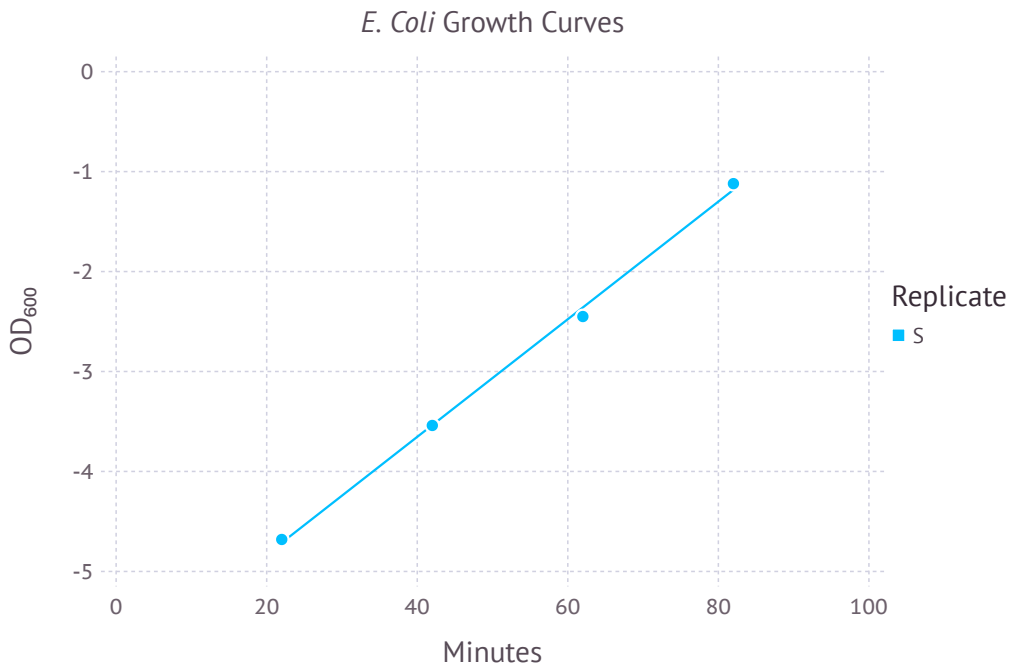
```
Coefficients:
```

	Coef.	Std. Error	t	Pr(> t )	Lower 95%	Upper 95%
(Intercept)	-6.00769	0.102749	-58.47	0.0003	-6.44979	-5.5656
Time	0.0588485	0.00181523	32.42	0.0010	0.0510382	0.0666588

- *# Perform and ordinary least-squares regression for a linear model*
- `sols = lm(@formula(OD ~ Time), tslogdf)`

```
[-4.71302, -3.53605, -2.35909, -1.18212]
```

- *# Insert a new column into our dataframe representing the model predictions*
- `tslogdf[:, :Model] = predict(sols)`



```

• # And then plot it on a log-scale
• plot(tslogdf, x=:Time, y=:OD, color=:Replicate,
•     Scale.color_discrete_hue, Geom.point,
•     Guide.xlabel("Minutes"), Guide.ylabel("OD600"),
•     Guide.title("<i>E. Coli</i> Growth Curves"),
•     # With the line-of-best-fit superimposed
•     layer(x=:Time, y=:Model, Geom.line))

```

The doubling time for S was ~17.0 minutes, with a growth rate of 0.0588

```

• begin
•   # Calculate doubling-time
•   sg = 1/coef(sols)[2]
•   # Format it into a nice string
•   md"The doubling time for S was ~$(round(sg, sigdigits=3)) minutes, with a growth
•     rate of $(round(coef(sols)[2], sigdigits=3))"
• end

```

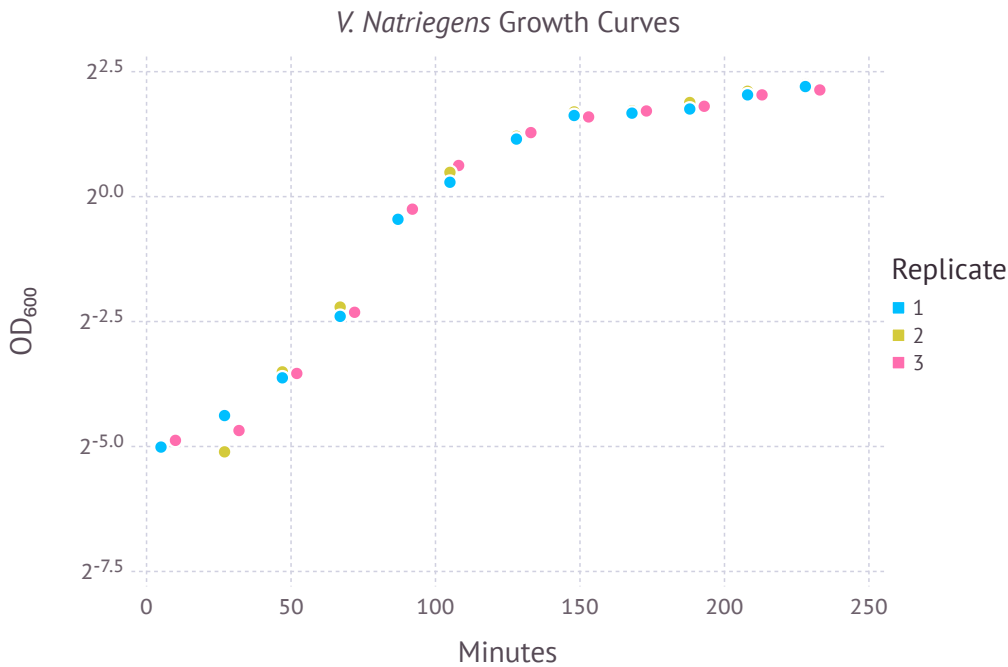
nglogdf =

---

	Time	Replicate	OD
<b>1</b>	5	"1"	0.031
<b>2</b>	5	"2"	0.031
<b>3</b>	10	"3"	0.034
<b>4</b>	27	"1"	0.048
<b>5</b>	27	"2"	0.029
<b>6</b>	32	"3"	0.039
<b>7</b>	47	"1"	0.081
<b>8</b>	47	"2"	0.088
<b>9</b>	52	"3"	0.086
<b>10</b>	67	"1"	0.19
more			
<b>36</b>	233	"3"	4.39

---

- *# Isolate the no glucose media*
- `nglogdf = filter(:Replicate => r -> r in ["1", "2", "3"], pdf)`



- *# Replot, but the isolated values so that we can pick out the exponential growth region*
- `plot(nglogdf, x=:Time, y=:OD, color=:Replicate,`
- `Scale.color_discrete_hue, Scale.y_log2,`
- `Guide.xlabel("Minutes"), Guide.ylabel("OD600"),`
- `Guide.title("<i>V. Natriegens</i> Growth Curves"))`

tnglogdf =

	Time	Replicate	OD
<b>1</b>	47	"1"	0.081
<b>2</b>	47	"2"	0.088
<b>3</b>	52	"3"	0.086
<b>4</b>	67	"1"	0.19
<b>5</b>	67	"2"	0.216
<b>6</b>	72	"3"	0.201
<b>7</b>	87	"1"	0.73
<b>8</b>	87	"2"	0.71
<b>9</b>	92	"3"	0.84

- *# Trim the data to take a closer look at log-phase*
- `tnglogdf = filter(:Time => t -> 47 <= t <= 92, nglogdf)`

	Time	Replicate	OD
1	47	"1"	-3.62593
2	47	"2"	-3.50635
3	52	"3"	-3.53952
4	67	"1"	-2.39593
5	67	"2"	-2.2109
6	72	"3"	-2.31473
7	87	"1"	-0.454032
8	87	"2"	-0.494109
9	92	"3"	-0.251539

- *# Log-transform the OD data*
- `transform!(tnglogdf, :OD => ByRow(log2) => :OD)`

```
ngols =
StatsModels.TableRegressionModel{LinearModel{GLM.LmResp{Vector{Float64}}, GLM.DensePredCho
```

OD ~ 1 + Time

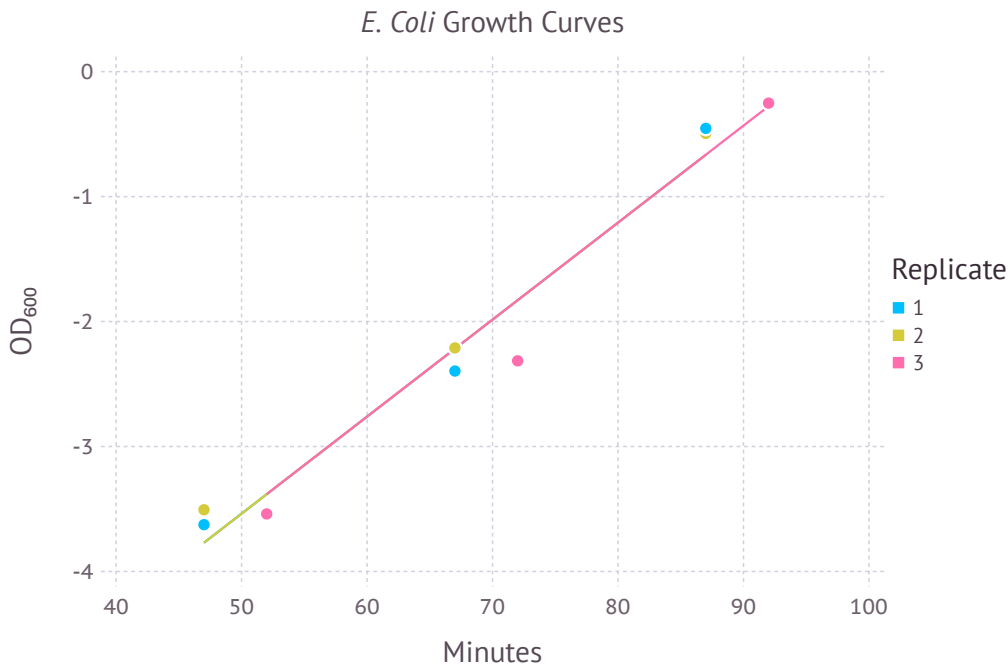
Coefficients:

	Coef.	Std. Error	t	Pr(> t )	Lower 95%	Upper 95%
(Intercept)	-7.41988	0.364521	-20.36	<1e-06	-8.28184	-6.55793
Time	0.0776471	0.00516165	15.04	<1e-05	0.0654417	0.0898524

- *# Perform and ordinary least-squares regression for a linear model*
- `ngols = lm(@formula(OD ~ Time), tnglogdf)`

```
[-3.77047, -3.77047, -3.38223, -2.21753, -2.21753, -1.82929, -0.664586, -0.664586, -0.2763
```

- *# Insert a new column into our dataframe representing the model predictions*
- `tnglogdf[:, :Model] = predict(ngols)`



```

• # And then plot it on a log-scale
• plot(tnglogdf, x=:Time, y=:OD, color=:Replicate,
•     Scale.color_discrete_hue, Geom.point,
•     Guide.xlabel("Minutes"), Guide.ylabel("OD600"),
•     Guide.title("<i>E. Coli</i> Growth Curves"),
•     # With the line-of-best-fit superimposed
•     layer(x=:Time, y=:Model, Geom.line))

```

The doubling time for no glucose was ~12.9 minutes, with a growth rate of 0.0776

```

• begin
•   # Calculate doubling-time
•   ngg = 1/coef(ngols)[2]
•   # Format it into a nice string
•   md"The doubling time for no glucose was ~$(round(ngg, sigdigits=3)) minutes, with
•     a growth rate of $(round(coef(ngols)[2], sigdigits=3))"
• end

```



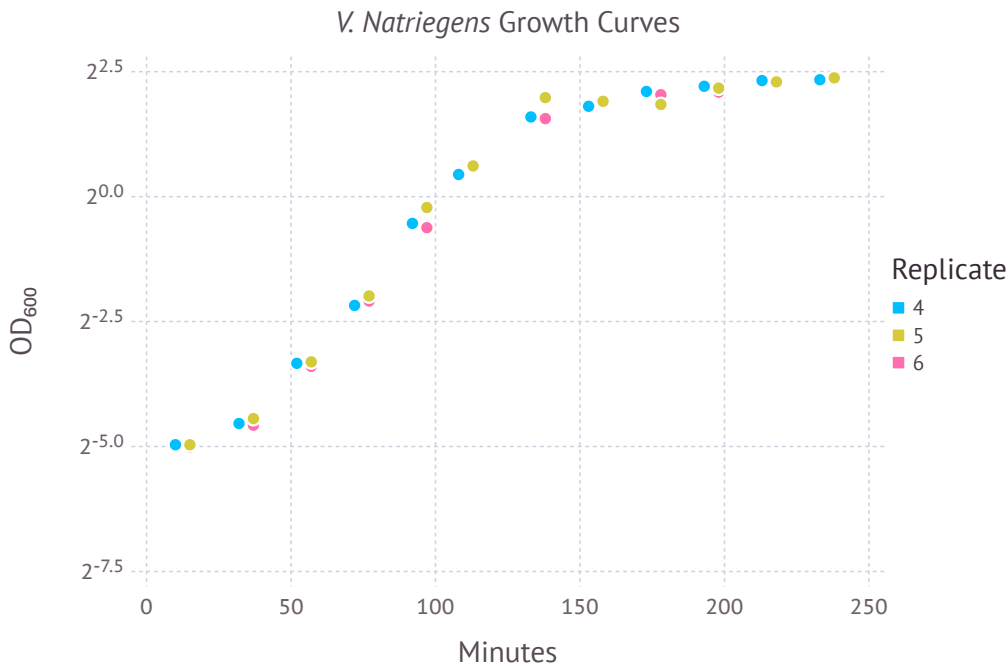
glogdf =

---

	Time	Replicate	OD
<b>1</b>	10	"4"	0.032
<b>2</b>	15	"5"	0.032
<b>3</b>	15	"6"	0.031
<b>4</b>	32	"4"	0.043
<b>5</b>	37	"5"	0.046
<b>6</b>	37	"6"	0.042
<b>7</b>	52	"4"	0.099
<b>8</b>	57	"5"	0.101
<b>9</b>	57	"6"	0.095
<b>10</b>	72	"4"	0.221
more			
<b>36</b>	238	"6"	5.1

---

- *# Isolate the glucose media*
- `glogdf = filter(:Replicate => r -> r in ["4", "5", "6"], pdf)`



- *# Replot, but the isolated values so that we can pick out the exponential growth region*
- `plot(glogdf, x=:Time, y=:OD, color=:Replicate,`
- `Scale.color_discrete_hue, Scale.y_log2,`
- `Guide.xlabel("Minutes"), Guide.ylabel("OD600"),`
- `Guide.title("<i>V. Natriegens</i> Growth Curves"))`

tglogdf =

	Time	Replicate	OD
1	52	"4"	0.099
2	57	"5"	0.101
3	57	"6"	0.095
4	72	"4"	0.221
5	77	"5"	0.252
6	77	"6"	0.235
7	92	"4"	0.69
8	97	"5"	0.86
9	97	"6"	0.65

- *# Trim the data to take a closer look at log-phase*
- `tglogdf = filter(:Time => t -> 52 <= t <= 97, glogdf)`

	Time	Replicate	OD
1	52	"4"	-3.33643
2	57	"5"	-3.30757
3	57	"6"	-3.39593
4	72	"4"	-2.17788
5	77	"5"	-1.9885
6	77	"6"	-2.08927
7	92	"4"	-0.535332
8	97	"5"	-0.217591
9	97	"6"	-0.621488

- *# Log-transform the OD data*
- `transform!(tglogdf, :OD => ByRow(log2) => :OD)`

```
gols =
StatsModels.TableRegressionModel{LinearModel{GLM.LmResp{Vector{Float64}}, GLM.DensePredCho
```

OD ~ 1 + Time

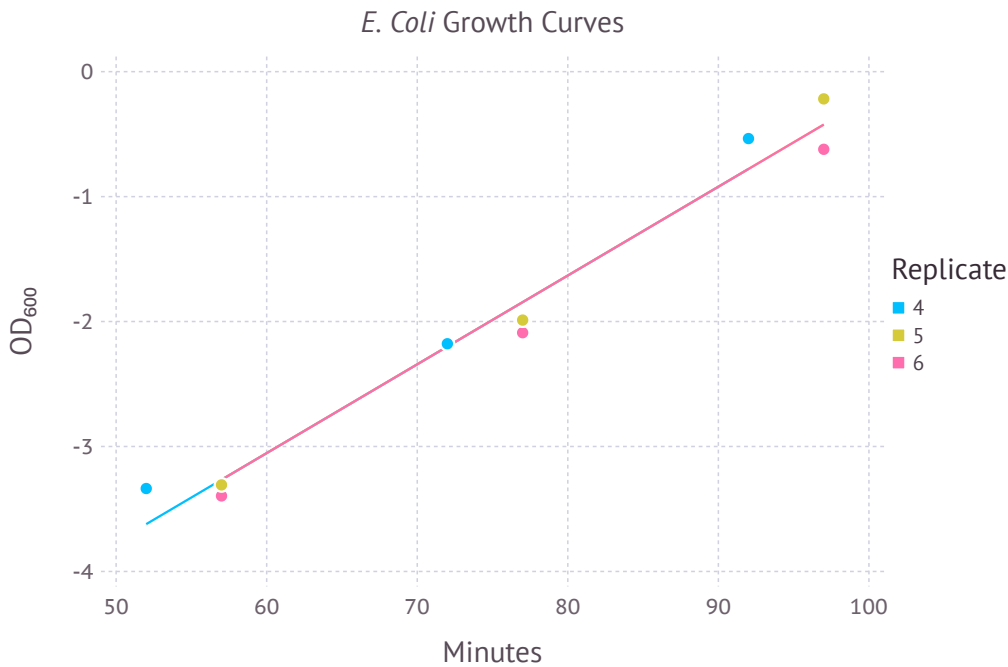
Coefficients:

	Coef.	Std. Error	t	Pr(> t )	Lower 95%	Upper 95%
(Intercept)	-7.31688	0.333952	-21.91	<1e-06	-8.10655	-6.5272
Time	0.0710647	0.00433035	16.41	<1e-06	0.0608251	0.0813044

- *# Perform and ordinary least-squares regression for a linear model*
- `gols = lm(@formula(OD ~ Time), tglogdf)`

```
[-3.62151, -3.26619, -3.26619, -2.20022, -1.84489, -1.84489, -0.778921, -0.423597, -0.4235
```

- *# Insert a new column into our dataframe representing the model predictions*
- `tglogdf[:, :Model] = predict(gols)`



```

• # And then plot it on a log-scale
• plot(tglogdf, x=:Time, y=:OD, color=:Replicate,
•     Scale.color_discrete_hue, Geom.point,
•     Guide.xlabel("Minutes"), Guide.ylabel("OD600"),
•     Guide.title("<i>E. Coli</i> Growth Curves"),
•     # With the line-of-best-fit superimposed
•     layer(x=:Time, y=:Model, Geom.line))

```

The doubling time for no glucose was ~14.1 minutes, with a growth rate of 0.0711

```

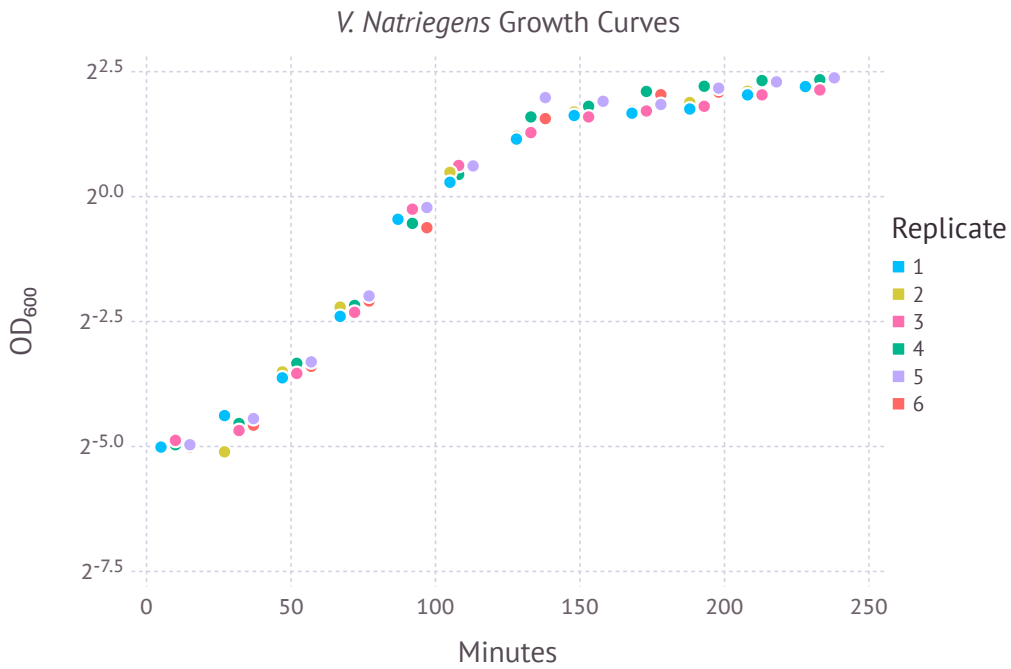
• begin
•   # Calculate doubling-time
•   gg = 1/coef(gols)[2]
•   # Format it into a nice string
•   md"The doubling time for no glucose was ~$(round(gg, sigdigits=3)) minutes, with
•     a growth rate of $(round(coef(gols)[2], sigdigits=3))"
• end

```

comparedf =

	Time	Replicate	OD
<b>1</b>	5	"1"	0.031
<b>2</b>	5	"2"	0.031
<b>3</b>	10	"3"	0.034
<b>4</b>	10	"4"	0.032
<b>5</b>	15	"5"	0.032
<b>6</b>	15	"6"	0.031
<b>7</b>	27	"1"	0.048
<b>8</b>	27	"2"	0.029
<b>9</b>	32	"3"	0.039
<b>10</b>	32	"4"	0.043
more			
<b>72</b>	238	"6"	5.1

- *# Isolate the with / without glucose media*
- `comparedf = filter(:Replicate => r -> isdigit(r[1]) , pdf)`



- *# Replot, but the isolated values so that we can pick out the exponential growth region*
- `plot(comparedf, x=:Time, y=:OD, color=:Replicate,`
- `Scale.color_discrete_hue, Scale.y_log2,`
- `Guide.xlabel("Minutes"), Guide.ylabel("OD600"),`
- `Guide.title("<i>V. Natriegens</i> Growth Curves"))`