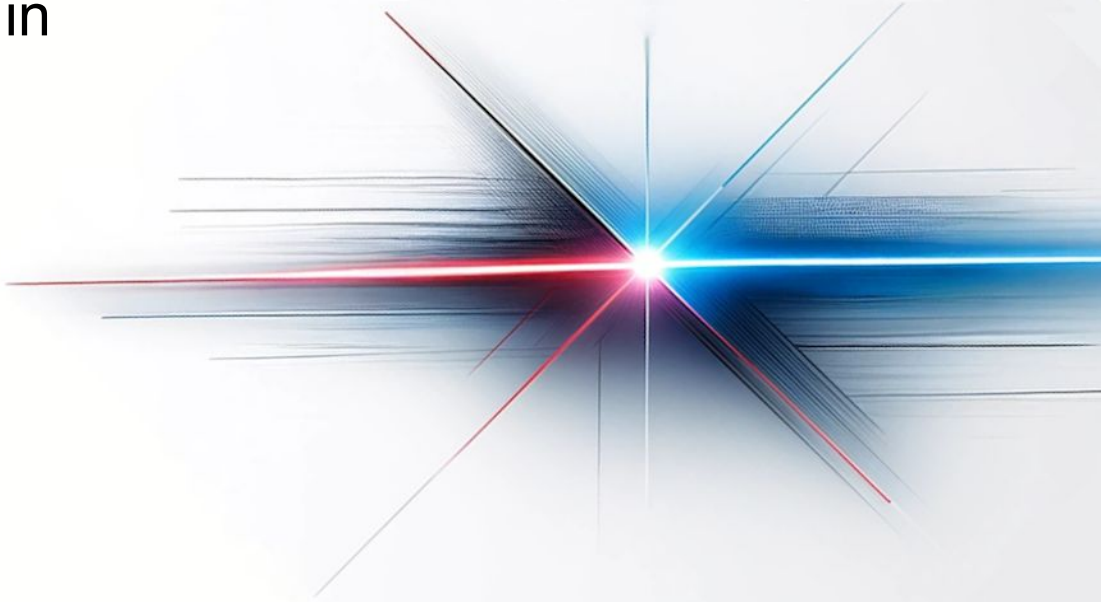
Representing Optical Density Growth Measurements to Assess Antimicrobial Susceptibility in Target Pathogens



R Tutorial Presentation

Tatiana McGarry

Guided and Unguided Antimicrobial Peptides (AMPs)

The antibiotic resistance crisis has prompted the search for new drugs that can combat infection.

Antimicrobial peptides are protein molecules that exhibit antimicrobial properties, and which can be used to destroy pathogens.

Guided (aka targeted) antimicrobial peptides are AMPs that have been fitted with a selective guide that allows them to attach directly to the pathogen of interest and leave other microbes largely unaffected.

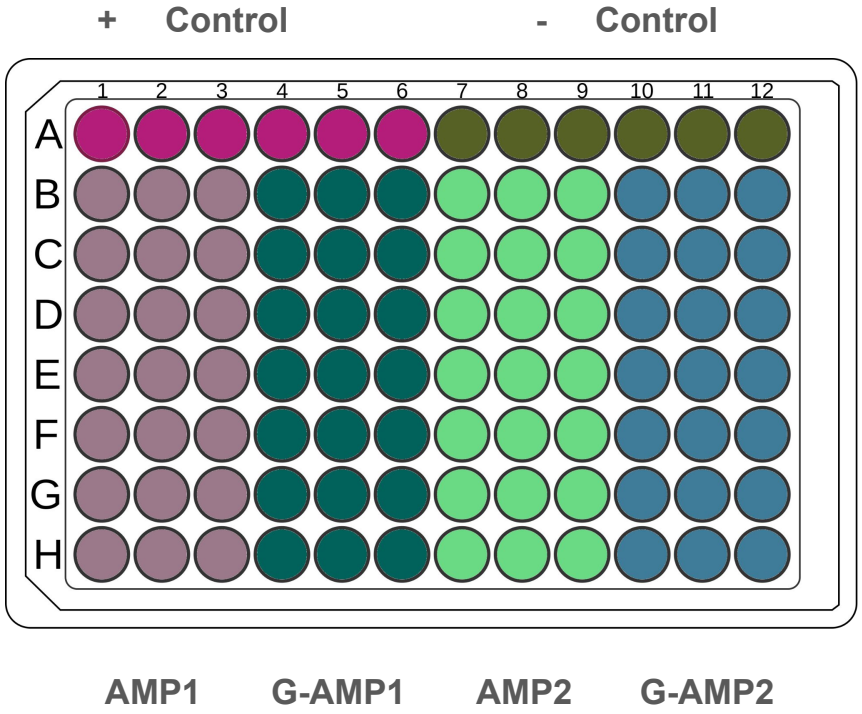
The experiment and data analysis pipeline presented here are used to assess the activity of guided and unguided AMPs against their target pathogens.

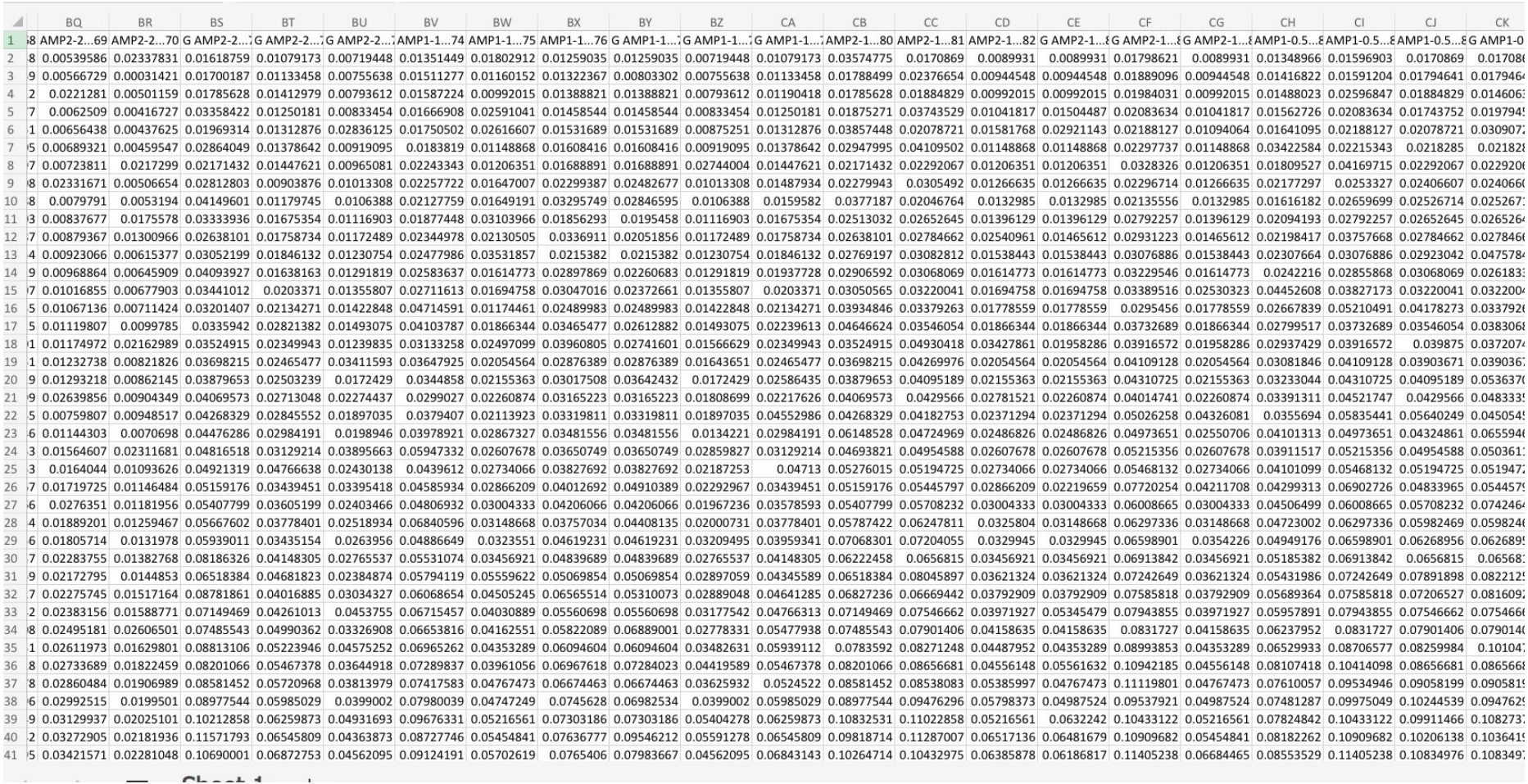
Experimental Design

96 well plate prepared with pathogen cultures in serial dilutions of targeted and untargeted AMPs – concentrations ranging from 0.5 to 32 μM

Placed in optical density reader programmed to take readings every 30 minutes over the course of a few days

Optical density data for each well is collected and analysed after the experiment.





	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE	CF	CG	CH	CI	CJ	CK
1	8	AMP2-2…69	AMP2-2…70	G AMP2-2…7	G AMP2-2…7	G AMP2-2…7	AMP1-1…74	AMP1-1…75	AMP1-1…76	G AMP1-1…7	G AMP1-1…7	G AMP1-1…7	AMP2-1…80	AMP2-1…81	AMP2-1…82	G AMP2-1…8	G AMP2-1…8	G AMP2-1…8	AMP1-0.5…8	AMP1-0.5…8	AMP1-0.5…8	G AMP1-0
2	8	0.00539586	0.02337831	0.01618759	0.01079173	0.00719448	0.01351449	0.01802912	0.01259035	0.01259035	0.00719448	0.01079173	0.03574775	0.0170869	0.0089931	0.0089931	0.01798621	0.0089931	0.01348966	0.01596903	0.0170869	0.01708
3	9	0.00566729	0.00031421	0.01700187	0.01133458	0.00755638	0.01511277	0.01160152	0.01322367	0.00803302	0.00755638	0.01133458	0.01788499	0.02376654	0.00944548	0.00944548	0.01889096	0.00944548	0.01416822	0.01591204	0.01794641	0.017946
4	2	0.0221281	0.00501159	0.01785628	0.01412979	0.00793612	0.01587224	0.00992015	0.01388821	0.01388821	0.00793612	0.01190418	0.01785628	0.01884829	0.00992015	0.00992015	0.01984031	0.00992015	0.01488023	0.02596847	0.01884829	0.014606
5	7	0.0062509	0.00416727	0.03358422	0.01250181	0.00833454	0.01666908	0.02591041	0.01458544	0.01458544	0.00833454	0.01250181	0.01875271	0.03743529	0.01041817	0.01504487	0.02083634	0.01041817	0.01562726	0.02083634	0.01743752	0.019794
6	1	0.00656438	0.00437625	0.01969314	0.01312876	0.02836125	0.01750502	0.02616607	0.01531689	0.01531689	0.00875251	0.01312876	0.03857448	0.02078721	0.01581768	0.02921143	0.02188127	0.01094064	0.01641095	0.02188127	0.02078721	0.030907
7	5	0.00689321	0.00459547	0.02864049	0.01378642	0.00919095	0.0183819	0.01148868	0.01608416	0.01608416	0.00919095	0.01378642	0.02947995	0.04109502	0.01148868	0.01148868	0.02297737	0.01148868	0.03422584	0.02215343	0.0218285	0.021828
8	7	0.00723811	0.0217299	0.02171432	0.01447621	0.00965081	0.02243343	0.01206351	0.01688891	0.01688891	0.02744004	0.01447621	0.02171432	0.02292067	0.01206351	0.01206351	0.0328326	0.01206351	0.01809527	0.04169715	0.02292067	0.022920
9	8	0.02331671	0.00506654	0.02812803	0.00903876	0.01013308	0.02257722	0.01647007	0.02299387	0.02482677	0.01013308	0.01487934	0.02279943	0.0305492	0.01266635	0.01266635	0.02296714	0.01266635	0.02177297	0.0253327	0.02406607	0.024066
10	8	0.0079791	0.0053194	0.04149601	0.01179745	0.0106388	0.02127759	0.01649191	0.03295749	0.02846595	0.0106388	0.0159582	0.0377187	0.02046764	0.0132985	0.0132985	0.02135556	0.0132985	0.01616182	0.02659699	0.02526714	0.025267
11	3	0.00837677	0.0175578	0.03333936	0.01675354	0.01116903	0.01877448	0.03103966	0.01856293	0.0195458	0.01116903	0.01675354	0.02513032	0.02652645	0.01396129	0.01396129	0.02792257	0.01396129	0.02094193	0.02792257	0.02652645	0.026526
12	7	0.00879367	0.01300966	0.02638101	0.01758734	0.01172489	0.02344978	0.02130505	0.0336911	0.02051856	0.01172489	0.01758734	0.02638101	0.02784662	0.02540961	0.01465612	0.02931223	0.01465612	0.02198417	0.03757668	0.02784662	0.027846
13	4	0.00923066	0.00615377	0.03052199	0.01846132	0.01230754	0.02477986	0.03531857	0.0215382	0.0215382	0.01230754	0.01846132	0.02769197	0.03082812	0.01538443	0.01538443	0.03076886	0.01538443	0.02307664	0.03076886	0.02923042	0.047578
14	9	0.00968864	0.00645909	0.04093927	0.01638163	0.01291819	0.02583637	0.01614773	0.02897869	0.02260683	0.01291819	0.01937728	0.02906592	0.03068069	0.01614773	0.01614773	0.03229546	0.01614773	0.0242216	0.02855868	0.03068069	0.026183
15	7	0.01016855	0.00677903	0.03441012	0.0203371	0.01355807	0.02711613	0.01694758	0.03047016	0.02372661	0.01355807	0.0203371	0.03050565	0.03220041	0.01694758	0.01694758	0.03389516	0.02530323	0.04452608	0.03827173	0.03220041	0.032200
16	5	0.01067136	0.00711424	0.03201407	0.02134271	0.01422848	0.04714591	0.01174461	0.02489983	0.02489983	0.01422848	0.02134271	0.03934846	0.03379263	0.01778559	0.01778559	0.0295456	0.01778559	0.02667839	0.05210491	0.04178273	0.033792
17	5	0.01119807	0.0099785	0.0335942	0.02821382	0.01493075	0.04103787	0.01866344	0.03465477	0.02612882	0.01493075	0.02239613	0.04646624	0.03546054	0.01866344	0.01866344	0.03732689	0.01866344	0.02799517	0.03732689	0.03546054	0.038306
18	1	0.01174972	0.02162989	0.03524915	0.02349943	0.01239835	0.03133258	0.02497099	0.03960805	0.02741601	0.01566629	0.02349943	0.03524915	0.04930418	0.03427861	0.01958286	0.03916572	0.01958286	0.02937429	0.03916572	0.039875	0.037207
19	1	0.01232738	0.00821826	0.03698215	0.02465477	0.03411593	0.03647925	0.02054564	0.02876389	0.02876389	0.01643651	0.02465477	0.03698215	0.04269976	0.02054564	0.02054564	0.04109128	0.02054564	0.03081846	0.04109128	0.03903671	0.039036
20	9	0.01293218	0.00862145	0.03879653	0.02503239	0.0172429	0.0344858	0.02155363	0.03017508	0.03642432	0.0172429	0.02586435	0.03879653	0.04095189	0.02155363	0.02155363	0.04310725	0.02155363	0.03233044	0.04310725	0.04095189	0.053637
21	9	0.02639856	0.00904349	0.04069573	0.02713048	0.02274437	0.0299027	0.02260874	0.03165223	0.03165223	0.01808699	0.02217626	0.04069573	0.0429566	0.02781521	0.02260874	0.04014741	0.02260874	0.03391311	0.04521747	0.0429566	0.048333
22	5	0.00759807	0.00948517	0.04268329	0.02845552	0.01897035	0.0379407	0.02113923	0.03319811	0.03319811	0.01897035	0.04552986	0.04268329	0.04182753	0.02371294	0.02371294	0.05026258	0.04326081	0.0355694	0.05835441	0.05640249	0.045054
23	6	0.01144303	0.0070698	0.04476286	0.02984191	0.0198946	0.03978921	0.02867327	0.03481556	0.03481556	0.0134221	0.02984191	0.06148528	0.04724969	0.02486826	0.02486826	0.04973651	0.02550706	0.04101313	0.04973651	0.04324861	0.065594
24	3	0.01564607	0.02311681	0.04816518	0.03129214	0.03895663	0.05947332	0.02607678	0.03650749	0.03650749	0.02859827	0.03129214	0.04693821	0.04954588	0.02607678	0.02607678	0.05215356	0.02607678	0.03911517	0.05215356	0.04954588	0.050361
25	3	0.0164044	0.01093626	0.04921319	0.04766638	0.02430138	0.0439612	0.02734066	0.03827692	0.03827692	0.02187253	0.04713	0.05276015	0.05194725	0.02734066	0.02734066	0.05468132	0.02734066	0.04101099	0.05468132	0.05194725	0.051947
26	7	0.01719725	0.01146484	0.05159176	0.03439451	0.03395418	0.04585934	0.02866209	0.04012692	0.04910389	0.02292967	0.03439451	0.05159176	0.05445797	0.02866209	0.02219659	0.07720254	0.04211708	0.04299313	0.06902726	0.04833965	0.054457
27	6	0.0276351	0.01181956	0.05407799	0.03605199	0.02403466	0.04806932	0.03004333	0.04206066	0.04206066	0.01967236	0.03578593	0.05407799	0.05708232	0.03004333	0.03004333	0.06008665	0.03004333	0.04506499	0.06008665	0.05708232	0.074246
28	4	0.01889201	0.01259467	0.05667602	0.03778401	0.02518934	0.06840596	0.03148668	0.03757034	0.04408135	0.02000731	0.03778401	0.05787422	0.06247811	0.0325804	0.03148668	0.06297336	0.03148668	0.04723002	0.06297336	0.05982469	0.059824
29	6	0.01805714	0.0131978	0.05939011	0.03435154	0.0263956	0.04886649	0.0323551	0.04619231	0.04619231	0.03209495	0.03959341	0.07068301	0.07204055	0.0329945	0.0329945	0.06598901	0.0354226	0.04949176	0.06598901	0.06268956	0.062689
30	7	0.02283755	0.01382768	0.08186326	0.04148305	0.02765537	0.05531074	0.03456921	0.04839689	0.04839689	0.02765537	0.04148305	0.06222458	0.0656815	0.03456921	0.03456921	0.06913842	0.03456921	0.05185382	0.06913842	0.0656815	0.06568
31	9	0.02172795	0.0144853	0.06518384	0.04681823	0.02384874	0.05794119	0.05559622	0.05069854	0.05069854	0.02897059	0.04345589	0.06518384	0.08045897	0.03621324	0.03621324	0.07242649	0.03621324	0.05431986	0.07242649	0.07891898	0.082212
32	7	0.02275745	0.01517164	0.08781861	0.04016885	0.03034327	0.06068654	0.04505245	0.06565514	0.05310073	0.02889048	0.04641285	0.06827236	0.06669442	0.03792909	0.03792909	0.07585818	0.03792909	0.05689364	0.07585818	0.07206527	0.081609
33	2	0.02383156	0.01588771	0.07149469	0.04261013	0.0453755	0.06715457	0.04030889	0.05560698	0.05560698	0.03177542	0.04766313	0.07149469	0.07546662	0.03971927	0.05345479	0.07943855	0.03971927	0.05957891	0.07943855	0.07546662	0.075466
34	8	0.02495181	0.02606501	0.07485543	0.04990362	0.03326908	0.06653816	0.04162551	0.05822089	0.06889001	0.02778331	0.05477938	0.07485543	0.07901406	0.04158635	0.04158635	0.0831727	0.04158635	0.06237952	0.0831727	0.07901406	0.079014
35	1	0.02611973	0.01629801	0.08813106	0.05223946	0.04575252	0.06965262	0.04353289	0.06094604	0.06094604	0.03482631	0.05939112	0.0783592	0.08271248	0.04487952	0.04353289	0.08993853	0.04353289	0.06529933	0.08706577	0.08259984	0.10104
36	8	0.02733689	0.01822459	0.08201066	0.05467378	0.03644918	0.07289837	0.03961056	0.06967618	0.07284023	0.04419589	0.05467378	0.08201066	0.08656681	0.04556148	0.05561632	0.10942185	0.04556148	0.08107418	0.10414098	0.08656681	0.085666
37	8	0.02860484	0.01906989	0.08581452	0.05720968	0.03813979	0.07417583	0.04767473	0.06674463	0.06674463	0.03625932	0.0524522	0.08581452	0.08538083	0.05385997	0.04767473	0.11119801	0.04767473	0.07610057	0.09534946	0.09058199	0.090581
38	6	0.02992515	0.0199501	0.08977544	0.05985029	0.0399002	0.07980039	0.04747249	0.0745628	0.06982534	0.0399002	0.05985029	0.08977544	0.09476296	0.05798373	0.04987524	0.09537921	0.04987524	0.07481287	0.09975049	0.10244539	0.094762
39	9	0.03129937	0.02025101	0.10212858	0.06259873	0.04931693	0.09676331	0.05216561	0.07303186	0.07303186	0.05404278	0.06259873	0.10832531	0.11022858	0.05216561	0.0632242	0.10433122	0.05216561	0.07824842	0.10433122	0.09911466	0.108273
40	2	0.03272905	0.02181936	0.11571793	0.06545809	0.04363873	0.08727746	0.05454841	0.07636777	0.09546212	0.05591278	0.06545809	0.09818714	0.11287007	0.06517136	0.06481679	0.10909682	0.05454841	0.08182262	0.10909682	0.10206138	0.103641
41	5	0.03421571	0.02281048	0.10690001	0.06872753	0.04562095	0.09124191	0.05702619	0.0765406	0.07983667	0.04562095	0.06843143	0.10264714	0.10432975	0.06385878	0.06186817	0.11405238	0.06684465	0.08553529	0.11405238	0.10834976	0.108349

96 * 161 = 15,456 optical density readings

Data Transformations

	Time	name	value
1	0.0	Blank...2	9.590443e-04
2	0.0	Blank...3	1.523397e-02
3	0.0	Blank...4	0.000000e+00
4	0.0	Blank...5	-1.672591e-03
5	0.0	Blank...6	1.254863e-02
6	0.0	Blank...7	1.080406e-02
7	0.0	Positive Control...8	2.723957e-02
8	0.0	Positive Control...9	1.798621e-02
9	0.0	Positive Control...10	3.218152e-02
10	0.0	Positive Control...11	1.798621e-02
11	0.0	Positive Control...12	1.798621e-02
12	0.0	Positive Control...13	1.798621e-02
13	0.0	AMP1-32...14	8.993105e-04
14	0.0	AMP1-32...15	0.000000e+00
15	0.0	AMP1-32...16	0.000000e+00
16	0.0	G AMP1-32...17	8.993105e-04
17	0.0	G AMP1-32...18	0.000000e+00
18	0.0	G AMP1-32...19	0.000000e+00
19	0.0	AMP2-32...20	-1.391547e-03
20	0.0	AMP2-32...21	8.993105e-04
21	0.0	AMP2-32...22	0.000000e+00
22	0.0	G AMP2-32...23	2.697931e-03
23	0.0	G AMP2-32...24	4.496552e-03

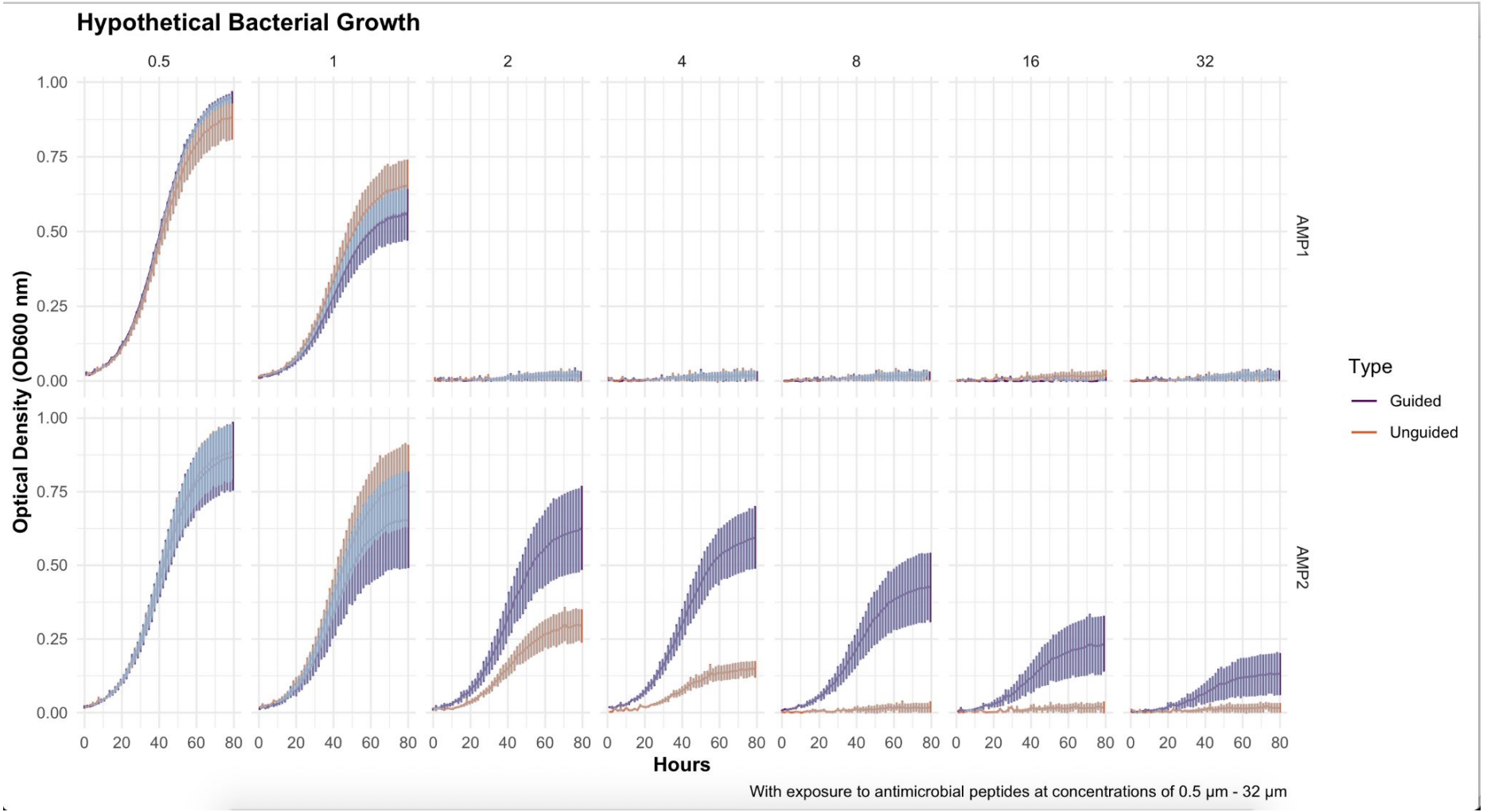
```
mutate(...gsub
("\\.\\.\\.\\d+",
"", name)) …

separate(...)

Data$Type <-
if_else(...)

Data$Type <-
if_else(...)

BASE_AMP=if
_else(grepl(...)

as.character(m
ap(strsplit(..))
```

	Time	AMP	Concentration (uM)	value	Type	BASE_AMP
1	0.0	Blank	*NA*	9.590443e-04		Blank
2	0.0	Blank	*NA*	1.523397e-02		Blank
3	0.0	Blank	*NA*	0.000000e+00		Blank
4	0.0	Blank	*NA*	-1.672591e-03		Blank
5	0.0	Blank	*NA*	1.254863e-02		Blank
6	0.0	Blank	*NA*	1.080406e-02		Blank
7	0.0	Positive Control	*NA*	2.723957e-02		Positive Control
8	0.0	Positive Control	*NA*	1.798621e-02		Positive Control
9	0.0	Positive Control	*NA*	3.218152e-02		Positive Control
10	0.0	Positive Control	*NA*	1.798621e-02		Positive Control
11	0.0	Positive Control	*NA*	1.798621e-02		Positive Control
12	0.0	Positive Control	*NA*	1.798621e-02		Positive Control
13	0.0	AMP1	32	8.993105e-04	Unguided	AMP1
14	0.0	AMP1	32	0.000000e+00	Unguided	AMP1
15	0.0	AMP1	32	0.000000e+00	Unguided	AMP1
16	0.0	G AMP1	32	8.993105e-04	Guided	AMP1
17	0.0	G AMP1	32	0.000000e+00	Guided	AMP1
18	0.0	G AMP1	32	0.000000e+00	Guided	AMP1
19	0.0	AMP2	32	-1.391547e-03	Unguided	AMP2
20	0.0	AMP2	32	8.993105e-04	Unguided	AMP2
21	0.0	AMP2	32	0.000000e+00	Unguided	AMP2
22	0.0	G AMP2	32	2.697931e-03	Guided	AMP2
23	0.0	G AMP2	32	4.496552e-03	Guided	AMP2

Group and Calculate Statistics

```
Data <- Data %>%
group_by(AMP,
BASE_AMP,
`Concentration (uM)`, Type,
Time)

Data <- summarize(Data,
Mean=mean(value),
        number=n(),

upper_confindence=mean(
value)+sd(value)/sqrt(n()),

lower_confindence=mean(v
alue)-sd(value)/sqrt(n()))

Data <- Data %>%
  mutate(...) =
as.numeric(`Concentration
(uM)`))
```

	AMP	BASE_AMP	Concentration (uM)	Type	Time	Mean	number	upper_confindence	lower_confindence
1	AMP1	AMP1	0.5	Unguided	0.0	0.01551520	3	0.01657813	0.01445226
2	AMP1	AMP1	0.5	Unguided	0.5	0.01600889	3	0.01710064	0.01491715
3	AMP1	AMP1	0.5	Unguided	1.0	0.01989900	3	0.02314272	0.01665527
4	AMP1	AMP1	0.5	Unguided	1.5	0.01796704	3	0.01949390	0.01644018
5	AMP1	AMP1	0.5	Unguided	2.0	0.01969314	3	0.02136435	0.01802193
6	AMP1	AMP1	0.5	Unguided	2.5	0.02606925	3	0.03014862	0.02198989
7	AMP1	AMP1	0.5	Unguided	3.0	0.02757103	3	0.03477014	0.02037192
8	AMP1	AMP1	0.5	Unguided	3.5	0.02372391	3	0.02476566	0.02268216
9	AMP1	AMP1	0.5	Unguided	4.0	0.02267532	3	0.02595462	0.01939602
10	AMP1	AMP1	0.5	Unguided	4.5	0.02513032	3	0.02726294	0.02299769
11	AMP1	AMP1	0.5	Unguided	5.0	0.02913582	3	0.03368291	0.02458873
12	AMP1	AMP1	0.5	Unguided	5.5	0.02769197	3	0.03004198	0.02534196
13	AMP1	AMP1	0.5	Unguided	6.0	0.02782032	3	0.02972110	0.02591955
14	AMP1	AMP1	0.5	Unguided	6.5	0.03833274	3	0.04189098	0.03477449
15	AMP1	AMP1	0.5	Unguided	7.0	0.04018868	3	0.04757183	0.03280553
16	AMP1	AMP1	0.5	Unguided	7.5	0.03359420	3	0.03644509	0.03074331
17	AMP1	AMP1	0.5	Unguided	8.0	0.03613834	3	0.03952655	0.03275012
18	AMP1	AMP1	0.5	Unguided	8.5	0.03698215	3	0.04012055	0.03384375
19	AMP1	AMP1	0.5	Unguided	9.0	0.03879653	3	0.04208890	0.03550416
20	AMP1	AMP1	0.5	Unguided	9.5	0.04069573	3	0.04414927	0.03724218
21	AMP1	AMP1	0.5	Unguided	10.0	0.05010877	3	0.05740026	0.04281728
22	AMP1	AMP1	0.5	Unguided	10.5	0.04466609	3	0.04728214	0.04205003
23	AMP1	AMP1	0.5	Unguided	11.0	0.04693821	3	0.05092150	0.04295491


Hypothetical Bacterial Growth
0.5
1
2
4
8
16
32
AMP1
AMP2
Optical Density (OD600 nm)
1.00
0.75
0.50
0.25
0.00
0
20
40
60
80
Hours
Type
Guided
Unguided
With exposure to antimicrobial peptides at concentrations of 0.5 µm - 32 µm

Questions?