

ALE of *Escherichia coli* to increased growth in $MgCl_2$

▶ Compared to growth in ideal medium...

- ▶ Cells formed worm like shape
- ▶ Significantly lower cell densities
- ▶ Growth rate depressed

Ancestor



Evolved lineage



1
00:00:25,589 --> 00:00:23,429
so chaotropic environments are hyper

2
00:00:25,830 --> 00:00:25,599
saline brines with a high concentration

3
00:00:29,189 --> 00:00:25,840
of

4
00:00:31,029 --> 00:00:29,199
ketotropes broadly speaking ko trips can

5
00:00:32,709 --> 00:00:31,039
be thought of as order breakers which

6
00:00:35,350 --> 00:00:32,719
disrupt h-bonding

7
00:00:37,430 --> 00:00:35,360
um in bulk solution these will denature

8
00:00:40,069 --> 00:00:37,440
biological macromolecules

9
00:00:41,030 --> 00:00:40,079
uh leading to cell death and high enough

10
00:00:43,270 --> 00:00:41,040
concentration

11
00:00:44,069 --> 00:00:43,280
so a common keotrope environmental

12
00:00:46,069 --> 00:00:44,079
chaotrope would be

13
00:00:47,990 --> 00:00:46,079

chloride so in a very high concentration

14

00:00:50,229 --> 00:00:48,000

of chloride this will inhibit

15

00:00:51,590 --> 00:00:50,239

cellular function via protein

16

00:00:54,630 --> 00:00:51,600

denaturation

17

00:00:56,389 --> 00:00:54,640

and enzyme inhibition and to a certain

18

00:00:57,990 --> 00:00:56,399

concentration and for example in this

19

00:01:00,470 --> 00:00:58,000

magnesium chloride brine

20

00:01:01,110 --> 00:01:00,480

southern california being largely

21

00:01:04,469 --> 00:01:01,120

considered

22

00:01:06,870 --> 00:01:04,479

sterile in these upper limits however

23

00:01:07,670 --> 00:01:06,880

there's some speculative evidence of

24

00:01:10,830 --> 00:01:07,680

active life

25

00:01:12,870 --> 00:01:10,840

within magnesium chloride saturated

26

00:01:15,590 --> 00:01:12,880

dhabs

27

00:01:17,190 --> 00:01:15,600

deep sea hyper saline and oxide basins

28

00:01:17,749 --> 00:01:17,200

these are noxic brine environments that

29

00:01:19,590 --> 00:01:17,759

are found

30

00:01:21,510 --> 00:01:19,600

in benthic depressions generally

31

00:01:22,870 --> 00:01:21,520

enclosed seas such as the eastern

32

00:01:26,789 --> 00:01:22,880

mediterranean

33

00:01:28,149 --> 00:01:26,799

and the red sea so there's a few dozen d

34

00:01:30,230 --> 00:01:28,159

habs that have been discovered

35

00:01:31,350 --> 00:01:30,240

most of them are sodium chloride

36

00:01:33,350 --> 00:01:31,360

saturated with

37

00:01:34,950 --> 00:01:33,360

um a swath of evidence showing active

38

00:01:36,789 --> 00:01:34,960

microbial communities

39

00:01:39,429 --> 00:01:36,799

while only three have been shown to be

40

00:01:42,310 --> 00:01:39,439

magnesium chloride dominated

41

00:01:43,990 --> 00:01:42,320

so within this interface layer so in

42

00:01:46,149 --> 00:01:44,000

this figure this is just showing a

43

00:01:47,429 --> 00:01:46,159

basic d hem so an ancient evaporate

44

00:01:49,030 --> 00:01:47,439

which then dissolves

45

00:01:50,469 --> 00:01:49,040

forming a brine that collects an

46

00:01:52,149 --> 00:01:50,479

adventic depression

47

00:01:53,749 --> 00:01:52,159

here we have a two to three meter

48

00:01:55,749 --> 00:01:53,759

interface layer where you have an

49

00:01:56,230 --> 00:01:55,759

extremely steep chemokline going from

50

00:01:59,190 --> 00:01:56,240

about

51
00:01:59,749 --> 00:01:59,200
zero millimolar um sorry zero molar all

52
00:02:02,230 --> 00:01:59,759
the way

53
00:02:03,749 --> 00:02:02,240
upwards of five molar magnesium chloride

54
00:02:05,030 --> 00:02:03,759
within the brine and member

55
00:02:07,270 --> 00:02:05,040
and that only two to three meter

56
00:02:09,469 --> 00:02:07,280
interface so so the current magnesium

57
00:02:12,710 --> 00:02:09,479
chloride limit of life is around

58
00:02:14,470 --> 00:02:12,720
2.3 some studies going as far to say

59
00:02:15,270 --> 00:02:14,480
that it's around three more in some of

60
00:02:18,869 --> 00:02:15,280
these

61
00:02:19,830 --> 00:02:18,879
dehabs whereas again as i mentioned this

62
00:02:21,990 --> 00:02:19,840
brine being

63
00:02:23,030 --> 00:02:22,000

four to five molar magnesium chloride

64

00:02:25,670 --> 00:02:23,040

and there's some

65

00:02:28,150 --> 00:02:25,680

speculative evidence in the cryosprine

66

00:02:29,670 --> 00:02:28,160

that shows sulfate reduction

67

00:02:32,790 --> 00:02:29,680

so this was just to really highlight

68

00:02:34,790 --> 00:02:32,800

this limit being around to it 2.3

69

00:02:36,949 --> 00:02:34,800

and then the brine itself being the 4

70

00:02:38,790 --> 00:02:36,959

fortified molar which is significantly

71

00:02:40,790 --> 00:02:38,800

more stressful

72

00:02:42,869 --> 00:02:40,800

the reason why we're interested in

73

00:02:43,910 --> 00:02:42,879

dehabs especially magnesium core id

74

00:02:46,309 --> 00:02:43,920

heads

75

00:02:47,910 --> 00:02:46,319

uh that they share some similarities in

76

00:02:50,869 --> 00:02:47,920

the hypothesized

77

00:02:53,030 --> 00:02:50,879

brine lenses on europa as well as some

78

00:02:55,509 --> 00:02:53,040

perchlorate brines on mars

79

00:02:57,030 --> 00:02:55,519

and then subsurface oceans of enceladus

80

00:02:58,710 --> 00:02:57,040

so we get this nice window to

81

00:03:01,509 --> 00:02:58,720

extraterrestrial brines

82

00:03:03,030 --> 00:03:01,519

via the scope of the dhabs and the

83

00:03:04,470 --> 00:03:03,040

motivation of this project that i'll be

84

00:03:06,949 --> 00:03:04,480

talking about today

85

00:03:09,030 --> 00:03:06,959

is to unders better understand micro

86

00:03:11,190 --> 00:03:09,040

microbial adaptation to magnesium

87

00:03:12,869 --> 00:03:11,200

chloride brines as the genes associated

88

00:03:14,869 --> 00:03:12,879

with life in these environments

89

00:03:15,990 --> 00:03:14,879

are virtually unknown there's some

90

00:03:17,910 --> 00:03:16,000

strategies that are

91

00:03:19,110 --> 00:03:17,920

associated with sodium chloride

92

00:03:21,190 --> 00:03:19,120

adaptation

93

00:03:22,309 --> 00:03:21,200

but specific to magnesium chloride there

94

00:03:24,070 --> 00:03:22,319

are some holes

95

00:03:25,350 --> 00:03:24,080

and the limits of life and magnesium

96

00:03:27,509 --> 00:03:25,360

chloride brines

97

00:03:28,550 --> 00:03:27,519

are poorly defined there's there's a lot

98

00:03:31,110 --> 00:03:28,560

of speculation

99

00:03:32,869 --> 00:03:31,120

of whether they're in a higher

100

00:03:34,149 --> 00:03:32,879

concentration brine that four to five

101

00:03:35,509 --> 00:03:34,159

molar zone

102

00:03:37,830 --> 00:03:35,519

so the study i'll talk about today is

103

00:03:40,070 --> 00:03:37,840

evolution of e coli to increase

104

00:03:41,830 --> 00:03:40,080

its magnesium chloride stress so the

105

00:03:44,309 --> 00:03:41,840

goal of this project was to engineer

106

00:03:46,390 --> 00:03:44,319

kyoto e coli strains using adaptive

107

00:03:48,949 --> 00:03:46,400

laboratory evolution hereby known as

108

00:03:50,550 --> 00:03:48,959

all for short with the purpose to

109

00:03:52,550 --> 00:03:50,560

identify genes that confer

110

00:03:54,630 --> 00:03:52,560

magnesium chloride tolerance with the

111

00:03:55,589 --> 00:03:54,640

big why to have this improved molecular

112

00:03:57,350 --> 00:03:55,599

understanding

113

00:03:58,710 --> 00:03:57,360

of the limits of life in chaotropic

114

00:04:01,509 --> 00:03:58,720

brines

115

00:04:03,110 --> 00:04:01,519

so adaptive laboratory evolution or ale

116

00:04:05,190 --> 00:04:03,120

again is based upon principles of

117

00:04:06,710 --> 00:04:05,200

darwinian evolution to develop evolved

118

00:04:07,910 --> 00:04:06,720

microbial strains

119

00:04:09,589 --> 00:04:07,920

so you can think of you'd have your

120

00:04:10,309 --> 00:04:09,599

original population of cells in your

121

00:04:12,630 --> 00:04:10,319

test tube

122

00:04:14,070 --> 00:04:12,640

if you increase a given stressor in my

123

00:04:15,750 --> 00:04:14,080

case magnesium chloride

124

00:04:17,670 --> 00:04:15,760

you'll have some rare survivors that

125

00:04:19,830 --> 00:04:17,680

might have a better adaptation

126
00:04:21,030 --> 00:04:19,840
just through random mutation to the

127
00:04:24,070 --> 00:04:21,040
changing environment

128
00:04:25,909 --> 00:04:24,080
this will then result in a population of

129
00:04:26,790 --> 00:04:25,919
cells that are slightly more tolerant to

130
00:04:28,310 --> 00:04:26,800
the stressor

131
00:04:30,550 --> 00:04:28,320
and as you keep going down the line

132
00:04:32,550 --> 00:04:30,560
you'll have more and more rare survivors

133
00:04:35,350 --> 00:04:32,560
until you eventually have a stress

134
00:04:37,590 --> 00:04:35,360
resistant lineage that is nearly ident

135
00:04:39,909 --> 00:04:37,600
genetically identical to that of the

136
00:04:43,189 --> 00:04:39,919
starting strain

137
00:04:44,710 --> 00:04:43,199
so once i completed my evolution

138
00:04:45,909 --> 00:04:44,720

experiment over a number of months i

139

00:04:47,510 --> 00:04:45,919

could then

140

00:04:50,150 --> 00:04:47,520

elucidate these mutations using a

141

00:04:52,150 --> 00:04:50,160

bioinformatics pipeline known as briseq

142

00:04:54,390 --> 00:04:52,160

so this involves taking the ancestral

143

00:04:56,550 --> 00:04:54,400

strain as well as your evolve strain

144

00:04:57,990 --> 00:04:56,560

throwing that through a uh sequencing

145

00:05:00,310 --> 00:04:58,000

company so i i went through

146

00:05:01,590 --> 00:05:00,320

illumina platform and then plugging into

147

00:05:03,590 --> 00:05:01,600

briseq which will then

148

00:05:05,510 --> 00:05:03,600

output a user summary file that

149

00:05:07,510 --> 00:05:05,520

essentially compares

150

00:05:08,550 --> 00:05:07,520

the ancestral genome to that of the

151
00:05:10,390 --> 00:05:08,560
evolved genome

152
00:05:11,990 --> 00:05:10,400
and identifies where mutations have

153
00:05:13,350 --> 00:05:12,000
occurred throughout the course of your

154
00:05:15,590 --> 00:05:13,360
ale experiment

155
00:05:17,909 --> 00:05:15,600
so the brief method here was to grow e

156
00:05:18,790 --> 00:05:17,919
coli and lb and three lineages shown

157
00:05:21,189 --> 00:05:18,800
here

158
00:05:23,830 --> 00:05:21,199
incubating them checking for cfus as

159
00:05:25,749 --> 00:05:23,840
well as phenotype on auger plates

160
00:05:27,350 --> 00:05:25,759
check tracking their ods over time and

161
00:05:29,029 --> 00:05:27,360
storing some in -80

162
00:05:31,110 --> 00:05:29,039
and again at every single subsequent

163
00:05:32,310 --> 00:05:31,120

transfer i'm increasing magnesium

164

00:05:34,550 --> 00:05:32,320

chloride concentration

165

00:05:36,150 --> 00:05:34,560

and repeating the process so after about

166

00:05:39,670 --> 00:05:36,160

200 generations

167

00:05:42,390 --> 00:05:39,680

i saw nearly a um

168

00:05:43,990 --> 00:05:42,400

tolerance growth sorry growth tolerance

169

00:05:47,110 --> 00:05:44,000

that nearly doubled

170

00:05:48,870 --> 00:05:47,120

through the course of ale from a macro

171

00:05:52,629 --> 00:05:48,880

perspective just looking at the cells

172

00:05:54,310 --> 00:05:52,639

phenotypically the ancestral strain

173

00:05:56,390 --> 00:05:54,320

form you know just a classic dense

174

00:05:58,390 --> 00:05:56,400

here's a classic dense e coli culture

175

00:06:00,870 --> 00:05:58,400

and lb whereas the evolve strains were

176
00:06:01,670 --> 00:06:00,880
forming this biofilm this large macro

177
00:06:03,830 --> 00:06:01,680
structure

178
00:06:05,749 --> 00:06:03,840
that was actually tying itself in knots

179
00:06:07,830 --> 00:06:05,759
from this image here so all the cells

180
00:06:08,870 --> 00:06:07,840
really aggregating and clumping up close

181
00:06:11,830 --> 00:06:08,880
together

182
00:06:13,749 --> 00:06:11,840
i also noticed a corresponding decrease

183
00:06:17,510 --> 00:06:13,759
in cell density and a

184
00:06:20,950 --> 00:06:19,350
so after the course of ale to confirm

185
00:06:22,629 --> 00:06:20,960
that i'm actually getting the phenotype

186
00:06:24,390 --> 00:06:22,639
of increased growth magnesium chloride

187
00:06:26,790 --> 00:06:24,400
so on the left here we have

188
00:06:28,870 --> 00:06:26,800

um growth of e coli just in zero

189

00:06:31,990 --> 00:06:28,880

millimolar magnesium chloride lb

190

00:06:34,469 --> 00:06:32,000

lb is just a rich protein broth that's

191

00:06:35,510 --> 00:06:34,479

commonly used for mesophiles such as e

192

00:06:38,469 --> 00:06:35,520

coli

193

00:06:39,510 --> 00:06:38,479

so in red i have the ancestor in green

194

00:06:41,189 --> 00:06:39,520

and blue i have the b

195

00:06:43,909 --> 00:06:41,199

and the c lineage these are the two

196

00:06:47,909 --> 00:06:43,919

evolved lineage after the chorus of ale

197

00:06:49,990 --> 00:06:47,919

i have od 600 on the the y-axis which is

198

00:06:50,629 --> 00:06:50,000

just a measure of optical density so

199

00:06:54,469 --> 00:06:50,639

this is

200

00:06:57,670 --> 00:06:54,479

in a culture

201
00:06:58,710 --> 00:06:57,680
with time on the x in hours and what i

202
00:07:01,430 --> 00:06:58,720
found was that

203
00:07:01,990 --> 00:07:01,440
the evolved strains had a slightly

204
00:07:03,510 --> 00:07:02,000
depressed

205
00:07:06,230 --> 00:07:03,520
growth rate compared to that of the

206
00:07:08,150 --> 00:07:06,240
ancestor whereas

207
00:07:09,589 --> 00:07:08,160
growing them at an elevated magnesium

208
00:07:11,749 --> 00:07:09,599
chloride concentration

209
00:07:13,510 --> 00:07:11,759
the ancestor never achieved growth as

210
00:07:13,990 --> 00:07:13,520
you can see here here by this flat red

211
00:07:17,990 --> 00:07:14,000
line

212
00:07:20,790 --> 00:07:18,000
reaching a significant

213
00:07:21,589 --> 00:07:20,800

cell density um with nearly similar

214

00:07:25,749 --> 00:07:21,599

growth rates

215

00:07:27,350 --> 00:07:25,759

at 375 millimolar magnesium chloride

216

00:07:28,790 --> 00:07:27,360

i was curious to see how the strains

217

00:07:29,270 --> 00:07:28,800

respond to other salts to see if there

218

00:07:31,589 --> 00:07:29,280

was a more

219

00:07:33,990 --> 00:07:31,599

universal chaotropic sorry more

220

00:07:34,469 --> 00:07:34,000

universal solute stress tolerance or if

221

00:07:36,469 --> 00:07:34,479

their

222

00:07:37,909 --> 00:07:36,479

adaptation was very specific to that of

223

00:07:39,589 --> 00:07:37,919

magnesium chloride

224

00:07:42,150 --> 00:07:39,599

i noticed that obviously the evolved

225

00:07:44,469 --> 00:07:42,160

strain is more ko tolerant

226

00:07:46,309 --> 00:07:44,479

in sodium chloride we saw that the

227

00:07:47,830 --> 00:07:46,319

greatest taunt was actually surprisingly

228

00:07:49,510 --> 00:07:47,840

with the ancestral strain which is

229

00:07:50,550 --> 00:07:49,520

probably indicating a very specific

230

00:07:52,710 --> 00:07:50,560

adaptation

231

00:07:54,469 --> 00:07:52,720

within magnesium sulfate brines again

232

00:07:58,550 --> 00:07:54,479

another environmentally

233

00:08:01,749 --> 00:07:58,560

relevant brine we're seeing the same

234

00:08:04,150 --> 00:08:01,759

trend of just a slightly depressed uh

235

00:08:05,670 --> 00:08:04,160

cell density so the blues being the

236

00:08:07,189 --> 00:08:05,680

evolved lineages

237

00:08:08,950 --> 00:08:07,199

whereas the ancestor growing to much

238

00:08:09,909 --> 00:08:08,960

higher cell densities and magnesium

239

00:08:12,309 --> 00:08:09,919

sulfate

240

00:08:13,270 --> 00:08:12,319

what was great to see is with um calcium

241

00:08:15,749 --> 00:08:13,280

chloride

242

00:08:18,150 --> 00:08:15,759

um we saw the same trend with that of

243

00:08:21,189 --> 00:08:18,160

magnesium chloride so we're seeing

244

00:08:22,150 --> 00:08:21,199

strong growth in calcium chloride at 350

245

00:08:24,629 --> 00:08:22,160

millimolar

246

00:08:26,390 --> 00:08:24,639

with that of the evolved lineage evolved

247

00:08:28,150 --> 00:08:26,400

lineage so the blue

248

00:08:30,150 --> 00:08:28,160

or as the ancestor never achieving

249

00:08:31,350 --> 00:08:30,160

growth so this told us our

250

00:08:33,909 --> 00:08:31,360

interpretation was that

251

00:08:36,790 --> 00:08:33,919

the cells have a very specific chloride

252

00:08:40,230 --> 00:08:39,829

um upon analysis of the breseq data so

253

00:08:43,509 --> 00:08:40,240

again

254

00:08:45,430 --> 00:08:43,519

comparing the two genomes the ancestral

255

00:08:47,190 --> 00:08:45,440

as well as the evolved lineages

256

00:08:49,430 --> 00:08:47,200

the two mutations that were shared

257

00:08:51,829 --> 00:08:49,440

amongst the evolved strains b

258

00:08:53,190 --> 00:08:51,839

and c were involved in the men's sea

259

00:08:54,470 --> 00:08:53,200

gene which is involved in the production

260

00:08:56,389 --> 00:08:54,480

of metaquinoids

261

00:08:57,990 --> 00:08:56,399

these have a role in electron transport

262

00:09:00,630 --> 00:08:58,000

in the cell membrane

263

00:09:01,030 --> 00:09:00,640

and ubiquinone has been previously shown

264

00:09:05,350 --> 00:09:01,040

to

265

00:09:07,430 --> 00:09:05,360

certain bacteria

266

00:09:09,190 --> 00:09:07,440

so the mnc gene we need to further

267

00:09:10,790 --> 00:09:09,200

elucidate this and really

268

00:09:12,470 --> 00:09:10,800

dive deep into literature to have a

269

00:09:13,269 --> 00:09:12,480

better understanding but just from first

270

00:09:15,509 --> 00:09:13,279

glance

271

00:09:18,630 --> 00:09:15,519

there's some previous research that

272

00:09:20,829 --> 00:09:18,640

suggests that this mensee mutation

273

00:09:22,230 --> 00:09:20,839

would confer and increase osmotic

274

00:09:24,630 --> 00:09:22,240

tolerance

275

00:09:27,110 --> 00:09:24,640

and the other mutation that was shared

276

00:09:29,269 --> 00:09:27,120

was in the rcs system so the rcs genes

277

00:09:29,910 --> 00:09:29,279

regulate clonic acid production also

278

00:09:32,790 --> 00:09:29,920

known as

279

00:09:34,630 --> 00:09:32,800

eps eps is this negatively charged

280

00:09:36,150 --> 00:09:34,640

polysaccharide that forms a protective

281

00:09:38,550 --> 00:09:36,160

sheath around the cell

282

00:09:40,550 --> 00:09:38,560

previously shown to desiccate sorry to

283

00:09:43,269 --> 00:09:40,560

protect cells from desiccation

284

00:09:44,150 --> 00:09:43,279

as well as osmotic stress so this brief

285

00:09:47,829 --> 00:09:44,160

figure

286

00:09:49,750 --> 00:09:47,839

um from this paper indicated here

287

00:09:51,750 --> 00:09:49,760

we have the environmental stimuli so if

288

00:09:53,990 --> 00:09:51,760

this is your cell membrane this is

289

00:09:55,990 --> 00:09:54,000

inside the cell this is the the

290

00:09:57,750 --> 00:09:56,000

peptidoglycan layer

291

00:10:00,150 --> 00:09:57,760

we would have an environmental stimuli

292

00:10:02,710 --> 00:10:00,160

represented by the black lightning bolts

293

00:10:03,750 --> 00:10:02,720

the rcs system is then activated

294

00:10:06,790 --> 00:10:03,760

phosphorus

295

00:10:08,710 --> 00:10:06,800

this phosphorylation cascade resulted in

296

00:10:10,870 --> 00:10:08,720

the production of eps which is this

297

00:10:13,030 --> 00:10:10,880

capsule indicated so then the eps would

298

00:10:13,990 --> 00:10:13,040

then be exuded outside of the cell and

299

00:10:17,190 --> 00:10:14,000

then protecting

300

00:10:18,150 --> 00:10:17,200

it from the osmotic stress so we

301
00:10:22,790 --> 00:10:18,160
identified

302
00:10:25,110 --> 00:10:22,800
rcsd and rcsb gene mutations

303
00:10:25,910 --> 00:10:25,120
the hypothesis right now simply put is

304
00:10:28,069 --> 00:10:25,920
just that the

305
00:10:29,990 --> 00:10:28,079
um evolve strain is probably producing

306
00:10:31,509 --> 00:10:30,000
excess eps so the magnesium chloride is

307
00:10:34,550 --> 00:10:31,519
perturbing the cell membrane

308
00:10:37,030 --> 00:10:34,560
activating the rcs fossil relay so the

309
00:10:38,630 --> 00:10:37,040
mutated rcs genes might be upregulating

310
00:10:40,470 --> 00:10:38,640
eps production

311
00:10:42,630 --> 00:10:40,480
and there's previous evidence showing

312
00:10:44,310 --> 00:10:42,640
the properties of eps binding heavy

313
00:10:47,750 --> 00:10:44,320

metals such as calcium

314

00:10:49,590 --> 00:10:47,760

or sorry not calcium zinc or arsenic

315

00:10:52,069 --> 00:10:49,600

so it's possible that this eps also has

316

00:10:53,590 --> 00:10:52,079

a magnesium binding effect

317

00:10:55,269 --> 00:10:53,600

and again this being consistent with

318

00:10:56,710 --> 00:10:55,279

that snotty phenotype of just this

319

00:11:01,430 --> 00:10:56,720

really large molecular

320

00:11:03,910 --> 00:11:01,440

sorry macro structure in the test tubes

321

00:11:05,430 --> 00:11:03,920

so now that we've seen the in silico

322

00:11:07,269 --> 00:11:05,440

data so the breeze data

323

00:11:08,790 --> 00:11:07,279

how do we find that smoking gun how do

324

00:11:10,710 --> 00:11:08,800

we determine if these genes

325

00:11:12,710 --> 00:11:10,720

actually confer magnesium chloride

326

00:11:15,670 --> 00:11:12,720

tolerance and this is now being done

327

00:11:17,750 --> 00:11:15,680

via genetics i'm using the lambda red

328

00:11:19,670 --> 00:11:17,760

system so i've successfully knocked out

329

00:11:21,350 --> 00:11:19,680

the genes of interest

330

00:11:24,389 --> 00:11:21,360

for the purpose of this talk i'll just

331

00:11:25,829 --> 00:11:24,399

talk about the rcsd gene

332

00:11:27,670 --> 00:11:25,839

and the lambda red system involves

333

00:11:29,670 --> 00:11:27,680

creating pcr products that have an

334

00:11:31,829 --> 00:11:29,680

antibiotic resistant cassette

335

00:11:34,069 --> 00:11:31,839

that will share homology to a target

336

00:11:37,430 --> 00:11:34,079

region so if the yellow

337

00:11:38,790 --> 00:11:37,440

indicates the homologous region of the

338

00:11:40,230 --> 00:11:38,800

target cell so like this would be the

339

00:11:41,190 --> 00:11:40,240

whole genome of the target cell for

340

00:11:43,430 --> 00:11:41,200

example

341

00:11:45,430 --> 00:11:43,440

i'm creating this linear this double

342

00:11:48,069 --> 00:11:45,440

stranded pcr product so i have my

343

00:11:51,030 --> 00:11:48,079

antibiotic resistant cassette here

344

00:11:52,790 --> 00:11:51,040

with the region of homology through

345

00:11:56,150 --> 00:11:52,800

recombination

346

00:11:59,030 --> 00:11:56,160

uh the antibiotic resistant cassette

347

00:12:00,790 --> 00:11:59,040

will replace itself with that of the the

348

00:12:01,509 --> 00:12:00,800

gene of interest so you'll excise the

349

00:12:03,350 --> 00:12:01,519

rbsd

350

00:12:06,150 --> 00:12:03,360

gene and these knockouts that then

351
00:12:09,269 --> 00:12:06,160
appear as drug-resistant colonies

352
00:12:11,590 --> 00:12:09,279
i then verified through pcr that i'm

353
00:12:12,790 --> 00:12:11,600
amplifying the rcsd gene product with my

354
00:12:15,990 --> 00:12:12,800
starting stream

355
00:12:17,430 --> 00:12:16,000
and i'm not amplifying the rcsd gene

356
00:12:19,670 --> 00:12:17,440
product with my knockouts

357
00:12:21,030 --> 00:12:19,680
so indicated here with the no

358
00:12:22,470 --> 00:12:21,040
amplification

359
00:12:24,069 --> 00:12:22,480
furthermore i just want to double check

360
00:12:25,990 --> 00:12:24,079
that i'm actually in the right spot so i

361
00:12:28,310 --> 00:12:26,000
designed primers that would

362
00:12:30,150 --> 00:12:28,320
design primers that would show this

363
00:12:33,030 --> 00:12:30,160

final construct

364

00:12:34,710 --> 00:12:33,040

to indicate that the antibiotic

365

00:12:35,910 --> 00:12:34,720

resistant cassette has been inserted in

366

00:12:37,509 --> 00:12:35,920

the correct spot

367

00:12:39,430 --> 00:12:37,519

in the starting strain not seeing that

368

00:12:41,990 --> 00:12:39,440

amplification whereas the knockout

369

00:12:43,670 --> 00:12:42,000

and also through sequencing showing that

370

00:12:44,949 --> 00:12:43,680

we actually have this correct final

371

00:12:46,310 --> 00:12:44,959

construct

372

00:12:49,030 --> 00:12:46,320

so the future work is to create this

373

00:12:50,949 --> 00:12:49,040

library as i've almost completed of all

374

00:12:51,750 --> 00:12:50,959

the mutations that i've identified via

375

00:12:55,190 --> 00:12:51,760

greasy

376

00:12:57,590 --> 00:12:55,200

to create double knockouts so to

377

00:12:58,629 --> 00:12:57,600

remove maybe the men's sea gene the rcsb

378

00:13:01,430 --> 00:12:58,639

gene meant

379

00:13:03,509 --> 00:13:01,440

rbsd gene removing those in tandem to

380

00:13:04,310 --> 00:13:03,519

identify the changes magnesium chloride

381

00:13:06,470 --> 00:13:04,320

tolerance

382

00:13:07,350 --> 00:13:06,480

and lastly would be the the best

383

00:13:09,829 --> 00:13:07,360

scenario

384

00:13:11,030 --> 00:13:09,839

would be to recreate the exact mutations

385

00:13:13,350 --> 00:13:11,040

that were identified

386

00:13:15,350 --> 00:13:13,360

via brie c and from there once i have

387

00:13:17,590 --> 00:13:15,360

this library constructed i can determine

388

00:13:20,949 --> 00:13:17,600

the ko tolerance of my mutants

389

00:13:21,990 --> 00:13:20,959

um which would then tell me exactly what

390

00:13:23,750 --> 00:13:22,000

the story is of

391

00:13:25,670 --> 00:13:23,760

how these genes are affecting the

392

00:13:26,629 --> 00:13:25,680

magnesium chloride tolerance of this e

393

00:13:29,269 --> 00:13:26,639

coli

394

00:13:30,870 --> 00:13:29,279

and for the purpose of this talk um

395

00:13:32,550 --> 00:13:30,880

obviously we're all interested

396

00:13:34,150 --> 00:13:32,560

in the halophiles are really interested

397

00:13:35,829 --> 00:13:34,160

in these

398

00:13:37,990 --> 00:13:35,839

these critters that have much more

399

00:13:40,550 --> 00:13:38,000

significance to an astrobiological

400

00:13:42,710 --> 00:13:40,560

astrobiological context and i want to

401
00:13:44,069 --> 00:13:42,720
mention that i'm continuing these same

402
00:13:46,069 --> 00:13:44,079
ale experiments with that of

403
00:13:47,030 --> 00:13:46,079
halobacterium salinarum a model

404
00:13:48,870 --> 00:13:47,040
halophile

405
00:13:51,750 --> 00:13:48,880
as well as methanohalophilus portico

406
00:13:54,069 --> 00:13:51,760
lenses so the same workflow

407
00:13:56,150 --> 00:13:54,079
of ale to increasing magnesium chloride

408
00:13:57,110 --> 00:13:56,160
tolerance with those model halophiles

409
00:13:58,949 --> 00:13:57,120
that i'm

410
00:14:01,350 --> 00:13:58,959
very much looking forward to i'm sharing

411
00:14:02,550 --> 00:14:01,360
the results with soon

412
00:14:04,470 --> 00:14:02,560
and lastly just wanted to make some

413
00:14:06,790 --> 00:14:04,480

acknowledgments to the bartlett lab

414

00:14:08,389 --> 00:14:06,800

um this is my lab at uc san diego at

415

00:14:09,269 --> 00:14:08,399

scripps institution of oceanography

416

00:14:11,590 --> 00:14:09,279

here's the

417

00:14:13,269 --> 00:14:11,600

lab pictured here um as well as oceans

418

00:14:15,189 --> 00:14:13,279

across space and time this is a

419

00:14:17,430 --> 00:14:15,199

recently awarded nasa project that i'm

420

00:14:18,949 --> 00:14:17,440

funded under um eric allen one of my

421

00:14:20,470 --> 00:14:18,959

committee members who's been extremely

422

00:14:23,750 --> 00:14:20,480

helpful in developing uh

423

00:14:25,189 --> 00:14:23,760

the knockout procedure uh a big shout

424

00:14:27,110 --> 00:14:25,199

out to marco allen for sharing

425

00:14:28,710 --> 00:14:27,120

his relevant strains with me and then

426

00:14:32,150 --> 00:14:28,720

benjamin klempe

427

00:14:32,949 --> 00:14:32,160

a great friend and graduate student that

428

00:14:35,110 --> 00:14:32,959

we've been

429

00:14:36,550 --> 00:14:35,120

i've been working very closely with and

430

00:14:38,949 --> 00:14:36,560

lastly jeff bowman