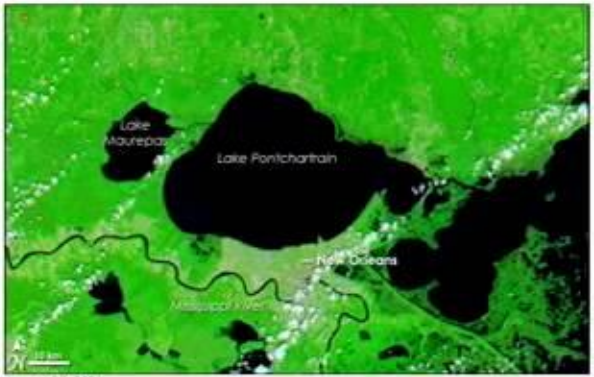
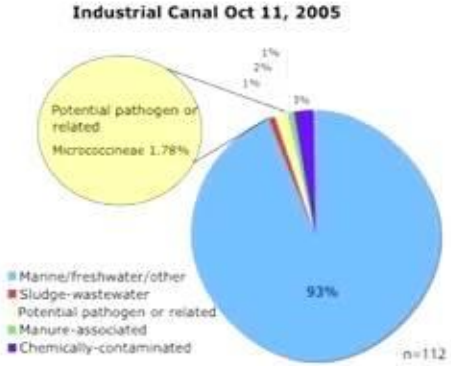
Microbial Response?

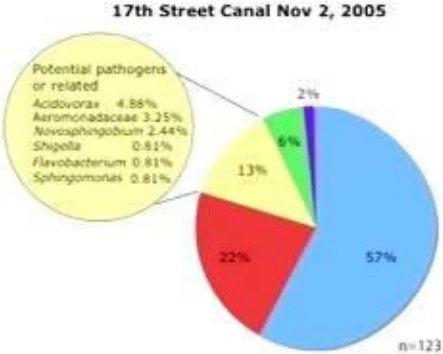


August 27, 2005

August 30, 2005

Earth Observatory, NASA





Linda Amaral-Zettler

1
00:00:03,409 --> 00:00:01,340
well good morning or good afternoon

2
00:00:05,090 --> 00:00:03,419
depending upon what time zone you're in

3
00:00:11,000 --> 00:00:05,100
I don't think we have any good evenings

4
00:00:13,999 --> 00:00:11,010
at the moment and I would just like to

5
00:00:16,010 --> 00:00:14,009
first of all thank Estelle and Marco for

6
00:00:17,960 --> 00:00:16,020
getting this all set up Estelle

7
00:00:19,460 --> 00:00:17,970
particularly for all her past service

8
00:00:21,529 --> 00:00:19,470
and doing this in Marco for all his

9
00:00:24,050 --> 00:00:21,539
current and future service in doing this

10
00:00:27,170 --> 00:00:24,060
and I'm sure that we are going to

11
00:00:28,849 --> 00:00:27,180
continue to have a very technically

12
00:00:32,089 --> 00:00:28,859
successful as well as scientifically

13
00:00:35,209 --> 00:00:32,099

successful a series of director seminars

14
00:00:37,819 --> 00:00:35,219
I'm also really really thrilled as the

15
00:00:40,880 --> 00:00:37,829
new director to be able to kick off this

16
00:00:44,240 --> 00:00:40,890
seminar series with a great talk by a

17
00:00:47,209 --> 00:00:44,250
great speaker I'm particularly excited

18
00:00:50,209 --> 00:00:47,219
about what Julie is going to talk about

19
00:00:52,189 --> 00:00:50,219
not only because it really is an

20
00:00:56,840 --> 00:00:52,199
important advance in our understanding

21
00:01:01,880 --> 00:00:56,850
of the microbial population of the

22
00:01:05,390 --> 00:01:01,890
oceans as a whole but also because this

23
00:01:10,340 --> 00:01:05,400
piece of work links together two very

24
00:01:12,200 --> 00:01:10,350
important things one is the things the

25
00:01:14,480 --> 00:01:12,210
timescales in particular that are

26
00:01:16,760 --> 00:01:14,490
important to astrobiology that is

27
00:01:19,280 --> 00:01:16,770
evolutionary time scales and it links

28
00:01:20,840 --> 00:01:19,290
those evolutionary timescales to the

29
00:01:23,120 --> 00:01:20,850
very short time scales that are of

30
00:01:25,429 --> 00:01:23,130
interest to nasa's arts science program

31
00:01:29,719 --> 00:01:25,439
in global change and we're going to hear

32
00:01:32,630 --> 00:01:29,729
about that from Julie Julie got her PhD

33
00:01:36,319 --> 00:01:32,640
in oceanography from the University of

34
00:01:38,420 --> 00:01:36,329
Washington in 2004 that followed her

35
00:01:40,969 --> 00:01:38,430
bachelor's in 1998 in marine science

36
00:01:43,780 --> 00:01:40,979
from eckerd college and the Masters from

37
00:01:49,120 --> 00:01:43,790
the University of Washington and 2000

38
00:01:51,620 --> 00:01:49,130

her dissertation title at u-dub was

39
00:01:54,109 --> 00:01:51,630
phylogenetic and physiological diversity

40
00:01:56,420 --> 00:01:54,119
of subsea floor microbial communities at

41
00:01:58,760 --> 00:01:56,430
deep sea sea mounts and that has in fact

42
00:02:00,620 --> 00:01:58,770
continued to be her principal research

43
00:02:02,209 --> 00:02:00,630
interest the work she's going to be

44
00:02:06,020 --> 00:02:02,219
talking about today was published

45
00:02:08,990 --> 00:02:06,030
recently in pnas in a paper by Mitch

46
00:02:10,109 --> 00:02:09,000
silgan and several other authors her

47
00:02:13,559 --> 00:02:10,119
talk today

48
00:02:16,140 --> 00:02:13,569
is co-authored by Mitch Hillary Morrison

49
00:02:18,330 --> 00:02:16,150
David Mark Welch sue Hughes and I

50
00:02:20,940 --> 00:02:18,340
apologize if I got the pronunciation of

51
00:02:22,949 --> 00:02:20,950
Sue's name wrong and Phil Neal and her

52
00:02:25,860 --> 00:02:22,959
title is microbial diversity in the deep

53
00:02:27,780 --> 00:02:25,870
sea and the underexplored rare biosphere

54
00:02:33,119 --> 00:02:27,790
and without further ado I turn it over

55
00:02:34,770 --> 00:02:33,129
to Julie all right thanks Carl I also

56
00:02:36,660 --> 00:02:34,780
want to thank you for the invitation to

57
00:02:39,780 --> 00:02:36,670
give this talk today and I especially

58
00:02:41,670 --> 00:02:39,790
want to thank Estelle and Marco the IT

59
00:02:44,339 --> 00:02:41,680
people here in Woods Hole it's been a

60
00:02:46,589 --> 00:02:44,349
pretty traumatic last couple of days

61
00:02:48,330 --> 00:02:46,599
without internet or email and I really

62
00:02:49,860 --> 00:02:48,340
appreciate all the efforts and a special

63
00:02:51,330 --> 00:02:49,870

the National Academy where I have a

64
00:02:53,970 --> 00:02:51,340
beautiful view of the ocean while I'm

65
00:02:56,460 --> 00:02:53,980
giving this talk I also want to thank my

66
00:02:58,589 --> 00:02:56,470
co-authors here at the NBL as Carl just

67
00:03:03,030 --> 00:02:58,599
mentioned and I think I'll just jump

68
00:03:04,440 --> 00:03:03,040
right in from there see so this is a

69
00:03:06,300 --> 00:03:04,450
brief outline of what I'm going to be

70
00:03:09,030 --> 00:03:06,310
talking about today I'm going to give

71
00:03:11,069 --> 00:03:09,040
you an overview of our motivation for

72
00:03:12,780 --> 00:03:11,079
doing this work and then spend a good

73
00:03:15,420 --> 00:03:12,790
bit of time talking about the approach

74
00:03:17,640 --> 00:03:15,430
through use this is a 454 tag sequencing

75
00:03:19,530 --> 00:03:17,650
approach which I'll talk about I'll also

76
00:03:21,420 --> 00:03:19,540
discuss sort of how we crunch our data

77
00:03:24,120 --> 00:03:21,430
since it's really integral part of

78
00:03:26,250 --> 00:03:24,130
understanding the system I'll go through

79
00:03:28,440 --> 00:03:26,260
some results discussion and implications

80
00:03:30,900 --> 00:03:28,450
and as Carl mentioned I'll talk about

81
00:03:34,650 --> 00:03:30,910
some future applications especially as

82
00:03:35,849 --> 00:03:34,660
they relate to earth sciences at NASA so

83
00:03:37,830 --> 00:03:35,859
we all know that we live on planet

84
00:03:40,589 --> 00:03:37,840
microbe and that they're the primary

85
00:03:43,439 --> 00:03:40,599
engines of Earth's biosphere that

86
00:03:45,960 --> 00:03:43,449
microbes mediate biogeochemical cycles

87
00:03:47,460 --> 00:03:45,970
that shape planetary habitability we

88
00:03:49,409 --> 00:03:47,470

know that microbes were likely the only

89
00:03:52,470 --> 00:03:49,419
form of life for most of our biological

90
00:03:54,900 --> 00:03:52,480
history and we know that microbial

91
00:03:56,849 --> 00:03:54,910
communities of untold diversity continue

92
00:03:59,879 --> 00:03:56,859
to dominate nearly every corner of our

93
00:04:02,009 --> 00:03:59,889
biosphere so understanding microbial

94
00:04:04,229 --> 00:04:02,019
diversity and community structure than

95
00:04:08,789 --> 00:04:04,239
our key to understanding our biosphere

96
00:04:10,379 --> 00:04:08,799
our habitability and our history I'm an

97
00:04:13,500 --> 00:04:10,389
oceanographer so today I'm going to be

98
00:04:15,479 --> 00:04:13,510
focusing on microbes in the sea and if

99
00:04:18,300 --> 00:04:15,489
we look at the relative abundance and

100
00:04:20,780 --> 00:04:18,310
productivity of marine life we can see

101
00:04:23,450 --> 00:04:20,790
that the smallest class

102
00:04:25,280 --> 00:04:23,460
organisms in the ocean the prokaryotes

103
00:04:27,860 --> 00:04:25,290
which are less than three micrometers in

104
00:04:30,470 --> 00:04:27,870
size make up almost eighty-two percent

105
00:04:32,390 --> 00:04:30,480
of the biomass in the ocean and in fact

106
00:04:35,570 --> 00:04:32,400
they account for over ninety percent of

107
00:04:38,440 --> 00:04:35,580
the primary productivity in the sea so

108
00:04:41,060 --> 00:04:38,450
if we actually take a look at what a

109
00:04:43,160 --> 00:04:41,070
some sea water looks like if you filter

110
00:04:45,650 --> 00:04:43,170
it and you stain it with a nucleic acid

111
00:04:48,500 --> 00:04:45,660
stain and take a look us is sort of what

112
00:04:51,710 --> 00:04:48,510
you sort of what you see and in fact

113
00:04:54,740 --> 00:04:51,720

these larger objects here are protists

114
00:04:56,780 --> 00:04:54,750
these of the bigger of the small green

115
00:04:59,600 --> 00:04:56,790
dots or bacteria and these very small

116
00:05:02,120 --> 00:04:59,610
ones in the background are viruses and

117
00:05:03,770 --> 00:05:02,130
in fact almost any place you look in the

118
00:05:07,280 --> 00:05:03,780
ocean you're going to get a picture like

119
00:05:09,380 --> 00:05:07,290
this and the amount of biomass in the

120
00:05:11,810 --> 00:05:09,390
ocean that is microbial is absolutely

121
00:05:15,110 --> 00:05:11,820
tremendous and Earth's by some estimates

122
00:05:19,610 --> 00:05:15,120
on an order of 10 to the 29th microbial

123
00:05:22,640 --> 00:05:19,620
cells in the ocean so I'm good you know

124
00:05:25,250 --> 00:05:22,650
right person somebody has right yeah

125
00:05:28,040 --> 00:05:25,260
you're getting close please all the

126
00:05:30,350 --> 00:05:28,050
polycom sites mute your microphones and

127
00:05:33,800 --> 00:05:30,360
Mike Fitzgerald if you would mute the

128
00:05:35,630 --> 00:05:33,810
conference I'll take care of it we have

129
00:05:44,300 --> 00:05:35,640
some open mics Berkeley I think your

130
00:05:47,960 --> 00:05:44,310
bike is open Thanks okay and Mike you

131
00:05:51,440 --> 00:05:47,970
okay Julie you should be ok but we I

132
00:05:53,000 --> 00:05:51,450
hear I hear someone coming in so if

133
00:05:57,649 --> 00:05:53,010
everyone can double-check we make sure

134
00:06:06,110 --> 00:06:01,159
go ahead Jill is not you look like

135
00:06:14,600 --> 00:06:12,119
okay yeah I keep I keep seeing Berkeley

136
00:06:19,589 --> 00:06:14,610
on my end I don't know why it may be a

137
00:06:21,749 --> 00:06:19,599
software problem okay so as I was saying

138
00:06:23,129 --> 00:06:21,759

the amount of cells in the ocean is

139
00:06:25,320 --> 00:06:23,139
absolutely enormous and it's on the

140
00:06:28,309 --> 00:06:25,330
order of somewhere around 10 to the 29th

141
00:06:30,779 --> 00:06:28,319
microbial cells and if we take a look at

142
00:06:33,450 --> 00:06:30,789
microbial interactions the ocean this is

143
00:06:35,879 --> 00:06:33,460
just a schematic showing some of the

144
00:06:38,490 --> 00:06:35,889
sources or ways of primary productivity

145
00:06:40,589 --> 00:06:38,500
in the sea from heterotroph e2 photo

146
00:06:42,779 --> 00:06:40,599
auto trophy and chemo autotrophs and

147
00:06:44,129 --> 00:06:42,789
while we have a pretty good sense of

148
00:06:47,010 --> 00:06:44,139
what's happening in the upper water

149
00:06:48,480 --> 00:06:47,020
column where photosynthesis dominates we

150
00:06:50,730 --> 00:06:48,490
have much less knowledge about what's

151
00:06:52,649 --> 00:06:50,740
happening in the deep sea for example in

152
00:06:54,480 --> 00:06:52,659
the bath people ajik in other zones and

153
00:06:56,790 --> 00:06:54,490
in fact when it comes to really

154
00:06:58,679 --> 00:06:56,800
microbial diversity community structure

155
00:07:00,719 --> 00:06:58,689
and how these populations change over

156
00:07:04,140 --> 00:07:00,729
time everywhere in the ocean we're

157
00:07:06,119 --> 00:07:04,150
lacking a great deal of knowledge so

158
00:07:08,219 --> 00:07:06,129
this lack of knowledge is really what

159
00:07:09,749 --> 00:07:08,229
motivated the formation of icon which is

160
00:07:12,749 --> 00:07:09,759
the international census of marine

161
00:07:15,209 --> 00:07:12,759
microbes and the goal of icon is to

162
00:07:17,730 --> 00:07:15,219
report what is known what is unknowable

163
00:07:19,050 --> 00:07:17,740

but knowable and what may be unknowable

164
00:07:22,740 --> 00:07:19,060
about the diversity of marine

165
00:07:25,439 --> 00:07:22,750
microorganisms by the year 2010 and the

166
00:07:27,450 --> 00:07:25,449
goal of our particular icon project is

167
00:07:29,399 --> 00:07:27,460
to determine the range of genetic

168
00:07:31,050 --> 00:07:29,409
diversity and relative numbers of

169
00:07:33,059 --> 00:07:31,060
different microbial organisms at

170
00:07:35,459 --> 00:07:33,069
sampling sites throughout the world's

171
00:07:37,649 --> 00:07:35,469
ocean so this project is headed up by

172
00:07:39,600 --> 00:07:37,659
Mitch here at the MBL and yonder Lou

173
00:07:41,010 --> 00:07:39,610
who's in the Netherlands and it's

174
00:07:42,929 --> 00:07:41,020
supported through the census of marine

175
00:07:46,050 --> 00:07:42,939
life program which is funded by the

176
00:07:47,879 --> 00:07:46,060
sloan foundation so over the past couple

177
00:07:51,659 --> 00:07:47,889
of years a number of working groups have

178
00:07:53,040 --> 00:07:51,669
gotten together for icon and have put

179
00:07:54,749 --> 00:07:53,050
together okay if we're going to do the

180
00:07:57,390 --> 00:07:54,759
census how are we going to do it and

181
00:08:00,029 --> 00:07:57,400
they've determined that determining the

182
00:08:02,579 --> 00:08:00,039
number of different types of organisms

183
00:08:04,310 --> 00:08:02,589
and their community composition is an

184
00:08:06,740 --> 00:08:04,320
essential first step in

185
00:08:08,570 --> 00:08:06,750
and they also decided that the metric

186
00:08:10,790 --> 00:08:08,580
for the census needs to be molecular

187
00:08:13,190 --> 00:08:10,800
this is not to say there isn't a place

188
00:08:14,840 --> 00:08:13,200

for culturing in the census but simply

189
00:08:16,850 --> 00:08:14,850
that it's unrealistic to think that we

190
00:08:20,210 --> 00:08:16,860
can catalog all of microbial life in the

191
00:08:22,610 --> 00:08:20,220
ocean using culturing alone and this is

192
00:08:24,560 --> 00:08:22,620
just exemplified by if we look simply at

193
00:08:27,530 --> 00:08:24,570
the bacterial known phylogenetic

194
00:08:29,840 --> 00:08:27,540
divisions over the last 15 years this is

195
00:08:33,100 --> 00:08:29,850
a figure from Noren paces lab you can

196
00:08:35,510 --> 00:08:33,110
see that in 2004 over half of the known

197
00:08:38,029 --> 00:08:35,520
bacterial phylogenetic divisions are

198
00:08:39,770 --> 00:08:38,039
represented only by sequences and no

199
00:08:43,880 --> 00:08:39,780
cultures and so it's very important that

200
00:08:45,920 --> 00:08:43,890
we use a molecular metric obviously the

201
00:08:48,460 --> 00:08:45,930
ocean is big there's a lot of cells and

202
00:08:51,410 --> 00:08:48,470
the census is a big job even if its most

203
00:08:53,330 --> 00:08:51,420
simple form conventional technology will

204
00:08:55,250 --> 00:08:53,340
be insufficient and it will clearly

205
00:08:56,870 --> 00:08:55,260
require the development of new

206
00:09:00,320 --> 00:08:56,880
computational tools which I'll be

207
00:09:02,540 --> 00:09:00,330
talking about today so we discussed some

208
00:09:04,520 --> 00:09:02,550
possible solutions and of course the

209
00:09:06,350 --> 00:09:04,530
first one we talked about was the one

210
00:09:09,430 --> 00:09:06,360
many of us are familiar with and that is

211
00:09:12,200 --> 00:09:09,440
DNA sequencing of either pcr-amplified

212
00:09:14,300 --> 00:09:12,210
and cloning or foz midst of some sort

213
00:09:16,670 --> 00:09:14,310

but really this technology is still

214
00:09:19,850 --> 00:09:16,680
pretty expensive depending on what your

215
00:09:22,160 --> 00:09:19,860
lab can put out and that that cost

216
00:09:24,140 --> 00:09:22,170
constraint also then constrains the size

217
00:09:25,910 --> 00:09:24,150
of our current surveys to less than

218
00:09:27,440 --> 00:09:25,920
about a thousand sequences from an

219
00:09:29,510 --> 00:09:27,450
environment and that really only

220
00:09:31,790 --> 00:09:29,520
captures a fraction of the community

221
00:09:33,560 --> 00:09:31,800
structure in addition because you're

222
00:09:35,480 --> 00:09:33,570
only looking at a thousand clones it can

223
00:09:38,120 --> 00:09:35,490
be difficult to detect underrepresented

224
00:09:40,310 --> 00:09:38,130
members of the community and it's also

225
00:09:41,840 --> 00:09:40,320
still pretty labor-intensive even if you

226
00:09:44,600 --> 00:09:41,850
have robots doing a lot of work for you

227
00:09:47,240 --> 00:09:44,610
it can still be pretty tough we also

228
00:09:49,190 --> 00:09:47,250
discussed using DNA microarrays but

229
00:09:51,650 --> 00:09:49,200
really you're limited by what is spotted

230
00:09:53,420 --> 00:09:51,660
or printed on your array we discussed a

231
00:09:54,770 --> 00:09:53,430
lot of other solutions but what we came

232
00:09:56,780 --> 00:09:54,780
up with and what I'll be talking about

233
00:09:58,400 --> 00:09:56,790
today is a tag sequencing strategy

234
00:10:02,060 --> 00:09:58,410
because we believe it has greater

235
00:10:04,820 --> 00:10:02,070
throughput and reduced costs so a number

236
00:10:07,670 --> 00:10:04,830
of investigators have examined using the

237
00:10:09,890 --> 00:10:07,680
small subunit ribosomal RNA for tag

238
00:10:11,930 --> 00:10:09,900

sequencing strategies and they focus

239
00:10:13,750 --> 00:10:11,940
mainly on hyper variable regions of the

240
00:10:16,330 --> 00:10:13,760
ribosomal structure and

241
00:10:18,160 --> 00:10:16,340
are shown here in red so this is the

242
00:10:20,620 --> 00:10:18,170
secondary structure and in it we have

243
00:10:22,990 --> 00:10:20,630
both conserved regions and variable

244
00:10:25,660 --> 00:10:23,000
regions and based on our own experience

245
00:10:28,510 --> 00:10:25,670
in our lab we chose to target this v6

246
00:10:30,520 --> 00:10:28,520
region of the ribosomal gene and so this

247
00:10:33,430 --> 00:10:30,530
by looking at one part of the gene we

248
00:10:35,350 --> 00:10:33,440
can infer taxonomic identity so we

249
00:10:37,390 --> 00:10:35,360
design primers which are shown here in

250
00:10:39,970 --> 00:10:37,400
yellow that are just to the highly

251
00:10:42,160 --> 00:10:39,980
conserved bacterial group we didn't even

252
00:10:46,060 --> 00:10:42,170
look at archaea in our initial our

253
00:10:47,200 --> 00:10:46,070
initial experiments we then applied what

254
00:10:49,780 --> 00:10:47,210
I'm going to be explaining which is

255
00:10:51,730 --> 00:10:49,790
called a 454 tag sequencing approach and

256
00:10:54,640 --> 00:10:51,740
this is just a basic environmental DNA

257
00:10:56,350 --> 00:10:54,650
pcr reaction where you take your DNA

258
00:10:58,540 --> 00:10:56,360
from seawater or whatever sample you're

259
00:11:01,180 --> 00:10:58,550
interested in and you amplify it with

260
00:11:03,490 --> 00:11:01,190
these v6 specific primers but

261
00:11:05,350 --> 00:11:03,500
synthesized onto these primers are these

262
00:11:06,970 --> 00:11:05,360
life science adapters and I'm going to

263
00:11:10,060 --> 00:11:06,980

explain why are those and why those are

264
00:11:13,270 --> 00:11:10,070
important in just a moment so we perform

265
00:11:16,000 --> 00:11:13,280
a PCR with these primers and we what we

266
00:11:18,820 --> 00:11:16,010
end up with is a v6 amplicon library and

267
00:11:21,610 --> 00:11:18,830
we sent that off to 454 life sciences

268
00:11:23,080 --> 00:11:21,620
which is located in Connecticut and what

269
00:11:25,770 --> 00:11:23,090
they give back to you is a number of

270
00:11:27,790 --> 00:11:25,780
tags from your different samples and

271
00:11:30,010 --> 00:11:27,800
because this is a relatively new

272
00:11:32,350 --> 00:11:30,020
technology and I know that quite a few

273
00:11:34,660 --> 00:11:32,360
people in the astrobiological community

274
00:11:36,250 --> 00:11:34,670
are actually using it I thought I would

275
00:11:38,740 --> 00:11:36,260
just briefly review how this process

276
00:11:41,110 --> 00:11:38,750
works it's also very different from

277
00:11:43,480 --> 00:11:41,120
traditional capillary DNA sequencing

278
00:11:46,000 --> 00:11:43,490
methods so I also thought I would

279
00:11:47,980 --> 00:11:46,010
explain it so these first two steps most

280
00:11:50,380 --> 00:11:47,990
of us are familiar with where we extract

281
00:11:52,870 --> 00:11:50,390
environmental DNA we amplify them with

282
00:11:55,840 --> 00:11:52,880
our specific primers of interest and in

283
00:11:57,460 --> 00:11:55,850
a normal microbial ecology lab from this

284
00:12:00,130 --> 00:11:57,470
stuff you would go on to cloning and

285
00:12:01,930 --> 00:12:00,140
sanger sequencing the 454 sequencing

286
00:12:04,240 --> 00:12:01,940
approach basically eliminates that

287
00:12:06,970 --> 00:12:04,250
cloning step so what you have is your

288
00:12:09,250 --> 00:12:06,980

pool of DNA amplicons your pcr amplified

289
00:12:11,140 --> 00:12:09,260
and these products are then denatured

290
00:12:13,030 --> 00:12:11,150
and a single strand is recovered and

291
00:12:15,490 --> 00:12:13,040
it's immobilized onto these very small

292
00:12:17,560 --> 00:12:15,500
beads that have a complementary primer

293
00:12:20,170 --> 00:12:17,570
to that a and B adapter that are

294
00:12:22,240 --> 00:12:20,180
synthesized to our own primers and these

295
00:12:25,600 --> 00:12:22,250
beads are then deposited and put into an

296
00:12:26,910 --> 00:12:25,610
oil-water emulsification pcr and that

297
00:12:29,220 --> 00:12:26,920
generates millions

298
00:12:32,610 --> 00:12:29,230
copies of that single DNA strand on each

299
00:12:34,440 --> 00:12:32,620
feed these this reaction is then

300
00:12:36,150 --> 00:12:34,450
denatured and they're putting to

301
00:12:38,490 --> 00:12:36,160
something called a picot tighter plate

302
00:12:40,620 --> 00:12:38,500
and these plates were developed just for

303
00:12:44,009 --> 00:12:40,630
this technology they have about I think

304
00:12:46,139 --> 00:12:44,019
1.2 million wells in the picot tighter

305
00:12:48,480 --> 00:12:46,149
plate and each one is just big enough to

306
00:12:51,030 --> 00:12:48,490
fit one bead and your sequencing

307
00:12:53,400 --> 00:12:51,040
reaction reagents then solid-phase

308
00:12:54,960 --> 00:12:53,410
pyrosequencing occurs I'm not going to

309
00:12:56,790 --> 00:12:54,970
go through that in a lot of detail but

310
00:12:59,370 --> 00:12:56,800
basically when a base is incorporated a

311
00:13:01,710 --> 00:12:59,380
flash of light occurs and in the end you

312
00:13:03,990 --> 00:13:01,720
get about 200 thousand reads per

313
00:13:06,269 --> 00:13:04,000

sequencing run and their average length

314
00:13:08,220 --> 00:13:06,279
is about a hundred base pairs and the

315
00:13:11,370 --> 00:13:08,230
cost is somewhere around two or five

316
00:13:13,530 --> 00:13:11,380
cents per read so remember the goal of

317
00:13:15,300 --> 00:13:13,540
this is really to identify organisms in

318
00:13:18,120 --> 00:13:15,310
an environmental sample we could so we

319
00:13:20,160 --> 00:13:18,130
can do a census so the key really is to

320
00:13:22,050 --> 00:13:20,170
identify the known universe and we've

321
00:13:23,939 --> 00:13:22,060
spent a lot of time building a reference

322
00:13:26,310 --> 00:13:23,949
data set so we can understand what we're

323
00:13:28,050 --> 00:13:26,320
studying so to do this we collected

324
00:13:30,540 --> 00:13:28,060
full-length ribosomal sequences that

325
00:13:32,220 --> 00:13:30,550
contain this v6 region we collected them

326
00:13:33,870 --> 00:13:32,230
from four major sources that are

327
00:13:36,449 --> 00:13:33,880
available freely on the internet and

328
00:13:39,360 --> 00:13:36,459
then we put them through the RDP the

329
00:13:41,009 --> 00:13:39,370
ribosomal database classifier to get

330
00:13:43,230 --> 00:13:41,019
taxonomy many of them are from

331
00:13:46,620 --> 00:13:43,240
uncultured bacteria have no affiliation

332
00:13:49,650 --> 00:13:46,630
no taxonomy and the identifiers and from

333
00:13:51,960 --> 00:13:49,660
those no one full-length sequences we

334
00:13:53,880 --> 00:13:51,970
extracted just the v6 so what we were

335
00:13:57,420 --> 00:13:53,890
able to do is build a database that has

336
00:14:00,120 --> 00:13:57,430
a full-length ribosomal sequence with an

337
00:14:02,310 --> 00:14:00,130
Associated v6 and a knowing taxonomy and

338
00:14:06,449 --> 00:14:02,320

those are included in both a blast able

339
00:14:08,280 --> 00:14:06,459
format and a searchable SQL database we

340
00:14:10,590 --> 00:14:08,290
then take our data through a number of

341
00:14:12,210 --> 00:14:10,600
different steps in the first that I just

342
00:14:15,059 --> 00:14:12,220
want to focus on right now is this

343
00:14:18,120 --> 00:14:15,069
quality control this is a very new

344
00:14:21,210 --> 00:14:18,130
technology and we've spent a lot of time

345
00:14:24,000 --> 00:14:21,220
trying to understand exactly how to

346
00:14:27,389 --> 00:14:24,010
evaluate it in terms of being able to

347
00:14:29,699 --> 00:14:27,399
remove low-quality reads it's important

348
00:14:31,860 --> 00:14:29,709
to note that this is very

349
00:14:33,389 --> 00:14:31,870
computationally expensive and so we've

350
00:14:35,730 --> 00:14:33,399
had to develop a lot of new tools to

351
00:14:37,590 --> 00:14:35,740
properly evaluate this and because of

352
00:14:37,910 --> 00:14:37,600
the general audience of my talk we're

353
00:14:41,090 --> 00:14:37,920
not

354
00:14:42,530 --> 00:14:41,100
talking about the details but we

355
00:14:44,210 --> 00:14:42,540
actually believe the error sequencing

356
00:14:46,790 --> 00:14:44,220
rate is quite low and if anybody has any

357
00:14:48,379 --> 00:14:46,800
questions about that at the end I'd be

358
00:14:51,019 --> 00:14:48,389
happy to answer them we've done a number

359
00:14:52,519 --> 00:14:51,029
of control experiments remember that

360
00:14:53,600 --> 00:14:52,529
we're going into an environment where we

361
00:14:56,389 --> 00:14:53,610
don't really know what we're going to

362
00:14:58,610 --> 00:14:56,399
find we can't benefit from like when you

363
00:15:00,860 --> 00:14:58,620

sequence the genome and you get 10 20 30

364
00:15:04,540 --> 00:15:00,870
X coverage so we're really trying to

365
00:15:09,199 --> 00:15:04,550
determine how well we can trust our data

366
00:15:10,759 --> 00:15:09,209
so i guess i'll go through these two

367
00:15:12,439 --> 00:15:10,769
different pipelines when we get to the

368
00:15:14,509 --> 00:15:12,449
data but first i want to talk to you

369
00:15:18,170 --> 00:15:14,519
about the pilot study that we performed

370
00:15:20,389 --> 00:15:18,180
for icon and we collected samples from

371
00:15:21,889 --> 00:15:20,399
two sets of collaborators and the first

372
00:15:24,530 --> 00:15:21,899
were our colleagues in the netherlands

373
00:15:26,629 --> 00:15:24,540
and these are transat samples from

374
00:15:28,220 --> 00:15:26,639
cruises in the North Atlantic the goal

375
00:15:30,110 --> 00:15:28,230
of these cruises is to track the

376
00:15:33,139 --> 00:15:30,120
formation of North Atlantic deep water

377
00:15:35,360 --> 00:15:33,149
from its source through through the

378
00:15:37,579 --> 00:15:35,370
oceanic conveyor belt and these yellow

379
00:15:40,129 --> 00:15:37,589
and red dots are two truce cruise tracks

380
00:15:42,019 --> 00:15:40,139
they've had and we chose just three

381
00:15:44,750 --> 00:15:42,029
sampling sites shown here in white and

382
00:15:46,970 --> 00:15:44,760
from each of those sites we chose both a

383
00:15:50,300 --> 00:15:46,980
sample from the oxygen minimum zone and

384
00:15:52,310 --> 00:15:50,310
also at greater depths in the ocean for

385
00:15:54,079 --> 00:15:52,320
our second study site which has a very

386
00:15:55,910 --> 00:15:54,089
different deep-sea setting and that is

387
00:15:59,120 --> 00:15:55,920
my own interest deep sea hydrothermal

388
00:16:01,280 --> 00:15:59,130

vents in this case axial seamount which

389
00:16:04,280 --> 00:16:01,290
is located about 300 miles off the coast

390
00:16:07,579 --> 00:16:04,290
of oregon on the Juan de Fuca mid-ocean

391
00:16:11,000 --> 00:16:07,589
ridge spreading Center and from axial we

392
00:16:12,470 --> 00:16:11,010
chose to particular diffuse flow events

393
00:16:13,819 --> 00:16:12,480
and i'm just showing one of them here

394
00:16:15,470 --> 00:16:13,829
which I'll be mentioning quite a bit

395
00:16:17,600 --> 00:16:15,480
because it's our largest data set and

396
00:16:20,060 --> 00:16:17,610
what you're looking at this is a diffuse

397
00:16:22,340 --> 00:16:20,070
flovent so there's warm fluids about 25

398
00:16:24,829 --> 00:16:22,350
degrees C leaking out at a sea floor you

399
00:16:27,199 --> 00:16:24,839
can see our sampler inside the vent

400
00:16:29,809 --> 00:16:27,209
trying to catch that flow and a bunch of

401
00:16:31,970 --> 00:16:29,819
charismatic macro phone around you can

402
00:16:33,590 --> 00:16:31,980
see two worms and limpets this is some

403
00:16:35,900 --> 00:16:33,600
stream ciliate that we don't really

404
00:16:39,680 --> 00:16:35,910
understand and this white matter is

405
00:16:42,620 --> 00:16:39,690
microbial mats so this is just a summary

406
00:16:44,930 --> 00:16:42,630
of those of those samples you can see

407
00:16:46,850 --> 00:16:44,940
the Payard transat samples from the

408
00:16:48,800 --> 00:16:46,860
north atlantic deep water each one from

409
00:16:51,199 --> 00:16:48,810
a different depth and then the to

410
00:16:55,249 --> 00:16:51,209
diffuse flow samples which are between

411
00:16:57,889 --> 00:16:55,259
25 and 30 degrees Celsius so the first

412
00:16:59,960 --> 00:16:57,899
part of our data processing when we got

413
00:17:01,879 --> 00:16:59,970

this data back I guess it was very very

414
00:17:03,279 --> 00:17:01,889
early this year I think it was two days

415
00:17:05,899 --> 00:17:03,289
after Christmas or something like that

416
00:17:07,579 --> 00:17:05,909
was we took all of our data and we

417
00:17:10,279 --> 00:17:07,589
blasted it against our reference

418
00:17:12,889 --> 00:17:10,289
database and you can use this side of

419
00:17:15,139 --> 00:17:12,899
the pipeline to get to the taxonomy so

420
00:17:17,449 --> 00:17:15,149
you you blast all of your sequences

421
00:17:19,549 --> 00:17:17,459
against the reference database and then

422
00:17:22,370 --> 00:17:19,559
you take your query and you align it

423
00:17:24,710 --> 00:17:22,380
with the top 250 best scoring sequences

424
00:17:27,049 --> 00:17:24,720
using a program called muscle and from

425
00:17:29,510 --> 00:17:27,059
that you can calculate the distances and

426
00:17:30,769 --> 00:17:29,520
identify the minimum the minimum

427
00:17:32,779 --> 00:17:30,779
distance and that helps you get the

428
00:17:35,960 --> 00:17:32,789
closest identify identity in your

429
00:17:39,019 --> 00:17:35,970
reference database you can also take

430
00:17:41,690 --> 00:17:39,029
that top last hit and use that as sort

431
00:17:43,549 --> 00:17:41,700
of an estimate of diversity in your

432
00:17:45,560 --> 00:17:43,559
sample so if you have a hundred

433
00:17:47,990 --> 00:17:45,570
sequences and eighty of them blast to

434
00:17:50,120 --> 00:17:48,000
one sequence and 22 the other you can

435
00:17:51,769 --> 00:17:50,130
try to get a rough estimate of diversity

436
00:17:53,360 --> 00:17:51,779
and that was the first thing we did

437
00:17:55,760 --> 00:17:53,370
simply because we didn't really know

438
00:17:58,190 --> 00:17:55,770

what else to do and so this is the first

439
00:18:00,500 --> 00:17:58,200
result that we got back when we did did

440
00:18:02,750 --> 00:18:00,510
that experiment so these are rare

441
00:18:04,730 --> 00:18:02,760
faction curves for those of you who

442
00:18:06,590 --> 00:18:04,740
aren't familiar with them they basically

443
00:18:09,760 --> 00:18:06,600
allow you to compare richness among

444
00:18:12,649 --> 00:18:09,770
samples that have been sampled at

445
00:18:14,930 --> 00:18:12,659
unequally and the Kurds result from

446
00:18:17,299 --> 00:18:14,940
basically averaging randomizations of

447
00:18:18,830 --> 00:18:17,309
the observed accumulation curve and so

448
00:18:20,659 --> 00:18:18,840
these are not an actual measure of

449
00:18:22,639 --> 00:18:20,669
confidence about diversity in a sample

450
00:18:24,950 --> 00:18:22,649
that they simply help you visualize how

451
00:18:26,860 --> 00:18:24,960
much more sampling needs to occur and if

452
00:18:29,240 --> 00:18:26,870
we had actually sampled these

453
00:18:31,100 --> 00:18:29,250
environments to extinction you would see

454
00:18:32,990 --> 00:18:31,110
these curves flattening out like this

455
00:18:34,789 --> 00:18:33,000
but in fact you can see they're still

456
00:18:37,519 --> 00:18:34,799
going way up and we were very surprised

457
00:18:39,049 --> 00:18:37,529
right by this result simply because no

458
00:18:41,029 --> 00:18:39,059
one had really seen this in the marine

459
00:18:43,789 --> 00:18:41,039
environment that's been discussed pretty

460
00:18:45,440 --> 00:18:43,799
thoroughly in the soil environment but

461
00:18:47,210 --> 00:18:45,450
we are we are quite surprised and

462
00:18:49,909 --> 00:18:47,220
especially since we knew this was an

463
00:18:52,130 --> 00:18:49,919

under estimate of diversity because two

464
00:18:55,399 --> 00:18:52,140
very different sequences can blast to

465
00:18:56,659 --> 00:18:55,409
the same to the same query and so we

466
00:18:58,250 --> 00:18:56,669
decided we are going to have to do

467
00:18:58,690 --> 00:18:58,260
something a little bit more rigorous and

468
00:19:01,270 --> 00:18:58,700
not

469
00:19:03,040 --> 00:19:01,280
so naive really and in fact that was

470
00:19:04,710 --> 00:19:03,050
backed up when we looked at how many

471
00:19:07,240 --> 00:19:04,720
blast hits we got for each sample

472
00:19:09,040 --> 00:19:07,250
compared to how many unique tags were

473
00:19:10,750 --> 00:19:09,050
actually in each sample and you can see

474
00:19:13,150 --> 00:19:10,760
that there are many more unique kept

475
00:19:14,140 --> 00:19:13,160
tags than there are blast hits and so we

476
00:19:17,260 --> 00:19:14,150
are new we are under estimating

477
00:19:19,510 --> 00:19:17,270
diversity using this approach so the

478
00:19:20,530 --> 00:19:19,520
second the second time around when we

479
00:19:22,870 --> 00:19:20,540
decide to do this a little more

480
00:19:24,910 --> 00:19:22,880
rigorously we used a program called odor

481
00:19:26,620 --> 00:19:24,920
which I'm going to explain so we take

482
00:19:28,480 --> 00:19:26,630
all of our sequences we align and we

483
00:19:30,910 --> 00:19:28,490
calculate distances and we pop them into

484
00:19:33,130 --> 00:19:30,920
this program called dotor and this was

485
00:19:34,930 --> 00:19:33,140
developed by patrick's laws and jo

486
00:19:37,540 --> 00:19:34,940
Handelsman slab he's now at UMass

487
00:19:40,180 --> 00:19:37,550
Amherst and what dotor does it's really

488
00:19:41,800 --> 00:19:40,190

a great program for all sorts of people

489
00:19:44,440 --> 00:19:41,810
who are working in different microbial

490
00:19:46,240 --> 00:19:44,450
systems it takes all your sequences and

491
00:19:48,700 --> 00:19:46,250
assigns them to operational taxonomic

492
00:19:50,890 --> 00:19:48,710
units based on the genetic distances

493
00:19:52,630 --> 00:19:50,900
between the sequences and then it looks

494
00:19:54,490 --> 00:19:52,640
at the frequency at which each of those

495
00:19:56,620 --> 00:19:54,500
OT use our observed and it constructs

496
00:19:58,120 --> 00:19:56,630
rarefaction and collectors curves for

497
00:20:01,570 --> 00:19:58,130
various measures of richness and

498
00:20:03,370 --> 00:20:01,580
diversity an in Patrick's paper he uses

499
00:20:05,260 --> 00:20:03,380
some example data sets and one of them

500
00:20:07,750 --> 00:20:05,270
that he looked at was craig Venter

501
00:20:10,660 --> 00:20:07,760
Sargasso Sea dataset which was generated

502
00:20:12,250 --> 00:20:10,670
by shotgun sequencing and what he did

503
00:20:14,800 --> 00:20:12,260
was you can see here at the three

504
00:20:17,020 --> 00:20:14,810
percent level after looking at about 700

505
00:20:21,070 --> 00:20:17,030
clones they estimate there's going to be

506
00:20:23,560 --> 00:20:21,080
about 150 maybe 200 species in in the

507
00:20:27,400 --> 00:20:23,570
Sargasso Sea so this is a nice point of

508
00:20:30,190 --> 00:20:27,410
comparison for our own samples and in

509
00:20:33,430 --> 00:20:30,200
fact when we're and odor on our on our

510
00:20:34,990 --> 00:20:33,440
samples this is just FS 396 you can see

511
00:20:37,150 --> 00:20:35,000
that these curves are still climbing

512
00:20:40,090 --> 00:20:37,160
rapidly even at this five percent level

513
00:20:43,330 --> 00:20:40,100

and if we look at some of the diversity

514
00:20:45,430 --> 00:20:43,340
estimates that donor gives out these are

515
00:20:47,290 --> 00:20:45,440
non parametric estimators that again

516
00:20:49,420 --> 00:20:47,300
have become a very common tool in

517
00:20:52,240 --> 00:20:49,430
microbial ecology they were actually

518
00:20:54,550 --> 00:20:52,250
adapted from mark release and recapture

519
00:20:57,430 --> 00:20:54,560
statistics that were used in you know

520
00:20:59,350 --> 00:20:57,440
counting rabbits and things like that so

521
00:21:00,700 --> 00:20:59,360
they consider the proportion of species

522
00:21:02,710 --> 00:21:00,710
that have already been observed or

523
00:21:04,390 --> 00:21:02,720
recaptured compared to those that are

524
00:21:07,180 --> 00:21:04,400
observed only once and they appear to be

525
00:21:09,160 --> 00:21:07,190
quite rigorous for for microbial

526
00:21:11,350 --> 00:21:09,170
estimates and you can see that at this

527
00:21:11,769 --> 00:21:11,360
three percent level for example just in

528
00:21:13,690 --> 00:21:11,779
this

529
00:21:15,940 --> 00:21:13,700
use vent they estimate that in a

530
00:21:19,269 --> 00:21:15,950
singular single leader event fluid

531
00:21:21,399 --> 00:21:19,279
there's probably over 20,000 species and

532
00:21:23,379 --> 00:21:21,409
I I use that term loosely because we're

533
00:21:26,529 --> 00:21:23,389
simply defining species by genetic

534
00:21:28,209 --> 00:21:26,539
distance in this case and so what we're

535
00:21:30,070 --> 00:21:28,219
doing here is we're able to sample very

536
00:21:33,700 --> 00:21:30,080
very deeply into the microbial world

537
00:21:34,659 --> 00:21:33,710
using this 454 sequencing strategy and

538
00:21:37,419 --> 00:21:34,669

we're getting a much more complete

539
00:21:39,999 --> 00:21:37,429
picture of what organisms might be there

540
00:21:41,560 --> 00:21:40,009
but really numbers are one thing and

541
00:21:43,810 --> 00:21:41,570
that's mostly what we reported in our

542
00:21:46,239 --> 00:21:43,820
pnas paper but we really want to know

543
00:21:48,009 --> 00:21:46,249
who the players in this ecosystem are so

544
00:21:50,289 --> 00:21:48,019
we can take a look at the taxonomic

545
00:21:52,329 --> 00:21:50,299
breakdown and again I'm just going to

546
00:21:53,950 --> 00:21:52,339
focus on the de to diffuse vent sites

547
00:21:55,810 --> 00:21:53,960
since that's what I study and we have

548
00:21:57,669 --> 00:21:55,820
the most data from and this is a

549
00:21:59,739 --> 00:21:57,679
breakdown in the taxonomy just from

550
00:22:01,659 --> 00:21:59,749
those to diffuse events this is at the

551
00:22:05,079 --> 00:22:01,669
three percent level and sort of that

552
00:22:06,940 --> 00:22:05,089
class level and you can see in fact that

553
00:22:09,159 --> 00:22:06,950
these two samples are quite different

554
00:22:10,810 --> 00:22:09,169
they have a lot of the same colors but

555
00:22:12,999 --> 00:22:10,820
the relative abundance of the colors are

556
00:22:14,379 --> 00:22:13,009
different and in fact this sample is

557
00:22:16,749 --> 00:22:14,389
dominated by these epsilon

558
00:22:19,269 --> 00:22:16,759
proteobacteria whereas this one has more

559
00:22:21,129 --> 00:22:19,279
gamma Proteobacteria and when I first

560
00:22:23,019 --> 00:22:21,139
saw this I immediately went and looked

561
00:22:25,509 --> 00:22:23,029
at the chemistry from these two vents

562
00:22:27,099 --> 00:22:25,519
and in fact it's quite different there

563
00:22:29,469 --> 00:22:27,109

are a number of indicators here that

564
00:22:32,739 --> 00:22:29,479
suggests that this vent on over here FS

565
00:22:35,200 --> 00:22:32,749
396 has a much higher carbon dioxide

566
00:22:37,539 --> 00:22:35,210
content and in fact this vent was

567
00:22:40,029 --> 00:22:37,549
effervescent or bubbling when these

568
00:22:42,579 --> 00:22:40,039
samples were taken and that's shown with

569
00:22:46,690 --> 00:22:42,589
this elevated hydrogen sulfide to temper

570
00:22:49,779 --> 00:22:46,700
ratio the GCV suppressed pH the higher

571
00:22:52,119 --> 00:22:49,789
alkalinity and also the higher iron and

572
00:22:53,889 --> 00:22:52,129
this is a characteristic of a lot events

573
00:22:56,979 --> 00:22:53,899
that we see at axial that have a high

574
00:22:59,139 --> 00:22:56,989
co2 content a low pH and an elevated

575
00:23:01,239 --> 00:22:59,149
alkalinity so what we're seeing here is

576
00:23:04,719 --> 00:23:01,249
a possible link between the different

577
00:23:07,839 --> 00:23:04,729
microbial composition and the 454

578
00:23:10,299 --> 00:23:07,849
sequences that we're getting back we can

579
00:23:12,519 --> 00:23:10,309
also just look at one aspect one group

580
00:23:14,200 --> 00:23:12,529
and look at the diversity within and so

581
00:23:17,560 --> 00:23:14,210
in this example we're looking at the

582
00:23:19,629 --> 00:23:17,570
epsilon proteobacteria and this is just

583
00:23:20,950 --> 00:23:19,639
again at that three percent level and

584
00:23:22,779 --> 00:23:20,960
what you can see is there's a huge

585
00:23:24,190 --> 00:23:22,789
amount of diversity just within this one

586
00:23:27,220 --> 00:23:24,200
group of organisms

587
00:23:29,379 --> 00:23:27,230
now epsilon Proteobacteria known to be

588
00:23:31,120 --> 00:23:29,389

key players at hydrothermal vents they

589
00:23:33,279 --> 00:23:31,130
were only recently cultured really in

590
00:23:35,500 --> 00:23:33,289
the last three or four years they're

591
00:23:37,779 --> 00:23:35,510
very phylogenetically physiologically

592
00:23:39,759 --> 00:23:37,789
diverse and they can be both mesophilic

593
00:23:41,560 --> 00:23:39,769
and thermophilic they can use a variety

594
00:23:43,899 --> 00:23:41,570
of electronic scepters and donors from

595
00:23:46,120 --> 00:23:43,909
both seawater and vent fluids allowing

596
00:23:48,879 --> 00:23:46,130
them to exploit both of those gradients

597
00:23:50,769 --> 00:23:48,889
and many of them are autotrophic in

598
00:23:53,350 --> 00:23:50,779
fixed carbon dioxide using the reverse

599
00:23:56,200 --> 00:23:53,360
TCA cycle but what's especially

600
00:23:59,049 --> 00:23:56,210
interesting about just this sort of data

601
00:24:02,230 --> 00:23:59,059
set is this pattern here we're a few

602
00:24:04,000 --> 00:24:02,240
tags dominate the samples but in fact

603
00:24:06,730 --> 00:24:04,010
the bulk of the diversity is made up by

604
00:24:08,350 --> 00:24:06,740
these very low abundant tags and I'd

605
00:24:11,680 --> 00:24:08,360
like to illustrate that point in our

606
00:24:12,940 --> 00:24:11,690
next few slides so this plot is a little

607
00:24:15,370 --> 00:24:12,950
bit confusing and so I'm going to walk

608
00:24:17,710 --> 00:24:15,380
you through it so this clutch basically

609
00:24:20,230 --> 00:24:17,720
shows the similarity of all of our 454

610
00:24:22,899 --> 00:24:20,240
tag sequences from all of the data to

611
00:24:25,180 --> 00:24:22,909
the v6 reference database so this red

612
00:24:27,009 --> 00:24:25,190
curve shows the distribution of all the

613
00:24:28,389 --> 00:24:27,019

tags to the percent difference from

614
00:24:31,120 --> 00:24:28,399
their best match in the reference

615
00:24:33,879 --> 00:24:31,130
database this blue line is cumulative

616
00:24:35,259 --> 00:24:33,889
and shows the percent of our tags and

617
00:24:37,629 --> 00:24:35,269
how different they are from the best

618
00:24:39,909 --> 00:24:37,639
match in our database and finally this

619
00:24:42,389 --> 00:24:39,919
green tag which shows the percent the

620
00:24:45,669 --> 00:24:42,399
unique reads and their distance from

621
00:24:47,110 --> 00:24:45,679
their best match in the database so if

622
00:24:50,279 --> 00:24:47,120
we just take a look at this blue line

623
00:24:53,440 --> 00:24:50,289
the cumulative curve you can see that

624
00:24:55,750 --> 00:24:53,450
about twenty-five percent of our tags

625
00:24:59,529 --> 00:24:55,760
are dead-on hits to something in in the

626
00:25:01,629 --> 00:24:59,539
database and if we keep climbing up that

627
00:25:03,370 --> 00:25:01,639
curve about forty percent of them are

628
00:25:05,649 --> 00:25:03,380
within three percent of something in our

629
00:25:07,750 --> 00:25:05,659
reference database and in fact over

630
00:25:09,549 --> 00:25:07,760
seventy-five percent of them are within

631
00:25:11,830 --> 00:25:09,559
ten percent of something in the database

632
00:25:14,110 --> 00:25:11,840
so this basically means we're covering

633
00:25:16,750 --> 00:25:14,120
the known universe very well using the

634
00:25:19,539 --> 00:25:16,760
sequencing strategy but if we take a

635
00:25:22,600 --> 00:25:19,549
look at this this green line the percent

636
00:25:24,909 --> 00:25:22,610
of unique reads we see that only twenty

637
00:25:26,889 --> 00:25:24,919
percent of our tags are within ten

638
00:25:29,529 --> 00:25:26,899

percent of something in the database and

639
00:25:31,629 --> 00:25:29,539
in fact the bulk of these unique tags

640
00:25:32,980 --> 00:25:31,639
over eighty percent of them are very

641
00:25:35,470 --> 00:25:32,990
different from anything we've seen

642
00:25:36,610 --> 00:25:35,480
before and are quite divergent and it's

643
00:25:39,220 --> 00:25:36,620
really this

644
00:25:41,650 --> 00:25:39,230
this these very divergent groups that

645
00:25:44,080 --> 00:25:41,660
also occur at low abundance as indicated

646
00:25:47,049 --> 00:25:44,090
by this red line where you can see that

647
00:25:49,180 --> 00:25:47,059
these very different sequences occur at

648
00:25:52,060 --> 00:25:49,190
low abundance this is what we're terming

649
00:25:53,710 --> 00:25:52,070
the rare biosphere in the deep sea we

650
00:25:56,080 --> 00:25:53,720
can look at this another way by looking

651
00:26:01,299 --> 00:25:56,090
again at rarefaction curves just from

652
00:26:03,700 --> 00:26:01,309
sample FS 396 and if we compare base

653
00:26:05,440 --> 00:26:03,710
sequences based on how distant they are

654
00:26:07,600 --> 00:26:05,450
from the reference database you get

655
00:26:09,460 --> 00:26:07,610
forward very different curves and this

656
00:26:11,140 --> 00:26:09,470
is sort of what the Sargasso Sea data

657
00:26:12,790 --> 00:26:11,150
set looks like right where it's

658
00:26:14,830 --> 00:26:12,800
flattening out at a relatively low

659
00:26:16,630 --> 00:26:14,840
number and these sequences are very

660
00:26:18,880 --> 00:26:16,640
close to things we already know about in

661
00:26:21,700 --> 00:26:18,890
the database but as you get increasingly

662
00:26:24,150 --> 00:26:21,710
divergent sequences you get increasing

663
00:26:26,890 --> 00:26:24,160

estimates of diversity in the deep sea

664
00:26:28,840 --> 00:26:26,900
so this concept of a rare biosphere

665
00:26:30,970 --> 00:26:28,850
isn't a new one but we now feel like we

666
00:26:34,210 --> 00:26:30,980
have a tool to regularly measure it in

667
00:26:35,980 --> 00:26:34,220
the environment and we're trying really

668
00:26:38,770 --> 00:26:35,990
to understand its significance and now

669
00:26:40,360 --> 00:26:38,780
we now possibly will be able to so the

670
00:26:42,730 --> 00:26:40,370
rare biosphere may simply reflect

671
00:26:45,070 --> 00:26:42,740
biogeography and many yet to be

672
00:26:46,900 --> 00:26:45,080
discovered habitats so what's rare and

673
00:26:50,020 --> 00:26:46,910
one habitat might be quite common in

674
00:26:52,150 --> 00:26:50,030
another and these rare organisms-- might

675
00:26:54,220 --> 00:26:52,160
also be a source of genomic innovation

676
00:26:56,140 --> 00:26:54,230
and this can help us understand how

677
00:26:57,940 --> 00:26:56,150
microbial communities seemed to recover

678
00:27:00,310 --> 00:26:57,950
from all sorts of environmental

679
00:27:02,680 --> 00:27:00,320
catastrophes and also it might help us

680
00:27:04,630 --> 00:27:02,690
explain the genetic novelty that we find

681
00:27:07,380 --> 00:27:04,640
in almost every genome and meta genome

682
00:27:09,430 --> 00:27:07,390
sequence to date the extreme

683
00:27:11,620 --> 00:27:09,440
phylogenetic diversity of the rare

684
00:27:14,230 --> 00:27:11,630
biosphere suggests that it's been around

685
00:27:16,450 --> 00:27:14,240
for a long time possibly over geological

686
00:27:18,820 --> 00:27:16,460
timescales and might have had a very

687
00:27:21,790 --> 00:27:18,830
important role in shaping planetary

688
00:27:23,830 --> 00:27:21,800

processes and finally low abundant

689
00:27:25,330 --> 00:27:23,840
populations at one site might become

690
00:27:28,419 --> 00:27:25,340
dominant in response to environmental

691
00:27:30,520 --> 00:27:28,429
change and in that regard the rare

692
00:27:34,630 --> 00:27:30,530
biosphere may serve a sentinel for

693
00:27:36,970 --> 00:27:34,640
global change so a recent paper in oh I

694
00:27:39,220 --> 00:27:36,980
don't think that came out on anybody's

695
00:27:41,470 --> 00:27:39,230
slide it didn't come out of mine but a

696
00:27:43,610 --> 00:27:41,480
recent paper and trends in microbiology

697
00:27:47,270 --> 00:27:43,620
tried to illustrate this and

698
00:27:50,630 --> 00:27:47,280
what you can see this on the x y axis

699
00:27:53,180 --> 00:27:50,640
here is the number and this is the tax

700
00:27:55,820 --> 00:27:53,190
on rank and this red part of the curve

701
00:27:58,130 --> 00:27:55,830
are those abundant organisms and this

702
00:28:03,980 --> 00:27:58,140
long tail here are the rare organisms--

703
00:28:05,870 --> 00:28:03,990
and in this paper at pedros a leo says

704
00:28:08,090 --> 00:28:05,880
that we've really only been able to

705
00:28:10,400 --> 00:28:08,100
detect these abundant organisms with

706
00:28:12,740 --> 00:28:10,410
current molecular methods and every once

707
00:28:14,840 --> 00:28:12,750
in a while with culturing we pick up

708
00:28:17,090 --> 00:28:14,850
some of these rare organisms-- and we

709
00:28:19,220 --> 00:28:17,100
believe with 454 sequencing we're now

710
00:28:22,580 --> 00:28:19,230
able to get the whole look at all of

711
00:28:24,230 --> 00:28:22,590
this biodiversity and so what I'd like

712
00:28:25,520 --> 00:28:24,240
to do really for the rest of my talk is

713
00:28:28,130 --> 00:28:25,530

what Carl would it's you in the

714
00:28:30,440 --> 00:28:28,140
beginning and I'd like to go through

715
00:28:32,240 --> 00:28:30,450
some examples of sites we might want to

716
00:28:34,580 --> 00:28:32,250
look at or situations we might want to

717
00:28:36,500 --> 00:28:34,590
look at and apply this technology not

718
00:28:38,330 --> 00:28:36,510
only in regard to the rare biosphere but

719
00:28:39,980 --> 00:28:38,340
also in microbial populations that are

720
00:28:42,560 --> 00:28:39,990
gonna be changing or responding to

721
00:28:44,090 --> 00:28:42,570
different environmental shifts and want

722
00:28:45,560 --> 00:28:44,100
to make this especially applicable to

723
00:28:47,600 --> 00:28:45,570
the science going on not only in

724
00:28:50,630 --> 00:28:47,610
astrobiology community but also NASA in

725
00:28:52,370 --> 00:28:50,640
general so we can take a very brief look

726
00:28:55,220 --> 00:28:52,380
at the history of life on our own planet

727
00:28:56,780 --> 00:28:55,230
as shown here and you can imagine major

728
00:28:59,330 --> 00:28:56,790
events in our history that really

729
00:29:00,919 --> 00:28:59,340
impacted life and I've obviously just

730
00:29:02,480 --> 00:29:00,929
highlighted a couple of them that we can

731
00:29:05,450 --> 00:29:02,490
think about including the build-up of

732
00:29:08,060 --> 00:29:05,460
atmospheric atmospheric oxygen and the

733
00:29:09,440 --> 00:29:08,070
origins and multicellularity but

734
00:29:11,299 --> 00:29:09,450
obviously there are more specific

735
00:29:14,720 --> 00:29:11,309
examples and one of these include

736
00:29:17,299 --> 00:29:14,730
snowball earth as we know snowball earth

737
00:29:19,880 --> 00:29:17,309
hypothesis is based on geological

738
00:29:22,610 --> 00:29:19,890

evidence from multiple glaciations at

739
00:29:25,430 --> 00:29:22,620
sea levels of sea level at low latitudes

740
00:29:27,650 --> 00:29:25,440
and really the leading explanation for

741
00:29:29,810 --> 00:29:27,660
these snowballs is a runaway ice albedo

742
00:29:31,760 --> 00:29:29,820
effect and basically we're putting so

743
00:29:34,760 --> 00:29:31,770
much solar energy back into space that

744
00:29:37,010 --> 00:29:34,770
we freeze over obviously life was not

745
00:29:39,680 --> 00:29:37,020
destroyed by these processes it was able

746
00:29:41,570 --> 00:29:39,690
to continue just fine but we really have

747
00:29:44,380 --> 00:29:41,580
to think about how it might have changed

748
00:29:46,790 --> 00:29:44,390
how evolution happens how organisms

749
00:29:50,299 --> 00:29:46,800
interact and respond to such a dramatic

750
00:29:52,970 --> 00:29:50,309
change globally and this is some work

751
00:29:55,820 --> 00:29:52,980
biker stink at all where they looked at

752
00:29:57,060 --> 00:29:55,830
sort of a post snowball earth setting

753
00:29:59,639 --> 00:29:57,070
and what might have been happy

754
00:30:02,789 --> 00:29:59,649
and it's just illustrated here so upon

755
00:30:04,889 --> 00:30:02,799
initial melting a cyanobacterial bloom

756
00:30:07,680 --> 00:30:04,899
may occur and then you have all this

757
00:30:10,529 --> 00:30:07,690
oxygen getting back into the ocean what

758
00:30:13,019 --> 00:30:10,539
used to be an anoxic metalliferous ocean

759
00:30:14,610 --> 00:30:13,029
is now reacting with this oxygen and you

760
00:30:16,799 --> 00:30:14,620
can imagine that they're both manganese

761
00:30:18,779 --> 00:30:16,809
oxidizing bacteria iron oxidizing

762
00:30:21,720 --> 00:30:18,789
bacteria other processes that are going

763
00:30:23,909 --> 00:30:21,730

to be mediated by micro microbes and so

764
00:30:26,639 --> 00:30:23,919
you can imagine that both at the onset

765
00:30:28,259 --> 00:30:26,649
during and after a snowball earth there

766
00:30:29,999 --> 00:30:28,269
are particular responses in the

767
00:30:33,119 --> 00:30:30,009
microbial communities to these different

768
00:30:35,909 --> 00:30:33,129
shifts of course we have modern-day

769
00:30:37,680 --> 00:30:35,919
phytoplankton blooms which are of great

770
00:30:40,619 --> 00:30:37,690
interest to a lot of people especially

771
00:30:42,810 --> 00:30:40,629
in an oceanography this is a picture of

772
00:30:45,919 --> 00:30:42,820
a phytoplankton bloom in the Arabian Sea

773
00:30:48,360 --> 00:30:45,929
this is the with the ocean chlorophyll

774
00:30:50,490 --> 00:30:48,370
concentrations the Arabian Sea has a

775
00:30:53,100 --> 00:30:50,500
very small window of time when

776
00:30:54,930 --> 00:30:53,110
phytoplankton can occur and it has to do

777
00:30:57,119 --> 00:30:54,940
with the monsoon seasons and the wind's

778
00:30:59,610 --> 00:30:57,129
coming in from the southwest which

779
00:31:01,289 --> 00:30:59,620
allows water to up well from the cold

780
00:31:03,539 --> 00:31:01,299
nutrient-rich water to up well from the

781
00:31:05,879 --> 00:31:03,549
deep and it also brings in nutrients

782
00:31:09,360 --> 00:31:05,889
from lamb and so these out in nutrients

783
00:31:12,600 --> 00:31:09,370
fee of the phytoplankton bloom and in

784
00:31:17,220 --> 00:31:12,610
response to phytoplankton blooms we also

785
00:31:19,110 --> 00:31:17,230
often see a response like this and here

786
00:31:20,580 --> 00:31:19,120
on the y-axis are just the number of

787
00:31:23,369 --> 00:31:20,590
bacterial cells and here we have

788
00:31:25,590 --> 00:31:23,379

chlorophyll and this is presumably due

789
00:31:28,440 --> 00:31:25,600
to heterotrophic bacteria which bloom in

790
00:31:30,060 --> 00:31:28,450
response to the increase of do C or

791
00:31:32,279 --> 00:31:30,070
dissolved organic carbon which is being

792
00:31:34,019 --> 00:31:32,289
leaked out of algal cells but his

793
00:31:36,240 --> 00:31:34,029
actions don't know that much about how

794
00:31:38,610 --> 00:31:36,250
diversity changes in response to

795
00:31:40,470 --> 00:31:38,620
phytoplankton blooms and we think this

796
00:31:44,580 --> 00:31:40,480
is now a great environment to fly our

797
00:31:46,139 --> 00:31:44,590
454 technology to in addition just this

798
00:31:48,930 --> 00:31:46,149
last summer you know we have things like

799
00:31:50,430 --> 00:31:48,940
dead zones and toxic algae blooms and so

800
00:31:52,680 --> 00:31:50,440
you can imagine we're just beginning to

801
00:31:55,289 --> 00:31:52,690
understand what causes those toxic algae

802
00:31:56,940 --> 00:31:55,299
blooms another human sort of impacted

803
00:32:00,509 --> 00:31:56,950
environments and we can begin to follow

804
00:32:03,060 --> 00:32:00,519
the microbial response here's another

805
00:32:05,759 --> 00:32:03,070
example of a marine environment that

806
00:32:07,799 --> 00:32:05,769
experienced both dramatic and spatial

807
00:32:09,659 --> 00:32:07,809
and temporal shifts and that is deep sea

808
00:32:11,669 --> 00:32:09,669
hydrothermal vents this is an

809
00:32:13,740 --> 00:32:11,679
illustration of a dyking eruptive event

810
00:32:16,320 --> 00:32:13,750
occurring at a mid-ocean ridge where you

811
00:32:18,450 --> 00:32:16,330
have a dike extrusion that results in

812
00:32:21,060 --> 00:32:18,460
eruption a lava eruption on the seafloor

813
00:32:25,409 --> 00:32:21,070

and this causes very extreme changes in

814
00:32:27,960 --> 00:32:25,419
heat flux in chemistry and in porosity

815
00:32:29,430 --> 00:32:27,970
and permeability of the crust and there

816
00:32:31,799 --> 00:32:29,440
have been some studies that will have

817
00:32:34,320 --> 00:32:31,809
looked at the response of the microbial

818
00:32:36,869 --> 00:32:34,330
system to this type of event and that's

819
00:32:38,759 --> 00:32:36,879
shown in the schematic here and you can

820
00:32:42,049 --> 00:32:38,769
imagine sort of a steady state system

821
00:32:45,210 --> 00:32:42,059
that sets up over longer periods of time

822
00:32:47,009 --> 00:32:45,220
with perhaps some deeper organisms that

823
00:32:49,740 --> 00:32:47,019
are living in the cross kind of just

824
00:32:51,990 --> 00:32:49,750
barely eking it out on those flew few

825
00:32:54,299 --> 00:32:52,000
hydrothermal nutrients that are around

826
00:32:55,889 --> 00:32:54,309
but in fact there's organisms that are

827
00:32:57,330 --> 00:32:55,899
living off of seawater maybe some of

828
00:32:59,789 --> 00:32:57,340
those epsilon that are making a good

829
00:33:02,100 --> 00:32:59,799
living but when you have this influx of

830
00:33:04,470 --> 00:33:02,110
hydrothermal nutrients and heat these

831
00:33:05,700 --> 00:33:04,480
isotherms get pushed up and organisms

832
00:33:08,100 --> 00:33:05,710
that can take advantage of that

833
00:33:09,840 --> 00:33:08,110
situation are going to bloom and that's

834
00:33:11,789 --> 00:33:09,850
what's shown here and in fact you might

835
00:33:13,980 --> 00:33:11,799
even have a secondary bloom then perhaps

836
00:33:16,289 --> 00:33:13,990
even see water organisms heterotrophic

837
00:33:18,330 --> 00:33:16,299
aerobes that can then feed on that

838
00:33:19,860 --> 00:33:18,340

primary gloom and eventually you're

839
00:33:21,840 --> 00:33:19,870
going to settle down to some new study

840
00:33:23,970 --> 00:33:21,850
state maybe those organisms that were

841
00:33:29,220 --> 00:33:23,980
just barely making it by now or dominant

842
00:33:31,830 --> 00:33:29,230
or vice versa we can also think about

843
00:33:33,480 --> 00:33:31,840
well I don't know if this was supposed

844
00:33:35,820 --> 00:33:33,490
to be a picture of Hurricane Katrina

845
00:33:37,649 --> 00:33:35,830
it's not on my slide but we can also

846
00:33:41,190 --> 00:33:37,659
think about natural disasters such as

847
00:33:43,470 --> 00:33:41,200
Hurricane Katrina and its aftermath and

848
00:33:45,690 --> 00:33:43,480
in response to Hurricane Katrina a

849
00:33:48,450 --> 00:33:45,700
number of microbial ecologist traveled

850
00:33:51,240 --> 00:33:48,460
to New Orleans to look at Lake

851
00:33:53,609 --> 00:33:51,250
Pontchartrain look at the canals and try

852
00:33:56,009 --> 00:33:53,619
to evaluate whether or not this water

853
00:33:59,149 --> 00:33:56,019
was really toxic as was being put out in

854
00:34:01,619 --> 00:33:59,159
the news media and here we can see

855
00:34:02,820 --> 00:34:01,629
before the hurricane and after the

856
00:34:05,490 --> 00:34:02,830
hurricane and where the flooding

857
00:34:07,230 --> 00:34:05,500
occurred Linda emerald Zettler here at

858
00:34:09,389 --> 00:34:07,240
the MDL was one of those scientists who

859
00:34:11,040 --> 00:34:09,399
went to New Orleans New Orleans to get

860
00:34:12,480 --> 00:34:11,050
follow-up samples

861
00:34:15,210 --> 00:34:12,490
she's particularly interested in looking

862
00:34:17,190 --> 00:34:15,220
for potentially pathogenic microbes and

863
00:34:19,500 --> 00:34:17,200

some of her data is shown here on the

864
00:34:21,230 --> 00:34:19,510
right these big blue chunks are

865
00:34:24,180 --> 00:34:21,240
basically a marine or freshwater

866
00:34:25,950 --> 00:34:24,190
bacteria and the small yellow of slices

867
00:34:27,960 --> 00:34:25,960
are microbes that she believed are

868
00:34:30,210 --> 00:34:27,970
potentially pathogenic or related to

869
00:34:32,370 --> 00:34:30,220
pathogenic microbes and so from this

870
00:34:35,790 --> 00:34:32,380
data set these look like low abundant

871
00:34:37,380 --> 00:34:35,800
organisms that maybe did bloom out right

872
00:34:39,030 --> 00:34:37,390
after the hurricane but it doesn't look

873
00:34:40,980 --> 00:34:39,040
like they are now but the fact that

874
00:34:43,080 --> 00:34:40,990
they're there suggest that under the

875
00:34:45,300 --> 00:34:43,090
right conditions perhaps something

876
00:34:47,490 --> 00:34:45,310
something more dangerous could happen

877
00:34:50,310 --> 00:34:47,500
and I think it emphasizes the need to

878
00:34:52,440 --> 00:34:50,320
monitor these types of systems so we

879
00:34:55,230 --> 00:34:52,450
don't just respond when something really

880
00:34:57,300 --> 00:34:55,240
terrible happens of course we have a

881
00:35:00,660 --> 00:34:57,310
very big human disaster looking on the

882
00:35:02,790 --> 00:35:00,670
horizon well actively occurring with

883
00:35:04,980 --> 00:35:02,800
increasing co2 levels and temperatures

884
00:35:06,720 --> 00:35:04,990
on our planet and we don't really know

885
00:35:09,360 --> 00:35:06,730
what the microbial response is going to

886
00:35:11,340 --> 00:35:09,370
be we know that this will impact co2

887
00:35:13,770 --> 00:35:11,350
solubility in the ocean it's certainly

888
00:35:16,320 --> 00:35:13,780

going to affect the biological pump but

889
00:35:17,880 --> 00:35:16,330
we don't know how microbial communities

890
00:35:20,100 --> 00:35:17,890
are going to respond and so it's

891
00:35:22,650 --> 00:35:20,110
essential that we begin some sort of

892
00:35:25,980 --> 00:35:22,660
census so we can track and follow these

893
00:35:28,910 --> 00:35:25,990
populations over time we can also think

894
00:35:31,110 --> 00:35:28,920
of another human impacted area where

895
00:35:33,450 --> 00:35:31,120
excuse me where we need to be able to

896
00:35:35,730 --> 00:35:33,460
assess and track microbial communities

897
00:35:37,620 --> 00:35:35,740
and where low abundant organisms might

898
00:35:39,930 --> 00:35:37,630
in fact be very important and that's

899
00:35:41,460 --> 00:35:39,940
with planetary protection so you can

900
00:35:43,590 --> 00:35:41,470
imagine applying this type of technology

901
00:35:45,590 --> 00:35:43,600
to make sure you know exactly what's

902
00:35:47,760 --> 00:35:45,600
going into space and then what comes out

903
00:35:50,220 --> 00:35:47,770
and we are in fact looking into

904
00:35:52,950 --> 00:35:50,230
collaborations with folks at both JPL

905
00:35:55,230 --> 00:35:52,960
and NASA to apply this technology for

906
00:35:58,410 --> 00:35:55,240
screening space materia for potential

907
00:36:01,980 --> 00:35:58,420
contaminants we can also think about

908
00:36:03,900 --> 00:36:01,990
life outside earth such as Mars i sat in

909
00:36:05,610 --> 00:36:03,910
on a workshop last fall for the

910
00:36:07,280 --> 00:36:05,620
microbial scientist exploration

911
00:36:10,380 --> 00:36:07,290
initiative and we talked a lot about

912
00:36:12,030 --> 00:36:10,390
sort of changes on Mars and Europa and

913
00:36:14,490 --> 00:36:12,040

how this might impact microbial

914
00:36:16,020 --> 00:36:14,500
populations and so I took from some of

915
00:36:17,370 --> 00:36:16,030
my notes some of the ideas that people

916
00:36:19,860 --> 00:36:17,380
had their which I thought were really

917
00:36:21,460 --> 00:36:19,870
interesting we know that Mars has what's

918
00:36:23,530 --> 00:36:21,470
called a chaotic liquidy

919
00:36:25,660 --> 00:36:23,540
that's showing here where it moves from

920
00:36:27,730 --> 00:36:25,670
a low mean obliquity period through a

921
00:36:30,099 --> 00:36:27,740
transition to a high mean of liquidy

922
00:36:31,690 --> 00:36:30,109
period and what this means is heating

923
00:36:34,290 --> 00:36:31,700
there's differential heating in the

924
00:36:37,060 --> 00:36:34,300
polar regions and this can cause a total

925
00:36:39,220 --> 00:36:37,070
redistribution of ice and water vapor on

926
00:36:41,200 --> 00:36:39,230
the planet and so we can see there might

927
00:36:43,420 --> 00:36:41,210
be important linkages between the solar

928
00:36:45,849 --> 00:36:43,430
forcing how the climate responds and

929
00:36:47,109 --> 00:36:45,859
geological consequences on Mars and we

930
00:36:50,140 --> 00:36:47,119
might want to think about for example

931
00:36:52,870 --> 00:36:50,150
how microbial populations could respond

932
00:36:54,820 --> 00:36:52,880
to those changes as well this is just an

933
00:36:57,250 --> 00:36:54,830
illustration of the ice evolution on

934
00:36:59,500 --> 00:36:57,260
Mars and this angle between the white

935
00:37:01,720 --> 00:36:59,510
arrow and the dotted line denotes that

936
00:37:04,150 --> 00:37:01,730
Martian of liquid e and so you could

937
00:37:06,400 --> 00:37:04,160
think about well is the martian biota in

938
00:37:08,830 --> 00:37:06,410

some sort of dormant stage when this

939
00:37:11,080 --> 00:37:08,840
obliquity winter and what might happen

940
00:37:13,930 --> 00:37:11,090
when the obliquity changes and there's

941
00:37:16,660 --> 00:37:13,940
much less ice would they hold on to some

942
00:37:18,400 --> 00:37:16,670
sort of genomic information what are the

943
00:37:21,400 --> 00:37:18,410
time constraints some of on that

944
00:37:24,190 --> 00:37:21,410
persistence we also know that your robo

945
00:37:26,109 --> 00:37:24,200
is a very dynamic system it has an

946
00:37:28,660 --> 00:37:26,119
extremely eccentric orbit which is shown

947
00:37:30,820 --> 00:37:28,670
here and it also has a complex geology

948
00:37:32,680 --> 00:37:30,830
and it's believed that the eccentric

949
00:37:35,140 --> 00:37:32,690
orbit the geology and these tidal

950
00:37:37,150 --> 00:37:35,150
heating are all tied together and in

951
00:37:39,880 --> 00:37:37,160
fact this is a model looking at Europa's

952
00:37:42,420 --> 00:37:39,890
ice thickness as it varies based on

953
00:37:45,310 --> 00:37:42,430
changes in both orbit and tidal heating

954
00:37:47,589 --> 00:37:45,320
at the workshop i was at we also talked

955
00:37:50,530 --> 00:37:47,599
about how the possibility of the

956
00:37:53,109 --> 00:37:50,540
convection occurring in the ice shell of

957
00:37:55,450 --> 00:37:53,119
Europa and also what I thought was

958
00:37:57,910 --> 00:37:55,460
really fascinating this idea that in the

959
00:38:00,220 --> 00:37:57,920
rope was very early stages it could have

960
00:38:02,170 --> 00:38:00,230
had a completely open ocean and so again

961
00:38:04,000 --> 00:38:02,180
this is another dynamic environment when

962
00:38:05,800 --> 00:38:04,010
we think about life on Europa what we

963
00:38:08,380 --> 00:38:05,810

might want to try to detect we have to

964
00:38:10,240 --> 00:38:08,390
think about all the stages that this

965
00:38:14,620 --> 00:38:10,250
planet or this moon might have gone

966
00:38:17,230 --> 00:38:14,630
through so in conclusion we believe that

967
00:38:19,599 --> 00:38:17,240
this fort by for tag sequencing approach

968
00:38:21,609 --> 00:38:19,609
provides an in-depth initial view on

969
00:38:24,040 --> 00:38:21,619
total diversity of microbes in an

970
00:38:25,599 --> 00:38:24,050
environment we think it's very efficient

971
00:38:27,940 --> 00:38:25,609
and it's high throughput which allows

972
00:38:30,460 --> 00:38:27,950
for intensive sampling of all sites of

973
00:38:33,220 --> 00:38:30,470
interest it can detect both major and

974
00:38:34,060 --> 00:38:33,230
minor population members and it offers a

975
00:38:35,830 --> 00:38:34,070
really neat

976
00:38:38,410 --> 00:38:35,840
tool to fingerprint microbial

977
00:38:40,120 --> 00:38:38,420
communities over time and space for

978
00:38:42,130 --> 00:38:40,130
correlations with biogeochemical

979
00:38:44,920 --> 00:38:42,140
activity to imagine doing a biogeography

980
00:38:47,020 --> 00:38:44,930
experiment as well we believe it's an

981
00:38:49,090 --> 00:38:47,030
important complement to metagenomic

982
00:38:51,940 --> 00:38:49,100
culturing and institute hybridization

983
00:38:53,980 --> 00:38:51,950
investigations and we're going to expand

984
00:38:56,140 --> 00:38:53,990
it expand it to include the archaea and

985
00:38:57,190 --> 00:38:56,150
the Eukarya we're in discussion about

986
00:39:00,040 --> 00:38:57,200
whether or not it's going to work for

987
00:39:02,620 --> 00:39:00,050
viruses and we recently received funding

988
00:39:04,660 --> 00:39:02,630

from the Keck foundation to begin our

989
00:39:08,740 --> 00:39:04,670
census and apply this in a variety of

990
00:39:11,170 --> 00:39:08,750
oceanic environments our GS 20 from 454

991
00:39:12,310 --> 00:39:11,180
was delivered about three weeks ago so

992
00:39:14,500 --> 00:39:12,320
we're just getting things up and running

993
00:39:16,660 --> 00:39:14,510
here but we already have samples in the

994
00:39:18,730 --> 00:39:16,670
pipeline with collaborators all over and

995
00:39:20,470 --> 00:39:18,740
that includes more samples from these

996
00:39:23,260 --> 00:39:20,480
complete crews track lines from the

997
00:39:24,850 --> 00:39:23,270
North Atlantic we're collaborating the

998
00:39:26,740 --> 00:39:24,860
people at the University of Hawaii to

999
00:39:28,450 --> 00:39:26,750
look at the Hawaii ocean time series at

1000
00:39:30,990 --> 00:39:28,460
station Aloha this is one of the best

1001
00:39:33,880 --> 00:39:31,000
long-term data sets we have in the ocean

1002
00:39:35,530 --> 00:39:33,890
we're going to be working more on human

1003
00:39:37,330 --> 00:39:35,540
impacted areas through the woods hole

1004
00:39:39,670 --> 00:39:37,340
center for oceans in human health that

1005
00:39:42,850 --> 00:39:39,680
includes collaborators in Woods Hole as

1006
00:39:44,470 --> 00:39:42,860
well as an MIT and we're also working

1007
00:39:46,480 --> 00:39:44,480
with colleagues through the ocean

1008
00:39:48,400 --> 00:39:46,490
drilling program and the ridge 2000

1009
00:39:51,180 --> 00:39:48,410
program to look at both subsea floor

1010
00:39:53,290 --> 00:39:51,190
basalts and subsea floor sediments and

1011
00:39:55,180 --> 00:39:53,300
of course we're going to be looking at

1012
00:39:57,430 --> 00:39:55,190
and in my favorite environment and

1013
00:39:59,460 --> 00:39:57,440

that's deep sea hydrothermal vents in

1014
00:40:01,840 --> 00:39:59,470
this case of the pacific ocean and

1015
00:40:04,150 --> 00:40:01,850
because i think i have a little bit of

1016
00:40:06,400 --> 00:40:04,160
time i'm going to show you a video from

1017
00:40:08,410 --> 00:40:06,410
a very dynamic environment that you can

1018
00:40:10,930 --> 00:40:08,420
imagine organisms responding to quite

1019
00:40:13,210 --> 00:40:10,940
rapidly and that is from this site here

1020
00:40:15,730 --> 00:40:13,220
in the western Pacific this is cruz i

1021
00:40:18,280 --> 00:40:15,740
participated in with noah ocean Explorer

1022
00:40:20,290 --> 00:40:18,290
this last spring and i'm going to show

1023
00:40:21,970 --> 00:40:20,300
you a video of this active eruption

1024
00:40:24,640 --> 00:40:21,980
taking place on the sea floor at about

1025
00:40:26,800 --> 00:40:24,650
500 meters depth and until last spring

1026
00:40:29,350 --> 00:40:26,810
this is something that had never been

1027
00:40:33,760 --> 00:40:29,360
seen before let me see if I can do this

1028
00:40:36,160 --> 00:40:33,770
right so while you're watching you

1029
00:40:38,500 --> 00:40:36,170
should look for gas bubbling you should

1030
00:40:39,880 --> 00:40:38,510
work look for sulfur you should look for

1031
00:40:43,509 --> 00:40:39,890
a lava bombs

1032
00:40:45,069 --> 00:40:43,519
and especially look for a red flash and

1033
00:40:47,440 --> 00:40:45,079
that's actually a magma extrusion and

1034
00:40:49,809 --> 00:40:47,450
that also is the first time it's been

1035
00:40:53,259 --> 00:40:49,819
seen on the seafloor so I'm just going

1036
00:40:56,769 --> 00:40:53,269
to wait a few more seconds and let this

1037
00:40:58,240 --> 00:40:56,779
finish loading up I should mention that

1038
00:41:01,359 --> 00:40:58,250

know people were harmed in the filming

1039
00:41:03,370 --> 00:41:01,369
of this eruption this was a remotely

1040
00:41:13,850 --> 00:41:03,380
operated vehicle Jason to which is

1041
00:41:19,190 --> 00:41:16,340
and if you could hear this we actually

1042
00:41:25,100 --> 00:41:19,200
have hydrophone data there's the red

1043
00:41:29,540 --> 00:41:25,110
flash we we actually have hydrophone

1044
00:41:33,380 --> 00:41:29,550
data that's tracking that listening as

1045
00:41:36,170 --> 00:41:33,390
we're viewing this eruption and we

1046
00:41:40,630 --> 00:41:36,180
actually got samples in 2004 from this

1047
00:41:44,870 --> 00:41:40,640
site we're not gonna see it take us off

1048
00:41:49,640 --> 00:41:44,880
um yeah we can request for her she can

1049
00:41:57,620 --> 00:41:49,650
email it it's about 150 it's your

1050
00:42:00,080 --> 00:41:57,630
microphone and so we actually got

1051
00:42:02,060 --> 00:42:00,090
samples from this site in 2004 beside

1052
00:42:05,000 --> 00:42:02,070
before it started going crazy and then

1053
00:42:07,220 --> 00:42:05,010
in 2006 we spent about a week watching

1054
00:42:09,170 --> 00:42:07,230
this volcano go through it's full of

1055
00:42:11,450 --> 00:42:09,180
rupted cycle so we have samples from

1056
00:42:14,690 --> 00:42:11,460
when it was just a wisp of 20 degree

1057
00:42:18,500 --> 00:42:14,700
fluid up to when it was full bang about

1058
00:42:21,260 --> 00:42:18,510
150 degree Celsius eruption occurring so

1059
00:42:22,850 --> 00:42:21,270
this is just an extreme example of the

1060
00:42:24,680 --> 00:42:22,860
type of environment that we're going to

1061
00:42:27,080 --> 00:42:24,690
be studying with the 454 sequencing

1062
00:42:28,610 --> 00:42:27,090
technology and this collection of

1063
00:42:30,770 --> 00:42:28,620

samples from all over the ocean is

1064
00:42:33,470 --> 00:42:30,780
really going to allow us to explore some

1065
00:42:35,660 --> 00:42:33,480
exciting things I think in the microbial

1066
00:42:39,350 --> 00:42:35,670
world in the sea and potentially in

1067
00:42:41,810 --> 00:42:39,360
other environments as well so just in

1068
00:42:43,700 --> 00:42:41,820
conclusion I want to acknowledge our

1069
00:42:45,380 --> 00:42:43,710
collaborators in the Netherlands Gerhard

1070
00:42:48,290 --> 00:42:45,390
handle and hey-zeus Arrieta for

1071
00:42:49,490 --> 00:42:48,300
providing those transaxles we have a lot

1072
00:42:52,370 --> 00:42:49,500
more of them in our freezer to get

1073
00:42:54,110 --> 00:42:52,380
working on Alfred P sloan Foundation

1074
00:42:56,780 --> 00:42:54,120
which has funded all of our pilot

1075
00:43:00,530 --> 00:42:56,790
studies the nasa astrobiology institute

1076
00:43:02,960 --> 00:43:00,540
that funds me the Keck foundation which

1077
00:43:04,820 --> 00:43:02,970
is helping us with our purchase of our

1078
00:43:08,030 --> 00:43:04,830
454 sequencing machine in the beginning

1079
00:43:09,740 --> 00:43:08,040
of our icom initiative and if you want

1080
00:43:12,170 --> 00:43:09,750
to learn more you can check out the

1081
00:43:15,380 --> 00:43:12,180
paper carl refer to or you can just drop

1082
00:43:25,150 --> 00:43:15,390
me an email so with that I'll take any

1083
00:43:31,820 --> 00:43:29,960
okay so just to review again because we

1084
00:43:36,110 --> 00:43:31,830
have a lot of sites connected please

1085
00:43:37,670 --> 00:43:36,120
raise your hand in the chat area or

1086
00:43:39,770 --> 00:43:37,680
please raise your hand in the

1087
00:43:41,840 --> 00:43:39,780
participant list or put a note in the

1088
00:43:44,930 --> 00:43:41,850

chat area to Marco bolt which is

1089
00:43:50,360 --> 00:43:44,940
actually Marco Bolton myself today and

1090
00:43:52,310 --> 00:43:50,370
and we will call on your site so I don't

1091
00:43:55,340 --> 00:43:52,320
see any time right now that are so now

1092
00:43:57,860 --> 00:43:55,350
just to open it up any questions oh wait

1093
00:43:59,630 --> 00:43:57,870
hold on sorry university of rhode island

1094
00:44:06,170 --> 00:43:59,640
you can now open your mic and talk

1095
00:44:16,310 --> 00:44:13,850
we're not hearing you okay great you're

1096
00:44:19,190 --> 00:44:16,320
dark though picking trance night yeah

1097
00:44:22,490 --> 00:44:19,200
we're just good working here you gonna

1098
00:44:25,760 --> 00:44:22,500
know how only one gets into this so yeah

1099
00:44:29,690 --> 00:44:25,770
on the 454 technology can you tell me

1100
00:44:31,430 --> 00:44:29,700
how only one one strand of DNA gets

1101
00:44:38,080 --> 00:44:31,440
incorporated on the bead that actually

1102
00:44:41,210 --> 00:44:38,090
has multiple receptors on it right so

1103
00:44:43,970 --> 00:44:41,220
you you have four possibilities that

1104
00:44:46,130 --> 00:44:43,980
could happen and they select just for

1105
00:44:48,890 --> 00:44:46,140
there's two steps they select just for

1106
00:44:51,530 --> 00:44:48,900
beads that only have one adapter picked

1107
00:44:54,110 --> 00:44:51,540
up on them but they they go through a

1108
00:44:57,560 --> 00:44:54,120
selection process to try to wean it down

1109
00:45:00,440 --> 00:44:57,570
but in fact you can get false reads that

1110
00:45:03,260 --> 00:45:00,450
have more than one amplicon on them and

1111
00:45:05,240 --> 00:45:03,270
the software is built to recognize that

1112
00:45:07,250 --> 00:45:05,250
your sequencing looks really really

1113
00:45:09,920 --> 00:45:07,260

messy because you can see multiple

1114
00:45:12,380 --> 00:45:09,930
incorporations in one flow gram and so

1115
00:45:14,270 --> 00:45:12,390
that it really happens a little bit in

1116
00:45:17,570 --> 00:45:14,280
the lab work part but mostly in the

1117
00:45:20,390 --> 00:45:17,580
bioinformatics and then you try and

1118
00:45:23,360 --> 00:45:20,400
control that by how much DNA you a deal

1119
00:45:25,130 --> 00:45:23,370
the ratio yeah so thursday things we do

1120
00:45:26,740 --> 00:45:25,140
a dilution series with your beads and

1121
00:45:32,200 --> 00:45:26,750
your amplicons and those sorts of things

1122
00:45:43,780 --> 00:45:36,250
hey thanks David uh University of

1123
00:45:48,210 --> 00:45:43,790
Arizona go ahead let wolf here very nice

1124
00:45:52,450 --> 00:45:48,220
talk Julie um coming at this from from

1125
00:45:56,380 --> 00:45:52,460
outside of biology I'm puzzled by the

1126
00:46:00,339 --> 00:45:56,390
following question since we know that

1127
00:46:04,780 --> 00:46:00,349
the same ribosomal RNA can be associated

1128
00:46:07,060 --> 00:46:04,790
with different metabolic processes how

1129
00:46:09,849 --> 00:46:07,070
do we know that the reverse can't happen

1130
00:46:12,790 --> 00:46:09,859
that what you'll see are essentially the

1131
00:46:18,460 --> 00:46:12,800
same Mike microbes with slightly

1132
00:46:22,180 --> 00:46:18,470
different ribosomal RNA that's a very

1133
00:46:23,589 --> 00:46:22,190
good question and the best tool we have

1134
00:46:26,680 --> 00:46:23,599
to look at that right now is our

1135
00:46:28,329 --> 00:46:26,690
reference database and so if we go

1136
00:46:30,370 --> 00:46:28,339
through our entire reference database

1137
00:46:33,099 --> 00:46:30,380
and have a hundred and twenty thousand

1138
00:46:36,190 --> 00:46:33,109

unique sequences we can only pull out

1139
00:46:38,680 --> 00:46:36,200
forty four thousand unique v6 regions

1140
00:46:40,750 --> 00:46:38,690
and so we already see a little bit of a

1141
00:46:43,300 --> 00:46:40,760
disparity there and so that what we're

1142
00:46:45,280 --> 00:46:43,310
trying to do is look at the diversity at

1143
00:46:47,140 --> 00:46:45,290
a level that we think is meaningful and

1144
00:46:48,910 --> 00:46:47,150
that's why we're not just looking at

1145
00:46:51,220 --> 00:46:48,920
individual sequences and we're using

1146
00:46:53,530 --> 00:46:51,230
this this distance approach where we

1147
00:46:55,540 --> 00:46:53,540
collapse thing into three percent five

1148
00:46:57,730 --> 00:46:55,550
percent levels so that we can hopefully

1149
00:46:59,950 --> 00:46:57,740
get a bit of lip rid of a little bit of

1150
00:47:02,470 --> 00:46:59,960
that wiggle room but actually this tells

1151
00:47:04,839 --> 00:47:02,480
us nothing about function right we have

1152
00:47:06,370 --> 00:47:04,849
we have no idea if these 20,000

1153
00:47:07,660 --> 00:47:06,380
estimated microbes are all doing

1154
00:47:09,730 --> 00:47:07,670
different things if they're all doing

1155
00:47:12,640 --> 00:47:09,740
the exact same thing this is a first

1156
00:47:14,230 --> 00:47:12,650
step in getting closer to a census and

1157
00:47:16,329 --> 00:47:14,240
what we're trying to come up with our

1158
00:47:18,310 --> 00:47:16,339
creative ways then to go back into the

1159
00:47:19,930 --> 00:47:18,320
environment and study those organisms to

1160
00:47:22,859 --> 00:47:19,940
find out what they're actually doing in

1161
00:47:36,609 --> 00:47:29,410
thank you thanks Nick ok MSU are you

1162
00:47:38,769 --> 00:47:36,619
there so my question is this tail end

1163
00:47:41,620 --> 00:47:38,779

this is very popular and we have seen it

1164
00:47:43,239 --> 00:47:41,630
in soil a lot tailing of microbial

1165
00:47:47,229 --> 00:47:43,249
communities where you see a lot of very

1166
00:47:49,989 --> 00:47:47,239
unique OT use how do you get a better

1167
00:47:53,499 --> 00:47:49,999
characterization of that now that 454 is

1168
00:47:57,489 --> 00:47:53,509
available right so the first thing we

1169
00:47:59,559 --> 00:47:57,499
want to do is our hope well one at one

1170
00:48:01,150 --> 00:47:59,569
of our hopes is that by doing a bunch of

1171
00:48:03,219 --> 00:48:01,160
different samples from in dump a bunch

1172
00:48:05,019 --> 00:48:03,229
of different environments we might be

1173
00:48:07,539 --> 00:48:05,029
able to see some patterns or at least

1174
00:48:10,630 --> 00:48:07,549
find places where those rare in one

1175
00:48:13,089 --> 00:48:10,640
sample occur in another sample so begin

1176
00:48:15,130 --> 00:48:13,099
to build a picture of these divergent Oh

1177
00:48:17,529 --> 00:48:15,140
to use instead of just one researcher

1178
00:48:19,689 --> 00:48:17,539
seeing them with their particular pcr

1179
00:48:21,699 --> 00:48:19,699
approach and then no one ever seeing

1180
00:48:23,259 --> 00:48:21,709
them again so the idea is if we treat

1181
00:48:25,779 --> 00:48:23,269
all the samples equally maybe we'll be

1182
00:48:27,939 --> 00:48:25,789
able to find them the second step and

1183
00:48:31,749 --> 00:48:27,949
something we're trying to show and a

1184
00:48:34,239 --> 00:48:31,759
proof of concept is to design a primer

1185
00:48:37,809 --> 00:48:34,249
based on that unique v6 sequences and

1186
00:48:42,039 --> 00:48:37,819
put it with a universal bacterial

1187
00:48:44,259 --> 00:48:42,049
sequence go back into into your sample

1188
00:48:49,359 --> 00:48:44,269

try to amplify out more of the genomic

1189
00:48:51,699 --> 00:48:49,369
DNA the 16s DNA in this case possibly

1190
00:48:54,219 --> 00:48:51,709
scream fosmon libraries for this gene

1191
00:48:57,249 --> 00:48:54,229
try to visualize it we're basically

1192
00:48:59,199 --> 00:48:57,259
we're trying to get to that point but

1193
00:49:01,209 --> 00:48:59,209
the first step is really to see how rare

1194
00:49:06,339 --> 00:49:01,219
is it actually or is it just that we

1195
00:49:08,199 --> 00:49:06,349
haven't been looking enough thank you

1196
00:49:10,449 --> 00:49:08,209
very much and if I could ask another

1197
00:49:12,910 --> 00:49:10,459
question it will be what if you actually

1198
00:49:14,739 --> 00:49:12,920
want to apply to specific functions

1199
00:49:18,189 --> 00:49:14,749
genes related to a specific functions

1200
00:49:20,739 --> 00:49:18,199
how would you change your approach well

1201
00:49:23,499 --> 00:49:20,749
right now 454 is really limited to about

1202
00:49:25,419 --> 00:49:23,509
a hundred base pairs so any primer

1203
00:49:27,069 --> 00:49:25,429
design you could think of where you're

1204
00:49:29,829 --> 00:49:27,079
going to get enough information out of

1205
00:49:31,059 --> 00:49:29,839
about a hundred base pairs that you go

1206
00:49:34,179 --> 00:49:31,069
for it you know you should give it a

1207
00:49:35,380 --> 00:49:34,189
shot the new generation of machines they

1208
00:49:37,450 --> 00:49:35,390
say are going to get about

1209
00:49:40,000 --> 00:49:37,460
200 base pairs so maybe that gives us a

1210
00:49:42,069 --> 00:49:40,010
little more lead way in the types of

1211
00:49:43,480 --> 00:49:42,079
genes we can design primers for but

1212
00:49:50,410 --> 00:49:43,490
that's sort of that's the constraint

1213
00:49:54,759 --> 00:49:50,420

right now thank you very much okay

1214
00:49:58,029 --> 00:49:54,769
University of Washington guys there hi

1215
00:50:00,430 --> 00:49:58,039
head saw Tom Quinn here um actually my

1216
00:50:02,349 --> 00:50:00,440
question was very similar to Nick's man

1217
00:50:04,299 --> 00:50:02,359
that is you know how can you tell

1218
00:50:06,910 --> 00:50:04,309
whether you've actually got diversity

1219
00:50:10,269 --> 00:50:06,920
and phenotype since you you're just

1220
00:50:12,970 --> 00:50:10,279
measuring diversity in genotype ah so

1221
00:50:16,960 --> 00:50:12,980
just to push you a little further on

1222
00:50:18,759 --> 00:50:16,970
that you know what ideas do have to try

1223
00:50:22,299 --> 00:50:18,769
to figure out the diversity of function

1224
00:50:24,309 --> 00:50:22,309
is it possible to get a hint of protein

1225
00:50:27,069 --> 00:50:24,319
structure from your sequences or are you

1226
00:50:31,630 --> 00:50:27,079
going to have to do more sort of sort of

1227
00:50:37,059 --> 00:50:31,640
whole cell culturing and things like

1228
00:50:39,220 --> 00:50:37,069
that yes using this technology we're not

1229
00:50:41,170 --> 00:50:39,230
going to get any sense of function we're

1230
00:50:43,660 --> 00:50:41,180
just going to get binning into taxonomic

1231
00:50:45,849 --> 00:50:43,670
identities we are though going to get

1232
00:50:47,650 --> 00:50:45,859
fingerprints for microbial communities

1233
00:50:49,299 --> 00:50:47,660
in all sorts of different environments

1234
00:50:54,279 --> 00:50:49,309
maybe we'll be able to pick up patterns

1235
00:50:56,470 --> 00:50:54,289
we only find certain talk tabs in oxygen

1236
00:50:58,599 --> 00:50:56,480
deplete domes or in high temperature

1237
00:51:00,549 --> 00:50:58,609
zones for example and that's how

1238
00:51:02,680 --> 00:51:00,559

microbial ecology has been going for the

1239
00:51:04,690 --> 00:51:02,690
last ten years and in the last few years

1240
00:51:07,420 --> 00:51:04,700
people have started using very creative

1241
00:51:09,009 --> 00:51:07,430
culturing techniques to get a sense to

1242
00:51:11,049 --> 00:51:09,019
find out what those organisms are

1243
00:51:12,970 --> 00:51:11,059
actually doing but really I think the

1244
00:51:15,069 --> 00:51:12,980
key is is going to be a combination of

1245
00:51:17,470 --> 00:51:15,079
culturing and doing some more functional

1246
00:51:19,359 --> 00:51:17,480
genomic methods to get a big a true

1247
00:51:21,490 --> 00:51:19,369
handle on that this is just a

1248
00:51:25,510 --> 00:51:21,500
cataloguing of diversity that's all it

1249
00:51:25,520 --> 00:51:30,430
thanks Tom anything else from Washington

1250
00:51:35,660 --> 00:51:32,810
that's it here thank you thank you julie

1251
00:51:39,680 --> 00:51:35,670
thanks yep ok Goddard Space Flight

1252
00:51:42,410 --> 00:51:39,690
Center you have a question there yeah I

1253
00:51:45,020 --> 00:51:42,420
think it's mike Mumma I'm interested in

1254
00:51:47,560 --> 00:51:45,030
the potential applicability to systems

1255
00:51:50,690 --> 00:51:47,570
that might be related to Mars or

1256
00:51:53,120 --> 00:51:50,700
subterranean life on Mars I wondered if

1257
00:51:57,110 --> 00:51:53,130
you had any plans to go to the lost city

1258
00:52:01,850 --> 00:51:57,120
formation it's not satisfied what unit

1259
00:52:05,120 --> 00:52:01,860
so forth uh well we we don't have any

1260
00:52:06,650 --> 00:52:05,130
plans to but we certainly know that a

1261
00:52:09,380 --> 00:52:06,660
lot of the data that's being generated

1262
00:52:11,990 --> 00:52:09,390
from lost city and other ecosystems

1263
00:52:13,520 --> 00:52:12,000

driven by serpent anization we know that

1264
00:52:16,640 --> 00:52:13,530
at Lost City actually the archaeal

1265
00:52:19,040 --> 00:52:16,650
community is very simple we made up only

1266
00:52:20,900 --> 00:52:19,050
a couple different organisms but the

1267
00:52:22,610 --> 00:52:20,910
bacterial story looks a little bit more

1268
00:52:24,440 --> 00:52:22,620
complicated and actually i think the

1269
00:52:29,700 --> 00:52:24,450
application of 454 to that environment

1270
00:52:37,320 --> 00:52:31,829
right here so if anyone has samples send

1271
00:52:39,630 --> 00:52:37,330
them along Ruby crusher thanks Mike okay

1272
00:52:41,490 --> 00:52:39,640
I don't see any more hands raised i just

1273
00:52:43,890 --> 00:52:41,500
want to give a chance to the people who

1274
00:52:46,859 --> 00:52:43,900
came in on the conference call number to

1275
00:52:51,400 --> 00:52:46,869
ask any questions any questions there

1276
00:53:01,510 --> 00:52:58,750
and Mike it should be unmuted good okay

1277
00:53:04,720 --> 00:53:01,520
great well thank you all very much for

1278
00:53:06,760 --> 00:53:04,730
attending and and again thanks to the

1279
00:53:09,220 --> 00:53:06,770
Johnson center for the use of their

1280
00:53:12,190 --> 00:53:09,230
video conferencing facilities I think it

1281
00:53:15,089 --> 00:53:12,200
worked wonderfully and I was very glad

1282
00:53:18,069 --> 00:53:15,099
to have ISDN connection today thanks for

1283
00:53:20,799 --> 00:53:18,079
everyone here and welcome to Carl puffer

1284
00:53:24,160 --> 00:53:20,809
Julie have a good time that C and we'll