AbSciCon
2019

1
00:00:00,790 --> 00:00:07,559
[Music]

2
00:00:13,020 --> 00:00:12,390
I thanks to the organizers for letting

3
00:00:15,750 --> 00:00:13,030
me be here

4
00:00:17,760 --> 00:00:15,760
the original tiled title was alternative

5
00:00:19,620 --> 00:00:17,770
biopolymers in the early evolution but I

6
00:00:22,769 --> 00:00:19,630
figured I should be a little more

7
00:00:25,079 --> 00:00:22,779
specific about what it is that I want to

8
00:00:27,150 --> 00:00:25,089
present today and so I chose this

9
00:00:29,790 --> 00:00:27,160
subtitle assessing the evolutionary

10
00:00:32,429 --> 00:00:29,800
potential of nucleic acid libraries with

11
00:00:34,740 --> 00:00:32,439
increasing increased information density

12
00:00:36,930 --> 00:00:34,750
and what I would like to show you today

13
00:00:41,189 --> 00:00:36,940

is a set of experiments that we started

14
00:00:45,330 --> 00:00:41,199
in the attempt to understand what is the

15
00:00:48,029 --> 00:00:45,340
real information density that is needed

16
00:00:50,750 --> 00:00:48,039
for a library to achieve evolution while

17
00:00:54,569 --> 00:00:50,760
still maintaining the least possible

18
00:00:57,829 --> 00:00:54,579
amount of well while still using the

19
00:01:01,740 --> 00:00:57,839
least possible amount of energy to do so

20
00:01:05,760 --> 00:01:01,750
so why would we want alternative

21
00:01:07,650 --> 00:01:05,770
biopolymers to to achieve that one of

22
00:01:10,020 --> 00:01:07,660
the reasons is of course that our innate

23
00:01:11,910 --> 00:01:10,030
prebiotic chemistry is hard and only

24
00:01:13,560 --> 00:01:11,920
works on under very specific sets of

25
00:01:16,020 --> 00:01:13,570
conditions and we heard about it all

26
00:01:16,740 --> 00:01:16,030
through this conference actually this

27
00:01:20,249 --> 00:01:16,750
morning

28
00:01:21,480 --> 00:01:20,259
Tyler Rush made a few good points about

29
00:01:24,420 --> 00:01:21,490
that

30
00:01:26,700 --> 00:01:24,430
also this one I should have raised it

31
00:01:28,590 --> 00:01:26,710
after this it's a fast talk this was

32
00:01:32,160 --> 00:01:28,600
very good actually

33
00:01:35,190 --> 00:01:32,170
so I think that a replicase is still our

34
00:01:38,219 --> 00:01:35,200
work in progress but people as we just

35
00:01:40,050 --> 00:01:38,229
saw in the Arab and in Holly girls love

36
00:01:42,709 --> 00:01:40,060
and enjoys lab actually they're doing

37
00:01:45,270 --> 00:01:42,719
great things with them but yet

38
00:01:47,670 --> 00:01:45,280

replicators are a key play player in a

39
00:01:50,340 --> 00:01:47,680
hypothetical RNA world and are still a

40
00:01:54,270 --> 00:01:50,350
little bit of a challenge that needs to

41
00:01:56,520 --> 00:01:54,280
be overcome another point is that alien

42
00:01:57,870 --> 00:01:56,530
life arising independently of life on

43
00:02:00,029 --> 00:01:57,880
Earth might have had a different

44
00:02:02,190 --> 00:02:00,039
molecular biology than what we see or

45
00:02:03,870 --> 00:02:02,200
was experienced here in the past and

46
00:02:06,420 --> 00:02:03,880
this is also something that people have

47
00:02:09,510 --> 00:02:06,430
been talking about quite a bit this past

48
00:02:11,970 --> 00:02:09,520
few days but even if the biopolymer of

49
00:02:13,290 --> 00:02:11,980
choice has been had been the same alien

50
00:02:14,820 --> 00:02:13,300
life might have chosen a different

51
00:02:18,360 --> 00:02:14,830
evolutionary pathway

52
00:02:19,880 --> 00:02:18,370
no matter what and even without having

53
00:02:24,110 --> 00:02:19,890
to go to all the planets

54
00:02:26,120 --> 00:02:24,120
or or before or after the evolution of

55
00:02:28,400 --> 00:02:26,130
life today's natural RNA

56
00:02:31,520 --> 00:02:28,410
post-transcriptional modifications are

57
00:02:33,440 --> 00:02:31,530
seen by many as remnants of an RNA like

58
00:02:36,980 --> 00:02:33,450
world and they might be considered as

59
00:02:39,500 --> 00:02:36,990
hints that in prebiotic times these

60
00:02:41,330 --> 00:02:39,510
modifications could have arise and

61
00:02:43,610 --> 00:02:41,340
during rough rather than after the

62
00:02:45,470 --> 00:02:43,620
establishment of the biopolymer so in

63
00:02:48,380 --> 00:02:45,480

other words these features that we call

64
00:02:49,670 --> 00:02:48,390
modifications or alternative now and

65
00:02:52,340 --> 00:02:49,680
here might have been the main

66
00:02:55,010 --> 00:02:52,350
evolutionary players in other times and

67
00:02:57,620 --> 00:02:55,020
players and in planets I did like these

68
00:02:59,780 --> 00:02:57,630
to add this quote from yesterday

69
00:03:02,720 --> 00:02:59,790
morning's talk from Christine Keating

70
00:03:04,250 --> 00:03:02,730
that I think submarines well what a lot

71
00:03:07,009 --> 00:03:04,260
of people have been saying these days

72
00:03:08,990 --> 00:03:07,019
what happens so there's this dichotomy

73
00:03:11,780 --> 00:03:09,000
in which what happens what happened who

74
00:03:14,509 --> 00:03:11,790
has versus what could happen or could

75
00:03:16,160 --> 00:03:14,519
have happened in this dichotomy the

76
00:03:19,190 --> 00:03:16,170
second is a much larger group of

77
00:03:23,240 --> 00:03:19,200
reactions so we need to start to

78
00:03:26,750 --> 00:03:23,250
investigate this I'll go back for a

79
00:03:28,580 --> 00:03:26,760
second so what as I said what we said

80
00:03:30,580 --> 00:03:28,590
what we decided to do is to set up a

81
00:03:34,340 --> 00:03:30,590
series of experiments a couple of

82
00:03:36,560 --> 00:03:34,350
initial in vitro selections starting

83
00:03:38,990 --> 00:03:36,570
with library so the the two selections

84
00:03:41,120 --> 00:03:39,000
that we want to do in parallel would go

85
00:03:43,160 --> 00:03:41,130
towards the same function but starting

86
00:03:46,090 --> 00:03:43,170
with two libraries that are the same

87
00:03:49,850 --> 00:03:46,100
length but carry different information

88
00:03:53,500 --> 00:03:49,860

contents so how do we achieve this these

89
00:03:55,699 --> 00:03:53,510
enrichment or depletion of information

90
00:03:58,039 --> 00:03:55,709
nothing would make me more happy than

91
00:04:02,360 --> 00:03:58,049
being able to do it with the naked

92
00:04:04,759 --> 00:04:02,370
systems or the neutral sites in the

93
00:04:06,770 --> 00:04:04,769
Taylor showed this morning but we are

94
00:04:10,280 --> 00:04:06,780
still lacking a molecular biology for

95
00:04:13,910 --> 00:04:10,290
those systems so what do we do have in

96
00:04:15,440 --> 00:04:13,920
the lab actually at home at Fame is this

97
00:04:17,449 --> 00:04:15,450
artificially expanded genetic

98
00:04:19,819 --> 00:04:17,459
information system that was developed in

99
00:04:21,979 --> 00:04:19,829
Boehner's lab in the last 20 years and

100
00:04:24,110 --> 00:04:21,989
for this system we do have a very

101
00:04:27,500 --> 00:04:24,120
well-established molecular biology

102
00:04:29,570 --> 00:04:27,510
structural biology synthetic biology and

103
00:04:31,430 --> 00:04:29,580
we have shown in the past years that we

104
00:04:32,910 --> 00:04:31,440
can actually use this system for

105
00:04:36,090 --> 00:04:32,920
evolutionary

106
00:04:38,280 --> 00:04:36,100
easily and effectively so the idea

107
00:04:40,620 --> 00:04:38,290
behind ages is to complete the

108
00:04:42,570 --> 00:04:40,630
watson-crick pairing concept as it's

109
00:04:44,400 --> 00:04:42,580
shown in this scheme by shuffling

110
00:04:46,860 --> 00:04:44,410
hydrogen bond donors and acceptors

111
00:04:49,950 --> 00:04:46,870
groups forming additional orthogonal

112
00:04:51,930 --> 00:04:49,960
nuclear base pairs they resemble net

113
00:04:53,520 --> 00:04:51,940

natural nucleotides in size shape and

114
00:04:55,530 --> 00:04:53,530
pairing geometries they are

115
00:04:57,390 --> 00:04:55,540
independently replicable do not

116
00:04:59,610 --> 00:04:57,400
interfere with the RNA folding or the

117
00:05:01,470 --> 00:04:59,620
DNA double helix structures they

118
00:05:03,120 --> 00:05:01,480
increase the information density and

119
00:05:05,130 --> 00:05:03,130
they have the potential to increase

120
00:05:08,130 --> 00:05:05,140
functionality and that's what we want to

121
00:05:10,800 --> 00:05:08,140
test so what we are going what we have

122
00:05:13,920 --> 00:05:10,810
done in this preliminary studies is we

123
00:05:16,650 --> 00:05:13,930
started out by using these pair Z's in

124
00:05:21,000 --> 00:05:16,660
peace where he has an extra nitrogen

125
00:05:25,560 --> 00:05:21,010
group and five while and also is has an

126
00:05:29,010 --> 00:05:25,570
extra hydrogen here at n 3 and P is

127
00:05:32,580 --> 00:05:29,020
lacking hydrogen right here and has an

128
00:05:34,290 --> 00:05:32,590
extra nitrogen in and v 2 so we pick

129
00:05:35,910 --> 00:05:34,300
this couple at first because it's the

130
00:05:37,470 --> 00:05:35,920
one that we have studied more and we

131
00:05:39,630 --> 00:05:37,480
know it works and it's a first step

132
00:05:44,100 --> 00:05:39,640
toward increasing the diversity and the

133
00:05:46,230 --> 00:05:44,110
information inside the library now the

134
00:05:47,940 --> 00:05:46,240
next step is to try to understand what

135
00:05:50,850 --> 00:05:47,950
is the sequence space coverage that we

136
00:05:53,480 --> 00:05:50,860
are trying to to achieve as I said we

137
00:05:56,520 --> 00:05:53,490
are going to start with same length

138
00:05:58,140 --> 00:05:56,530

libraries but one will have six letters

139
00:06:00,180 --> 00:05:58,150
DNA and the other one will have four

140
00:06:03,330 --> 00:06:00,190
letters DNA if you look at this table

141
00:06:04,830 --> 00:06:03,340
well signal space is defined for us at

142
00:06:07,830 --> 00:06:04,840
least his define is the number of

143
00:06:10,230 --> 00:06:07,840
library window block blocks to the N

144
00:06:12,660 --> 00:06:10,240
where n is the length of the library so

145
00:06:15,390 --> 00:06:12,670
if we have 19 nucleotides and six letter

146
00:06:17,880 --> 00:06:15,400
DNA we will achieve 100 percent coverage

147
00:06:20,310 --> 00:06:17,890
when we use one nano mole of material

148
00:06:22,140 --> 00:06:20,320
which is what you usually are able to

149
00:06:25,110 --> 00:06:22,150
handle in a in a laboratory experiment

150
00:06:27,720 --> 00:06:25,120
while if we have four letters DNA for

151
00:06:30,510 --> 00:06:27,730
the same amount you will actually have

152
00:06:33,240 --> 00:06:30,520
it about a thousand molecules a thousand

153
00:06:35,100 --> 00:06:33,250
copies for each sequence that you have

154
00:06:37,190 --> 00:06:35,110
in your library so obviously our for

155
00:06:39,570 --> 00:06:37,200
light of DNA always has an advantage

156
00:06:41,610 --> 00:06:39,580
compared to a six letter DNA when you're

157
00:06:43,439 --> 00:06:41,620
trying to do wet lab experiments

158
00:06:45,570 --> 00:06:43,449
evolutionary experiment at

159
00:06:47,519 --> 00:06:45,580
at least so if you look at the table and

160
00:06:49,649 --> 00:06:47,529
you keep going through the numbers you

161
00:06:52,640 --> 00:06:49,659
will actually see I'll do the math for

162
00:06:57,390 --> 00:06:52,650
you you will actually see that for every

163
00:06:58,980 --> 00:06:57,400

there is a the column space coverage

164
00:07:02,100 --> 00:06:58,990
decreases of one order of magnitude

165
00:07:03,869 --> 00:07:02,110
every five nucleotide lengths increase

166
00:07:06,899 --> 00:07:03,879
in the library for two bits of

167
00:07:09,299 --> 00:07:06,909
information difference so the longer you

168
00:07:12,929 --> 00:07:09,309
go the more you actually have the four

169
00:07:16,070 --> 00:07:12,939
letters DNA have an advantage versus the

170
00:07:19,170 --> 00:07:16,080
six letters the six letter DNA that is

171
00:07:21,200 --> 00:07:19,180
unless the functionality and the extra

172
00:07:24,809 --> 00:07:21,210
nucleotides that you are adding are

173
00:07:27,989 --> 00:07:24,819
actually more powerful for more

174
00:07:32,040 --> 00:07:27,999
effective for the type of phenomenon

175
00:07:33,899 --> 00:07:32,050
that you're trying to evolve down here

176
00:07:36,119 --> 00:07:33,909
we actually have the the actual

177
00:07:37,950 --> 00:07:36,129
libraries that we have used for these

178
00:07:40,769 --> 00:07:37,960
preliminary studies we started with an n

179
00:07:43,889 --> 00:07:40,779
25 for the full nucleotides we started

180
00:07:47,070 --> 00:07:43,899
with 0.5 nanomoles just this was a first

181
00:07:50,550 --> 00:07:47,080
run so we covered 25% of sequence page

182
00:07:52,969 --> 00:07:50,560
space for the standard DNA library while

183
00:07:57,899 --> 00:07:52,979
for the ages library we actually covered

184
00:08:00,749 --> 00:07:57,909
0.00 11% of the sequence space so we did

185
00:08:02,429 --> 00:08:00,759
the next step would be which reaction

186
00:08:04,409 --> 00:08:02,439
are we gonna pick as a proof of

187
00:08:08,070 --> 00:08:04,419
principle probably one of the easiest

188
00:08:10,589 --> 00:08:08,080

reaction to pick is selecting for DNA

189
00:08:12,629 --> 00:08:10,599
RNA cleaving DN enzymes raghava talked

190
00:08:16,050 --> 00:08:12,639
about it a little bit this morning it's

191
00:08:18,029 --> 00:08:16,060
a very well-known type of molecule it's

192
00:08:20,100 --> 00:08:18,039
very well studied so we have a lot of

193
00:08:22,139 --> 00:08:20,110
literature to compare to once we get

194
00:08:25,139 --> 00:08:22,149
some results and people have actually

195
00:08:27,959 --> 00:08:25,149
been done selection on the n enzymes for

196
00:08:32,189 --> 00:08:27,969
the past maybe decade and they were of

197
00:08:35,309 --> 00:08:32,199
course first selected by and in the

198
00:08:38,159 --> 00:08:35,319
Joyce lab Santora enjoys 1997 this is

199
00:08:40,800 --> 00:08:38,169
Craig Craig jerem did all of it is he's

200
00:08:42,930 --> 00:08:40,810
a technician in in at Fame he's a very

201
00:08:45,809 --> 00:08:42,940
skillet guy but he needs still a little

202
00:08:47,879 --> 00:08:45,819
training this is the schematic of the

203
00:08:50,550 --> 00:08:47,889
selection so we start with an RNA target

204
00:08:52,740 --> 00:08:50,560
that is here in blue that is attached to

205
00:08:54,990 --> 00:08:52,750
a primer that will amplify a library and

206
00:08:56,090 --> 00:08:55,000
at the Phi Prime and actually has a

207
00:09:01,069 --> 00:08:56,100
biotin

208
00:09:03,680 --> 00:09:01,079
that you see after the you got the

209
00:09:05,900 --> 00:09:03,690
double-stranded DNA you can remove the

210
00:09:07,550 --> 00:09:05,910
the one of the strands and everything

211
00:09:10,069 --> 00:09:07,560
all your system will be attached to it

212
00:09:13,309 --> 00:09:10,079
back to the biotin this portion which is

213
00:09:15,710 --> 00:09:13,319

your evolving ribozyme can fold over the

214
00:09:18,230 --> 00:09:15,720
target cleave it and you will actually

215
00:09:21,769 --> 00:09:18,240
have in the supernatant your evolving

216
00:09:23,540 --> 00:09:21,779
library that you can amplify and this is

217
00:09:26,059 --> 00:09:23,550
just a slightly modified from the

218
00:09:27,949 --> 00:09:26,069
original protocol to make it a little

219
00:09:30,199 --> 00:09:27,959
more effective and remove a few steps

220
00:09:31,340 --> 00:09:30,209
these are the conditions that we used is

221
00:09:34,579 --> 00:09:31,350
to millimolar magnesium because

222
00:09:39,290 --> 00:09:34,589
everybody cares about magnesium and what

223
00:09:41,329 --> 00:09:39,300
we care about is that we use pH 7.8 for

224
00:09:44,900 --> 00:09:41,339
a very specific reason that I will tell

225
00:09:47,180 --> 00:09:44,910
that I will say in a moment and okay so

226
00:09:49,610 --> 00:09:47,190
this is the the big deal we did the

227
00:09:53,150 --> 00:09:49,620
parallel in-vitro selection and what we

228
00:09:55,970 --> 00:09:53,160
could see is that while the standard DNA

229
00:09:59,150 --> 00:09:55,980
had a really hard time growing at least

230
00:10:01,340 --> 00:09:59,160
in our conditions at 25 nucleotide long

231
00:10:03,710 --> 00:10:01,350
library library and with only two

232
00:10:08,120 --> 00:10:03,720
millimolar magnesium and instead the

233
00:10:12,110 --> 00:10:08,130
ages they the known standard library

234
00:10:15,170 --> 00:10:12,120
grew very well and and and was happy so

235
00:10:17,509 --> 00:10:15,180
these by itself is already a surprising

236
00:10:20,960 --> 00:10:17,519
result because while we did expect that

237
00:10:23,449 --> 00:10:20,970
ages might have some some advantages

238
00:10:26,960 --> 00:10:23,459

because of the extra groups that we had

239
00:10:29,780 --> 00:10:26,970
we didn't expect that we would have such

240
00:10:31,490 --> 00:10:29,790
a such a big diverse difference also I'm

241
00:10:32,960 --> 00:10:31,500
a little disappointed we did these two

242
00:10:35,090 --> 00:10:32,970
or three times I think three times in

243
00:10:37,160 --> 00:10:35,100
total we really can't grow this DN

244
00:10:39,470 --> 00:10:37,170
enzymes with standard nucleotides it

245
00:10:41,210 --> 00:10:39,480
really looks like only four it's not

246
00:10:42,829 --> 00:10:41,220
enough for two millimolar magnesium

247
00:10:49,429 --> 00:10:42,839
thank you

248
00:10:53,360 --> 00:10:49,439
and for 25 nucleotides long sequence one

249
00:10:56,420 --> 00:10:53,370
reason might be in the Z nucleotide

250
00:10:58,429 --> 00:10:56,430
which I reported here again which has a

251
00:11:01,069 --> 00:10:58,439
pKa of seven point eight which is

252
00:11:04,759 --> 00:11:01,079
exactly the pKa then the pH that we use

253
00:11:05,850 --> 00:11:04,769
during selection so ideally you can see

254
00:11:08,370 --> 00:11:05,860
here the

255
00:11:10,650 --> 00:11:08,380
the output of the deep sequencing that

256
00:11:14,610 --> 00:11:10,660
we did so we actually were able to we

257
00:11:17,670 --> 00:11:14,620
have a technique to transform our ages

258
00:11:19,949 --> 00:11:17,680
DNA into standard DNA submitted to deep

259
00:11:22,410 --> 00:11:19,959
sequencing and then keep track of where

260
00:11:23,550 --> 00:11:22,420
the original season peas were so we

261
00:11:25,740 --> 00:11:23,560
applied that technique to these

262
00:11:28,970 --> 00:11:25,750
libraries and we actually sequenced each

263
00:11:32,190 --> 00:11:28,980

cycle of the selection and what was

264
00:11:34,350 --> 00:11:32,200
surprising surprising but maybe not is

265
00:11:36,660 --> 00:11:34,360
that there was a preponderance with the

266
00:11:39,240 --> 00:11:36,670
up Z's in all the major clusters that

267
00:11:41,250 --> 00:11:39,250
were that survived the selection here

268
00:11:44,100 --> 00:11:41,260
there's just a graphic representation of

269
00:11:45,780 --> 00:11:44,110
how the various different clusters

270
00:11:48,630 --> 00:11:45,790
appear and disappear during the

271
00:11:51,180 --> 00:11:48,640
selection this is a table that shows you

272
00:11:54,030 --> 00:11:51,190
at which psychology cluster will will

273
00:11:55,920 --> 00:11:54,040
appear but back to the Z's so our main

274
00:11:59,220 --> 00:11:55,930
sequence the cluster that actually

275
00:12:01,440 --> 00:11:59,230
showed most prominently in the deep

276
00:12:04,530 --> 00:12:01,450
sequencing analysis actually carries two

277
00:12:09,660 --> 00:12:04,540
for Z's and it has two sides of cleavage

278
00:12:12,540 --> 00:12:09,670
so in an ideal in in the hypothesis that

279
00:12:14,490 --> 00:12:12,550
the pKa of Z is actually very important

280
00:12:16,590 --> 00:12:14,500
here you can see how half of the

281
00:12:18,660 --> 00:12:16,600
molecules will be protonated half of the

282
00:12:21,690 --> 00:12:18,670
molecules will be deprotonated and they

283
00:12:24,870 --> 00:12:21,700
will act each of them every couple

284
00:12:30,180 --> 00:12:24,880
around one of the size of cleavage to to

285
00:12:34,230 --> 00:12:30,190
achieve cleavage so this is one example

286
00:12:36,360 --> 00:12:34,240
of all the of all this and she's acting

287
00:12:39,389 --> 00:12:36,370
clusters that we found we got three

288
00:12:42,150 --> 00:12:39,399

families of Ages DN enzymes when one as

289
00:12:44,130 --> 00:12:42,160
I just said is cuts at you nine and you

290
00:12:46,769 --> 00:12:44,140
thirteen see one two six and seven

291
00:12:49,230 --> 00:12:46,779
cluster one two six and seven then we

292
00:12:54,150 --> 00:12:49,240
have cleavage at a sixteen and then we

293
00:12:55,949 --> 00:12:54,160
have creases at a in a a ten sorry so we

294
00:12:58,010 --> 00:12:55,959
we got these three families and we

295
00:13:00,660 --> 00:12:58,020
decided to go ahead and and try to

296
00:13:02,819 --> 00:13:00,670
characterize exactly what was happening

297
00:13:04,530 --> 00:13:02,829
in each of these clusters we took a

298
00:13:06,990 --> 00:13:04,540
cluster one to begin with

299
00:13:09,180 --> 00:13:07,000
so in sis it will just have a you know a

300
00:13:12,180 --> 00:13:09,190
decent activity but this is a really big

301
00:13:13,980 --> 00:13:12,190
molecule you do see that the evolving

302
00:13:16,769 --> 00:13:13,990
portion of the library actually does

303
00:13:17,230 --> 00:13:16,779
best pairs very nicely with the RNA and

304
00:13:19,389 --> 00:13:17,240
then

305
00:13:21,010 --> 00:13:19,399
you have the Z's over here so I'm what I

306
00:13:23,079 --> 00:13:21,020
think is happening is actually that

307
00:13:25,300 --> 00:13:23,089
we're getting a very big 3d

308
00:13:27,430 --> 00:13:25,310
restructuring around the double

309
00:13:29,769 --> 00:13:27,440
partially double stranded area this is

310
00:13:31,150 --> 00:13:29,779
actually there's a GU bubble and here

311
00:13:34,870 --> 00:13:31,160
it's probably open because there's this

312
00:13:38,290 --> 00:13:34,880
huge structure area but we then so we

313
00:13:39,970 --> 00:13:38,300

checked that see one with no Z would not

314
00:13:42,070 --> 00:13:39,980
actually have the same activity and

315
00:13:43,720 --> 00:13:42,080
that's what is shown here in two really

316
00:13:45,970 --> 00:13:43,730
different representations so here you're

317
00:13:47,769 --> 00:13:45,980
monitoring the appearance of the bands

318
00:13:50,740 --> 00:13:47,779
that will show you cleavage here you're

319
00:13:53,019 --> 00:13:50,750
monitoring the disappearance of of the

320
00:13:55,000 --> 00:13:53,029
full-length product and the red one is

321
00:13:57,040 --> 00:13:55,010
the one that does not contain Z and the

322
00:13:58,930 --> 00:13:57,050
black one is the one that does contain

323
00:14:00,960 --> 00:13:58,940
see I'm running out of time so I'm just

324
00:14:04,150 --> 00:14:00,970
gonna go very fast

325
00:14:06,910 --> 00:14:04,160
we did find yeah this is like it's a

326
00:14:09,970 --> 00:14:06,920
very dense slide that basically we did a

327
00:14:12,730 --> 00:14:09,980
bunch of biochemistry assays in which we

328
00:14:15,519 --> 00:14:12,740
separated it two strands and we finally

329
00:14:17,980 --> 00:14:15,529
could see that we got a cake out of 10

330
00:14:20,139 --> 00:14:17,990
to the nine we get a km of 10 to the 5

331
00:14:22,840 --> 00:14:20,149
and a catalytic efficiency of about 10

332
00:14:26,139 --> 00:14:22,850
to the fifth per mole per minute that is

333
00:14:28,210 --> 00:14:26,149
very very comparable to I don't know if

334
00:14:31,930 --> 00:14:28,220
you guys know the work of David Perrin

335
00:14:34,269 --> 00:14:31,940
he works with heavily modified DN

336
00:14:37,120 --> 00:14:34,279
enzymes and he got exactly the same type

337
00:14:39,130 --> 00:14:37,130
well but we got similar catalytic

338
00:14:41,380 --> 00:14:39,140

efficiencies just with one extra

339
00:14:43,870 --> 00:14:41,390
nucleotide so that's that's very

340
00:14:47,050 --> 00:14:43,880
interesting conclusion conclusions and

341
00:14:50,500 --> 00:14:47,060
perspectives this is just a summary what

342
00:14:53,560 --> 00:14:50,510
we would like to do after is actually

343
00:14:56,380 --> 00:14:53,570
start to perform multiple parallel

344
00:14:58,540 --> 00:14:56,390
strand ages standard ages selections

345
00:15:00,280 --> 00:14:58,550
with increased and decreased lens number

346
00:15:02,889 --> 00:15:00,290
of nucleotides and extra nuclear base

347
00:15:07,290 --> 00:15:02,899
modifications so two four six seven

348
00:15:10,150 --> 00:15:07,300
eight and and whatnot I am going to

349
00:15:12,699 --> 00:15:10,160
thank of course Craig Jerome who is the

350
00:15:14,740 --> 00:15:12,709
one who did all of these all our funding

351
00:15:18,310 --> 00:15:14,750
agencies although this specific project

352
00:15:20,290 --> 00:15:18,320
is not yet funded and everybody at fame

353
00:15:24,180 --> 00:15:20,300
and firebird thank you I'll take any

354
00:15:29,230 --> 00:15:27,790
if there's time for one question okay

355
00:15:48,520 --> 00:15:29,240
all right

356
00:15:58,330 --> 00:15:48,530
we're gonna go to the back yes can you

357
00:16:00,640 --> 00:15:58,340
repeat that I'm sorry well see in our

358
00:16:03,210 --> 00:16:00,650
life studies actually we did have we did

359
00:16:06,580 --> 00:16:03,220
found that when these MPs and other

360
00:16:09,600 --> 00:16:06,590
pairs in the ages system are not best

361
00:16:12,370 --> 00:16:09,610
paired they actually contribute into

362
00:16:14,140 --> 00:16:12,380
into secondary and tertiary structures

363
00:16:17,530 --> 00:16:14,150

that we are still investigating but seem

364
00:16:20,620 --> 00:16:17,540
to be extremely interesting and not seen

365
00:16:23,500 --> 00:16:20,630
before in DNA and RNA what what I meant

366
00:16:25,000 --> 00:16:23,510
with that point was that we have also a

367
00:16:26,800 --> 00:16:25,010
lot of data that shows that when you

368
00:16:28,780 --> 00:16:26,810
substitute C and P in like

369
00:16:32,020 --> 00:16:28,790
double-stranded structures you actually

370
00:16:34,240 --> 00:16:32,030
do get the same the same foldings that

371
00:16:35,980 --> 00:16:34,250
you expect from known molecules you

372
00:16:37,960 --> 00:16:35,990
might have seen you know they hatch much

373
00:16:41,020 --> 00:16:37,970
in DNA paper with the spinach septum ER

374
00:16:43,600 --> 00:16:41,030
and some ribose which that we actually

375
00:16:45,250 --> 00:16:43,610
substituted these in piece for but yes

376
00:16:48,400 --> 00:16:45,260
it's true some of these nucleotides will

377
00:16:50,230 --> 00:16:48,410
actually provide I think we'll we don't

378
00:16:51,430 --> 00:16:50,240
have confirmation yet but I really think

379
00:16:56,050 --> 00:16:51,440
we'll have actually provided new

380
00:16:58,060 --> 00:16:56,060
structures for DNA and RNA molecules yep

381
00:16:59,380 --> 00:16:58,070
all right great let's thank ELISA again