AbSciCon
2019

1
00:00:00,790 --> 00:00:07,320
[Music]

2
00:00:11,350 --> 00:00:08,999
[Applause]

3
00:00:13,660 --> 00:00:11,360
so most of you are familiar with the

4
00:00:15,879 --> 00:00:13,670
ironing world hypothesis which proposes

5
00:00:17,679 --> 00:00:15,889
that early in the evolution of life RNA

6
00:00:20,230 --> 00:00:17,689
serve both genetic and catalytic roles

7
00:00:22,870 --> 00:00:20,240
roles which have been taken over mostly

8
00:00:25,030 --> 00:00:22,880
by DNA and enzymatic proteins so one

9
00:00:27,999 --> 00:00:25,040
important tool for the RNA world would

10
00:00:30,549 --> 00:00:28,009
be having an RNA polymerase ribozyme not

11
00:00:32,709 --> 00:00:30,559
only able of self replication but also a

12
00:00:36,459 --> 00:00:32,719
more general transcription of an RNA

13
00:00:39,310 --> 00:00:36,469

template so a number of labs are looking

14
00:00:41,169 --> 00:00:39,320
into this and it all started with the in

15
00:00:43,239 --> 00:00:41,179
vitro selected class one ligase by

16
00:00:45,160 --> 00:00:43,249
Bartel and Shaw stack and it can

17
00:00:47,529 --> 00:00:45,170
catalyze the 3 prime to 5 prime

18
00:00:51,189 --> 00:00:47,539
phosphodiester bond formation of two RNA

19
00:00:53,410 --> 00:00:51,199
molecules with that is a starting point

20
00:00:55,450 --> 00:00:53,420
a second functional domain was in vitro

21
00:00:57,489 --> 00:00:55,460
selected and that donate domain was

22
00:00:59,559 --> 00:00:57,499
called an accessory domain and this

23
00:01:02,379 --> 00:00:59,569
transformed it from ligase to a

24
00:01:04,630 --> 00:01:02,389
polymerase and what this polymerase can

25
00:01:07,420 --> 00:01:04,640
do now is extend an RNA primer that's

26
00:01:10,270 --> 00:01:07,430
been hybridized on an RNA template in a

27
00:01:11,890 --> 00:01:10,280
template dependent manner however it

28
00:01:15,010 --> 00:01:11,900
does have a preference for gc-rich

29
00:01:17,590 --> 00:01:15,020
templates so the example shown here is B

30
00:01:20,020 --> 00:01:17,600
six point six one it was a polymerase

31
00:01:22,600 --> 00:01:20,030
ribozyme evolved later in our lab and

32
00:01:24,310 --> 00:01:22,610
what it can do is extend the primer by

33
00:01:26,320 --> 00:01:24,320
about twenty nucleotides in twenty-four

34
00:01:30,030 --> 00:01:26,330
hours so a major problem with these

35
00:01:32,710 --> 00:01:30,040
polymerases is a lack of processivity so

36
00:01:34,780 --> 00:01:32,720
they tend to dissociate from the primer

37
00:01:37,800 --> 00:01:34,790
template complex in between nucleotide

38
00:01:40,270 --> 00:01:37,810

additions so one solution that has been

39
00:01:42,220 --> 00:01:40,280
found and used to increase processivity

40
00:01:43,990 --> 00:01:42,230
by a number of labs such as the

41
00:01:46,750 --> 00:01:44,000
Hollinger and the Joyce lab was to

42
00:01:49,360 --> 00:01:46,760
directly hybridize that the polymerase

43
00:01:51,100 --> 00:01:49,370
or the ribozyme straight to the template

44
00:01:53,650 --> 00:01:51,110
that way you can dissociate away and

45
00:01:56,890 --> 00:01:53,660
this has resulted in extensions of up to

46
00:01:58,930 --> 00:01:56,900
200 nucleotides over seven days and this

47
00:02:02,710 --> 00:01:58,940
again is on GC rich templates with

48
00:02:05,800 --> 00:02:02,720
multiple repeats this is the latest

49
00:02:08,559 --> 00:02:05,810
Joyce ribozyme 24.3 and it was able to

50
00:02:12,009 --> 00:02:08,569
transcribe small functional RNAs

51
00:02:14,170 --> 00:02:12,019
but even thither it has there's a

52
00:02:17,860 --> 00:02:14,180
trade-off between template complexity

53
00:02:18,940 --> 00:02:17,870
that it can transcribe and process

54
00:02:21,750 --> 00:02:18,950
tivity so we can see that the

55
00:02:25,259 --> 00:02:21,760
full-length product is not in a

56
00:02:28,619 --> 00:02:25,269
and there's a number of stalling that

57
00:02:30,479 --> 00:02:28,629
happens during transcription so at this

58
00:02:32,550 --> 00:02:30,489
point we thought would it be possible

59
00:02:34,740 --> 00:02:32,560
and it would be interesting to make a

60
00:02:37,199 --> 00:02:34,750
polymerase ribozyme that has high

61
00:02:39,740 --> 00:02:37,209
processivity without that feathering and

62
00:02:42,330 --> 00:02:39,750
also that has more biological features

63
00:02:46,130 --> 00:02:42,340

so what I mean by biological features is

64
00:02:48,690 --> 00:02:46,140
looking at bacterial DNA dependent RNA

65
00:02:51,000 --> 00:02:48,700
polymerases and one thing that they have

66
00:02:54,420 --> 00:02:51,010
is specificity factors so they use Sigma

67
00:02:56,190 --> 00:02:54,430
factors which recognize promoter region

68
00:02:58,710 --> 00:02:56,200
on the double-stranded DNA template and

69
00:03:00,839 --> 00:02:58,720
it localizes the polymerase to this

70
00:03:02,729 --> 00:03:00,849
promoter region so similarly we're

71
00:03:05,250 --> 00:03:02,739
thinking that we can do the same with an

72
00:03:07,710 --> 00:03:05,260
RNA specificity factor termed here in

73
00:03:10,160 --> 00:03:07,720
RNA primer and this primer can recognize

74
00:03:13,319 --> 00:03:10,170
a promoter region on an RNA template by

75
00:03:17,160 --> 00:03:13,329
via hybridization and again localize

76
00:03:18,509 --> 00:03:17,170
this polymerase to the template another

77
00:03:20,940 --> 00:03:18,519
thing that the DNA dependent RNA

78
00:03:22,440 --> 00:03:20,950
polymerase is have this process Savini

79
00:03:24,479 --> 00:03:22,450
and the way they get this is either by

80
00:03:28,289 --> 00:03:24,489
clamping onto the DNA template so they

81
00:03:31,710 --> 00:03:28,299
don't associate or by in training the

82
00:03:35,069 --> 00:03:31,720
RNA product so again we think we can do

83
00:03:37,379 --> 00:03:35,079
this by selecting - in vitro selection a

84
00:03:40,229 --> 00:03:37,389
third functional domain a clamped domain

85
00:03:41,640 --> 00:03:40,239
and this domain would be in an open clam

86
00:03:43,710 --> 00:03:41,650
conformation when bound to the

87
00:03:46,020 --> 00:03:43,720
specificity factor the RNA primer and

88
00:03:48,089 --> 00:03:46,030

once the primer is displaced onto the

89
00:03:52,379 --> 00:03:48,099
promoter region of the template this

90
00:03:54,030 --> 00:03:52,389
open clamp domain can close and circling

91
00:03:55,860 --> 00:03:54,040
the template and being able to slide

92
00:04:00,390 --> 00:03:55,870
back and forth on the template in an

93
00:04:02,699 --> 00:04:00,400
untethered fashion so to select for such

94
00:04:06,479 --> 00:04:02,709
a polymerase ribozyme I started with B

95
00:04:08,280 --> 00:04:06,489
six point six one as my progenitor to

96
00:04:09,539 --> 00:04:08,290
make the pool and it has the ligase

97
00:04:11,400 --> 00:04:09,549
course only and in blue

98
00:04:14,699 --> 00:04:11,410
the accessory domain in green and this

99
00:04:16,979 --> 00:04:14,709
is a little cartoon signifying that so

100
00:04:18,990 --> 00:04:16,989
first I designed a five prime primer

101
00:04:21,120 --> 00:04:19,000
binding site shown here in orange and

102
00:04:23,370 --> 00:04:21,130
this is partially complementary to this

103
00:04:25,710 --> 00:04:23,380
RNA primer or the specificity factor and

104
00:04:28,770 --> 00:04:25,720
this serves as the first arm of the

105
00:04:30,300 --> 00:04:28,780
clamp domain for the second arm of the

106
00:04:32,520 --> 00:04:30,310
clamp domain this is where the in vitro

107
00:04:34,440 --> 00:04:32,530
selection comes in handy and I've

108
00:04:35,190 --> 00:04:34,450
appended random sequence so the three

109
00:04:36,990 --> 00:04:35,200
prime end of

110
00:04:39,210 --> 00:04:37,000
the accessory domain and this gave me an

111
00:04:43,680 --> 00:04:39,220
RNA pool with a diversity about 10 to

112
00:04:45,210 --> 00:04:43,690
the 13th this is the general schematic

113
00:04:47,160 --> 00:04:45,220

of the in vitro selection where I

114
00:04:49,410 --> 00:04:47,170
started with a B six point six one DNA

115
00:04:51,600 --> 00:04:49,420
pool that has been fused with random

116
00:04:54,060 --> 00:04:51,610
nucleotides it's being transcribed to

117
00:04:57,060 --> 00:04:54,070
make an RNA pool then multiple selective

118
00:04:59,340 --> 00:04:57,070
steps are undertaken to remove inactive

119
00:05:02,250 --> 00:04:59,350
RNAs while selecting for sliding and

120
00:05:04,200 --> 00:05:02,260
active RNA clamp polymerases which are

121
00:05:07,110 --> 00:05:04,210
then reverse transcribed to make a cDNA

122
00:05:10,320 --> 00:05:07,120
library and then PCR amplified to feed

123
00:05:11,940 --> 00:05:10,330
back into the cycle so to go over in

124
00:05:14,370 --> 00:05:11,950
more details about the Selective steps

125
00:05:16,760 --> 00:05:14,380
I've used the first elliptic selective

126
00:05:19,980 --> 00:05:16,770
step looked for clamping domains alone

127
00:05:22,500 --> 00:05:19,990
so primer was given to the pool to

128
00:05:24,900 --> 00:05:22,510
create this open clam conformation and

129
00:05:27,270 --> 00:05:24,910
then it was incubated on circular

130
00:05:31,680 --> 00:05:27,280
template that has been immobilized on

131
00:05:35,040 --> 00:05:31,690
streptavidin magnetic beads so ribozymes

132
00:05:37,280 --> 00:05:35,050
with functioning clamps would be

133
00:05:39,480 --> 00:05:37,290
directed to the promoter region by the

134
00:05:41,580 --> 00:05:39,490
specificity factor or the RNA primer

135
00:05:43,260 --> 00:05:41,590
where the primer would be displaced and

136
00:05:44,580 --> 00:05:43,270
the clamping domain would go from an

137
00:05:46,680 --> 00:05:44,590
open conformation to a closed

138
00:05:49,590 --> 00:05:46,690

conformation being trapped on the

139
00:05:51,090 --> 00:05:49,600
circular template and now all non

140
00:05:53,760 --> 00:05:51,100
functioning claps would be able to be

141
00:05:58,020 --> 00:05:53,770
washed away at this point to recover

142
00:06:02,550 --> 00:05:58,030
this functioning active ribozymes fresh

143
00:06:04,860 --> 00:06:02,560
primer is added to ask the the clamp to

144
00:06:07,080 --> 00:06:04,870
open back up and this allows collection

145
00:06:09,600 --> 00:06:07,090
of them off the circular template and

146
00:06:11,910 --> 00:06:09,610
we're able to monitor this process by

147
00:06:14,580 --> 00:06:11,920
radio leaving the radio label in the

148
00:06:16,830 --> 00:06:14,590
pool and then the whole process can be

149
00:06:20,610 --> 00:06:16,840
carried out multiple times to increase

150
00:06:22,320 --> 00:06:20,620
the stringency so this is what we did so

151
00:06:24,720 --> 00:06:22,330
by monitoring the pool that comes off

152
00:06:26,400 --> 00:06:24,730
with fresh primer off the beads we're

153
00:06:29,000 --> 00:06:26,410
able to look these are the last two

154
00:06:31,980 --> 00:06:29,010
rounds that used this selection protocol

155
00:06:33,630 --> 00:06:31,990
and the Selective steps hasn't been used

156
00:06:35,610 --> 00:06:33,640
every single round that's why I round

157
00:06:37,260 --> 00:06:35,620
twenty is missing so while we see that

158
00:06:39,450 --> 00:06:37,270
primer recovery is higher than just

159
00:06:41,370 --> 00:06:39,460
buffer recovery and this is the first on

160
00:06:45,600 --> 00:06:41,380
and off event the second and then in

161
00:06:48,090 --> 00:06:45,610
between rounds this amount increases the

162
00:06:50,040 --> 00:06:48,100
second selections scheme looked

163
00:06:52,620 --> 00:06:50,050

both clamping and Templi dependent

164
00:06:56,430 --> 00:06:52,630
extension of a primer so again an open

165
00:06:58,230 --> 00:06:56,440
clamp confirmation was it was done by

166
00:07:00,720 --> 00:06:58,240
adding primer to the pool and then

167
00:07:03,480 --> 00:07:00,730
incubated on a circular template with an

168
00:07:06,210 --> 00:07:03,490
TPS but instead of using a regular CTP

169
00:07:09,840 --> 00:07:06,220
we used an analog upaya tune latest CTP

170
00:07:11,880 --> 00:07:09,850
this way an active polymerase or an

171
00:07:14,430 --> 00:07:11,890
active ribozyme would be able to

172
00:07:17,580 --> 00:07:14,440
incorporate this by 2 in latest CTP into

173
00:07:19,800 --> 00:07:17,590
the extended primer this allows

174
00:07:21,930 --> 00:07:19,810
purification of active by molecular

175
00:07:24,240 --> 00:07:21,940
complexes on script Avenue marinated

176
00:07:26,600 --> 00:07:24,250
beets anything that isn't active is

177
00:07:29,040 --> 00:07:26,610
washed away and again we can monitor the

178
00:07:31,770 --> 00:07:29,050
active guys by recovering them with a

179
00:07:35,220 --> 00:07:31,780
primer focusing on this opening the

180
00:07:37,650 --> 00:07:35,230
clamp one more time so these are the

181
00:07:40,290 --> 00:07:37,660
last three rounds that we did with this

182
00:07:41,820 --> 00:07:40,300
selective step so you can see that the

183
00:07:44,730 --> 00:07:41,830
amount of pool recovered with primer

184
00:07:46,200 --> 00:07:44,740
increases in between each round so this

185
00:07:48,630 --> 00:07:46,210
is the final round of selection that

186
00:07:53,370 --> 00:07:48,640
that we've done so far and we looked at

187
00:07:56,670 --> 00:07:53,380
the activity of this round 27 so first

188
00:07:58,230 --> 00:07:56,680

we looked at at its processivity on the

189
00:07:59,970 --> 00:07:58,240
template that it was selected so I

190
00:08:02,340 --> 00:07:59,980
mentioned that be six point six one was

191
00:08:04,200 --> 00:08:02,350
able to extend 20 nucleotides on a GC

192
00:08:06,750 --> 00:08:04,210
rich template however we used an 8 you

193
00:08:08,010 --> 00:08:06,760
reach template and B six point six one

194
00:08:10,950 --> 00:08:08,020
can only extend about three to four

195
00:08:15,290 --> 00:08:10,960
nucleotides wide around 27 pool is able

196
00:08:19,830 --> 00:08:17,820
we then switched the system from a red

197
00:08:22,110 --> 00:08:19,840
primer red templates to a secondary

198
00:08:24,510 --> 00:08:22,120
green primer green template with a

199
00:08:27,630 --> 00:08:24,520
different promoter and we were able to

200
00:08:29,940 --> 00:08:27,640
see extension over 24 hours quite

201
00:08:32,280 --> 00:08:29,950
precisely and we see about an extension

202
00:08:35,760 --> 00:08:32,290
about 3035 nucleotides on the left side

203
00:08:39,900 --> 00:08:35,770
on template 1 and about 20 to 25 on the

204
00:08:43,320 --> 00:08:39,910
right side on templates 2 and again both

205
00:08:45,240 --> 00:08:43,330
these templates are au rich so then we

206
00:08:47,670 --> 00:08:45,250
wanted to see if a specificity factor is

207
00:08:50,910 --> 00:08:47,680
actually working and we are doing

208
00:08:52,980 --> 00:08:50,920
driving template specific extension so

209
00:08:54,540 --> 00:08:52,990
we took the pool and incubated them with

210
00:08:57,030 --> 00:08:54,550
either the red primer or the green

211
00:08:58,830 --> 00:08:57,040
primer and gave them both templates at

212
00:09:00,780 --> 00:08:58,840
the same time and based on the extension

213
00:09:02,460 --> 00:09:00,790

pattern we can see that with the red

214
00:09:04,920 --> 00:09:02,470
primer there's a preference for the red

215
00:09:09,450 --> 00:09:04,930
template with a green primer there's a

216
00:09:11,610 --> 00:09:09,460
preference for the green template so

217
00:09:13,650 --> 00:09:11,620
I've showed so far that red primer red

218
00:09:15,930 --> 00:09:13,660
templates being extended green primer

219
00:09:17,220 --> 00:09:15,940
green templates being extended we wanted

220
00:09:20,250 --> 00:09:17,230
to see if we're able to make the pool

221
00:09:23,340 --> 00:09:20,260
switch in between templates so once

222
00:09:25,380 --> 00:09:23,350
given into a complex we're able to want

223
00:09:28,560 --> 00:09:25,390
it to see if we can add fresh primer to

224
00:09:31,230 --> 00:09:28,570
open up the clamp give the primer of the

225
00:09:33,780 --> 00:09:31,240
different color and then make a switch

226
00:09:36,630 --> 00:09:33,790
to a secondary template this would allow

227
00:09:38,330 --> 00:09:36,640
for multiple rounds of transcription if

228
00:09:41,790 --> 00:09:38,340
it works properly

229
00:09:44,160 --> 00:09:41,800
now one activity we did not want was the

230
00:09:45,480 --> 00:09:44,170
same complex but if given a primer

231
00:09:47,220 --> 00:09:45,490
that's already hybridized to the

232
00:09:50,450 --> 00:09:47,230
template the clamp domain should remain

233
00:09:53,970 --> 00:09:50,460
closed and stay on the primary template

234
00:09:55,560 --> 00:09:53,980
so the stable at the top just is exactly

235
00:09:58,080 --> 00:09:55,570
like the images from the previous slide

236
00:10:00,390 --> 00:09:58,090
so red primer red template we see

237
00:10:03,870 --> 00:10:00,400
extension green primer green template we

238
00:10:05,670 --> 00:10:03,880

see extension we also see that by

239
00:10:08,240 --> 00:10:05,680
switching primers and templates we're

240
00:10:10,710 --> 00:10:08,250
able to see extensions on the secondary

241
00:10:14,340 --> 00:10:10,720
templates were able to switch templates

242
00:10:17,790 --> 00:10:14,350
however the pool doesn't perfectly stop

243
00:10:19,800 --> 00:10:17,800
the undesired behavior of switching to a

244
00:10:22,890 --> 00:10:19,810
template that's already been primed I'll

245
00:10:25,740 --> 00:10:22,900
be at a lesser extent then when properly

246
00:10:27,690 --> 00:10:25,750
primed and switched over the last thing

247
00:10:29,160 --> 00:10:27,700
we wanted to test since we're selecting

248
00:10:31,680 --> 00:10:29,170
for a sliding clamp domain we

249
00:10:33,390 --> 00:10:31,690
hypothesized that they should see more

250
00:10:36,180 --> 00:10:33,400
processivity with the pool on a circular

251
00:10:39,690 --> 00:10:36,190
template over a linear template as on a

252
00:10:41,400 --> 00:10:39,700
circular template clamped ribozyme would

253
00:10:43,290 --> 00:10:41,410
be able to slide back and forth and not

254
00:10:45,120 --> 00:10:43,300
dissociate from the template where on a

255
00:10:46,710 --> 00:10:45,130
linear it would be able to slide off the

256
00:10:49,170 --> 00:10:46,720
ends and it would lose its primer

257
00:10:51,030 --> 00:10:49,180
template remarkably that's exactly what

258
00:10:52,860 --> 00:10:51,040
we see or there's more processivity on

259
00:10:55,290 --> 00:10:52,870
the circular template in the linear

260
00:10:57,480 --> 00:10:55,300
we're able to see this with the second

261
00:10:59,340 --> 00:10:57,490
green primer and green template and we

262
00:11:00,990 --> 00:10:59,350
also tested five other templates for the

263
00:11:03,900 --> 00:11:01,000

same behavior with different lengths and

264
00:11:05,280 --> 00:11:03,910
we see the same behavior so it's

265
00:11:07,020 --> 00:11:05,290
important to note at this point that all

266
00:11:10,020 --> 00:11:07,030
the data has been done with the

267
00:11:11,640 --> 00:11:10,030
selection pool so what we see is an

268
00:11:13,650 --> 00:11:11,650
average of activities of the different

269
00:11:14,690 --> 00:11:13,660
individuals and just last week we cloned

270
00:11:17,820 --> 00:11:14,700
in sequenced

271
00:11:21,390 --> 00:11:17,830
45 clones of the pool and we have to

272
00:11:23,160 --> 00:11:21,400
further characterized the individuals

273
00:11:27,060 --> 00:11:23,170
inside the pool to look for the optimal

274
00:11:29,190 --> 00:11:27,070
activity so just to conclude comparing

275
00:11:31,800 --> 00:11:29,200
our in-vitro selected polymerase

276
00:11:33,630 --> 00:11:31,810
ribozyme with the DNA dependent RNA

277
00:11:35,280 --> 00:11:33,640
polymerase although RNA might never be

278
00:11:37,530 --> 00:11:35,290
able to get the proximity of a protein

279
00:11:39,330 --> 00:11:37,540
we're able to increase the profit eekum

280
00:11:41,430 --> 00:11:39,340
pair to the progenitor be six point six

281
00:11:43,860 --> 00:11:41,440
one on a you rich templates we're also

282
00:11:47,250 --> 00:11:43,870
able to show promoter recognition using

283
00:11:49,530 --> 00:11:47,260
an RNA specificity factor and we were

284
00:11:50,910 --> 00:11:49,540
partially able to show a switching of

285
00:11:53,220 --> 00:11:50,920
templates which can lead to multiple

286
00:11:55,260 --> 00:11:53,230
replication events and we believe that

287
00:11:58,290 --> 00:11:55,270
the process civet e and the switching of

288
00:11:59,640 --> 00:11:58,300

templates are linked and then we have to

289
00:12:01,590 --> 00:11:59,650
characterize the pool to find

290
00:12:03,780 --> 00:12:01,600
individuals that are better at doing

291
00:12:05,880 --> 00:12:03,790
both these processes and the last

292
00:12:07,470 --> 00:12:05,890
question that remains unanswered even in

293
00:12:09,360 --> 00:12:07,480
the field is how do we do strand

294
00:12:13,830 --> 00:12:09,370
displacement perhaps with a fourth

295
00:12:16,380 --> 00:12:13,840
fourth domain but yes so once RNA is

296
00:12:19,100 --> 00:12:16,390
transcribed of an RNA template how do we

297
00:12:21,570 --> 00:12:19,110
get it to come off without he donation

298
00:12:24,060 --> 00:12:21,580
with that I would like to thank my

299
00:12:31,800 --> 00:12:24,070
supervisor dr. Ron Rao my lab member is

300
00:12:44,380 --> 00:12:41,260
time for one question maybe two yeah so

301
00:12:45,820 --> 00:12:44,390
your list of features on protein enzymes

302
00:12:48,640 --> 00:12:45,830
I think there's something really missing

303
00:12:51,040 --> 00:12:48,650
there in that they are motors they they

304
00:12:53,800 --> 00:12:51,050
take chemical energy and they do work

305
00:12:57,010 --> 00:12:53,810
with it in a really directed way and I

306
00:12:58,690 --> 00:12:57,020
and I think on your list where you you

307
00:13:00,670 --> 00:12:58,700
know you're just not you're not doing

308
00:13:02,380 --> 00:13:00,680
that being possessive by locking it onto

309
00:13:06,130 --> 00:13:02,390
the template is not the same thing as

310
00:13:07,810 --> 00:13:06,140
making it into a directed motor yes so

311
00:13:10,840 --> 00:13:07,820
there's been multiple publications where

312
00:13:13,180 --> 00:13:10,850
even the protein polymerases have had

313
00:13:16,300 --> 00:13:13,190

their clamps removed and their proximity

314
00:13:19,030 --> 00:13:16,310
goes way lower by thousandfold so

315
00:13:21,220 --> 00:13:19,040
clamping does play a major role in being

316
00:13:23,710 --> 00:13:21,230
processive but they're they're

317
00:13:26,590 --> 00:13:23,720
definitely more complex and can go much

318
00:13:30,700 --> 00:13:26,600
further distances than an RNA polymerase

319
00:13:34,180 --> 00:13:30,710
would ever be able to I'm allowed to

320
00:13:35,860 --> 00:13:34,190
have one question so first I think he

321
00:13:39,520 --> 00:13:35,870
does have some form of a motor because

322
00:13:41,410 --> 00:13:39,530
the hydrolysis of each ntp drives it

323
00:13:43,630 --> 00:13:41,420
forward as long as you bind to this

324
00:13:46,090 --> 00:13:43,640
interface so you that could be an

325
00:13:48,580 --> 00:13:46,100
analogy to motor my question or

326
00:13:50,080 --> 00:13:48,590
suggestion is in our hands high

327
00:13:52,090 --> 00:13:50,090
throughput sequencing has been

328
00:13:54,370 --> 00:13:52,100
incredibly powerful in analyzing these

329
00:13:56,530 --> 00:13:54,380
rounds and that not only allows you to

330
00:13:58,870 --> 00:13:56,540
cluster sequences but also to find

331
00:14:01,150 --> 00:13:58,880
sequences that are getting enriched

332
00:14:04,120 --> 00:14:01,160
within one cluster and Irene Chen has

333
00:14:05,590 --> 00:14:04,130
been teaching us how to do that are you

334
00:14:07,030 --> 00:14:05,600
planning to do that yes so we're

335
00:14:08,920 --> 00:14:07,040
planning to do high throughput

336
00:14:10,660 --> 00:14:08,930
sequencing so again this was just the

337
00:14:14,950 --> 00:14:10,670
data from last week I was analyzing them

338
00:14:18,030 --> 00:14:14,960

in in the hotel room but yes and we

339
00:14:21,460 --> 00:14:18,040
might carry out a couple more selective

340
00:14:23,730 --> 00:14:21,470
schemes to get them a little further and

341
00:14:26,260 --> 00:14:23,740
then we do high throughput sequencing

342
00:14:28,190 --> 00:14:26,270
all right great let's thank Rosalyn

343
00:14:28,700 --> 00:14:28,200
again next time