E.ADEM v0.0.5.0

2009.8.8

2009.5.3

96 days

2009.8.8

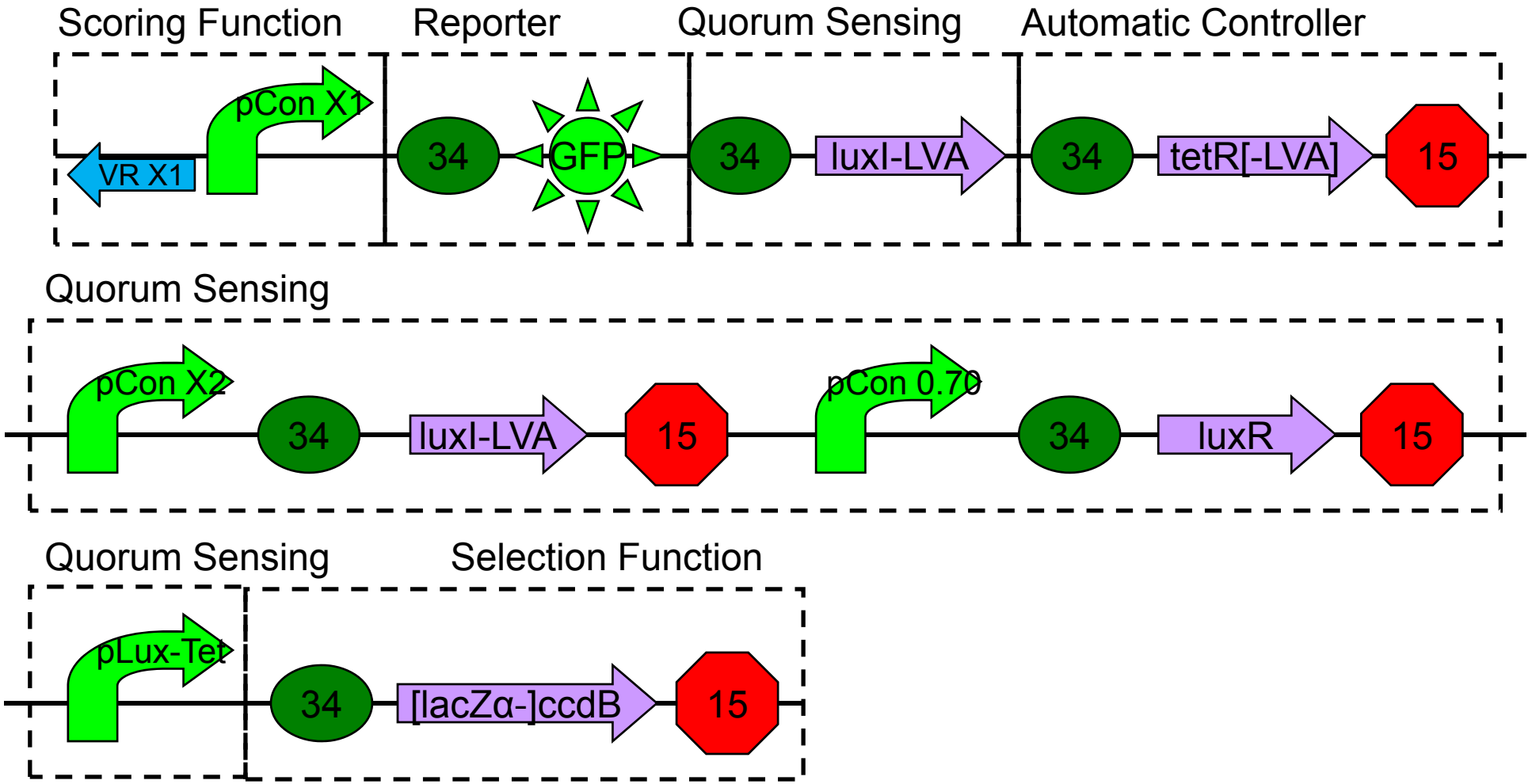
82 days

2009.10.30

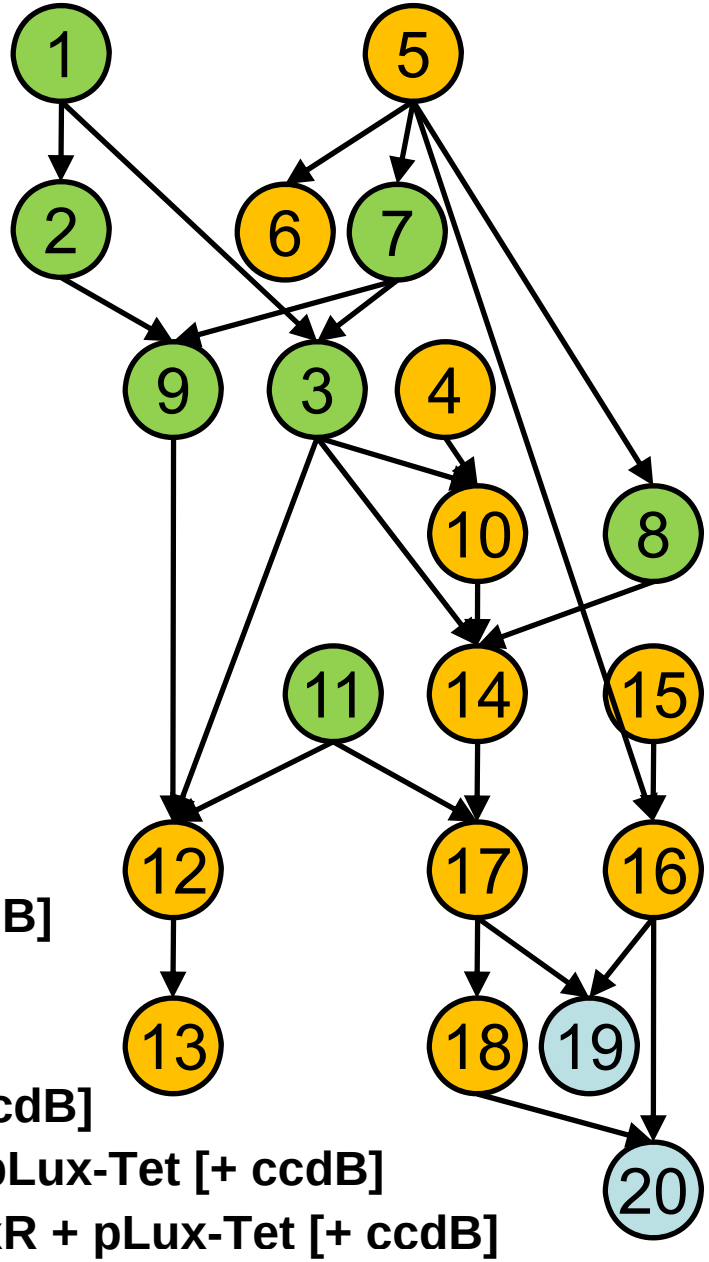
Outline

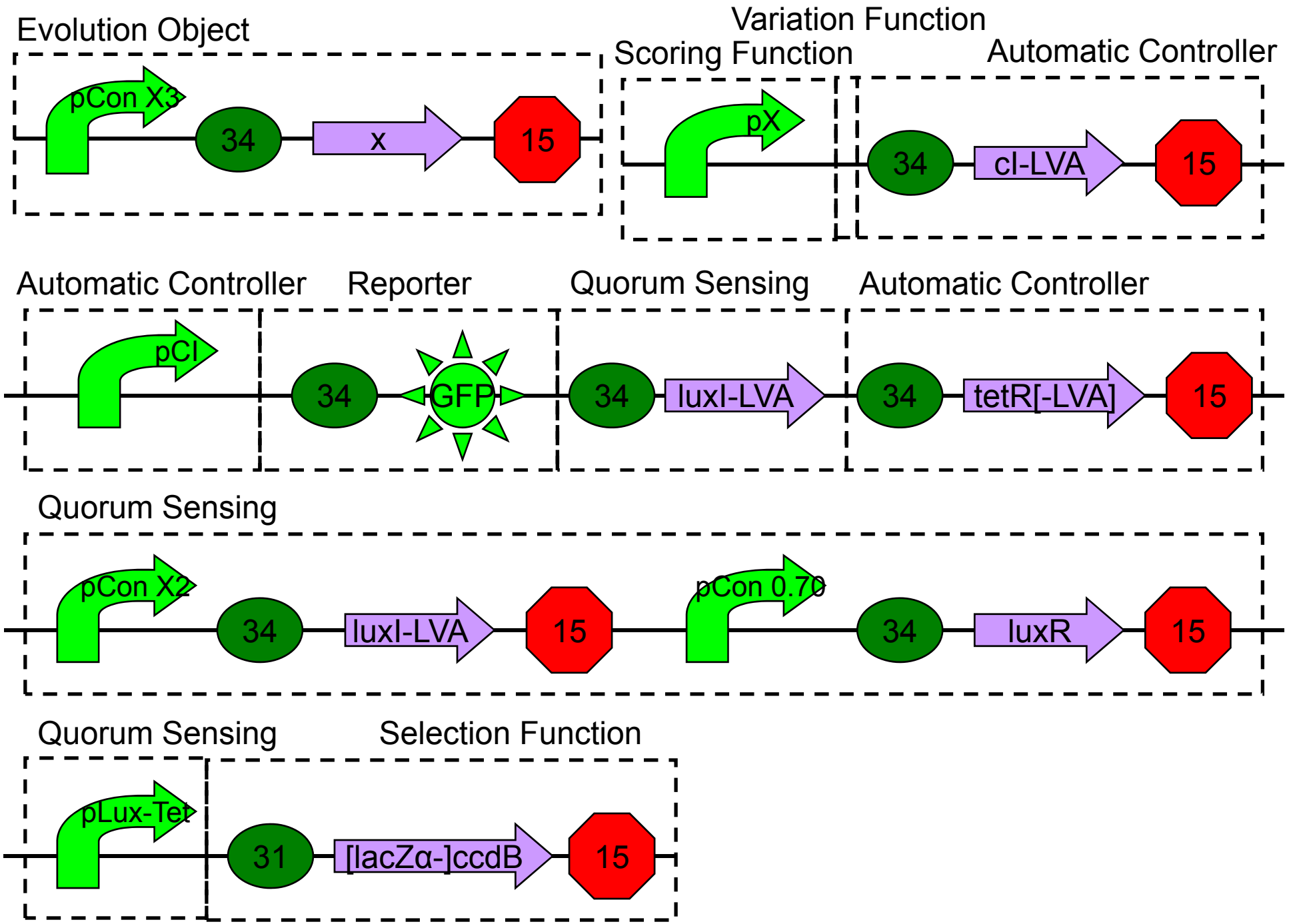
- Assembly
- Measurement
  - General Conditions
  - GFP
  - AHL
  - CcdB
  - LacZα
- Wiki
  - Team project description
  - Notebook
- Instructional Videos

Assembly

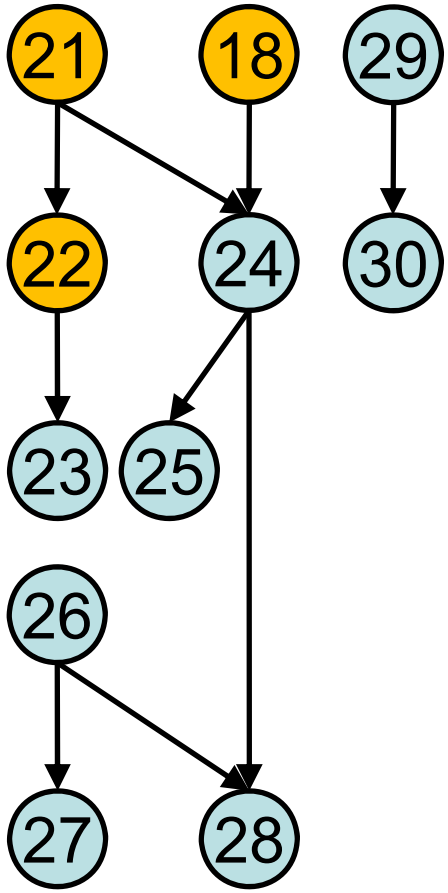

Scoring Function
Reporter
Quorum Sensing
Automatic Controller
pCon X1
VR X1
34
GFP
34
luxI-LVA
34
tetR[-LVA]
15
Quorum Sensing
pCon X2
34
luxI-LVA
15
pCon 0.70
34
luxR
15
Quorum Sensing
Selection Function
pLux-Tet
34
[lacZα-]ccdB
15

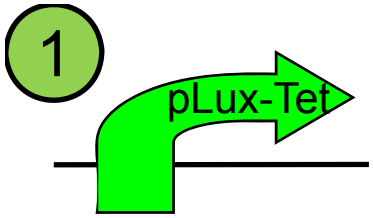
1. pLux-Tet
2. pLux-Tet + GFP
3. pCon + luxR + pLux-Tet
4. ccdB×4
5. pCon×8
6. pCon×8 + GFP
7. pCon + luxR
8. pCon×7 + luxI (AHL detection by 9 or GC-MS ?)
9. pCon + luxR + pLux-Tet + GFP (AHL)
10. pCon + luxR + pLux-Tet + ccdB×4 (AHL)
11. tetR×2
12. tetR×2 + pCon + luxR + pLux-Tet [+ GFP]
13. pCon×4 + tetR×2 + pCon + luxR + pLux-Tet [+ GFP] (AHL/aTc)
14. [pCon×7 +] luxI + pCon + luxR + pLux-Tet [+ ccdB]
15. VR×10
16. (VR + pCon)×7
17. tetR + pCon + luxI + pCon + luxR + pLux-Tet [+ ccdB]
18. GFP + luxI + tetR + pCon + luxI + pCon + luxR + pLux-Tet [+ ccdB]
19. (VR + pCon)×8 + tetR + pCon + luxI + pCon + luxR + pLux-Tet [+ ccdB]
20. (VR + pCon)×8 + GFP + luxI + tetR + pCon + luxI + pCon + luxR + pLux-Tet [+ ccdB]



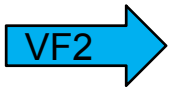

Evolution Object
pCon X3
34
x
15
Variation Function
Scoring Function
pX
Automatic Controller
34
cI-LVA
15
Automatic Controller
pCI
Reporter
34
GFP
Quorum Sensing
34
luxI-LVA
Automatic Controller
34
tetR[-LVA]
15
Quorum Sensing
pCon X2
34
luxI-LVA
15
pCon 0.70
34
luxR
15
Quorum Sensing
pLux-Tet
Selection Function
31
[lacZα-]ccdB
15

21. cI + pCI
22. cI + pCI + GFP
23. (VR + pCon)×7 + cI + pCI + GFP
24. cI + pCI + GFP + luxI + tetR + pCon + luxI + pCon + luxR + pLux-Tet [+ ccdB]
25. (VR + pCon)×7 + cI + pCI + GFP + luxI + tetR + pCon + luxI + pCon + luxR + pLux-Tet [+ ccdB]
26. pX
27. pX + GFP
28. pX + cI + pCI + GFP + luxI + tetR + pCon + luxI + pCon + luxR + pLux-Tet [+ ccdB]
29. x
30. pCon×7 + x



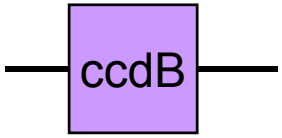


K176000 X+P with nicks
Sequence OK



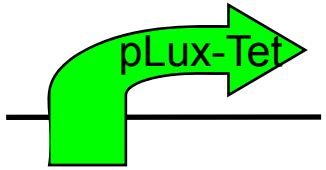
G00100
Synthesis OK

G00101
Synthesis OK



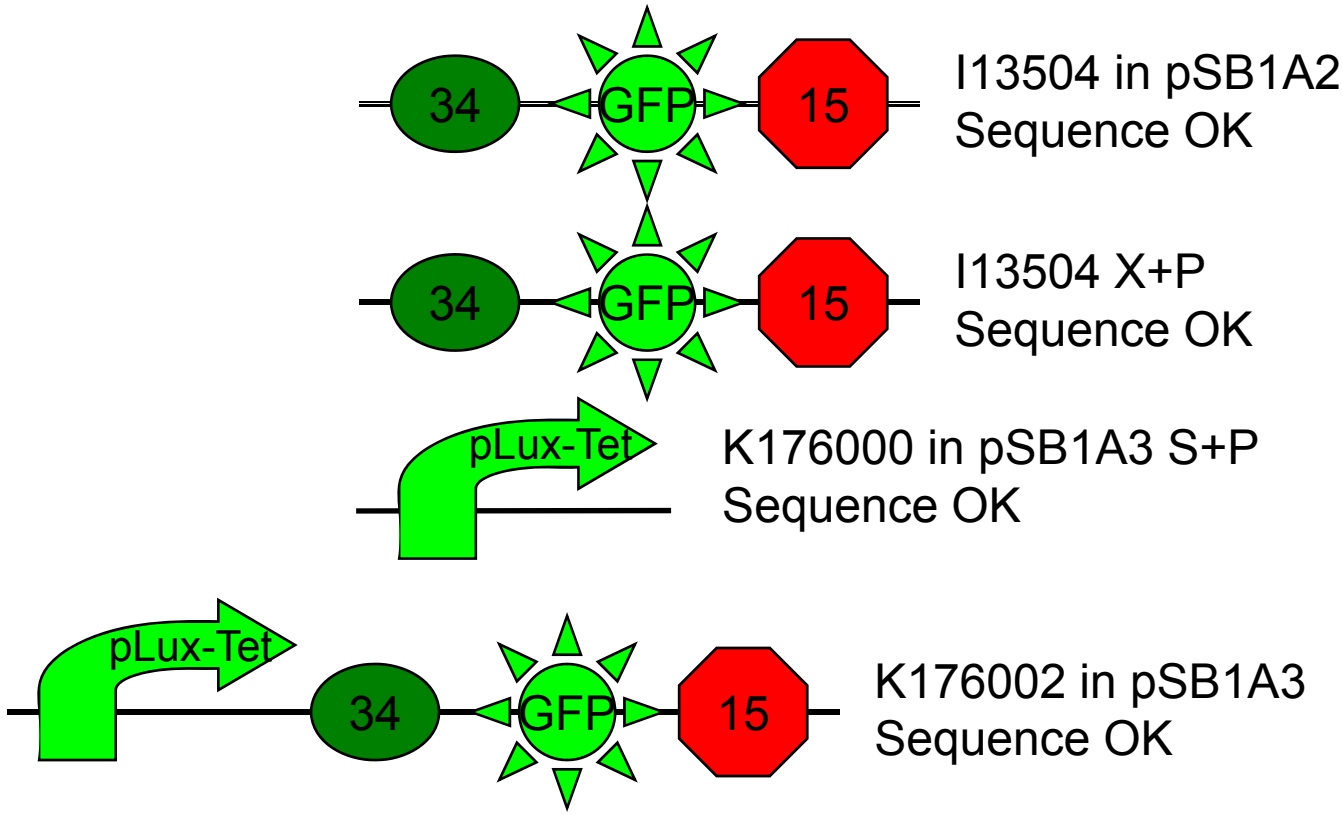
P1010 in pSB1A3 in DB 3.1
Length OK

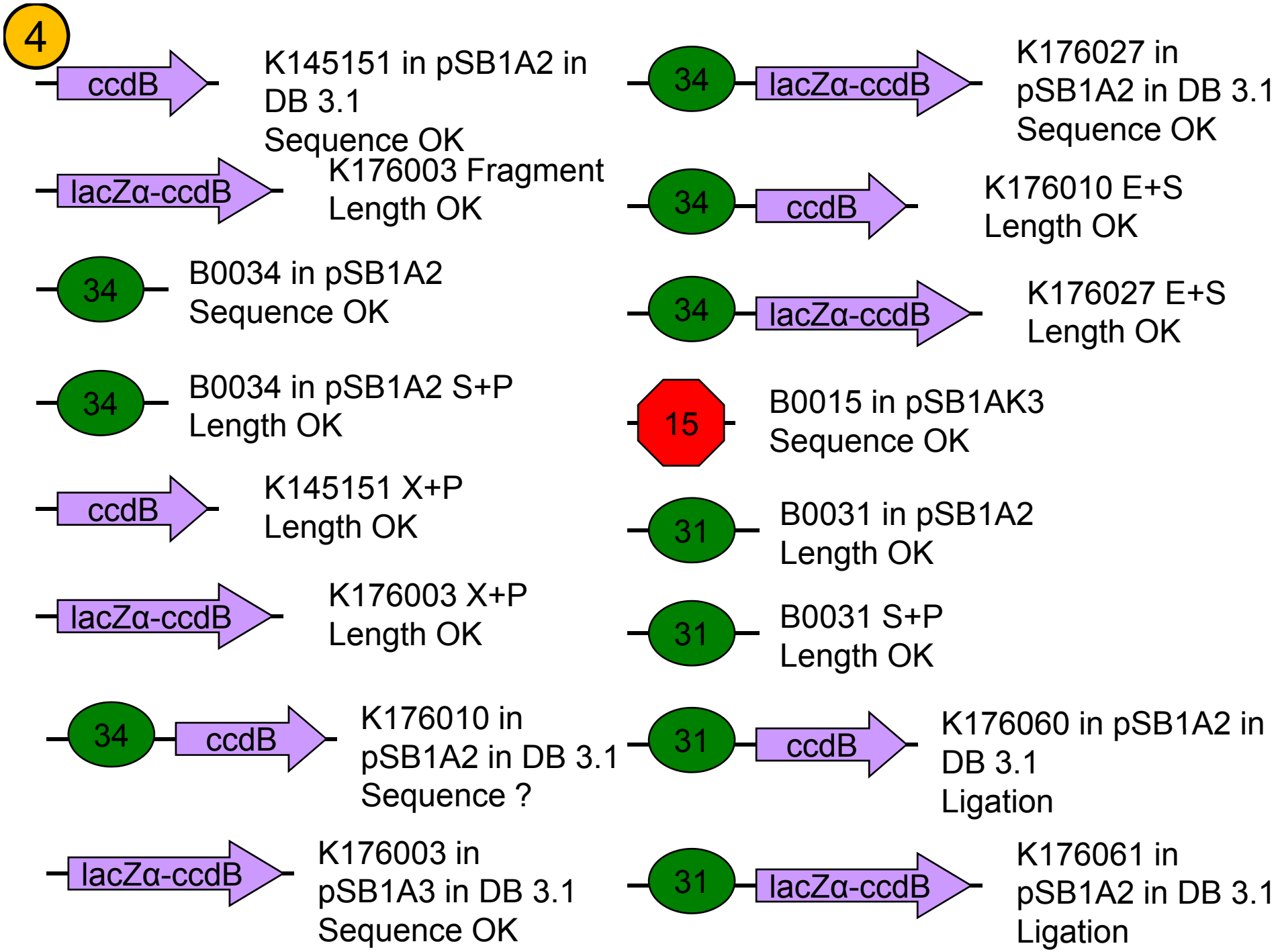
pSB1A3 X+P
Length OK

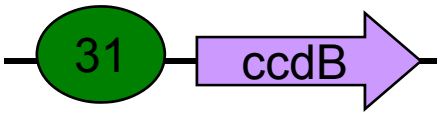


K176000 in pSB1A3
Sequence OK

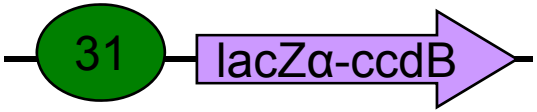
2


34
GFP
15
I13504 in pSB1A2
Sequence OK
34
GFP
15
I13504 X+P
Sequence OK
pLux-Tet
K176000 in pSB1A3 S+P
Sequence OK
pLux-Tet
34
GFP
15
K176002 in pSB1A3
Sequence OK

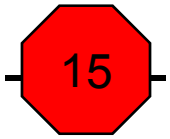

4
ccdB
K145151 in pSB1A2 in DB 3.1 Sequence OK
lacZα-ccdB
K176003 Fragment Length OK
34
B0034 in pSB1A2 Sequence OK
34
B0034 in pSB1A2 S+P Length OK
ccdB
K145151 X+P Length OK
lacZα-ccdB
K176003 X+P Length OK
34
ccdB
K176010 in pSB1A2 in DB 3.1 Sequence ?
lacZα-ccdB
K176003 in pSB1A3 in DB 3.1 Sequence OK
34
lacZα-ccdB
K176027 in pSB1A2 in DB 3.1 Sequence OK
34
ccdB
K176010 E+S Length OK
34
lacZα-ccdB
K176027 E+S Length OK
15
B0015 in pSB1AK3 Sequence OK
31
B0031 in pSB1A2 Length OK
31
B0031 S+P Length OK
31
ccdB
K176060 in pSB1A2 in DB 3.1 Ligation
31
lacZα-ccdB
K176061 in pSB1A2 in DB 3.1 Ligation



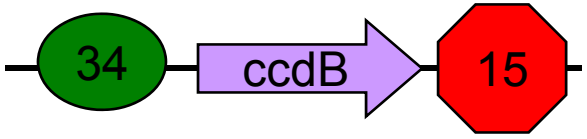
K176060 E+S
Waiting



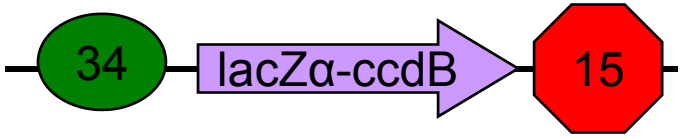
K176061 E+S
Waiting



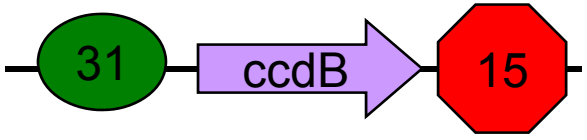
B0015 in pSB1AK3 E+X
Length OK



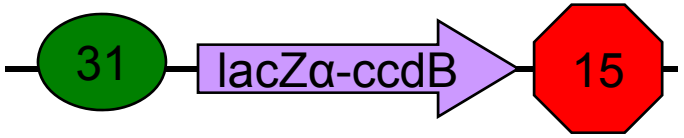
K176033 in pSB1AK3 in DB 3.1
Sequence OK



K176034 in pSB1AK3 in DB 3.1
Sequence OK

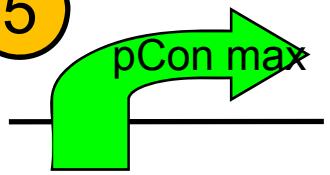


K176062 in pSB1AK3 in DB 3.1
Waiting

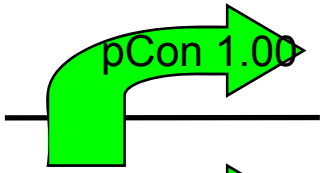


K176063 in pSB1AK3 in DB 3.1
Waiting

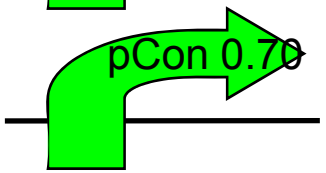
5



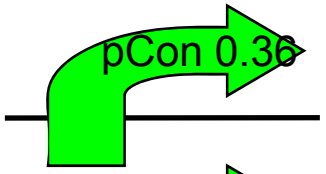
J23119 in pSB1A2
Sequence OK



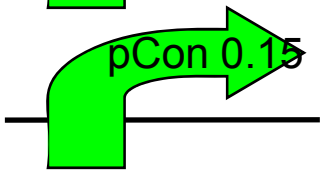
J23100 in J61002
Sequence ?



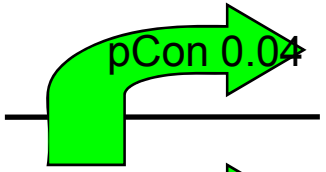
J23101 in J61002
Sequence OK



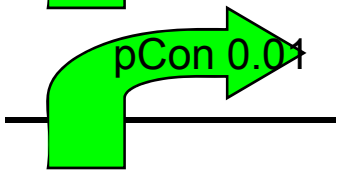
K176009 in J61002
Sequence OK



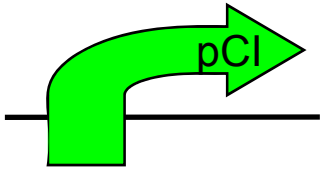
K176008 in J61002
Sequence OK



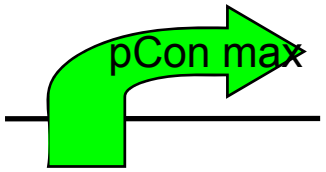
J23109 in J61002
Sequence OK



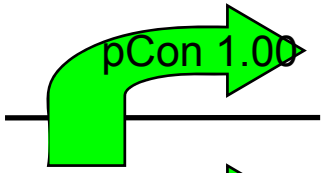
J23103 in J61002
Sequence OK



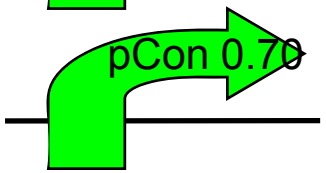
R0051 in pSB1A2
Sequence ?



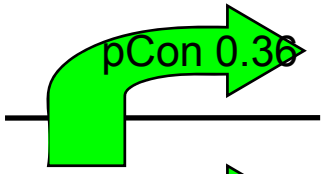
J23119 X+P
Sequence OK



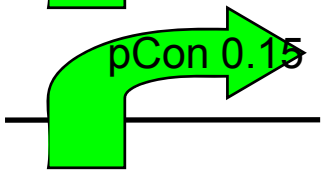
J23100-J61002SF X+P
Sequence ?



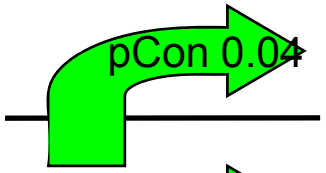
J23101-J61002SF X+P
Sequence OK



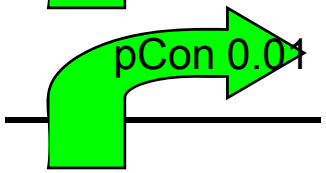
K176009-J61002SF X+P
Sequence OK



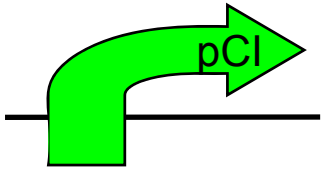
K176008-J61002SF X+P
Sequence OK



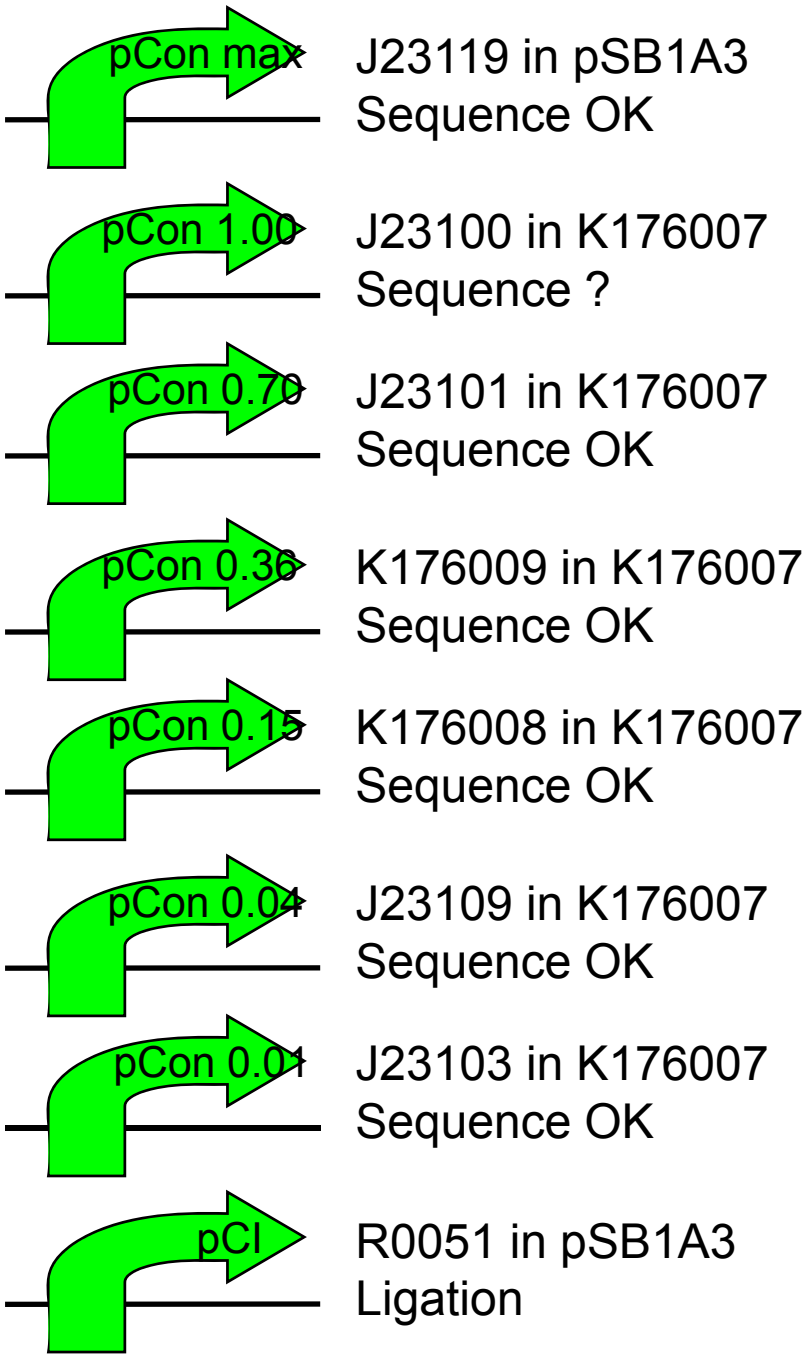
J23109-J61002SF X+P
Sequence OK



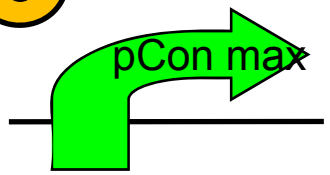
J23103-J61002SF X+P
Sequence OK



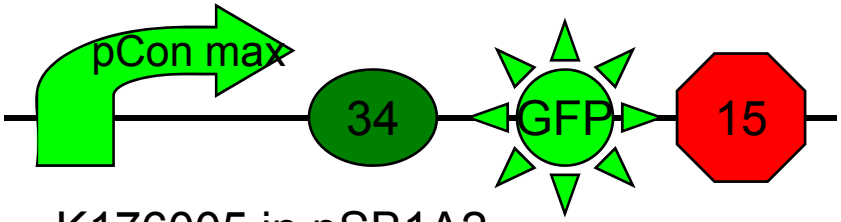
R0051 X+P
Length ?


pCon max
J23119 in pSB1A3
Sequence OK
pCon 1.00
J23100 in K176007
Sequence ?
pCon 0.70
J23101 in K176007
Sequence OK
pCon 0.36
K176009 in K176007
Sequence OK
pCon 0.15
K176008 in K176007
Sequence OK
pCon 0.04
J23109 in K176007
Sequence OK
pCon 0.01
J23103 in K176007
Sequence OK
pCI
R0051 in pSB1A3
Ligation

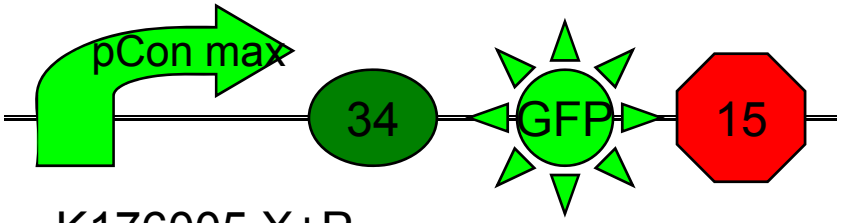
6



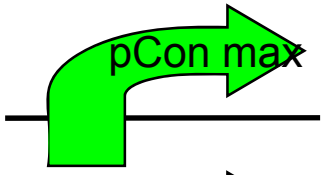
J23119 in pSB1A2 S+P
Sequence OK



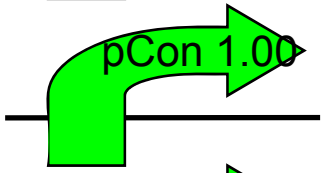
K176005 in pSB1A2
Sequence OK



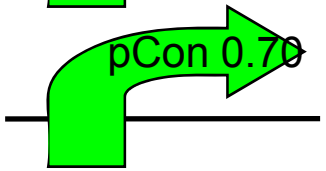
K176005 X+P
Length OK



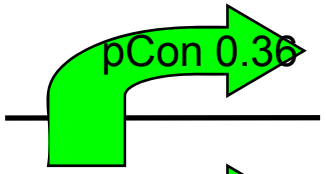
J23119 in pSB1A3 S+P
Length OK



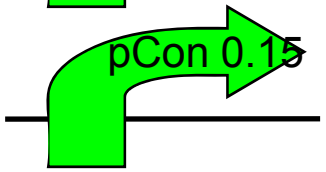
J23100 in pSB1A3 S+P
Length OK



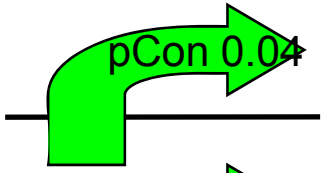
J23101 in pSB1A3 S+P
Length OK



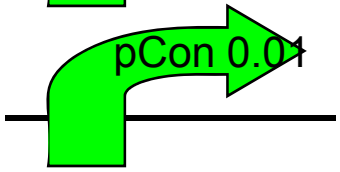
K176009 in pSB1A3 S+P
Length OK



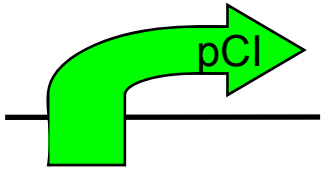
K176008 in pSB1A3 S+P
Length OK



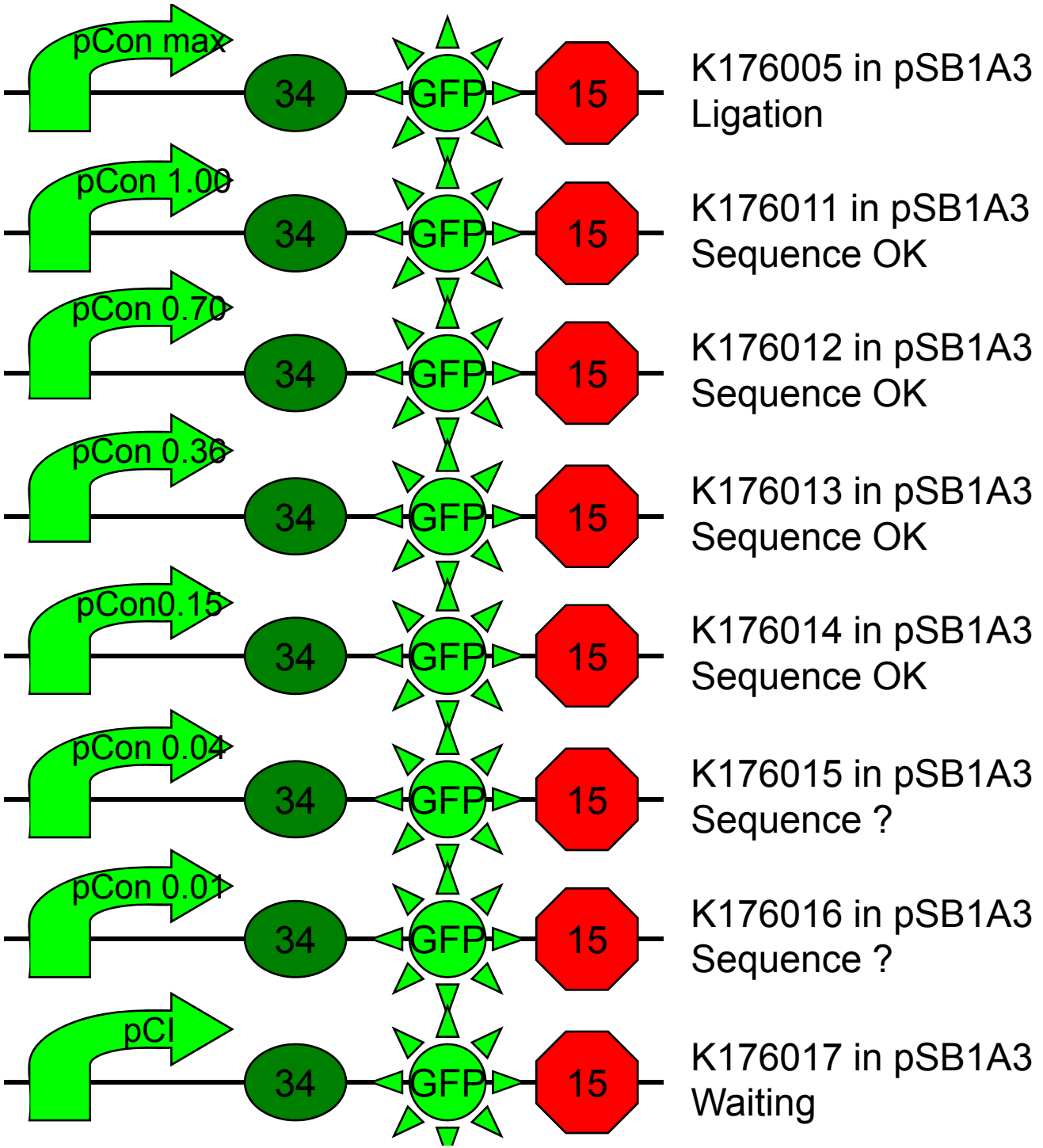
J23109 in pSB1A3 S+P
Length OK

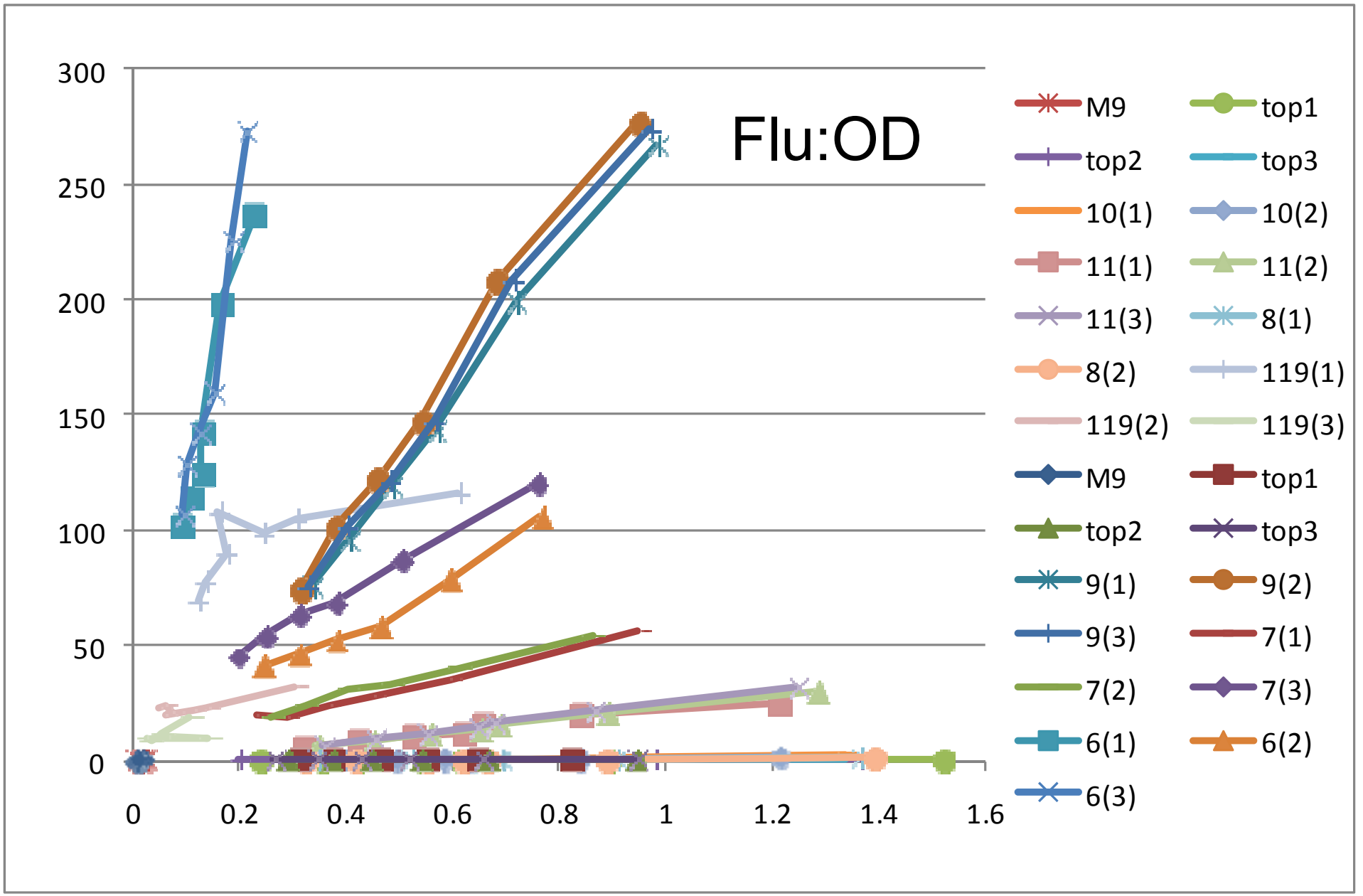


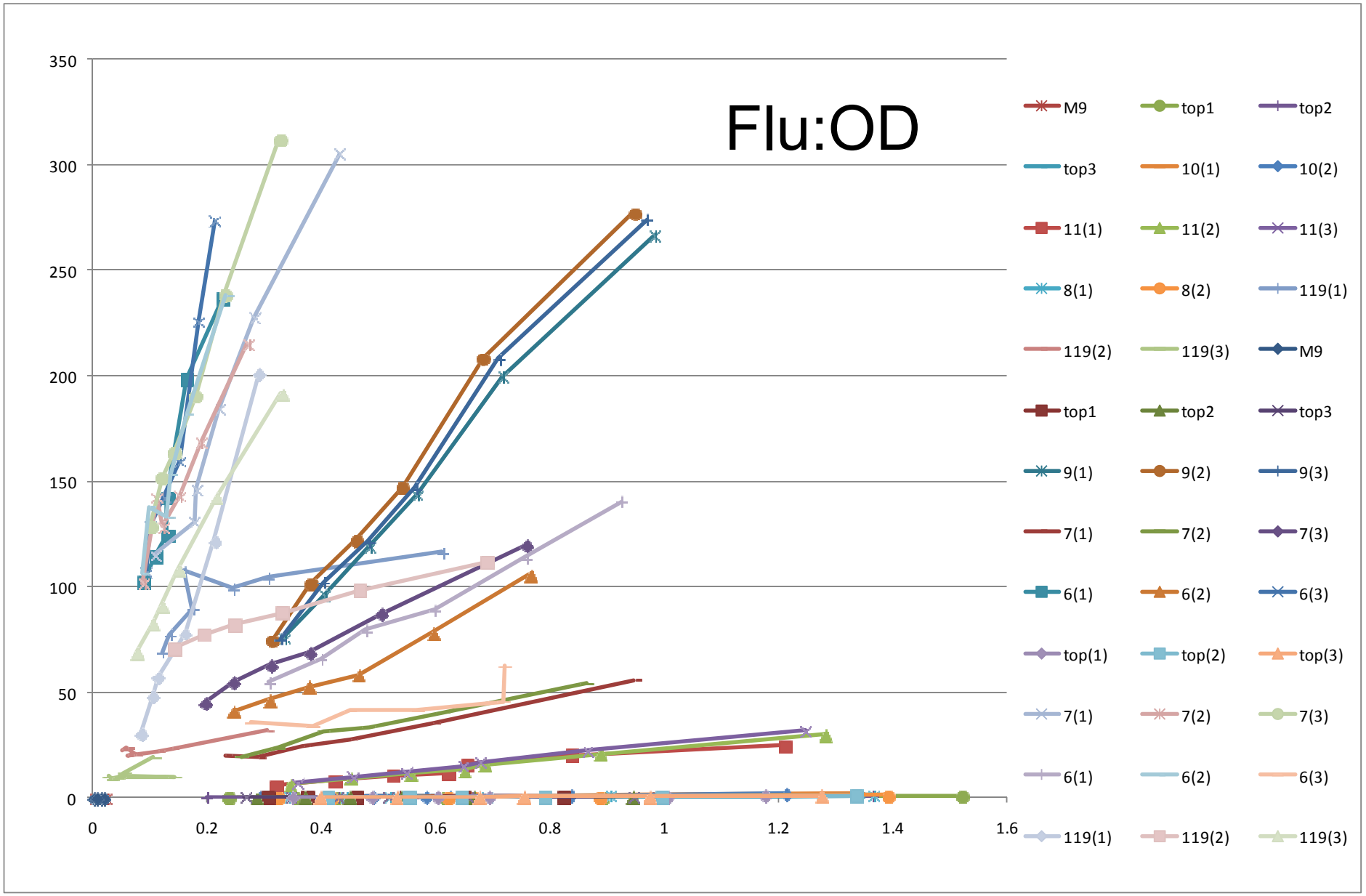
J23103 in pSB1A3 S+P
Length OK

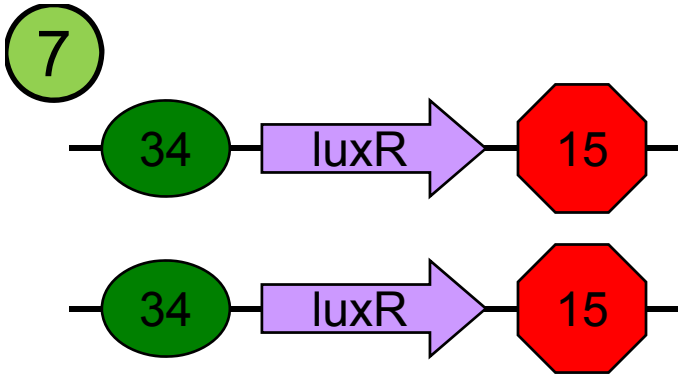


R0051 in pSB1A3 S+P
Length OK


pCon max
34
GFP
15
K176005 in pSB1A3
Ligation
pCon 1.00
34
GFP
15
K176011 in pSB1A3
Sequence OK
pCon 0.70
34
GFP
15
K176012 in pSB1A3
Sequence OK
pCon 0.36
34
GFP
15
K176013 in pSB1A3
Sequence OK
pCon0.15
34
GFP
15
K176014 in pSB1A3
Sequence OK
pCon 0.04
34
GFP
15
K176015 in pSB1A3
Sequence ?
pCon 0.01
34
GFP
15
K176016 in pSB1A3
Sequence ?
pCl
34
GFP
15
K176017 in pSB1A3
Waiting

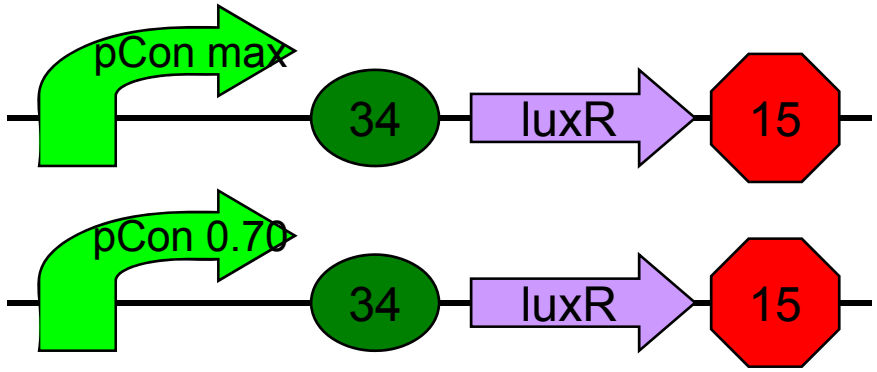

Flu:OD
300
250
200
150
100
50
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
M9
top1
top2
top3
10(1)
10(2)
11(1)
11(2)
11(3)
8(1)
8(2)
119(1)
119(2)
119(3)
M9
top1
top2
top3
9(1)
9(2)
9(3)
7(1)
7(2)
7(3)
6(1)
6(2)
6(3)


Flu:OD
350
300
250
200
150
100
50
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
M9
top1
top2
top3
10(1)
10(2)
11(1)
11(2)
11(3)
8(1)
8(2)
119(1)
119(2)
119(3)
M9
top1
top2
top3
9(1)
9(2)
9(3)
7(1)
7(2)
7(3)
6(1)
6(2)
6(3)
top(1)
top(2)
top(3)
7(1)
7(2)
7(3)
6(1)
6(2)
6(3)
119(1)
119(2)
119(3)



I0462 in pSB1A2
Sequence OK

I0462 X+P
Length OK

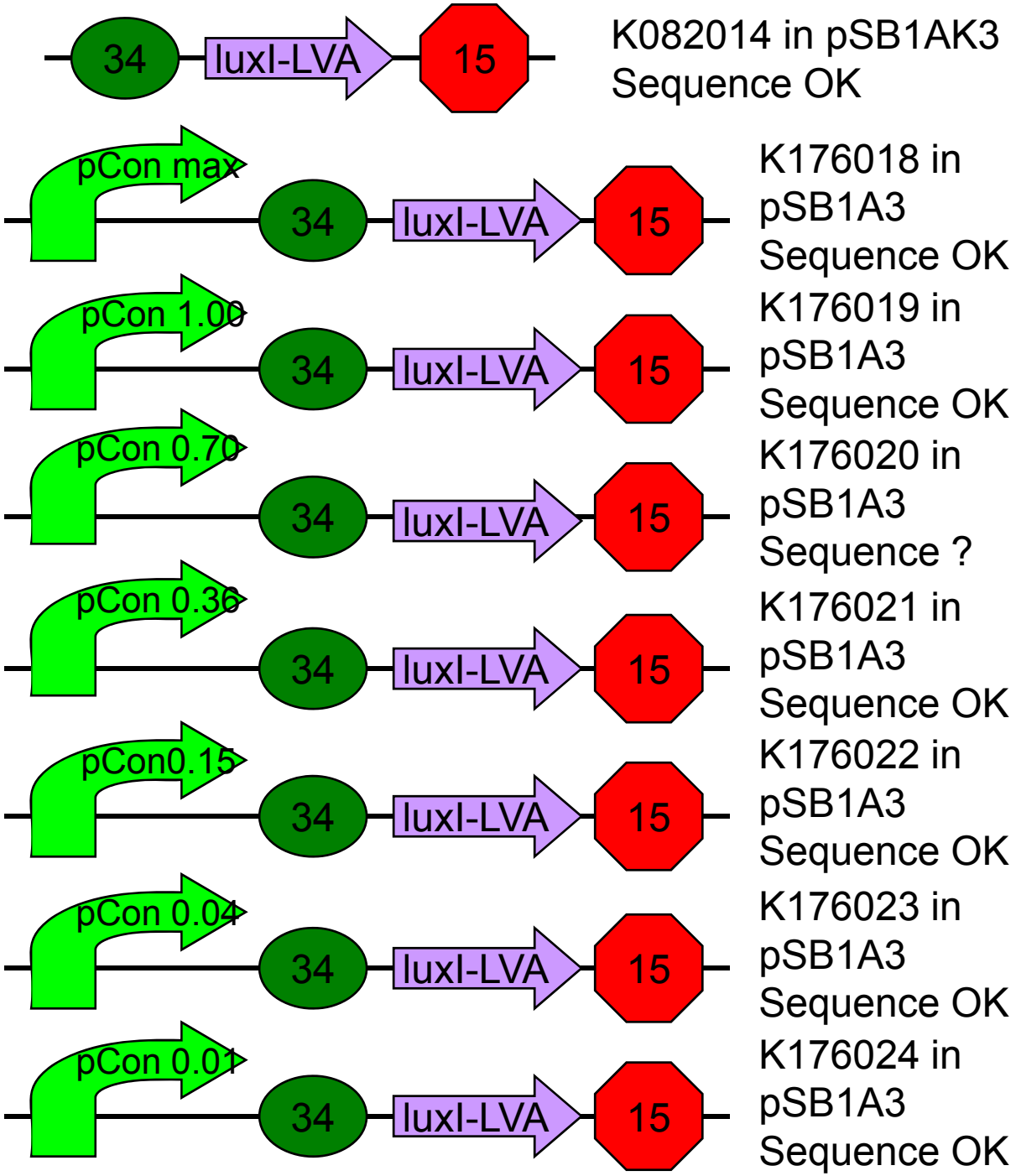


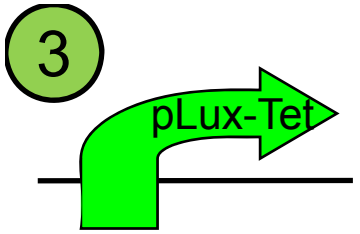
K176004 in pSB1A2
Sequence OK
Length ?

K176025 in pSB1A3
Sequence ?

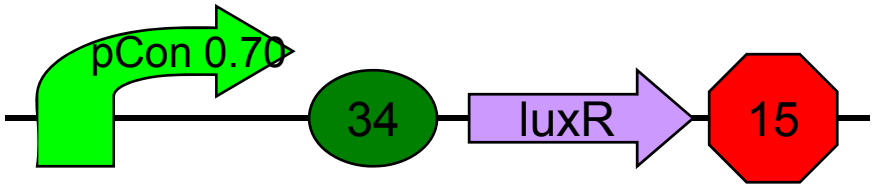
8

Measurement

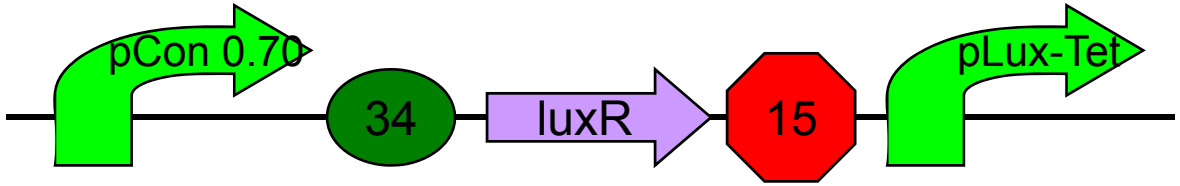




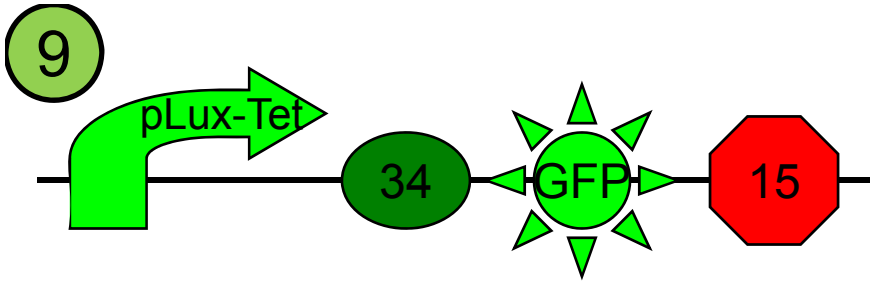
K176000 in pSB1A3 E+X
Length OK



K176025 E+S
Length OK

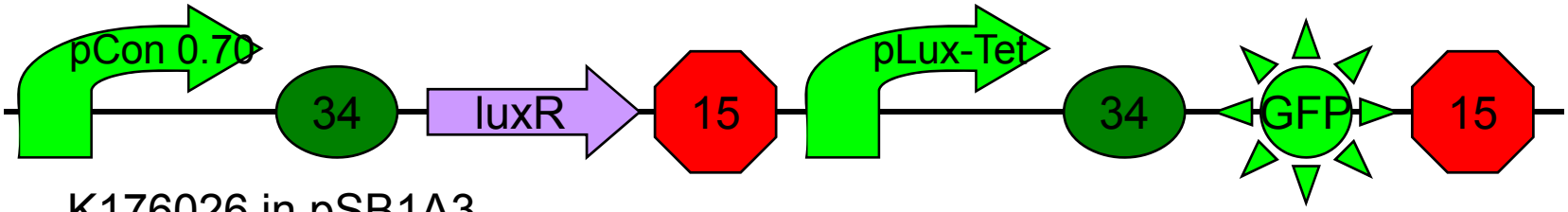


K176028 in pSB1A3
Sequence ?



K176002 in pSB1A3 E+X
Length OK

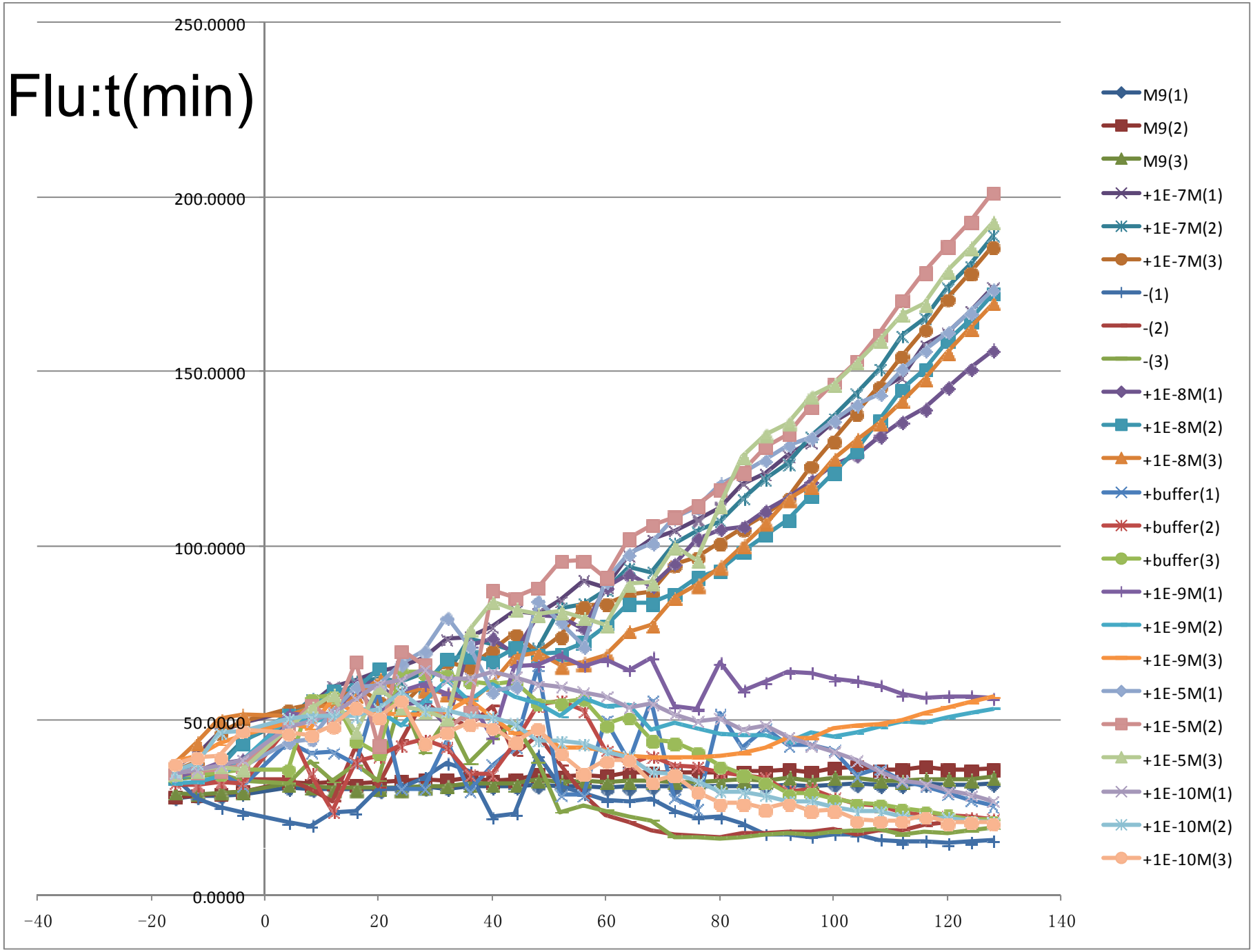
G00201
Synthesis OK

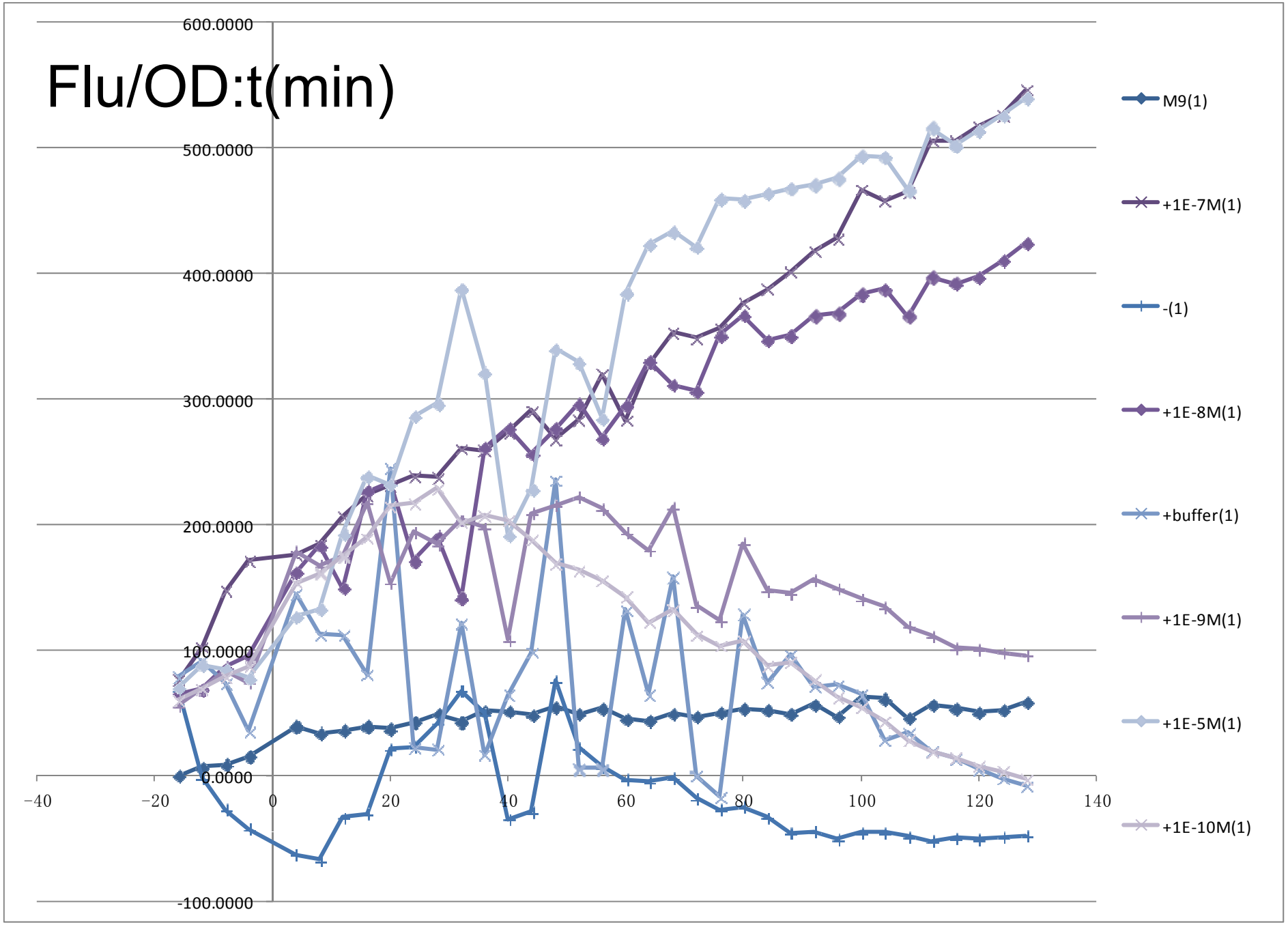


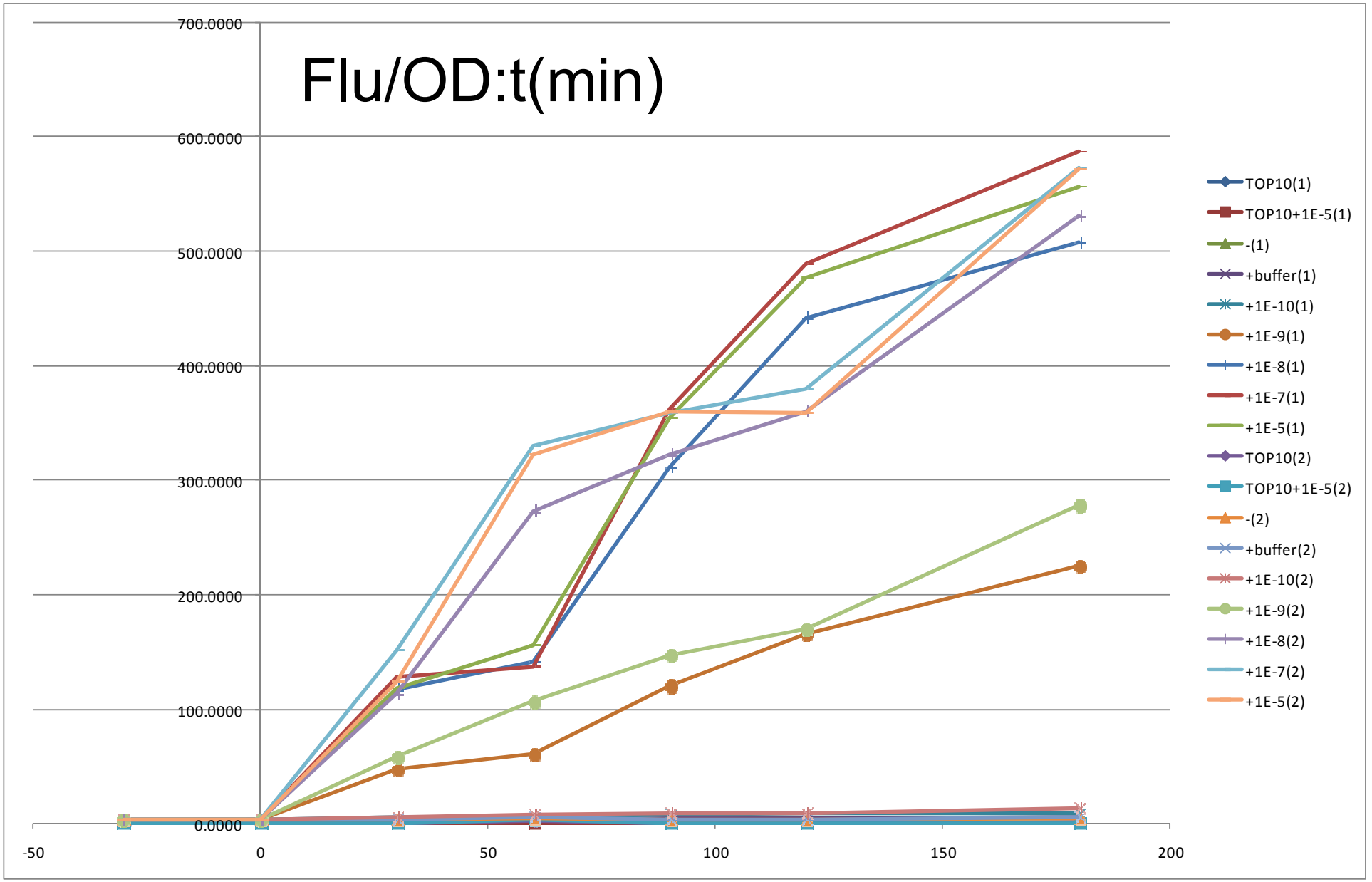
K176026 in pSB1A3
Sequence ?

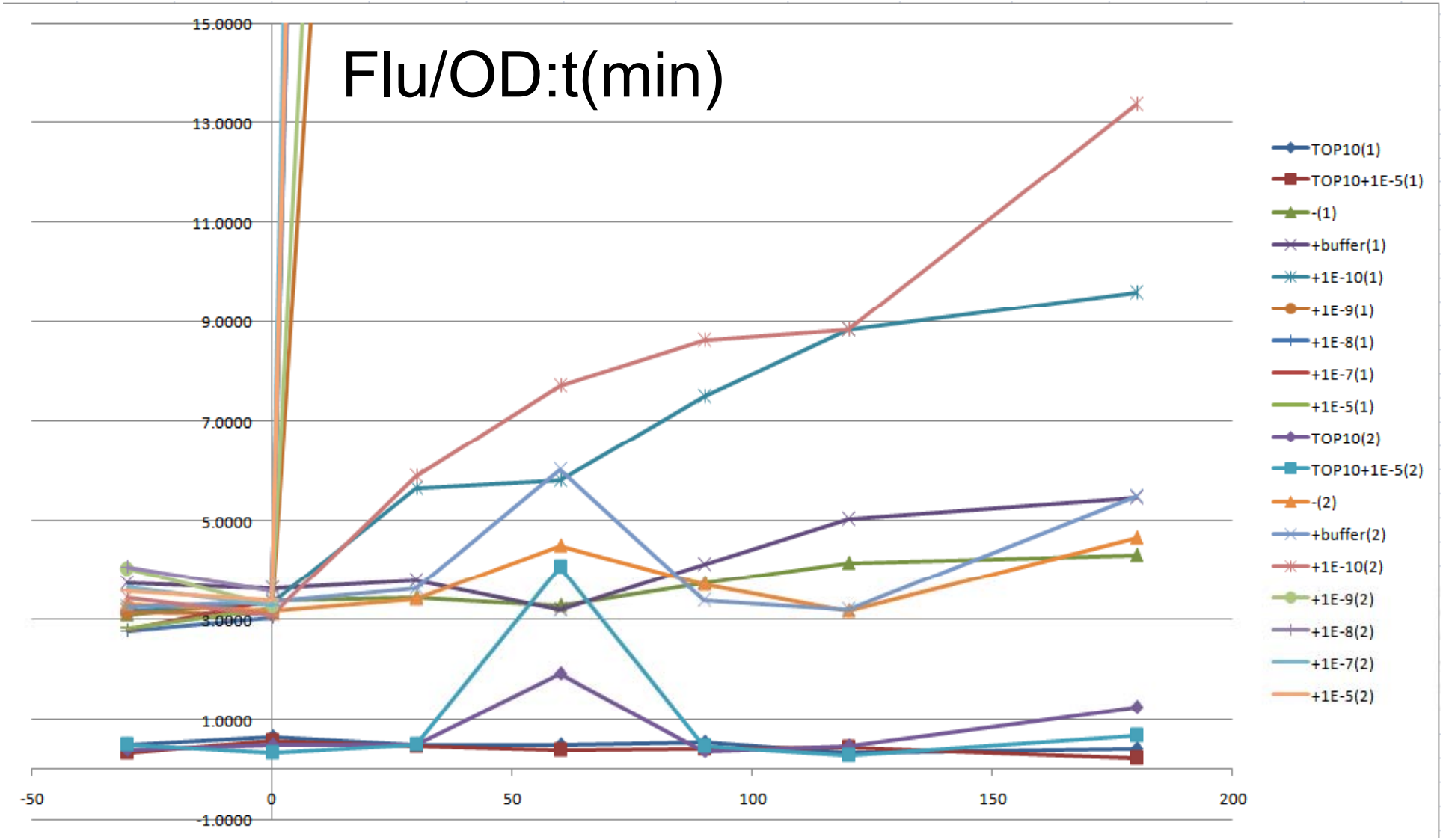
Methods

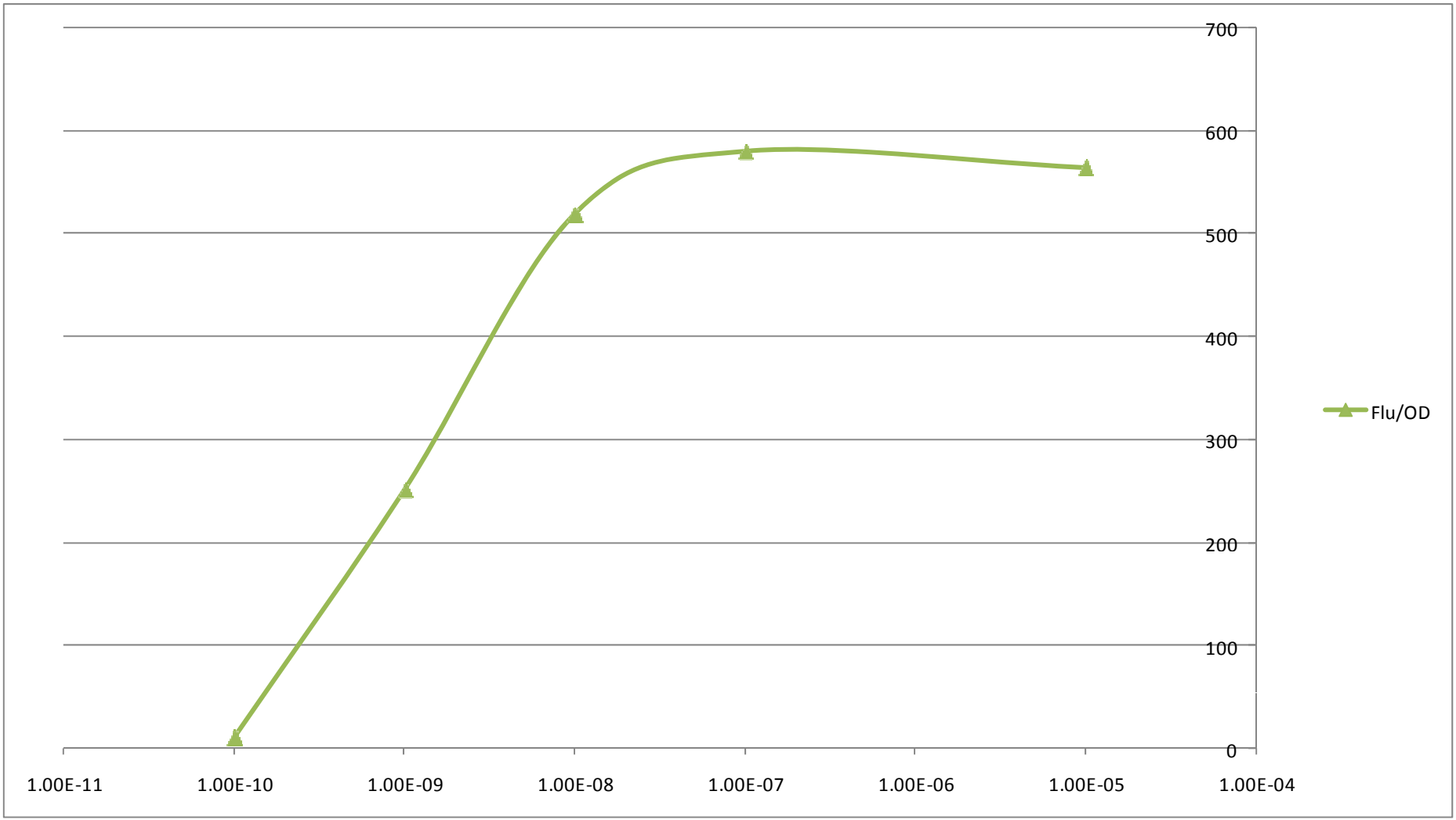
- Plate Reader
- Spectrophotometer


Flu:t(min)
250.0000
200.0000
150.0000
100.0000
50.0000
0.0000
-40
-20
0
20
40
60
80
100
120
140
M9(1)
M9(2)
M9(3)
+1E-7M(1)
+1E-7M(2)
+1E-7M(3)
-(1)
-(2)
-(3)
+1E-8M(1)
+1E-8M(2)
+1E-8M(3)
+buffer(1)
+buffer(2)
+buffer(3)
+1E-9M(1)
+1E-9M(2)
+1E-9M(3)
+1E-5M(1)
+1E-5M(2)
+1E-5M(3)
+1E-10M(1)
+1E-10M(2)
+1E-10M(3)

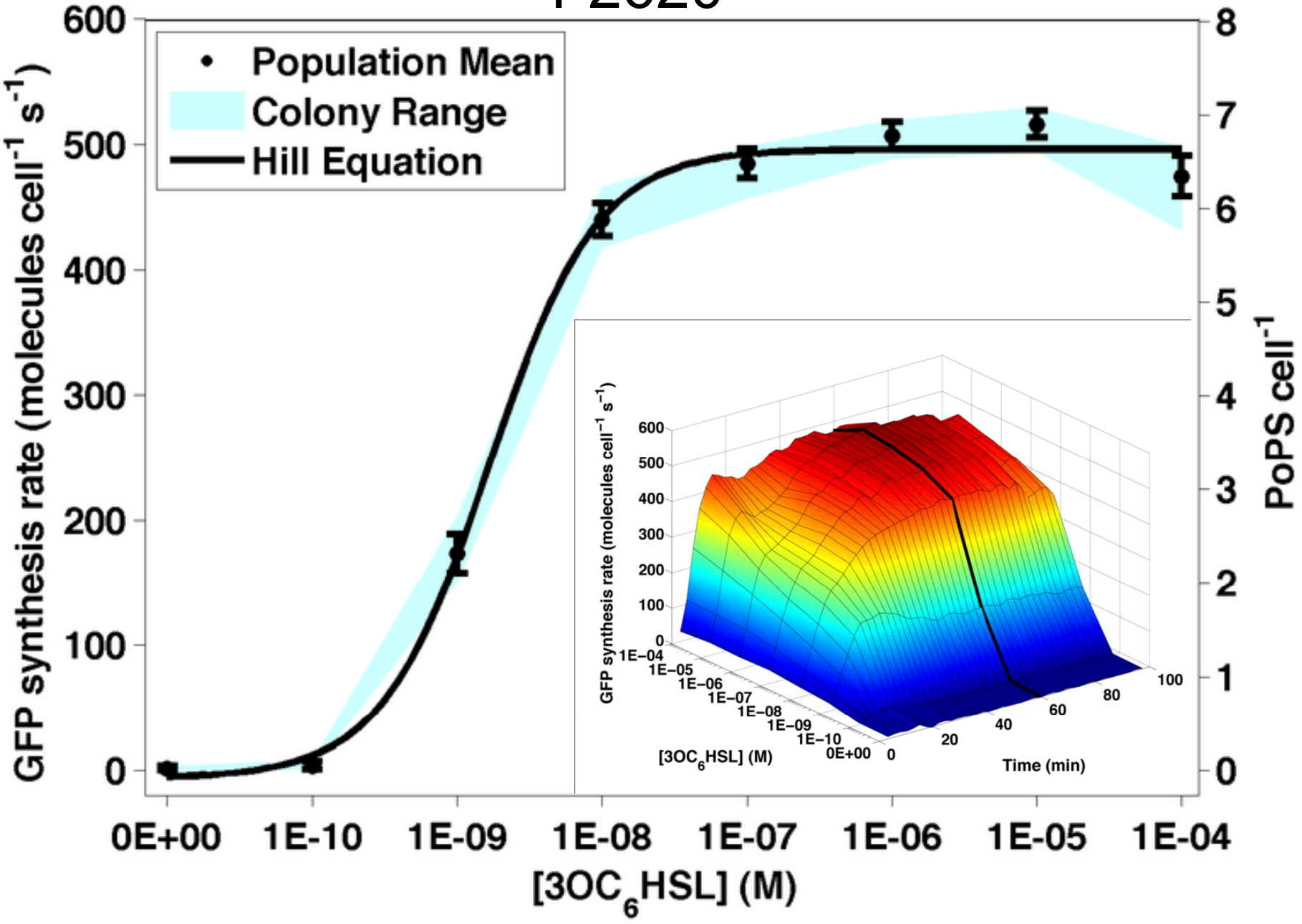

Flu/OD:t(min)
600.0000
500.0000
400.0000
300.0000
200.0000
100.0000
0.0000
-100.0000
−40
−20
0
20
40
60
80
100
120
140
M9(1)
+1E-7M(1)
-(1)
+1E-8M(1)
+buffer(1)
+1E-9M(1)
+1E-5M(1)
+1E-10M(1)

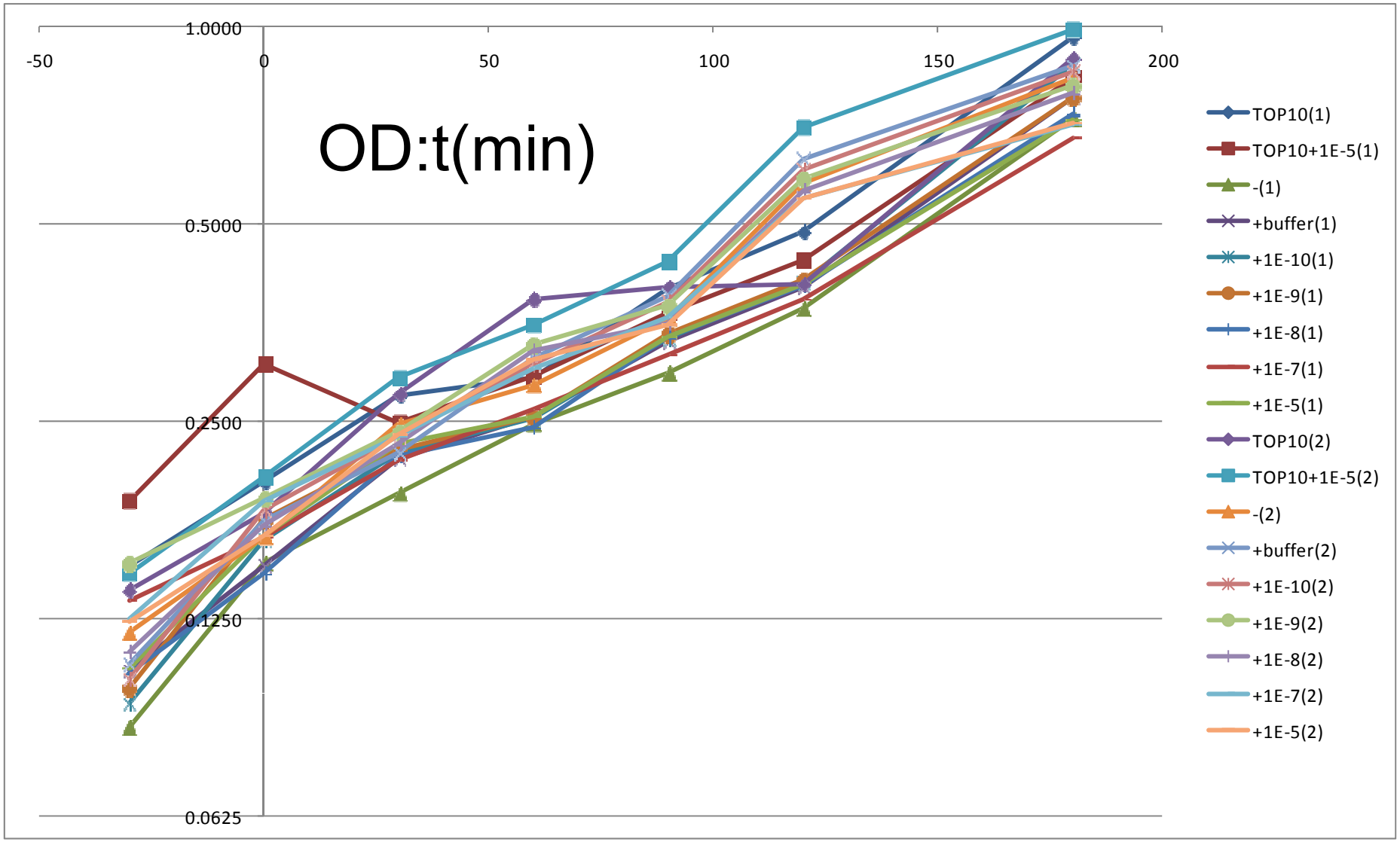

Flu/OD:t(min)
700.0000
600.0000
500.0000
400.0000
300.0000
200.0000
100.0000
0.0000
-50
0
50
100
150
200
TOP10(1)
TOP10+1E-5(1)
-(1)
+buffer(1)
+1E-10(1)
+1E-9(1)
+1E-8(1)
+1E-7(1)
+1E-5(1)
TOP10(2)
TOP10+1E-5(2)
-(2)
+buffer(2)
+1E-10(2)
+1E-9(2)
+1E-8(2)
+1E-7(2)
+1E-5(2)

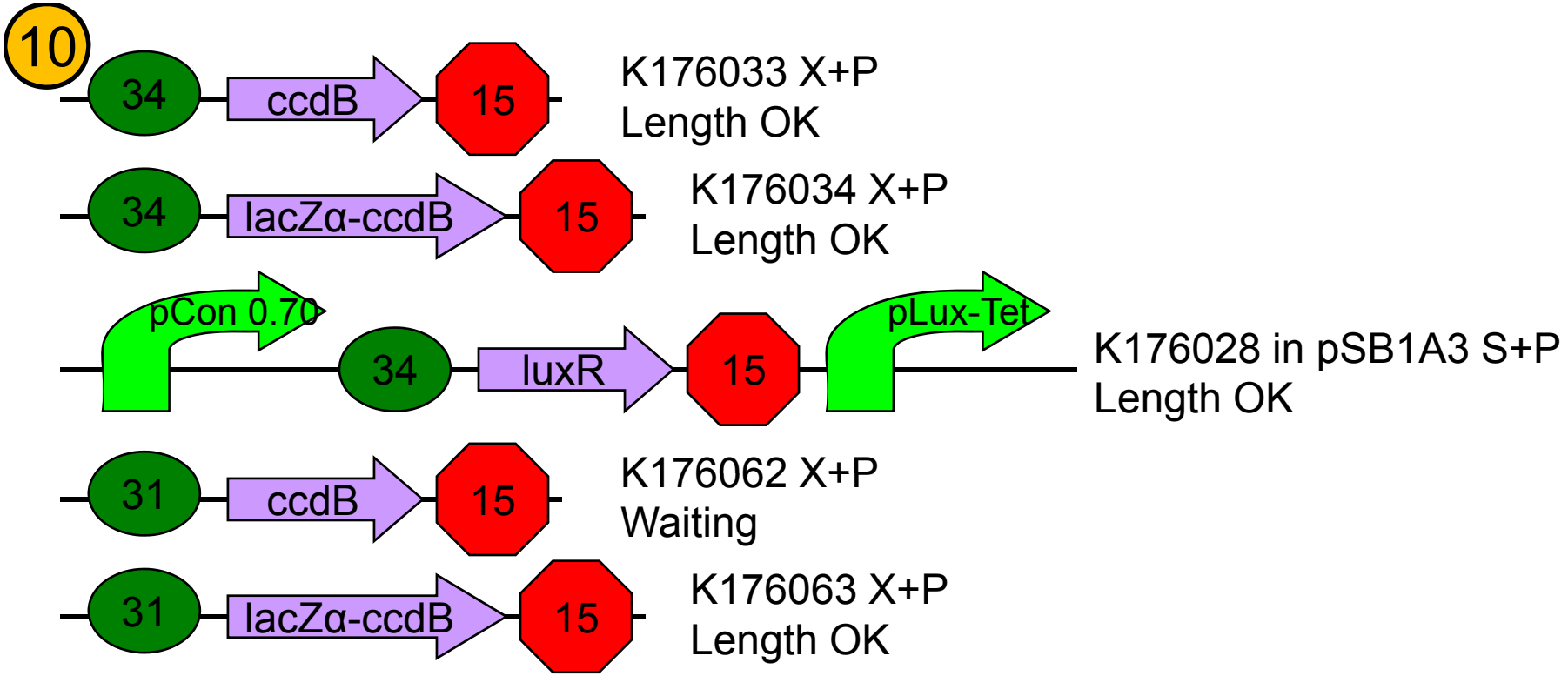

Flu/OD:t(min)
15.0000
13.0000
11.0000
9.0000
7.0000
5.0000
3.0000
1.0000
-1.0000
-50
0
50
100
150
200
TOP10(1)
TOP10+1E-5(1)
-(1)
+buffer(1)
+1E-10(1)
+1E-9(1)
+1E-8(1)
+1E-7(1)
+1E-5(1)
TOP10(2)
TOP10+1E-5(2)
-(2)
+buffer(2)
+1E-10(2)
+1E-9(2)
+1E-8(2)
+1E-7(2)
+1E-5(2)

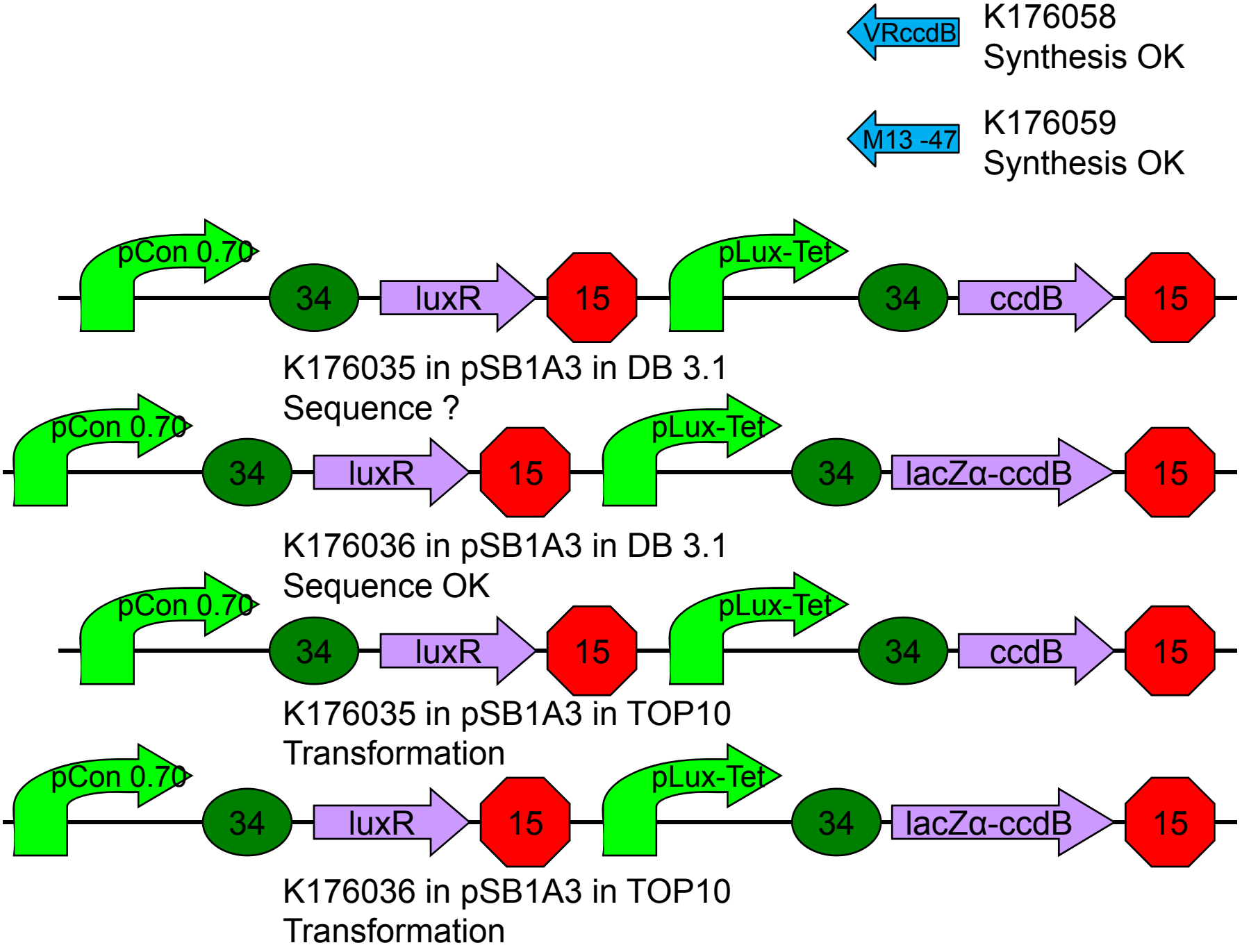

700
600
500
400
300
200
100
0
1.00E-11
1.00E-10
1.00E-09
1.00E-08
1.00E-07
1.00E-06
1.00E-05
1.00E-04
Flu/OD

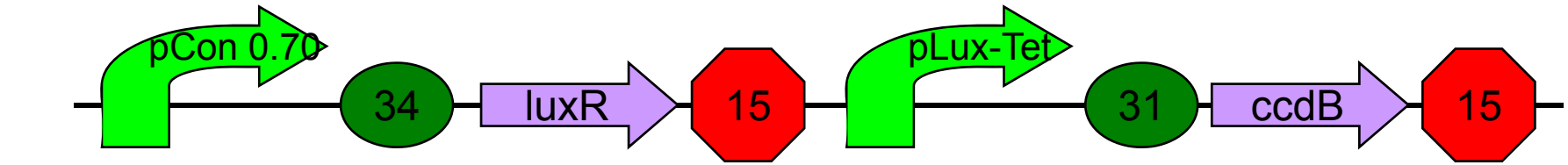
F2620


Population Mean
Colony Range
Hill Equation
GFP synthesis rate (molecules cell^{-1} s^{-1})
PoPS cell^{-1}
[3OC$_6$HSL] (M)
0E+00
1E-10
1E-09
1E-08
1E-07
1E-06
1E-05
1E-04
0
100
200
300
400
500
600
0
1
2
3
4
5
6
7
8
GFP synthesis rate (molecules cell^{-1} s^{-1})
[3OC$_6$HSL] (M)
Time (min)
0
20
40
60
80
100

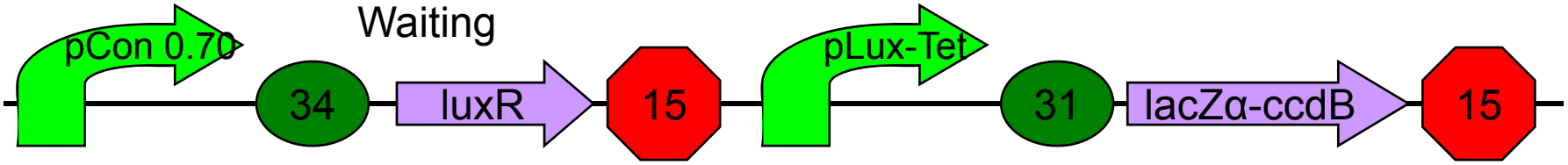

OD:t(min)
1.0000
0.5000
0.2500
0.1250
0.0625
-50
0
50
100
150
200
TOP10(1)
TOP10+1E-5(1)
-(1)
+buffer(1)
+1E-10(1)
+1E-9(1)
+1E-8(1)
+1E-7(1)
+1E-5(1)
TOP10(2)
TOP10+1E-5(2)
-(2)
+buffer(2)
+1E-10(2)
+1E-9(2)
+1E-8(2)
+1E-7(2)
+1E-5(2)


10
34
ccdB
15
K176033 X+P
Length OK
34
lacZα-ccdB
15
K176034 X+P
Length OK
pCon 0.70
34
luxR
15
pLux-Tet
K176028 in pSB1A3 S+P
Length OK
31
ccdB
15
K176062 X+P
Waiting
31
lacZα-ccdB
15
K176063 X+P
Length OK

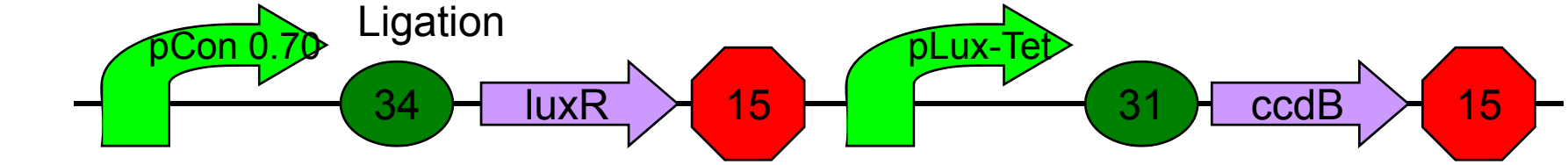

VRccdB
K176058
Synthesis OK
M13 -47
K176059
Synthesis OK
pCon 0.70
34
luxR
15
pLux-Tet
34
ccdB
15
K176035 in pSB1A3 in DB 3.1
Sequence ?
pCon 0.70
34
luxR
15
pLux-Tet
34
lacZα-ccdB
15
K176036 in pSB1A3 in DB 3.1
Sequence OK
pCon 0.70
34
luxR
15
pLux-Tet
34
ccdB
15
K176035 in pSB1A3 in TOP10
Transformation
pCon 0.70
34
luxR
15
pLux-Tet
34
lacZα-ccdB
15
K176036 in pSB1A3 in TOP10
Transformation



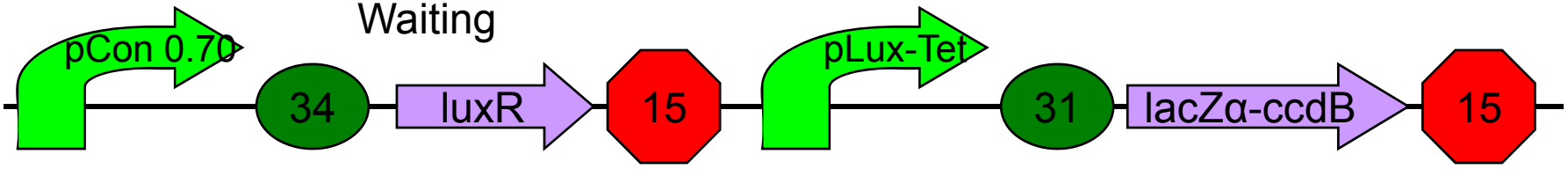
K176089 in pSB1A3 in DB 3.1
Waiting



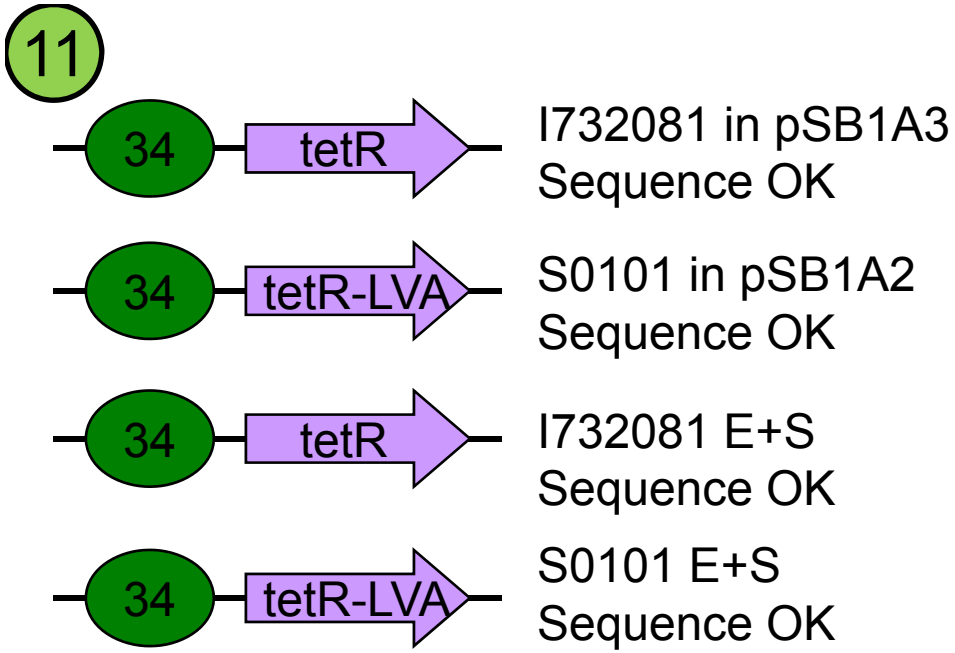
K176090 in pSB1A3 in DB 3.1
Ligation

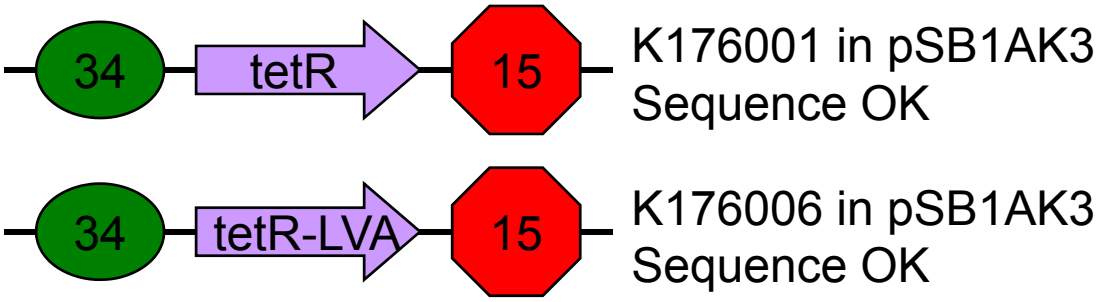


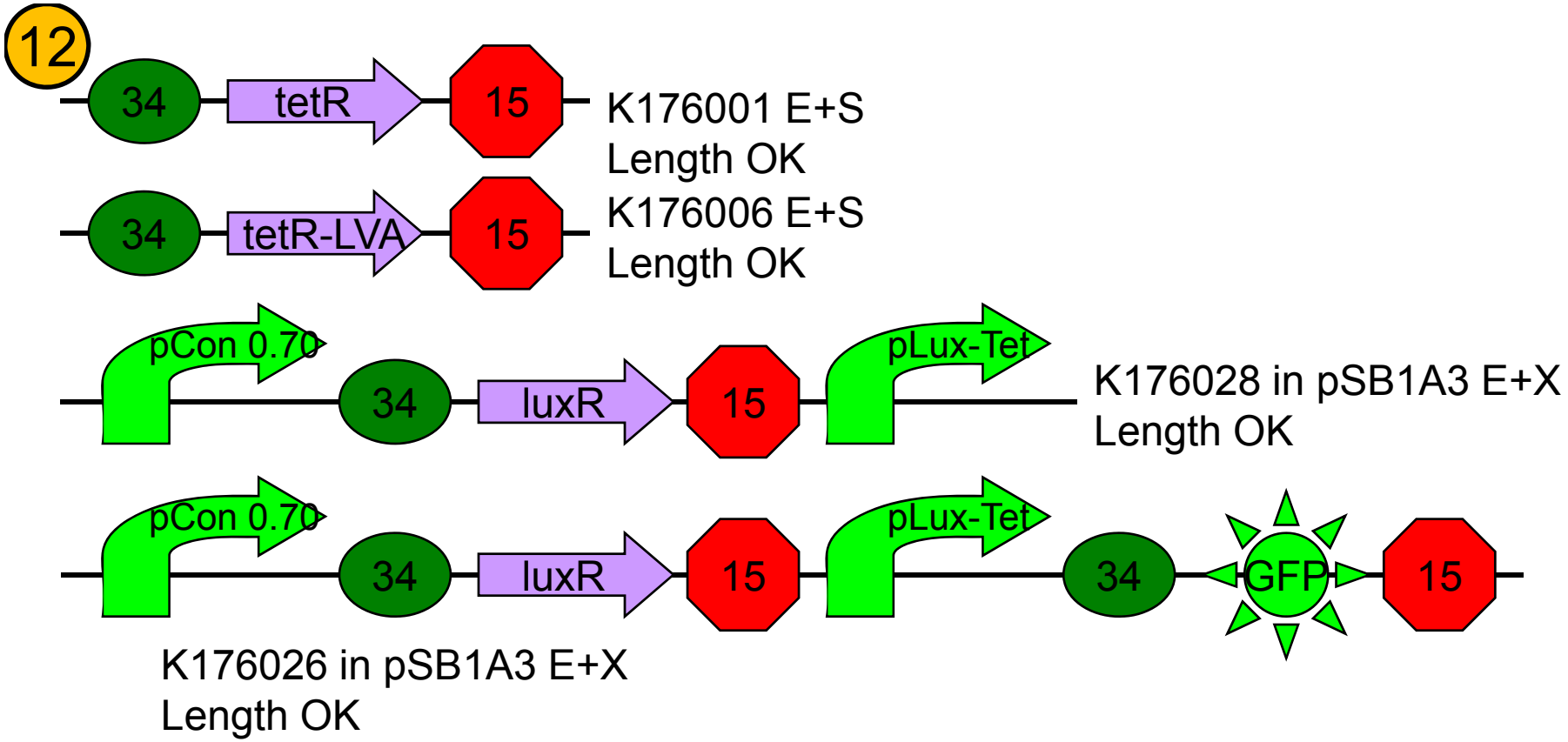
K176089 in pSB1A3 in TOP10
Waiting

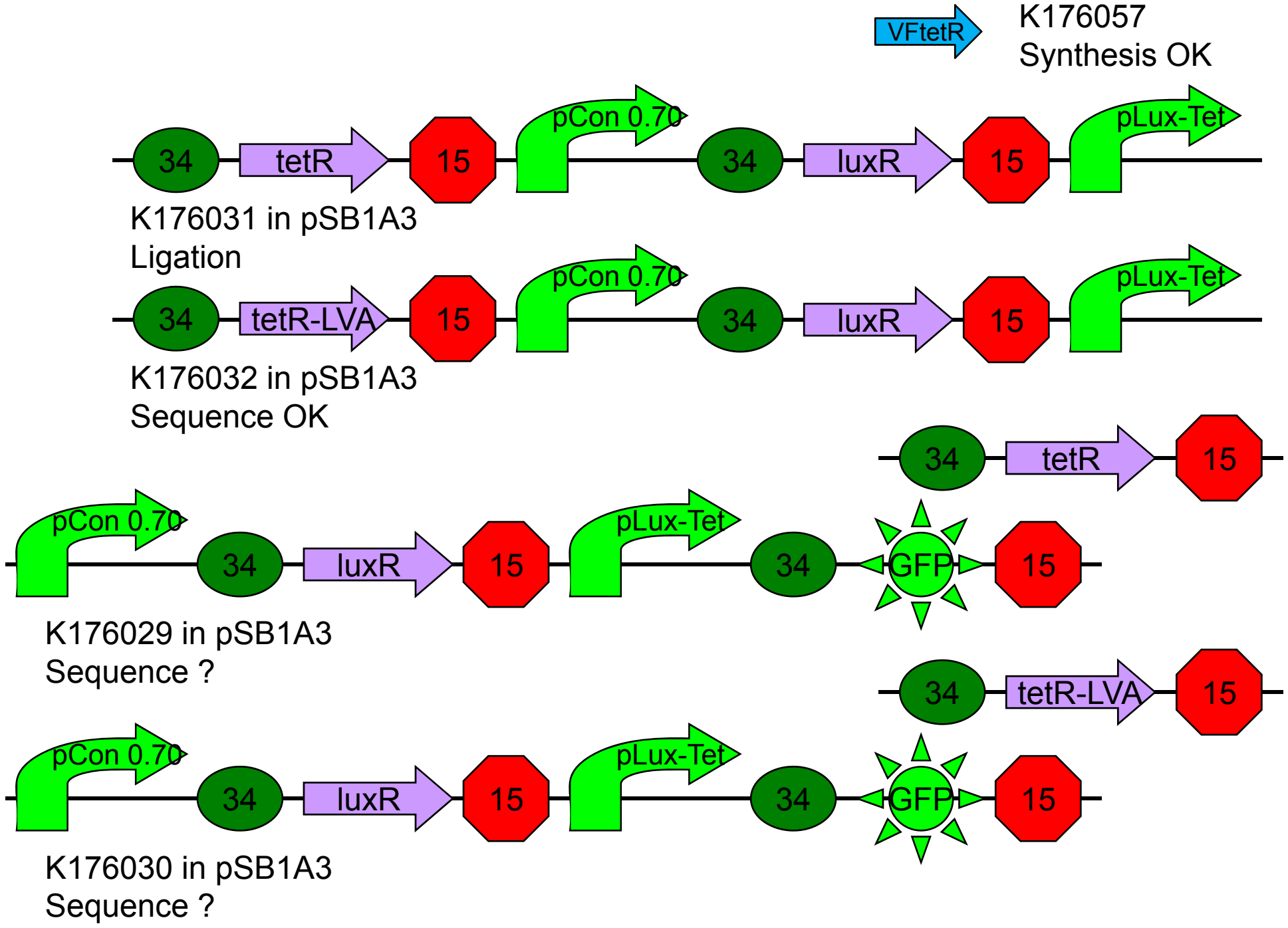


K176090 in pSB1A3 in TOP10
Waiting

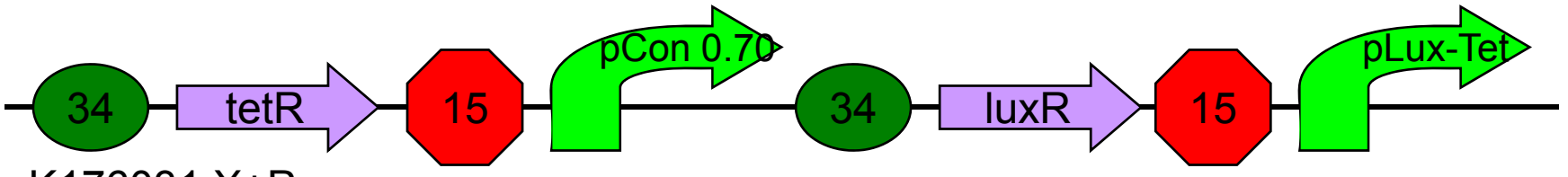

11
34
tetR
I732081 in pSB1A3
Sequence OK
34
tetR-LVA
S0101 in pSB1A2
Sequence OK
34
tetR
I732081 E+S
Sequence OK
34
tetR-LVA
S0101 E+S
Sequence OK


34
tetR
15
K176001 in pSB1AK3
Sequence OK
34
tetR-LVA
15
K176006 in pSB1AK3
Sequence OK

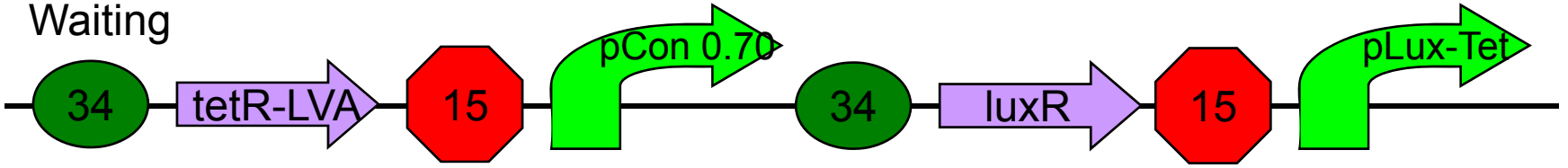

12
34
tetR
15
K176001 E+S
Length OK
34
tetR-LVA
15
K176006 E+S
Length OK
pCon 0.70
34
luxR
15
pLux-Tet
K176028 in pSB1A3 E+X
Length OK
pCon 0.70
34
luxR
15
pLux-Tet
34
GFP
15
K176026 in pSB1A3 E+X
Length OK


VFtetR
K176057
Synthesis OK
34
tetR
15
pCon 0.70
34
luxR
15
pLux-Tet
K176031 in pSB1A3
Ligation
34
tetR-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K176032 in pSB1A3
Sequence OK
34
tetR
15
pCon 0.70
34
luxR
15
pLux-Tet
34
GFP
15
K176029 in pSB1A3
Sequence ?
34
tetR-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
34
GFP
15
K176030 in pSB1A3
Sequence ?

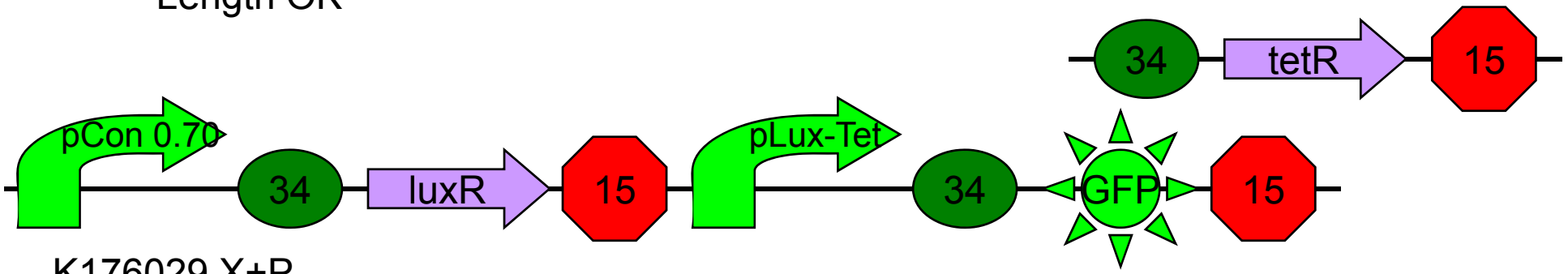
13



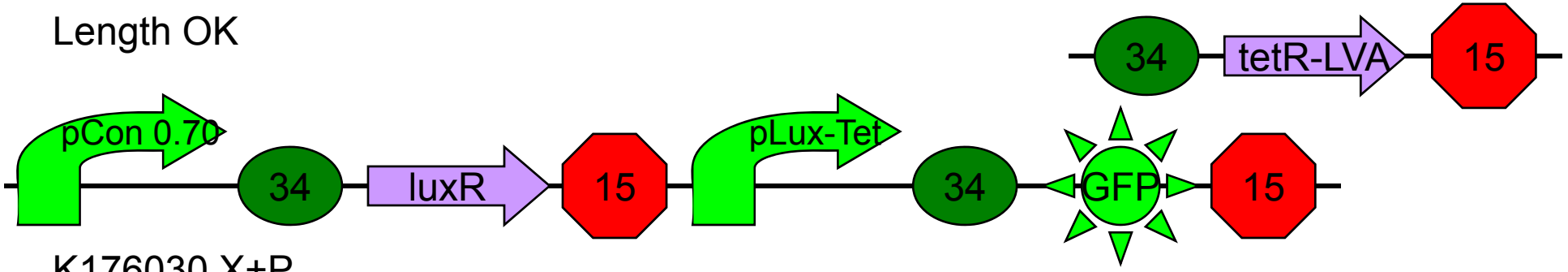
K176031 X+P
Waiting



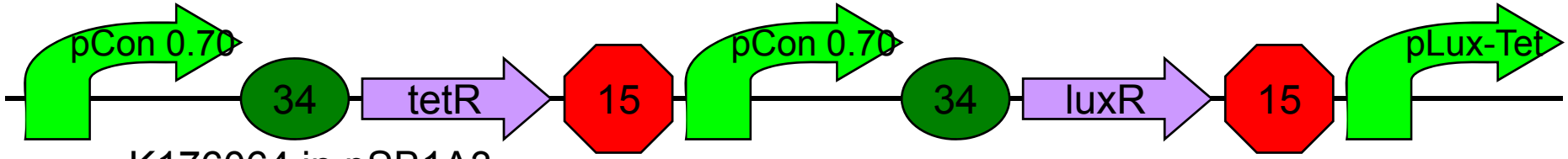
K176032 X+P
Length OK



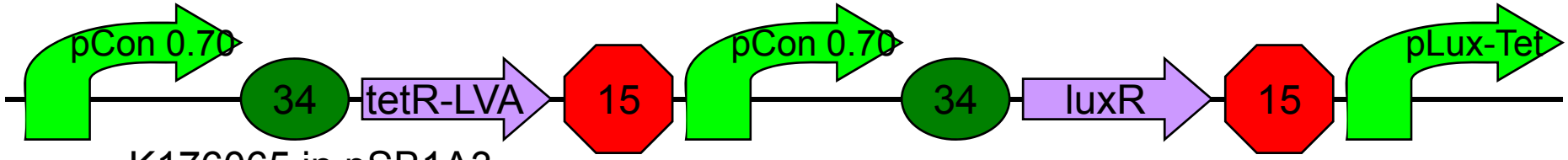
K176029 X+P
Length OK



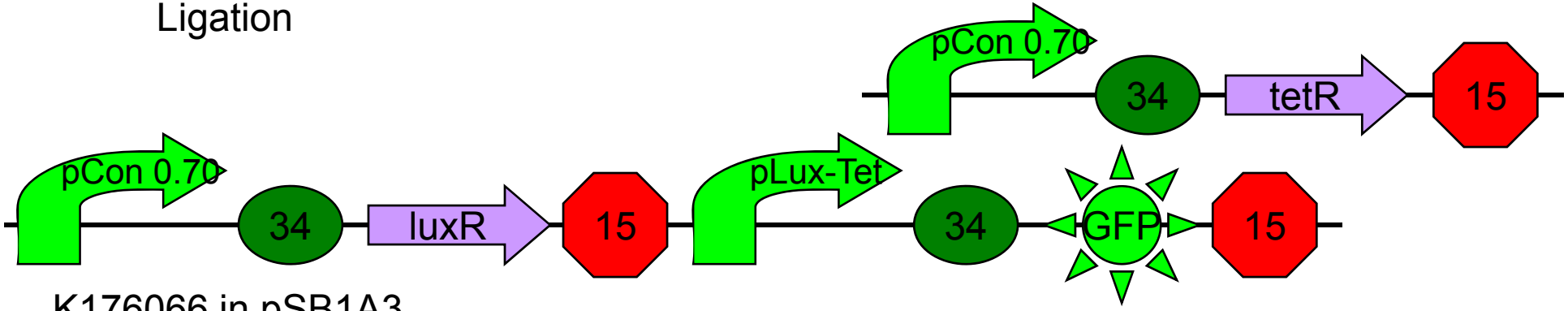
K176030 X+P
Length OK



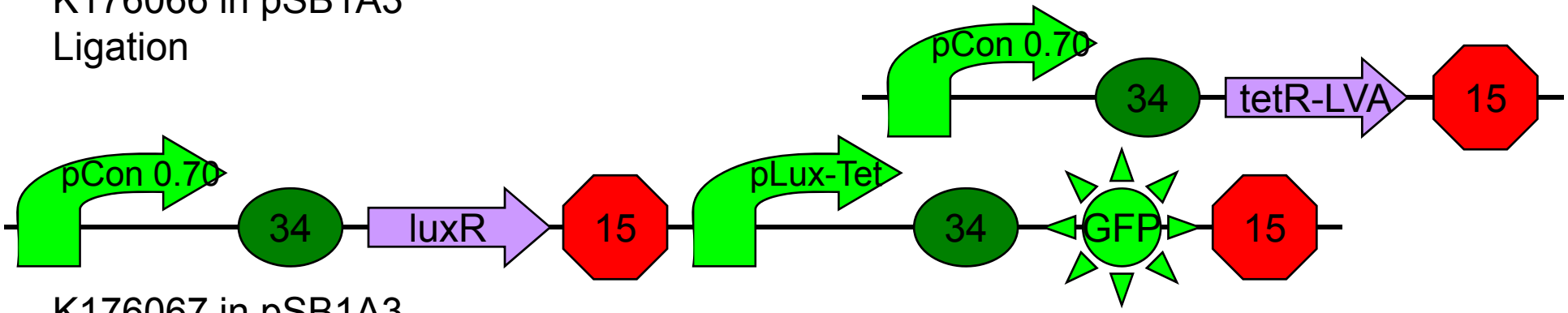
K176064 in pSB1A3
Waiting



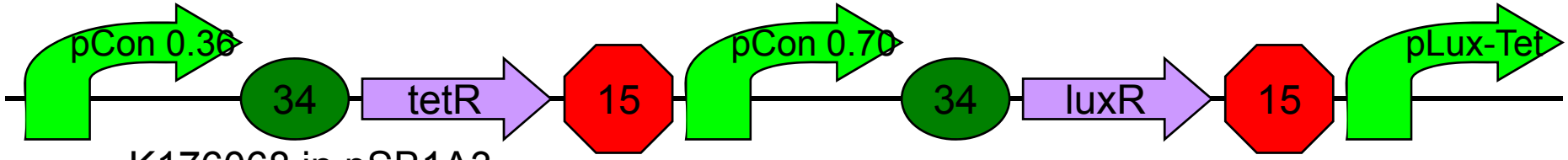
K176065 in pSB1A3
Ligation



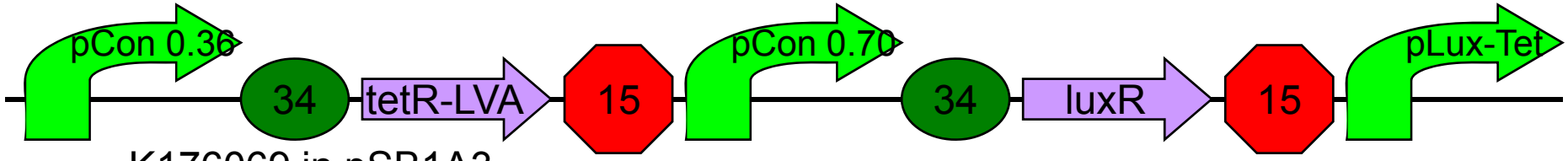
K176066 in pSB1A3
Ligation



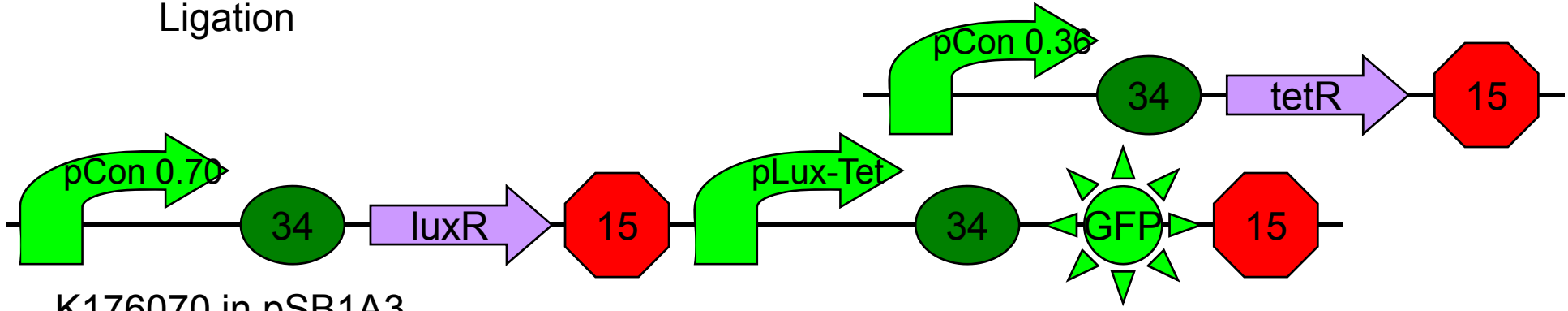
K176067 in pSB1A3
Ligation



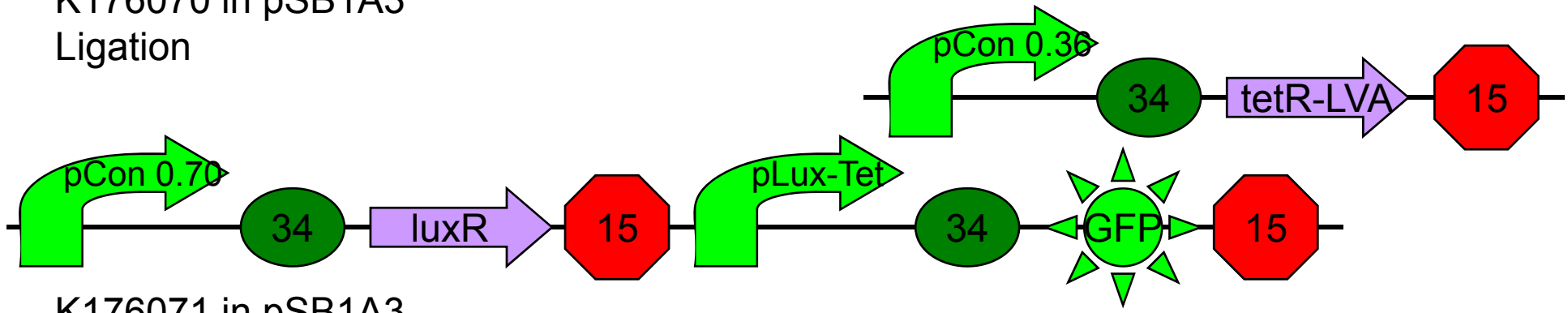
K176068 in pSB1A3
Waiting



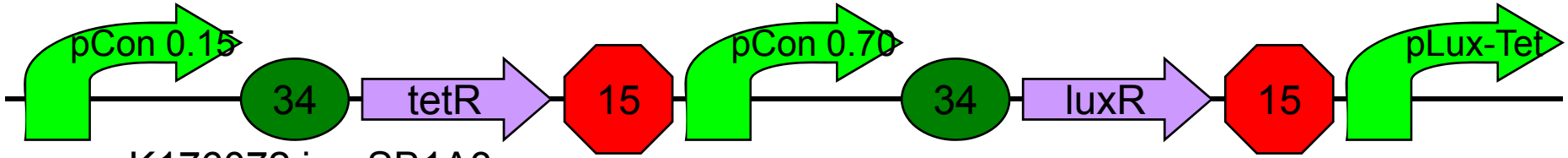
K176069 in pSB1A3
Ligation



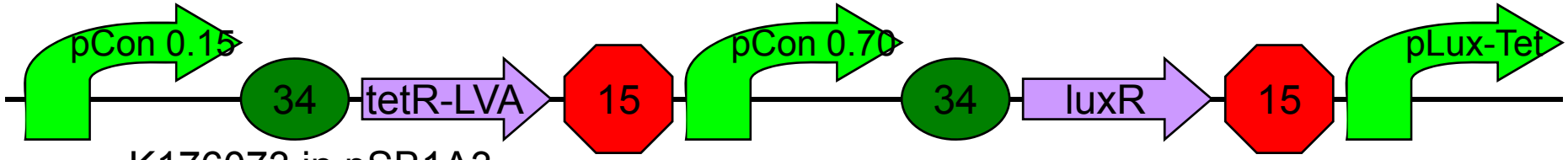
K176070 in pSB1A3
Ligation



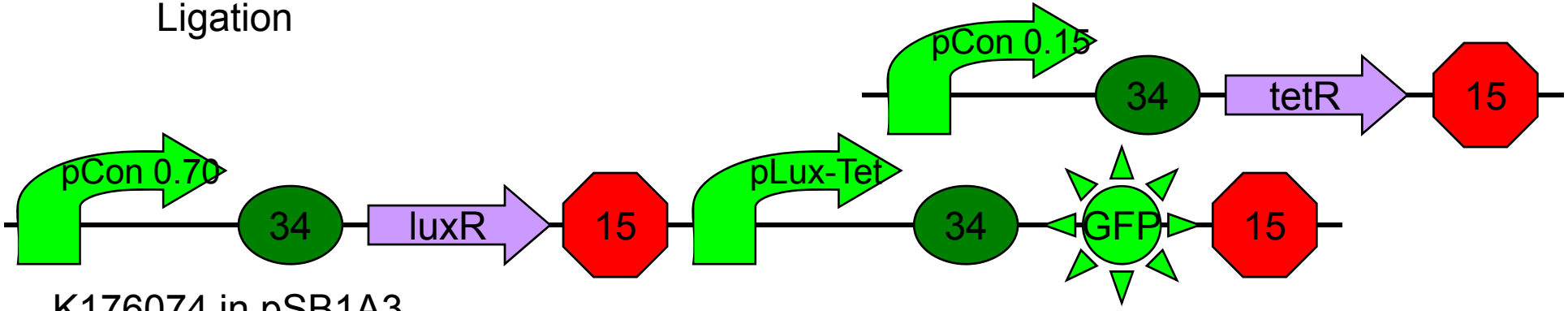
K176071 in pSB1A3
Ligation



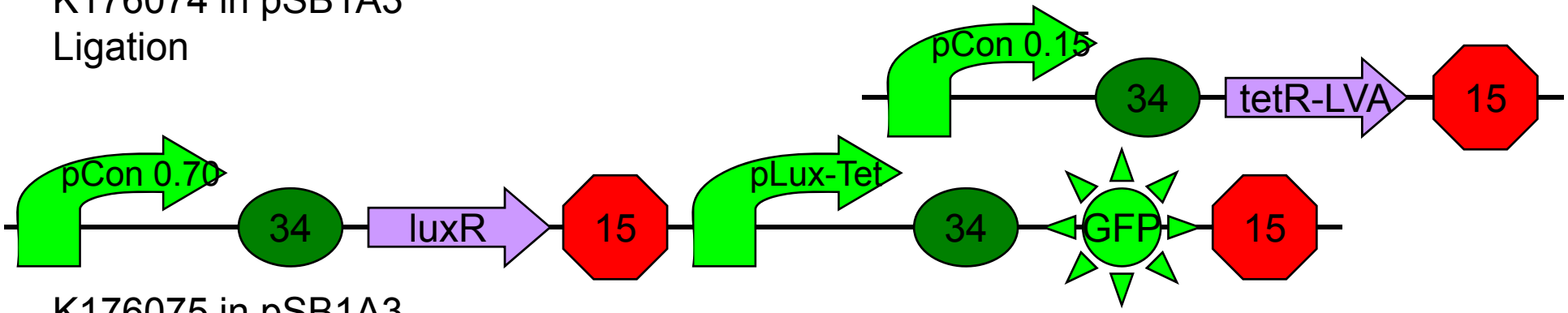
K176072 in pSB1A3
Waiting



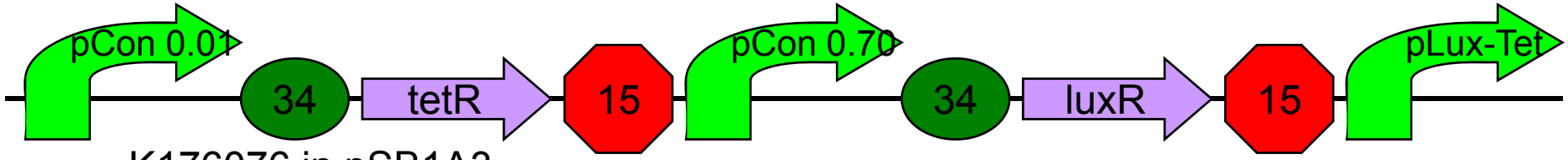
K176073 in pSB1A3
Ligation



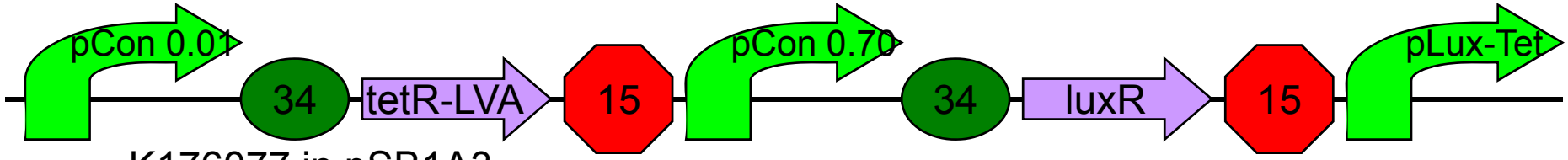
K176074 in pSB1A3
Ligation



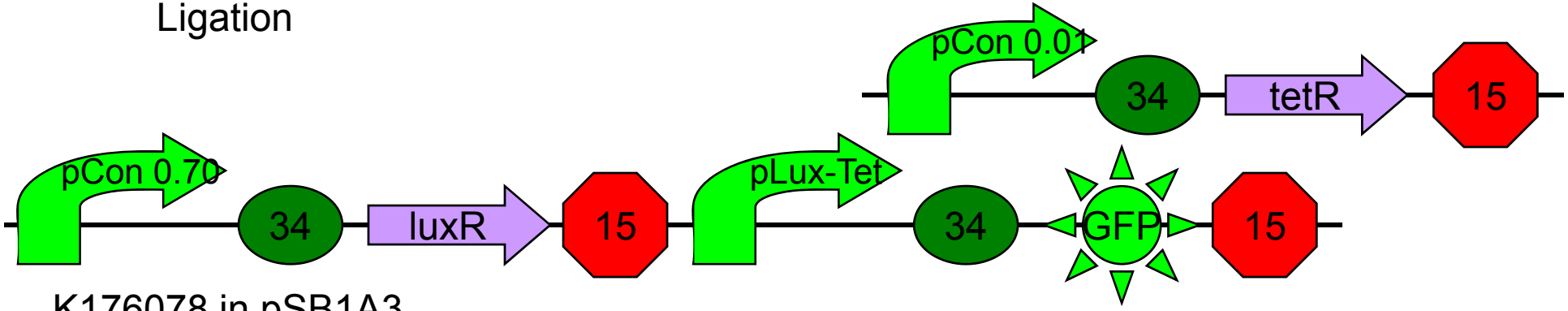
K176075 in pSB1A3
Sequencing



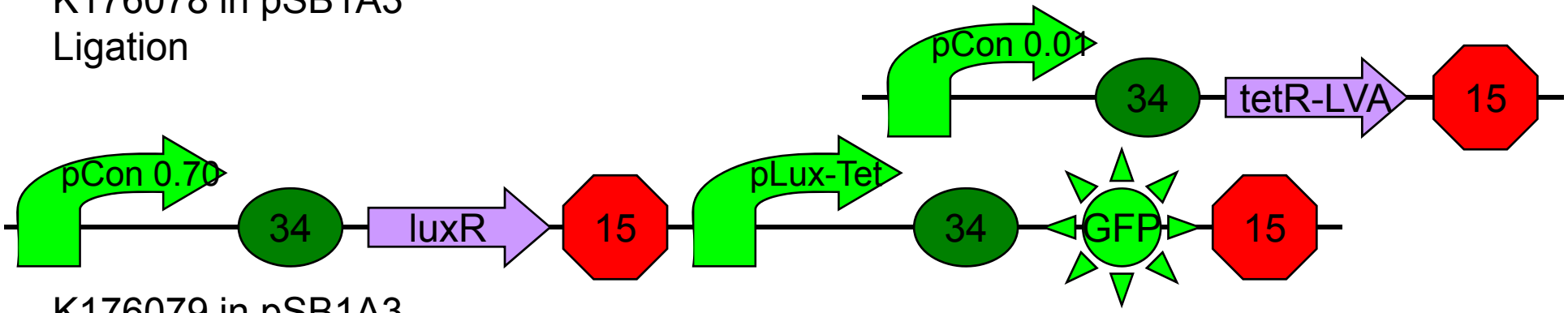
K176076 in pSB1A3
Waiting



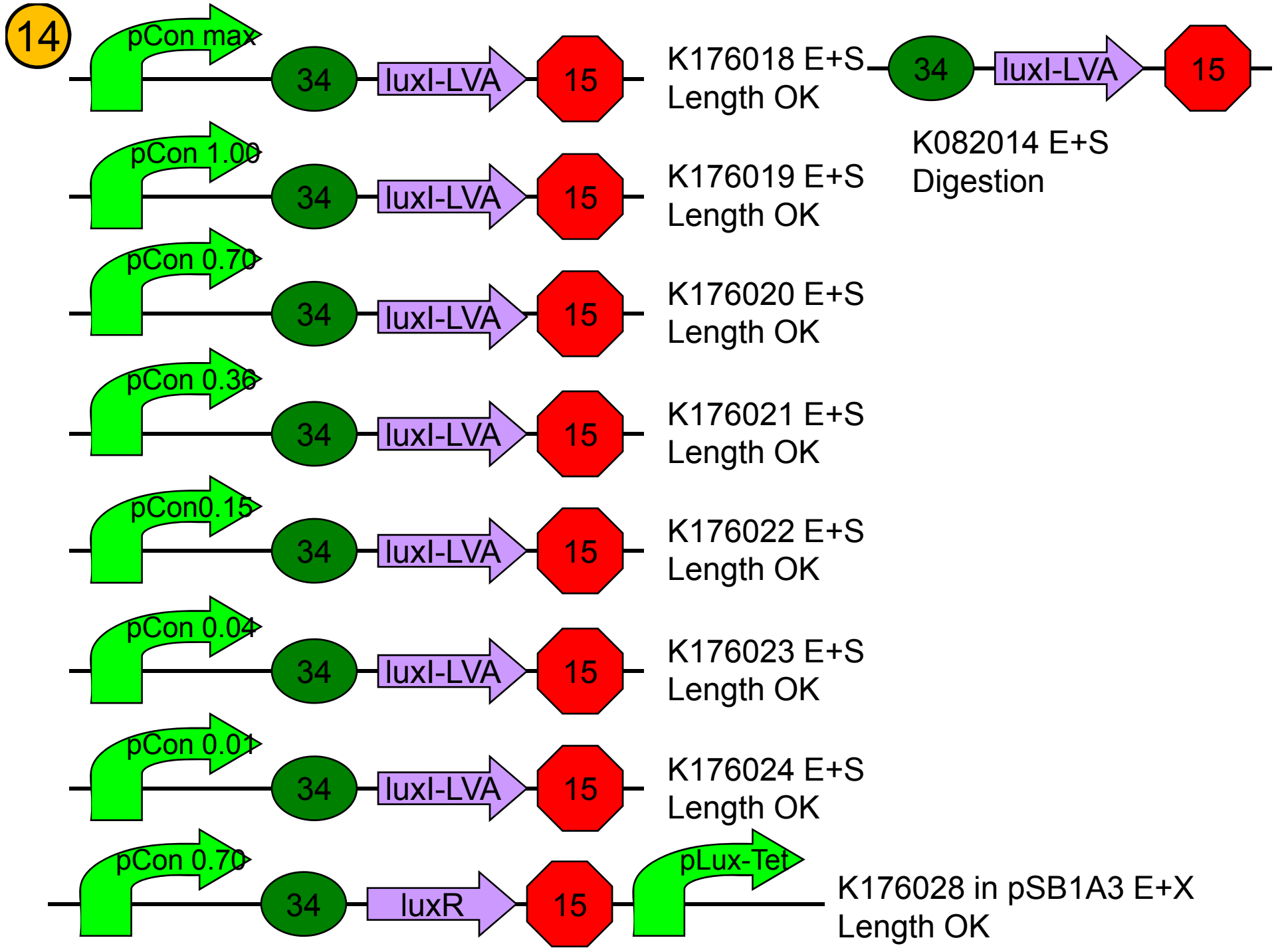
K176077 in pSB1A3
Ligation

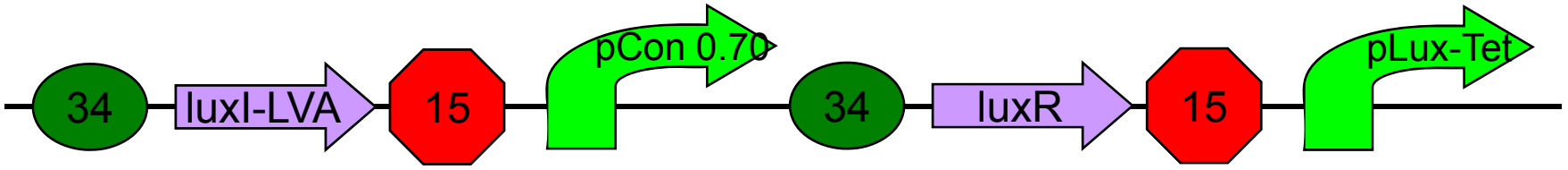


K176078 in pSB1A3
Ligation

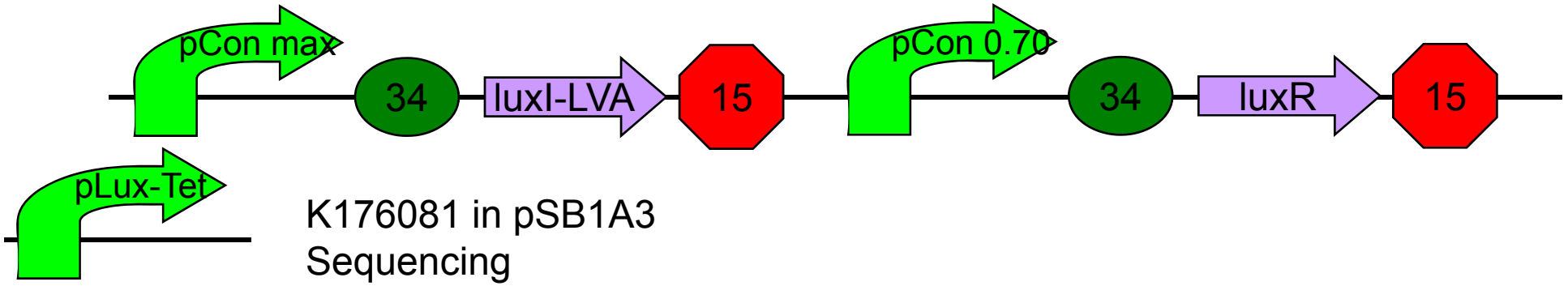


K176079 in pSB1A3
Sequence ?

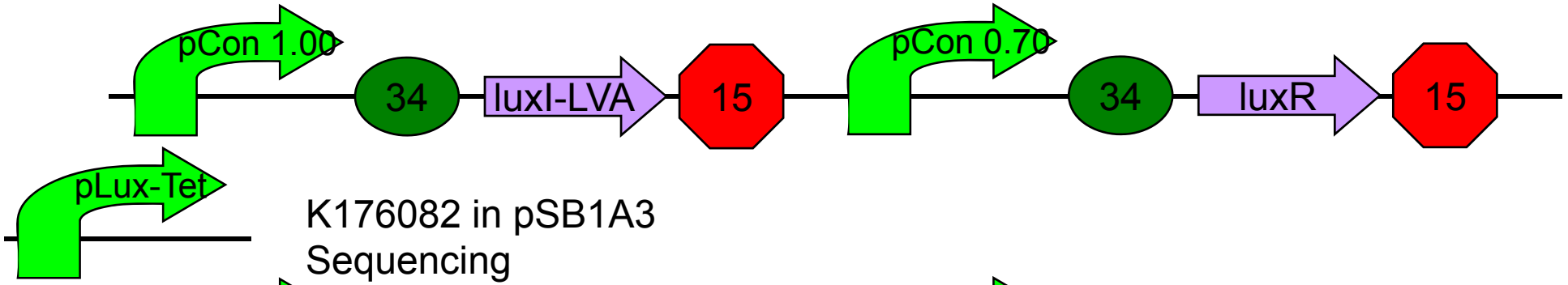

14
pCon max
34
luxI-LVA
15
K176018 E+S
Length OK
34
luxI-LVA
15
K082014 E+S
Digestion
pCon 1.00
34
luxI-LVA
15
K176019 E+S
Length OK
pCon 0.70
34
luxI-LVA
15
K176020 E+S
Length OK
pCon 0.36
34
luxI-LVA
15
K176021 E+S
Length OK
pCon0.15
34
luxI-LVA
15
K176022 E+S
Length OK
pCon 0.04
34
luxI-LVA
15
K176023 E+S
Length OK
pCon 0.01
34
luxI-LVA
15
K176024 E+S
Length OK
pCon 0.70
34
luxR
15
pLux-Tet
K176028 in pSB1A3 E+X
Length OK



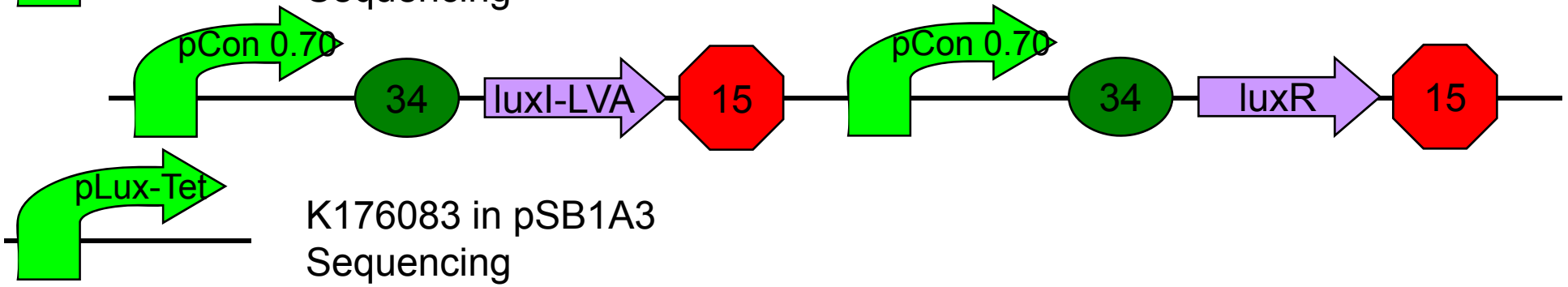
K176080 in pSB1A3
Waiting



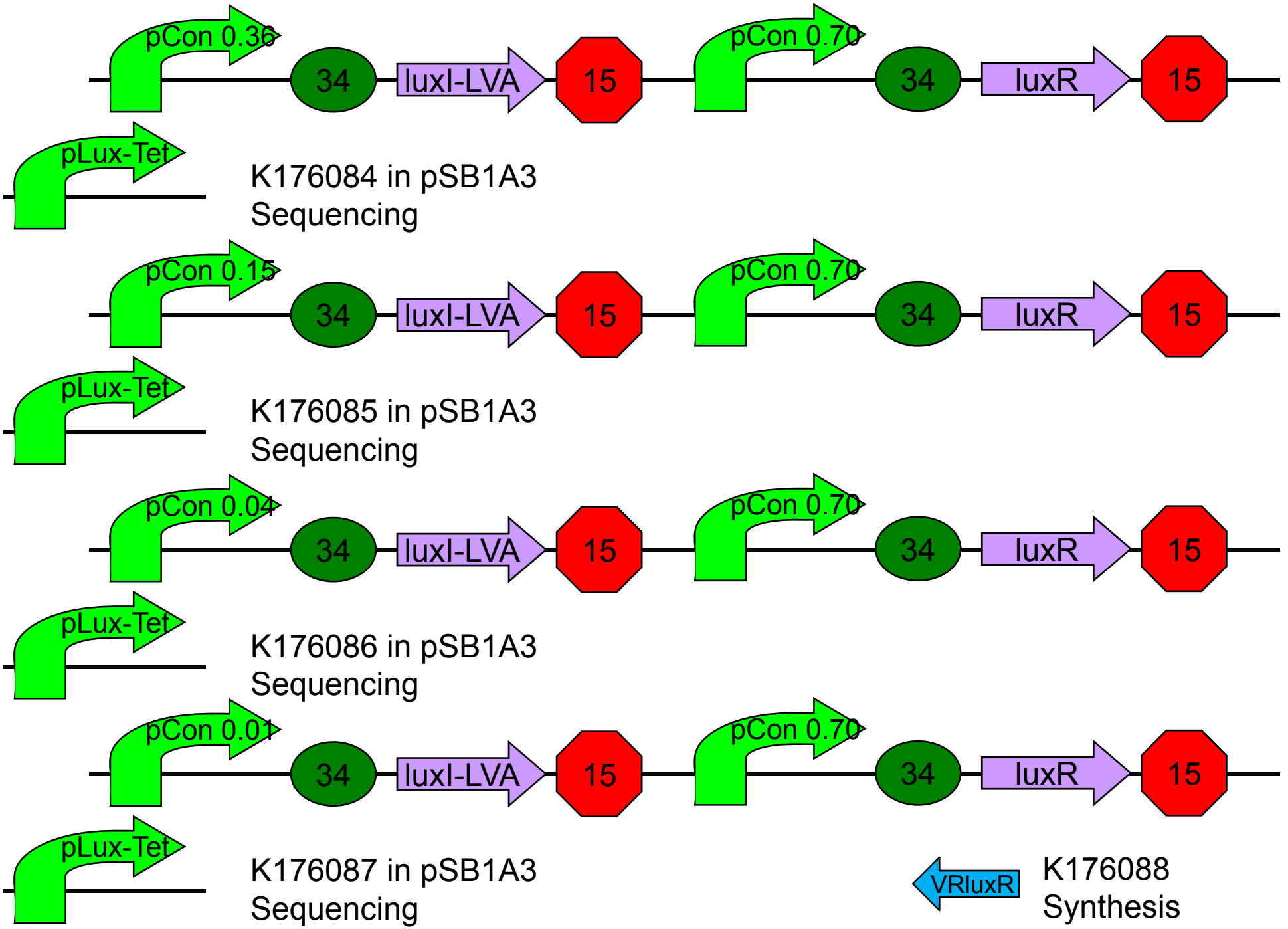
K176081 in pSB1A3
Sequencing

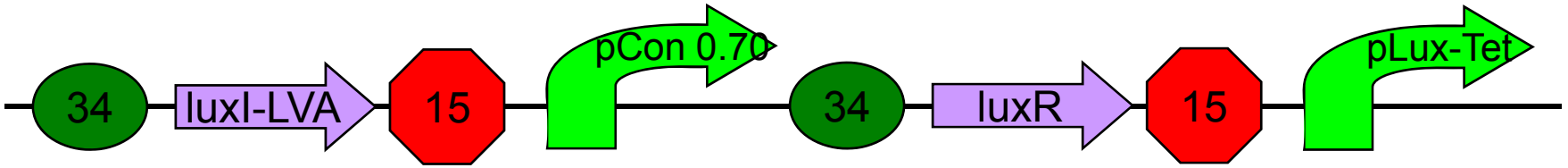


K176082 in pSB1A3
Sequencing

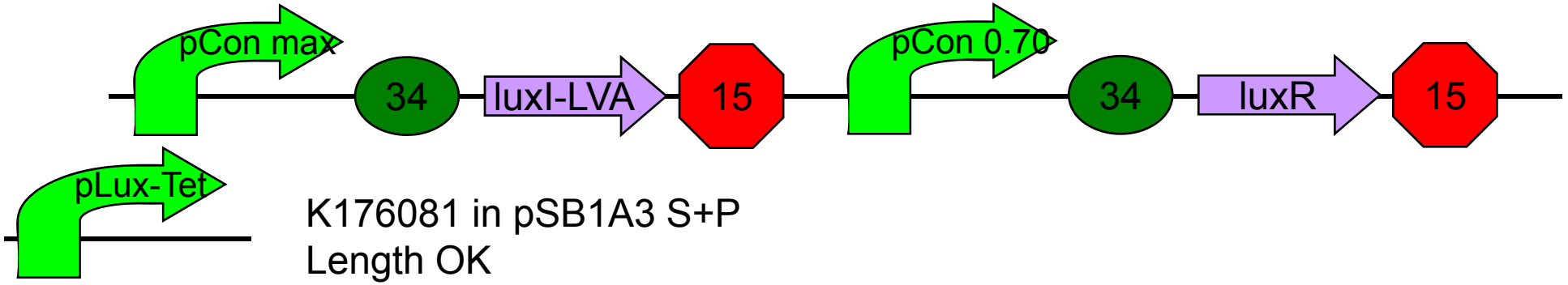


K176083 in pSB1A3
Sequencing

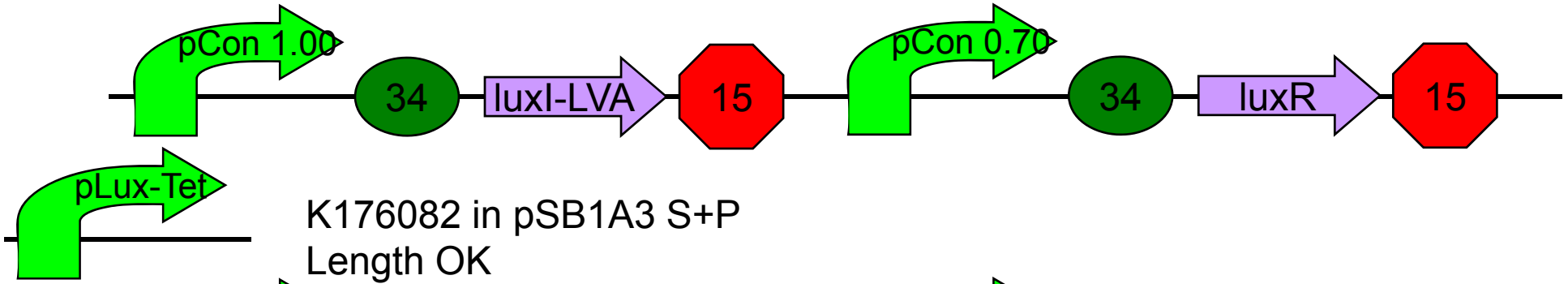

pCon 0.36
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K176084 in pSB1A3
Sequencing
pCon 0.15
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K176085 in pSB1A3
Sequencing
pCon 0.04
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K176086 in pSB1A3
Sequencing
pCon 0.01
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K176087 in pSB1A3
Sequencing
VRluxR
K176088
Synthesis



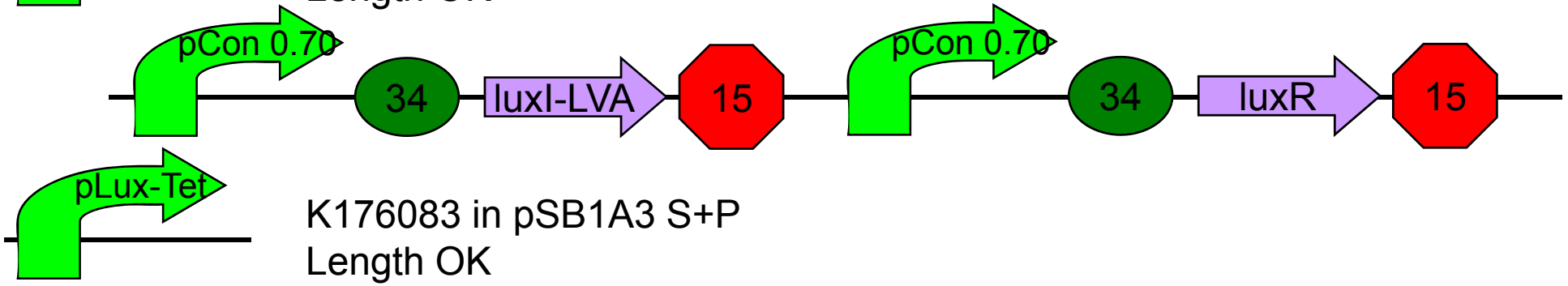
K176080 in pSB1A3 S+P
Waiting



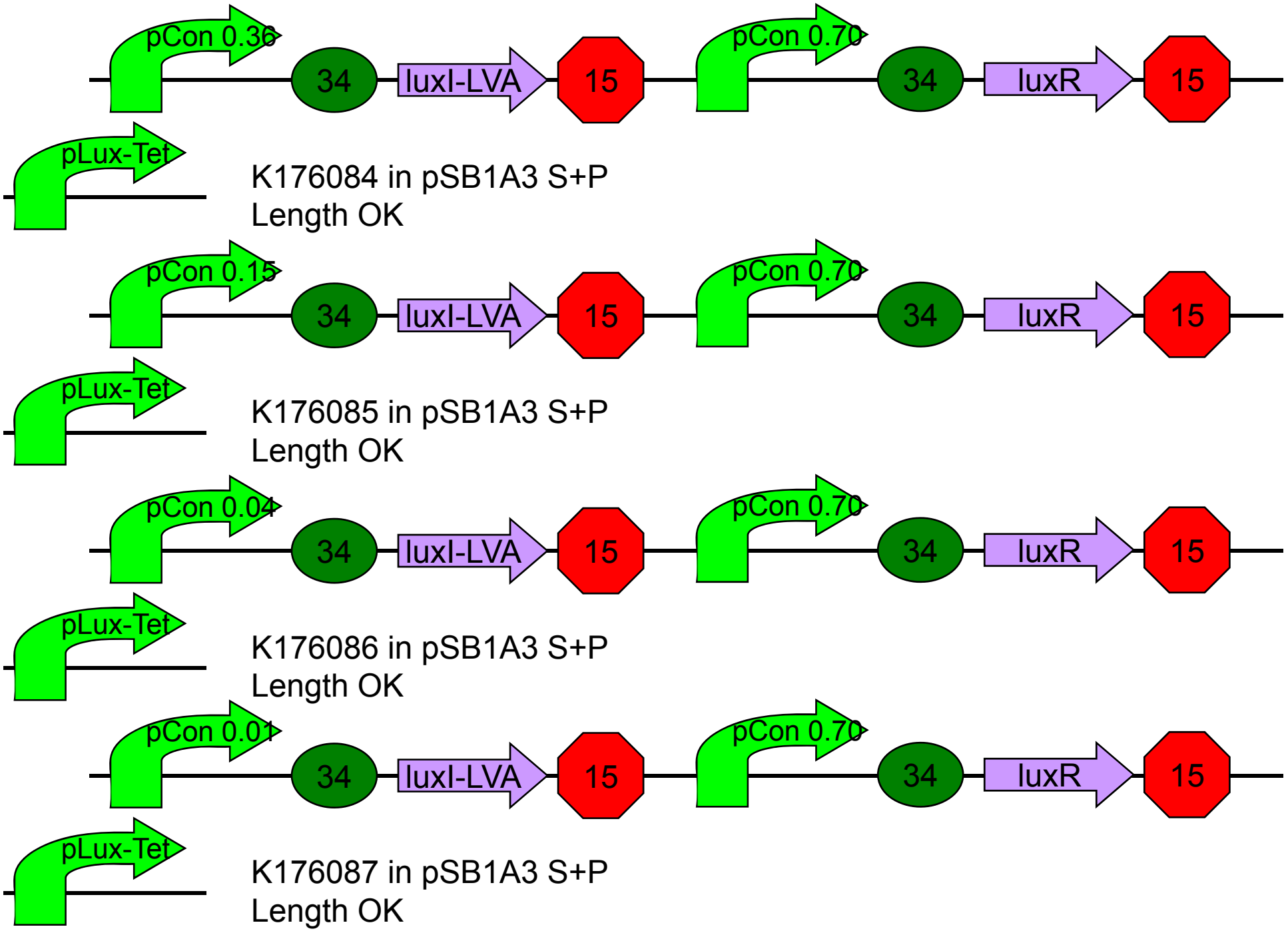
K176081 in pSB1A3 S+P
Length OK

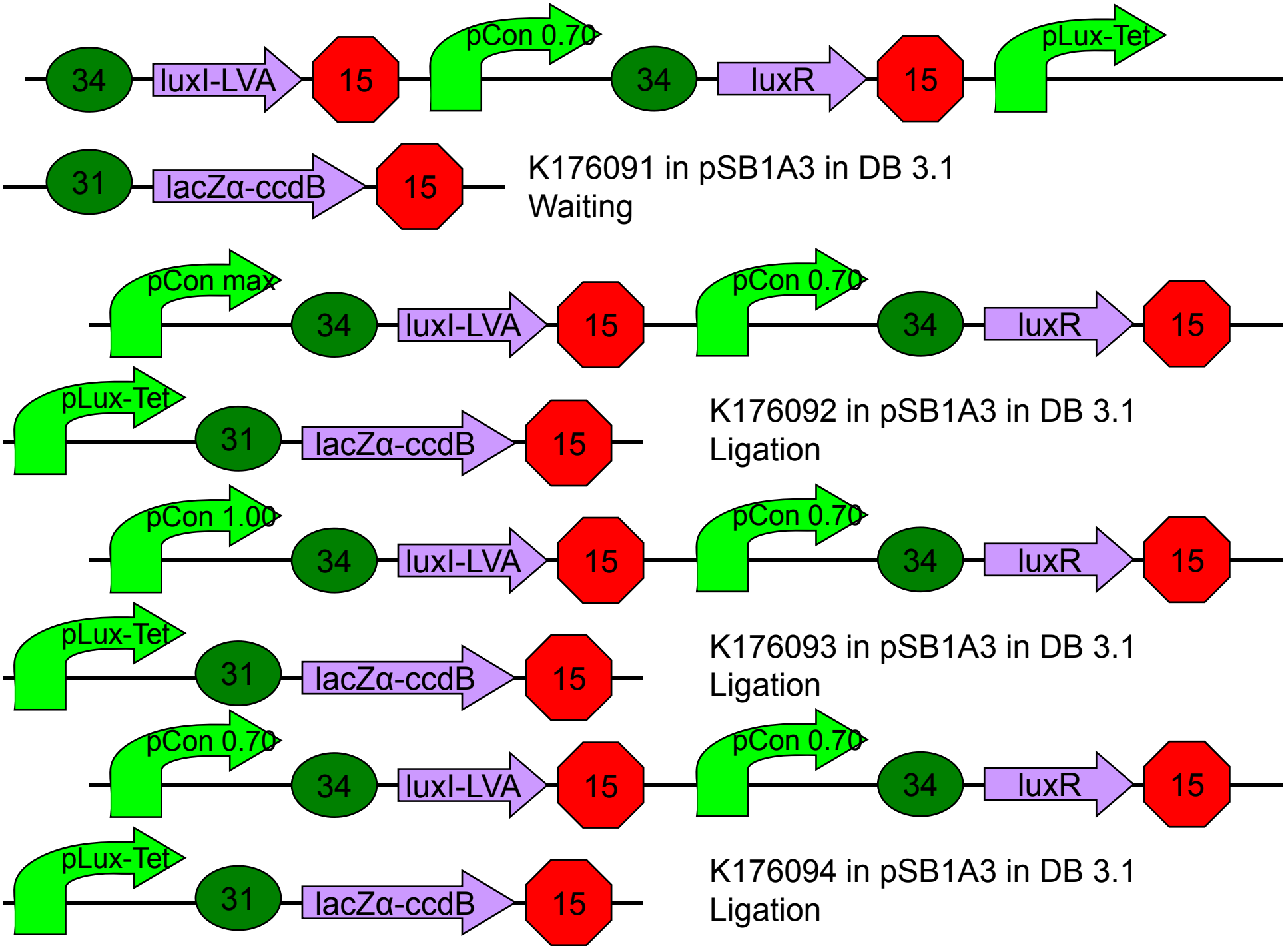


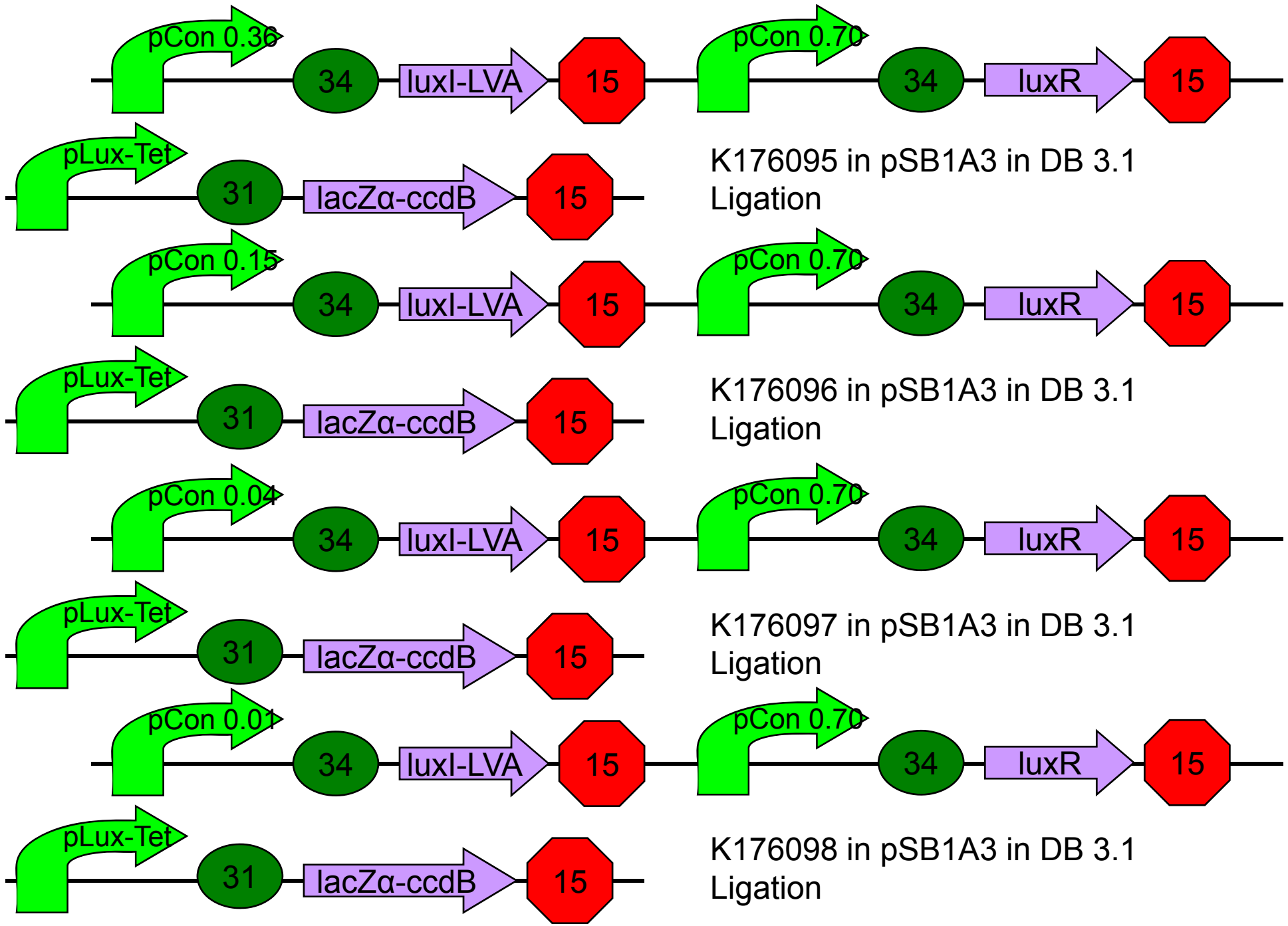
K176082 in pSB1A3 S+P
Length OK

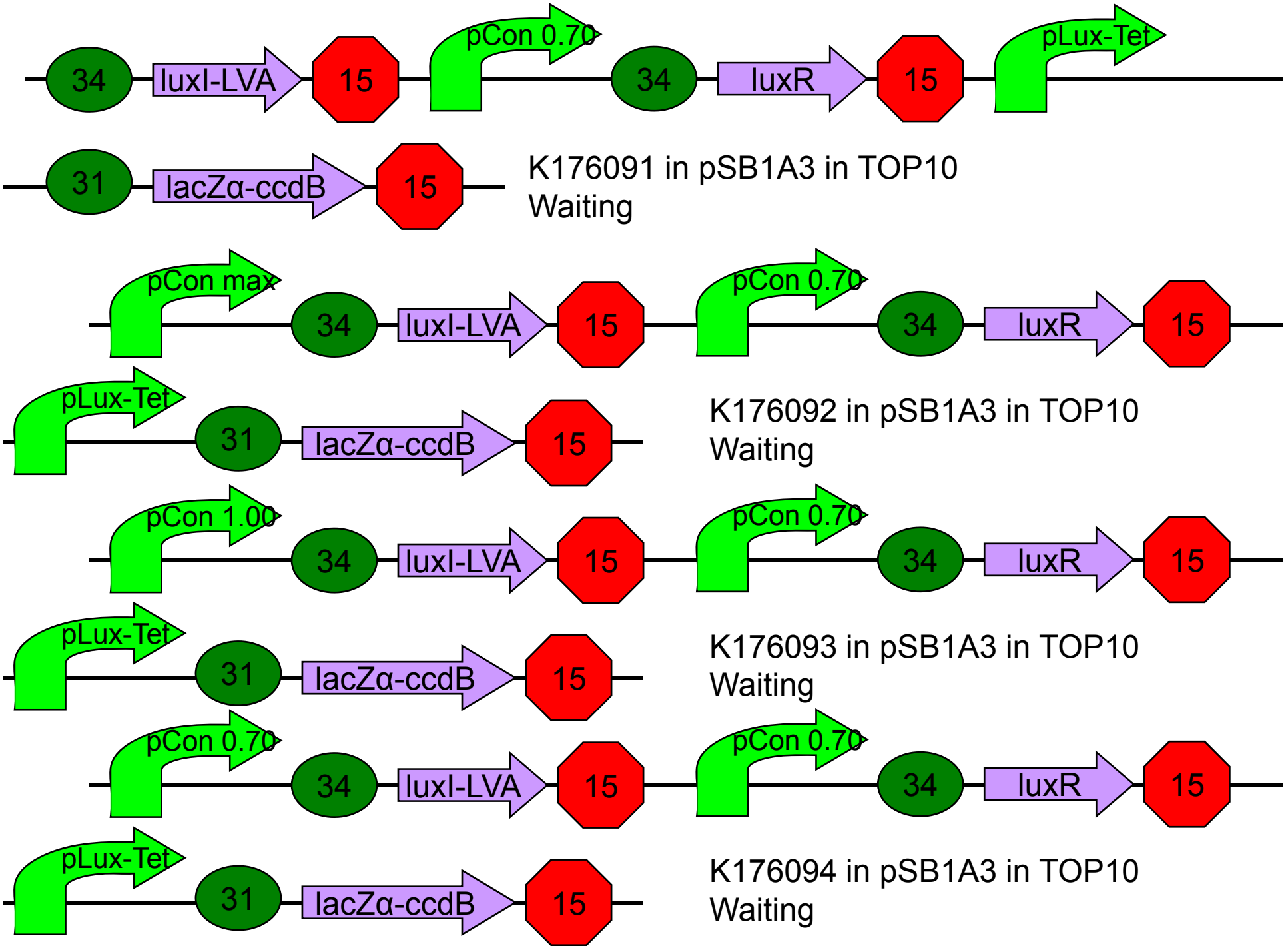


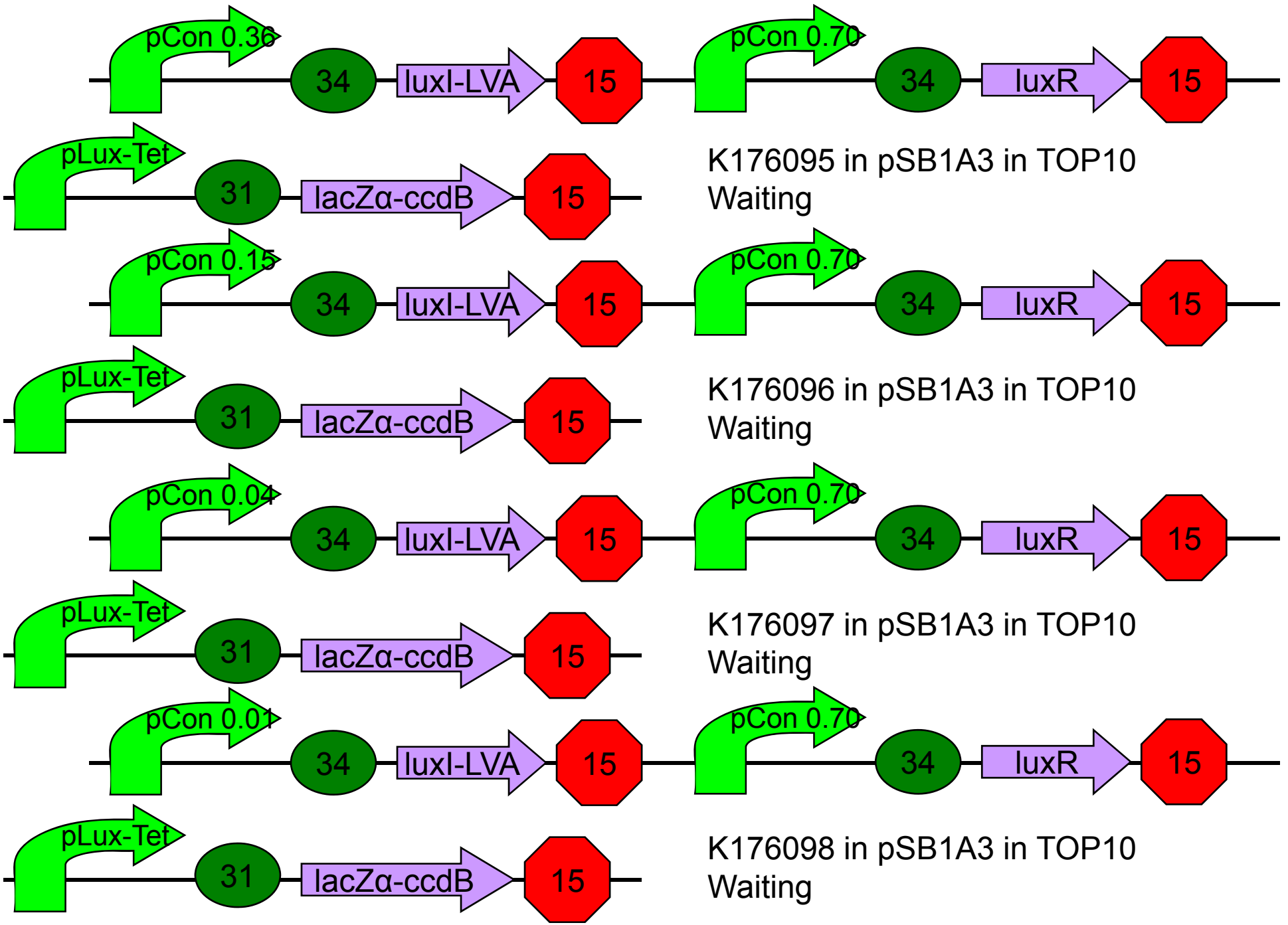
K176083 in pSB1A3 S+P
Length OK

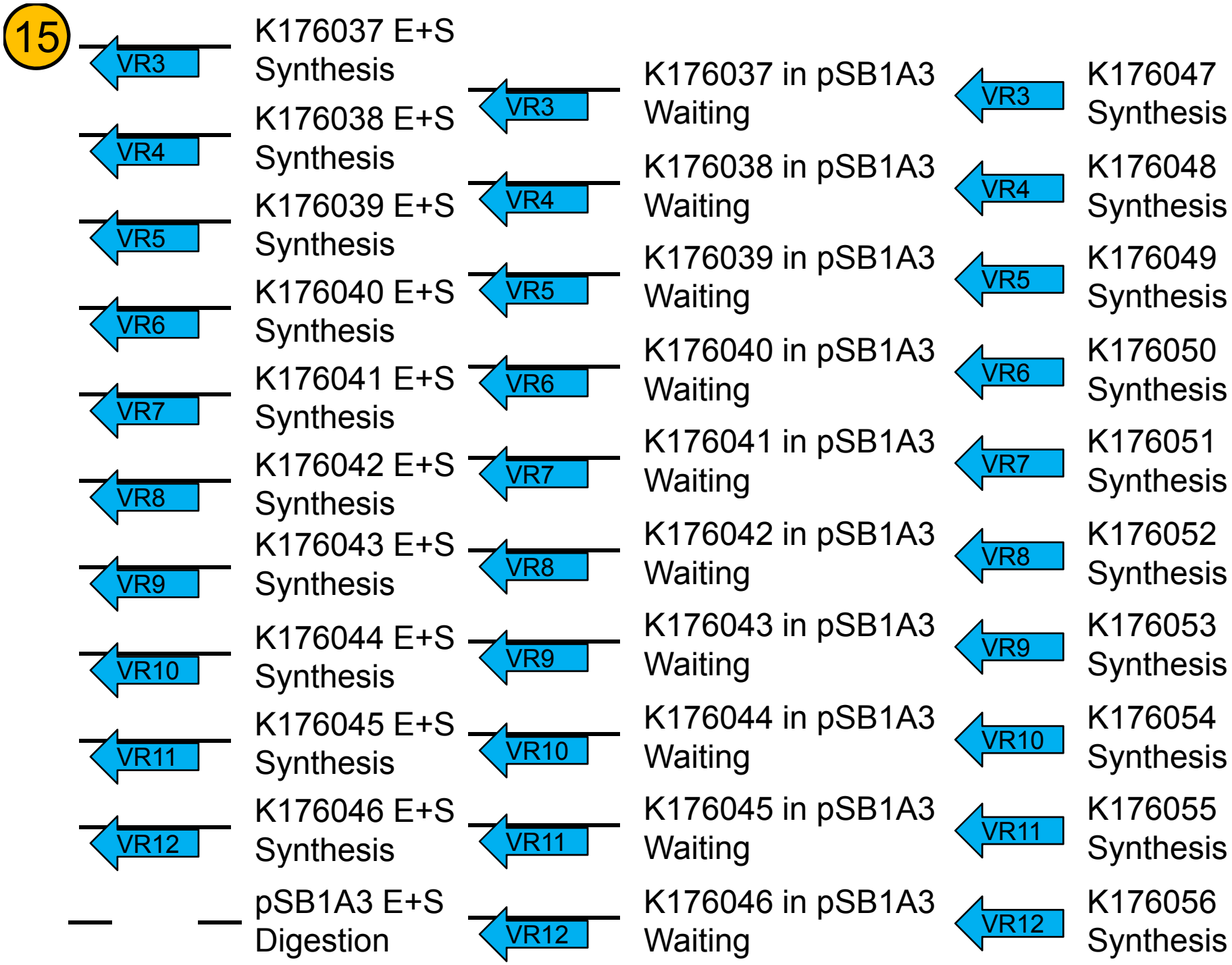

pCon 0.36
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K176084 in pSB1A3 S+P
Length OK
pCon 0.15
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K176085 in pSB1A3 S+P
Length OK
pCon 0.04
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K176086 in pSB1A3 S+P
Length OK
pCon 0.01
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K176087 in pSB1A3 S+P
Length OK


34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176091 in pSB1A3 in DB 3.1
Waiting
pCon max
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176092 in pSB1A3 in DB 3.1
Ligation
pCon 1.00
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176093 in pSB1A3 in DB 3.1
Ligation
pCon 0.70
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176094 in pSB1A3 in DB 3.1
Ligation

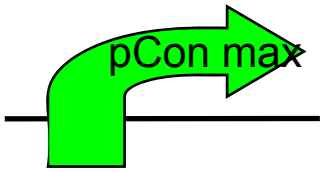

pCon 0.36
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176095 in pSB1A3 in DB 3.1 Ligation
pCon 0.15
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176096 in pSB1A3 in DB 3.1 Ligation
pCon 0.04
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176097 in pSB1A3 in DB 3.1 Ligation
pCon 0.01
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176098 in pSB1A3 in DB 3.1 Ligation


34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176091 in pSB1A3 in TOP10
Waiting
pCon max
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176092 in pSB1A3 in TOP10
Waiting
pCon 1.00
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176093 in pSB1A3 in TOP10
Waiting
pCon 0.70
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176094 in pSB1A3 in TOP10
Waiting

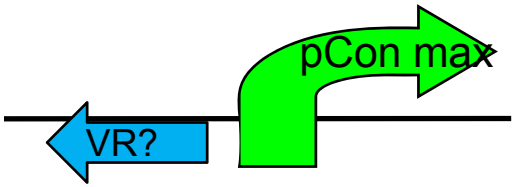

pCon 0.36
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176095 in pSB1A3 in TOP10
Waiting
pCon 0.15
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176096 in pSB1A3 in TOP10
Waiting
pCon 0.04
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176097 in pSB1A3 in TOP10
Waiting
pCon 0.01
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
lacZα-ccdB
15
K176098 in pSB1A3 in TOP10
Waiting


15
VR3
K176037 E+S Synthesis
VR4
K176038 E+S Synthesis
VR5
K176039 E+S Synthesis
VR6
K176040 E+S Synthesis
VR7
K176041 E+S Synthesis
VR8
K176042 E+S Synthesis
VR9
K176043 E+S Synthesis
VR10
K176044 E+S Synthesis
VR11
K176045 E+S Synthesis
VR12
K176046 E+S Synthesis
pSB1A3 E+S Digestion
VR3
K176037 in pSB1A3 Waiting
VR4
K176038 in pSB1A3 Waiting
VR5
K176039 in pSB1A3 Waiting
VR6
K176040 in pSB1A3 Waiting
VR7
K176041 in pSB1A3 Waiting
VR8
K176042 in pSB1A3 Waiting
VR9
K176043 in pSB1A3 Waiting
VR10
K176044 in pSB1A3 Waiting
VR11
K176045 in pSB1A3 Waiting
VR12
K176046 in pSB1A3 Waiting
VR3
K176047 Synthesis
VR4
K176048 Synthesis
VR5
K176049 Synthesis
VR6
K176050 Synthesis
VR7
K176051 Synthesis
VR8
K176052 Synthesis
VR9
K176053 Synthesis
VR10
K176054 Synthesis
VR11
K176055 Synthesis
VR12
K176056 Synthesis

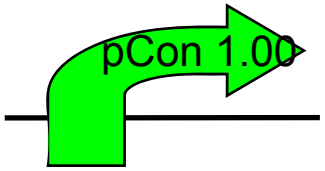
16



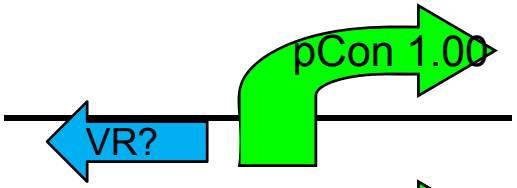
J23119 in pSB1A3 E+X
Digestion



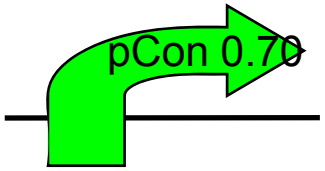
K176099 in pSB1A3
Waiting



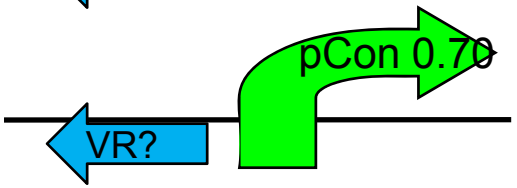
J23100 in K176007 E+X
Digestion



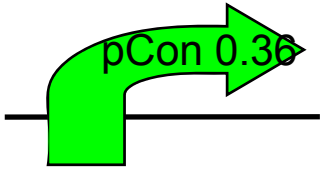
K176100 in K176007
Waiting



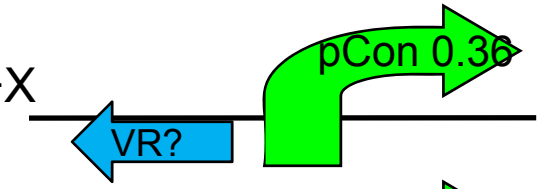
J23101 in K176007 E+X
Digestion



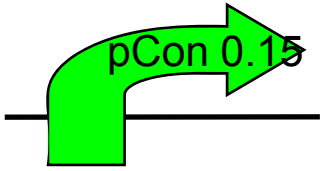
K176101 in K176007
Waiting



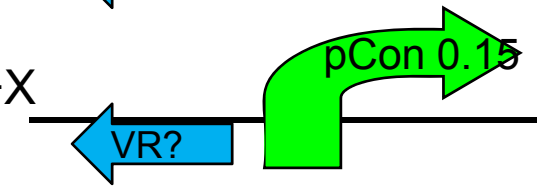
K176009 in K176007 E+X
Digestion



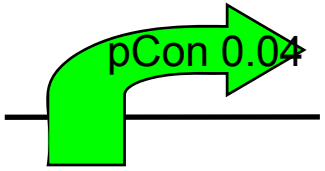
K176102 in K176007
Waiting



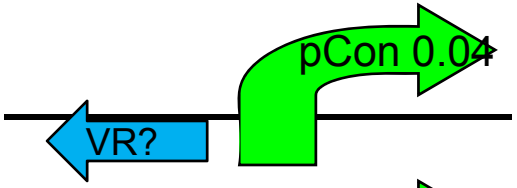
K176008 in K176007 E+X
Digestion



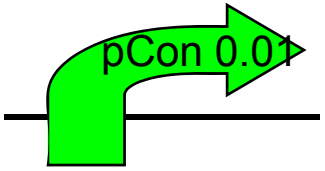
K176103 in K176007
Waiting



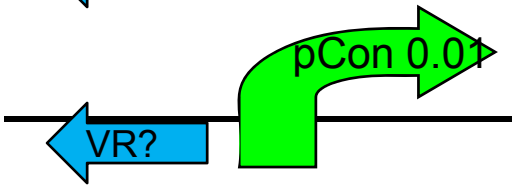
J23109 in K176007 E+X
Digestion



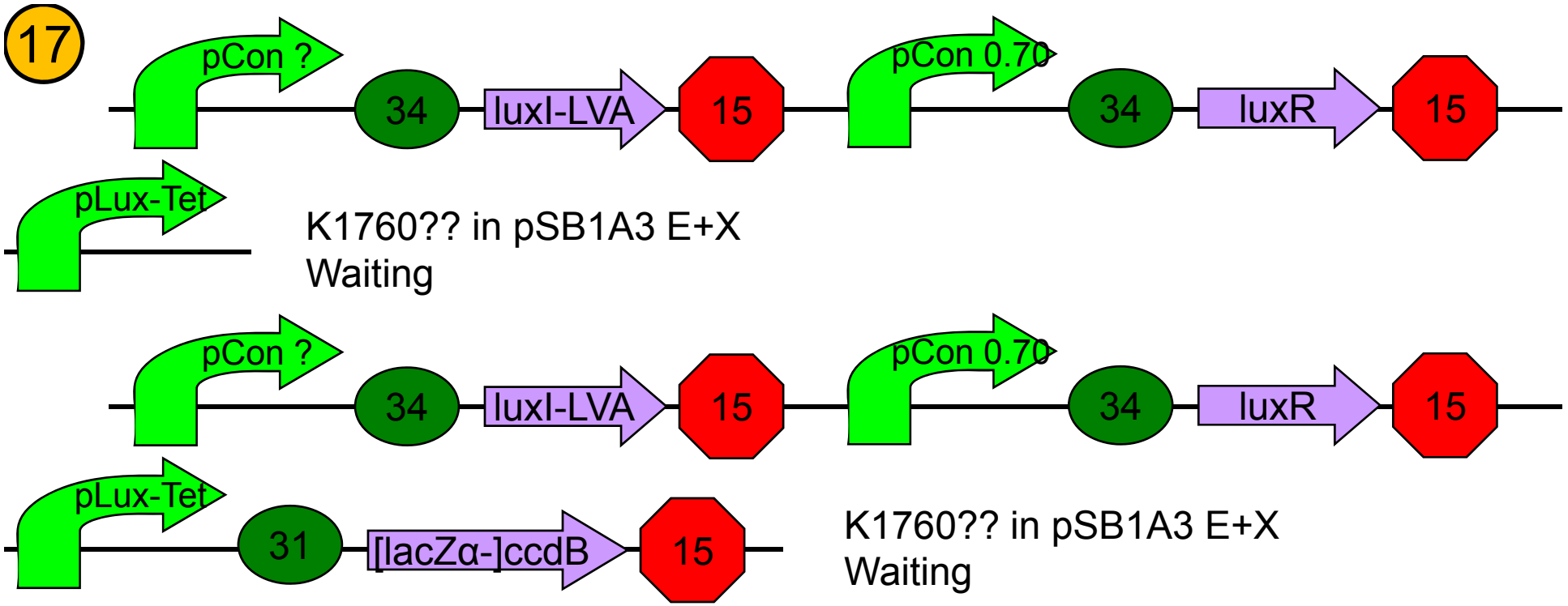
K176104 in K176007
Waiting

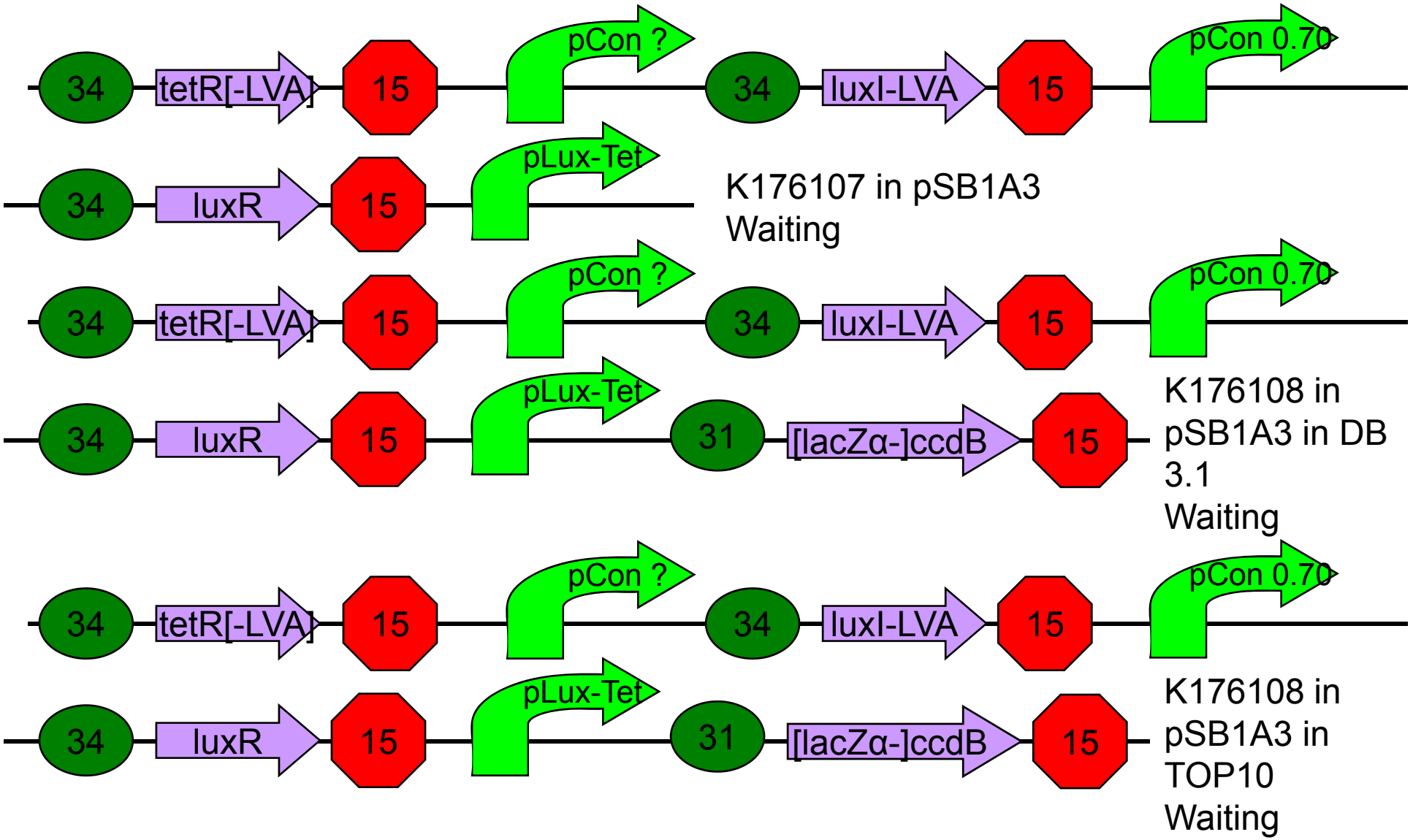


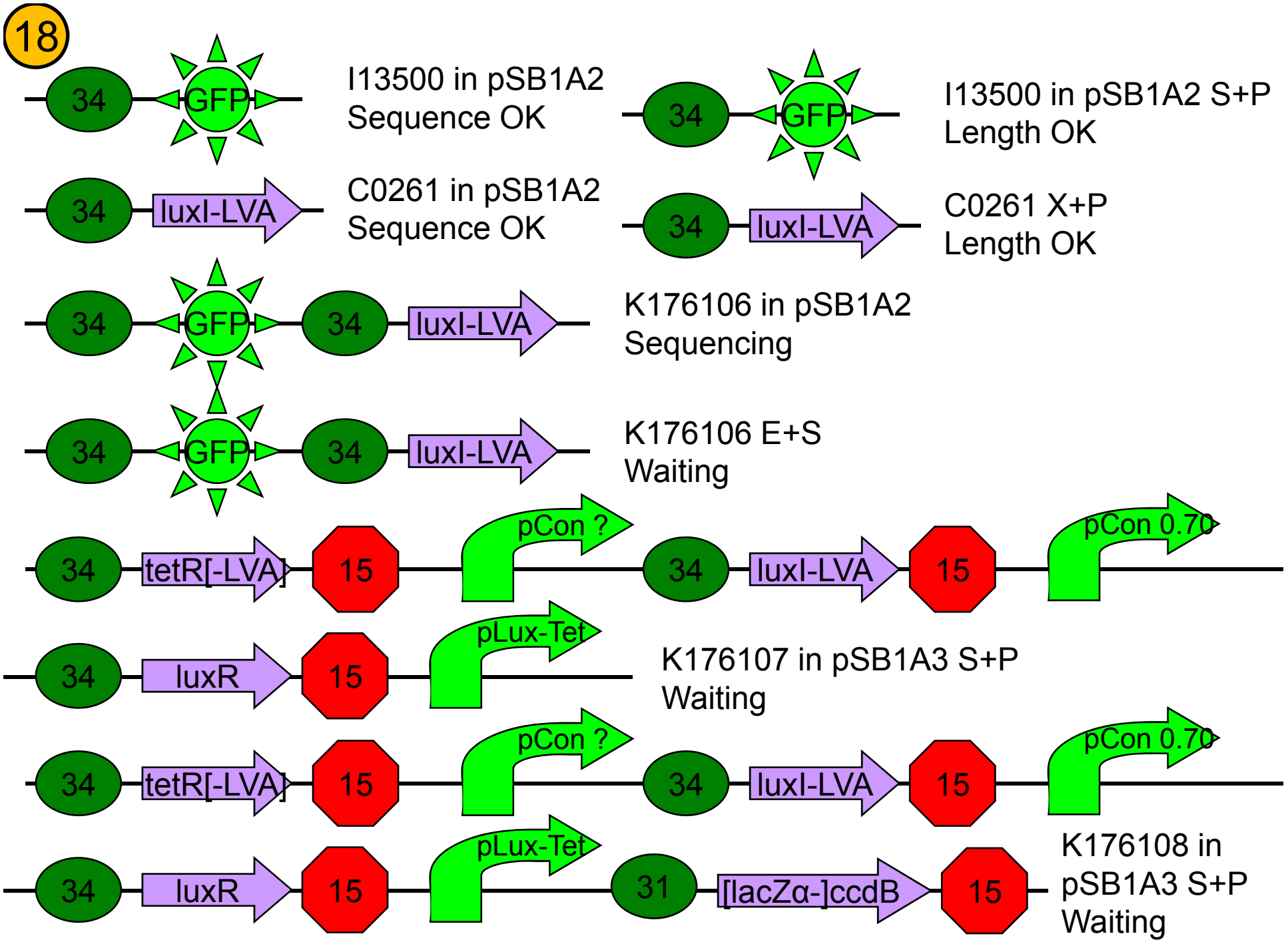
J23103 in K176007 E+X
Digestion

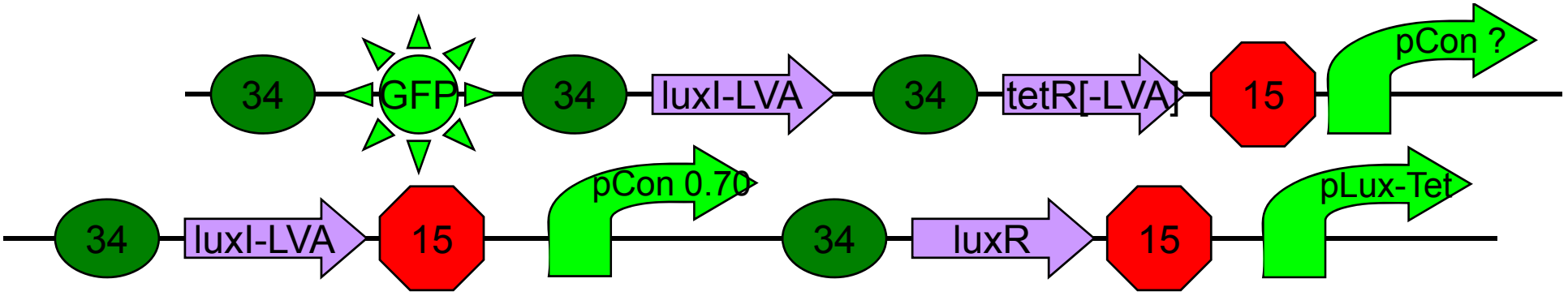


K176105 in K176007
Waiting

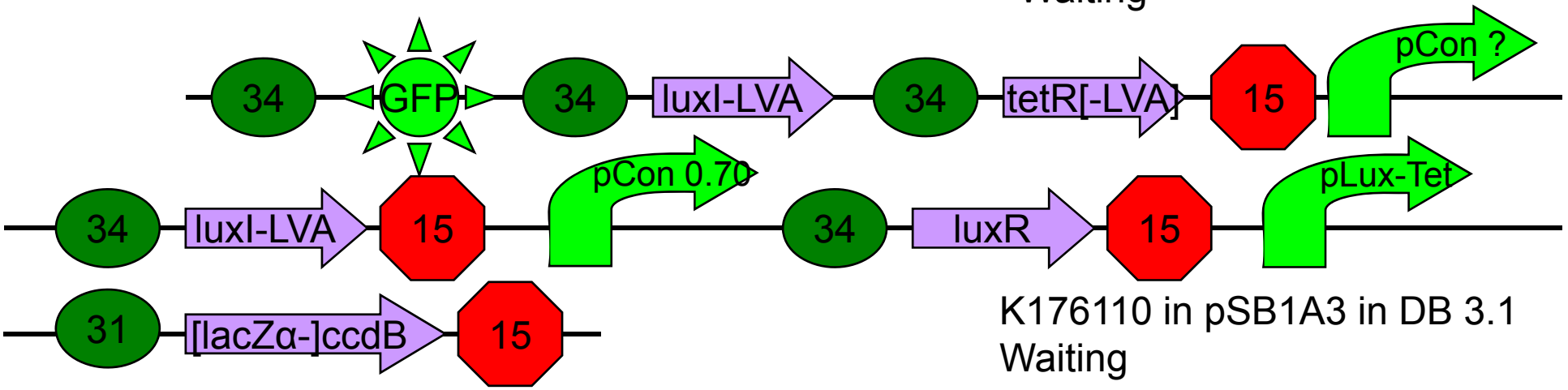

17
pCon ?
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K1760?? in pSB1A3 E+X
Waiting
pCon ?
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
[lacZα-]ccdB
15
K1760?? in pSB1A3 E+X
Waiting


34
tetR[-LVA]
15
pCon ?
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K176107 in pSB1A3
Waiting
34
tetR[-LVA]
15
pCon ?
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
[lacZα-]ccdB
15
K176108 in
pSB1A3 in DB
3.1
Waiting
34
tetR[-LVA]
15
pCon ?
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
[lacZα-]ccdB
15
K176108 in
pSB1A3 in
TOP10
Waiting

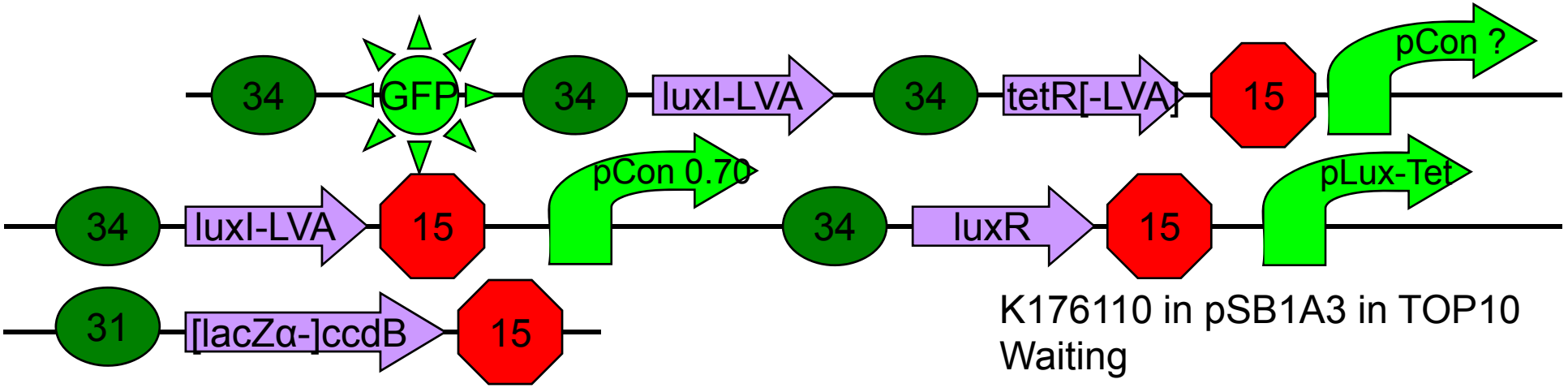

18
34
GFP
I13500 in pSB1A2
Sequence OK
34
GFP
I13500 in pSB1A2 S+P
Length OK
34
luxI-LVA
C0261 in pSB1A2
Sequence OK
34
luxI-LVA
C0261 X+P
Length OK
34
GFP
34
luxI-LVA
K176106 in pSB1A2
Sequencing
34
GFP
34
luxI-LVA
K176106 E+S
Waiting
34
tetR[-LVA]
15
pCon ?
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
K176107 in pSB1A3 S+P
Waiting
34
tetR[-LVA]
15
pCon ?
34
luxI-LVA
15
pCon 0.70
34
luxR
15
pLux-Tet
31
[lacZα-]ccdB
15
K176108 in
pSB1A3 S+P
Waiting



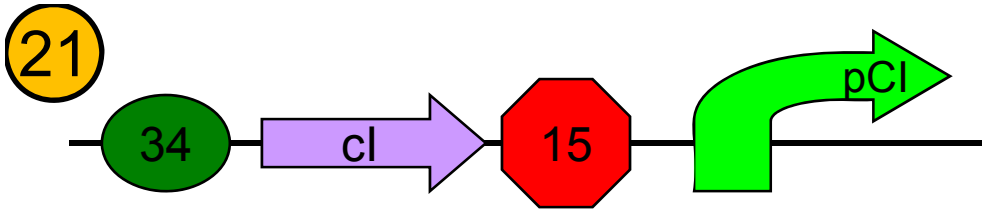
K176109 in pSB1A3
Waiting



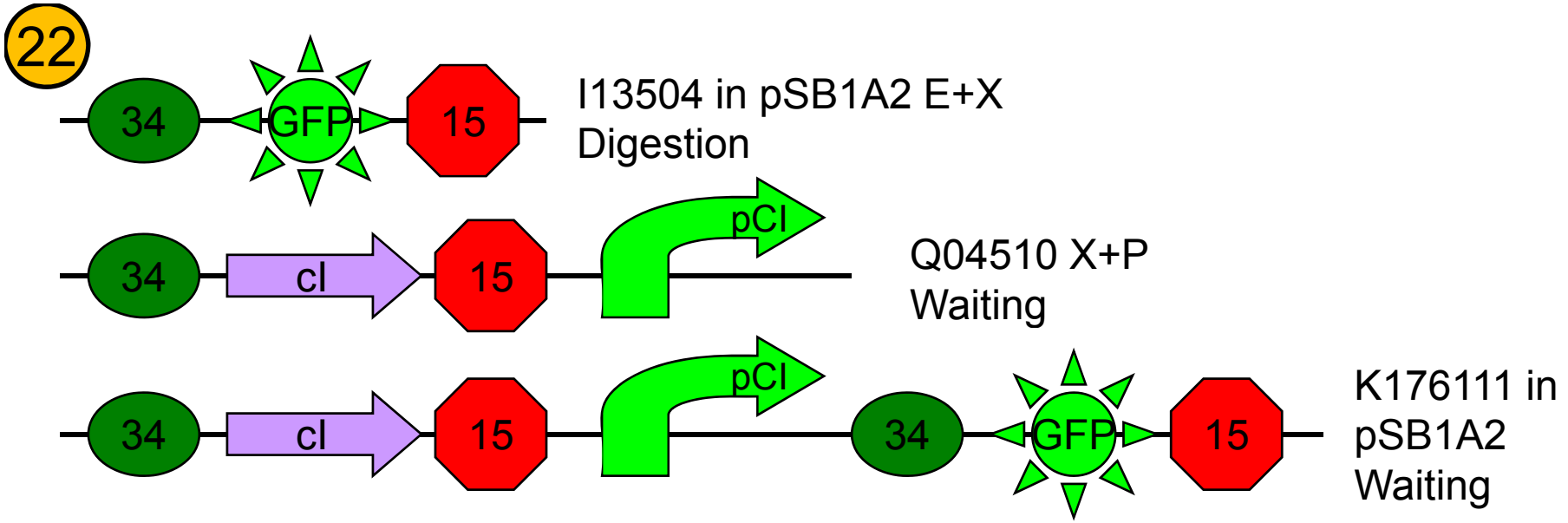
K176110 in pSB1A3 in DB 3.1
Waiting



K176110 in pSB1A3 in TOP10
Waiting



Q04510 in pSB2K3
Transformation


22
34
GFP
15
I13504 in pSB1A2 E+X
Digestion
34
cI
15
pCI
Q04510 X+P
Waiting
34
cI
15
pCI
34
GFP
15
K176111 in
pSB1A2
Waiting

USTC 2009 iGEM Team Parts

Favorite USTC 2009 iGEM Team Parts

Edit

-?-	Name	Type	Description	Designer	Length

USTC 2009 iGEM Team Parts Sandbox

Edit

-?-		Name	Type	Description	Designer	Length
		BBa_K176000	Regulatory	pLux/Tet Hybrid Promoter: (LuxR+,TetR-)->PoPS	Danqian Liu, Chao Li, Hao Jiang	72
		BBa_K176001	Generator	PoPS->RBS+tetR(no LVA)+T	Chao Li,Danqian Liu,Hao Jiang	782
		BBa_K176002	Reporter	pLux/Tet(K176000)(LuxR+,TetR-)->RBS+GFP+T	Chao Li,Danqian Liu,Hao Jiang	955
		BBa_K176003	Coding	lacZalpha-ccdB coding sequence	Zongxiao He, Hao Jiang	480
		BBa_K176004	Generator	pCon max(J23119)->RBS+luxR+T	Chao Li,Danqian Liu,Hao Jiang	979
		BBa_K176005	Reporter	pCon max(J23119)->RBS+GFP+T	Chao Li,Danqian Liu,Hao Jiang	910
		BBa_K176006	Generator	PoPS->RBS+tetR-LVA+T	Chao Li,Danqian Liu,Hao Jiang	840
	W	BBa_K176007	Plasmid_Backbone	pSB1A3 with the suffix of J61002 (mRFP)	Hao Jiang, Danqian Liu, Chao Li	3026
		BBa_K176008	Regulatory	constitutive promoter family member J23115 actual sequence	Hao Jiang, Danqian Liu, Chao Li	35
		BBa_K176009	Regulatory	constitutive promoter family member J23107 actual sequence	Hao Jiang, Danqian Liu, Chao Li	35
		BBa_K176010	Translational_Unit	PoPS->RBS+ccdB->PoPS	Zongxiao He, Hao Jiang	324
		BBa_K176011	Reporter	pCon 1.00(J23100)->RBS+GFP+T	Chao Li, Danqian Liu, Hao Jiang	918
		BBa_K176012	Reporter	pCon 0.70(J23101)->RBS+GFP+T	Chao Li, Danqian Liu, Hao Jiang	918
		BBa_K176013	Reporter	pCon 0.36(K176009)->RBS+GFP+T	Chao Li, Danqian Liu, Hao Jiang	918
		BBa_K176014	Reporter	pCon 0.15(K176008)->RBS+GFP+T	Chao Li, Danqian Liu, Hao Jiang	918
		BBa_K176015	Reporter	pCon 0.04(J23109)->RBS+GFP+T	Chao Li, Danqian Liu, Hao Jiang	918
		BBa_K176016	Reporter	pCon 0.01(J23103)->RBS+GFP+T	Chao Li, Danqian Liu, Hao Jiang	918
		BBa_K176017	Reporter	pCI(R0051)(lambda CI-)->RBS+GFP+T	Chao Li, Danqian Liu, Hao Jiang	932
		BBa_K176018	Signalling	pCon max(J23119)->RBS+luxI-LVA+T	Danqian Liu, Chao Li, Hao Jiang	841
		BBa_K176019	Signalling	pCon 1.00(J23100)->RBS+luxI-LVA+T	Danqian Liu, Chao Li, Hao Jiang	841
		BBa_K176020	Signalling	pCon 0.70(J23101)->RBS+luxI-LVA+T	Danqian Liu, Chao Li, Hao Jiang	841
		BBa_K176021	Signalling	pCon 0.36(K176009)->RBS+luxI-LVA+T	Danqian Liu, Chao Li, Hao Jiang	841
		BBa_K176022	Signalling	pCon 0.15(K176008)->RBS+luxI-LVA+T	Danqian Liu, Chao Li, Hao Jiang	841
		BBa_K176023	Signalling	pCon 0.04(J23109)->RBS+luxI-LVA+T	Danqian Liu, Chao Li, Hao Jiang	841
		BBa_K176024	Signalling	pCon 0.01(J23103)->RBS+luxI-LVA+T	Danqian Liu, Chao Li, Hao Jiang	841

Measurement

- Strain
  - TOP10
  - DH5α
  - MG1655
  - MDS 42 recA Blue
- Plasmid
- Medium
  - LB
  - M9
    - Minimal
    - Supplemented
  - EZ Rich Define
  - pH-buffered TBK
  - pH-buffered LBK
- pH
- Temperature
  - 37℃
  - 30℃
  - 34℃
- Pre-warm
- Shake
- Dilution
- Wash

http://openwetware.org/wiki/M9_medium
http://openwetware.org/wiki/M9_medium/minimal
http://openwetware.org/wiki/M9_medium/supplemented
http://openwetware.org/wiki/Neidhardt_EZ_Rich_Defined
http://www.genome.wisc.edu/resources/protocols/ezmedium.htm

Emergent Properties of Reduced-Genome *Escherichia coli*

György Pósfai,[1,2]* Guy Plunkett III,[2,3,4] Tamás Fehér,[1] David Frisch,[2,4] Günther M. Keil,[5] Kinga Umenhoffer,[1] Vitaliy Kolisnychenko,[1]† Buffy Stahl,[2] Shamik S. Sharma,[6]‡ Monika de Arruda,[2] Valerie Burland,[2,3] Sarah W. Harcum,[7] Frederick R. Blattner[2,3,4]*

With the use of synthetic biology, we reduced the *Escherichia coli* K-12 genome by making planned, precise deletions. The multiple-deletion series (MDS) strains, with genome reductions up to 15%, were designed by identifying nonessential genes and sequences for elimination, including recombinogenic or mobile DNA and cryptic virulence genes, while preserving good growth profiles and protein production. Genome reduction also led to unanticipated beneficial properties: high electroporation efficiency and accurate propagation of recombinant genes and plasmids that were unstable in other strains. Eradication of stress-induced transposition evidently stabilized the MDS genomes and provided some of the new properties.

Escherichia coli K-12 is one of the best understood and most thoroughly analyzed organisms and is the platform of choice for genetic, biochemical, and metabolic simulation research. Commercially, it is used for production of metabolites such as amino acids and proteins of therapeutic or commercial interest. K-12 is also gaining ground for production of DNA for gene therapy, DNA vaccines, and interference RNA. The genomes of two closely related K-12 strains, MG1655 and W3110, have been sequenced (*1–3*), and 87% of their genes have functional assignments (*4*). mobile DNA elements that mediate recombination events such as transposition and horizontal gene transfer, including insertion sequence (IS) elements, transposases, defective phages, integrases, and site-specific recombinases (*5*). Multiple elements also provide DNA sequence repeats that mediate inversions, duplications, and deletions by homologous recombination even without transposase. To stabilize the genome and streamline metabolism, these elements must be deleted and unwanted functions removed, such as those specific for human hosts or particular envi- ated by the phage lambda Red system. Beginning with prototype strain MDS12 (*9*), "scarless" deletions were made by removing the targeted segment and resealing the genome so that markers used in the construction were eliminated. Resulting strains were tested for robust growth on minimal medium, and deletions were serially accumulated into a single strain by P1 transduction. Deletion endpoints were verified by sequencing and by DNA microarray hybridization (Fig. 1) (*8*). Physical characteristics of the MDS strains are summarized in Table 1; deletion endpoints are in table S1, deleted genes in table S3, and strain request information in (*8*). Generation of double-strand breaks (DSBs) in each deletion process might have induced error-prone repair, but experiments designed to detect this showed that a single transient break would have no detectable effect on the accumulation of point mutations.

MDS39, the first in the series designed to be IS-free, was examined by genomic DNA hybridization to NimbleGen genome scanning microarrays, which included IS elements, phages, and plasmids absent from K-12 (*8*) as well as the K-12 genomic sequence in the form of 24-base oligonucleotides tiled about every 50 bases on both strands. Alarmingly, we found five unexpected copies of IS that had transposed to new locations (*8*) since the project began

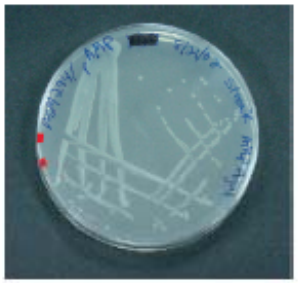
GFP (PoPS)

- Fluorospectrophotometer
- Plate Reader
- Flow Cytometer (FACS)
- Blotting
  - Northern
  - Western
- Realtime RT-PCR

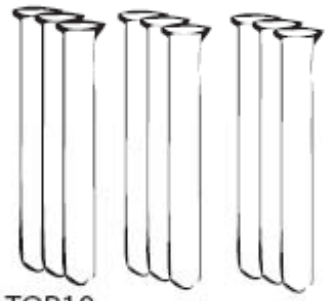
- http://partsregistry.org/Measurement
- http://openwetware.org/wiki/The_BioBricks_Foundation:Standards/Technical/Measurement
- http://openwetware.org/wiki/Standardized_GFP_quantification
- Engineering the interface between cellular chassis (Barry Canton PhD thesis)
- Applying engineering principles to the design and construction of transcriptional devices (Reshma P. Shetty PhD thesis)

STEP1: Streak 3 plates

A: TOP10
B: BBa_I20260
C: Your promoter!



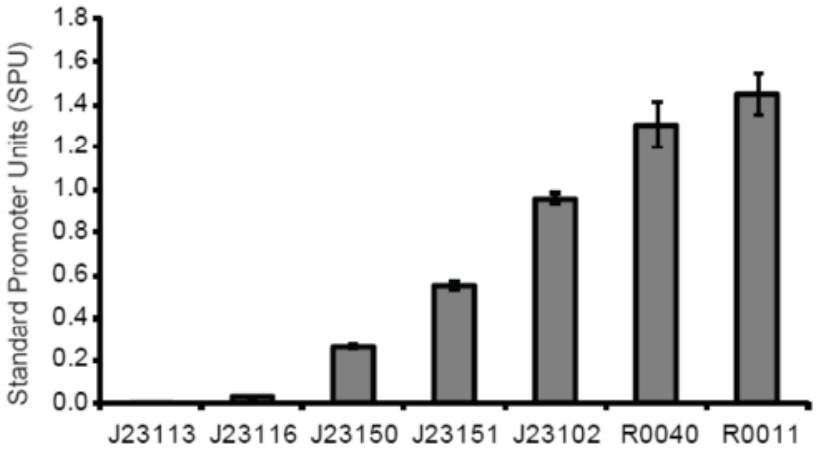
STEP 2: Pick 3 colonies from each plate to start overnight cultures in Supplemented M9 Media at 37 C (9 tubes)



TOP10
BBa_I20260
Your Promoter

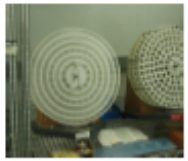
37C

16 hours



Standard Promoter Units

STEP 3: Dilute 1/100 into fresh, pre-warmed media incubate at 37C (9 tubes)



37C

STEP 4: After 3 hours measure GFP and OD

3 hours
GFP
OD

STEP 5: After another half hour measure GFP and OD again

1/2 hour
GFP
OD

2006 Berkeley **J23100~J23119** Reported activities of the promoters are given as the relative fluorescence of these plasmids in strain TG1 grown in LB media to **saturation**. See part J61002 for details on their use.

AHL

- Rapid Screening of Quorum-Sensing Signal N-Acyl Homoserine Lactones by an In Vitro Cell-Free Assay
- Detection of N-acylhomoserine lactones in lung tissues of mice infected with Pseudomonas aeruginosa
- Detecting and characterizing N-acyl-homoserine lactone signal molecules by thin-layer chromatography
- Detection of N-acyl homoserine lactones using a traI-luxCDABE-based biosensor as a high-throughput screening tool
- On-line high-performance liquid chromatography-mass spectrometric detection and quantification of N-acylhomoserine lactones, quorum sensing signal molecules, in the presence of biological matrices
- Detection of quorum-sensing N-acyl homoserine lactone signal molecules by bacterial biosensors

CcdB & LacZα

- Programmed population control by cell–cell communication and regulated killing
- A synthetic Escherichia coli predator–prey ecosystem
- LacZα
  - X-gal
  - ONPG
    - http://parts.mit.edu/igem07/index.php/USTC/BetaGalactosidaseAssay

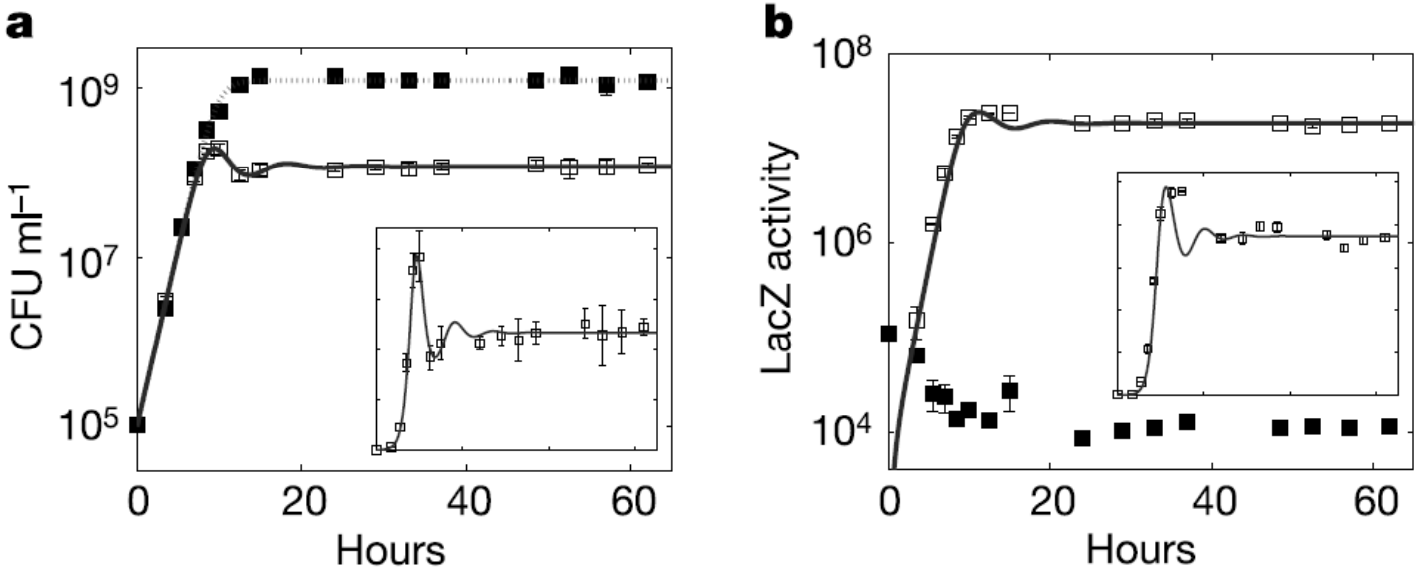


Figure S2:

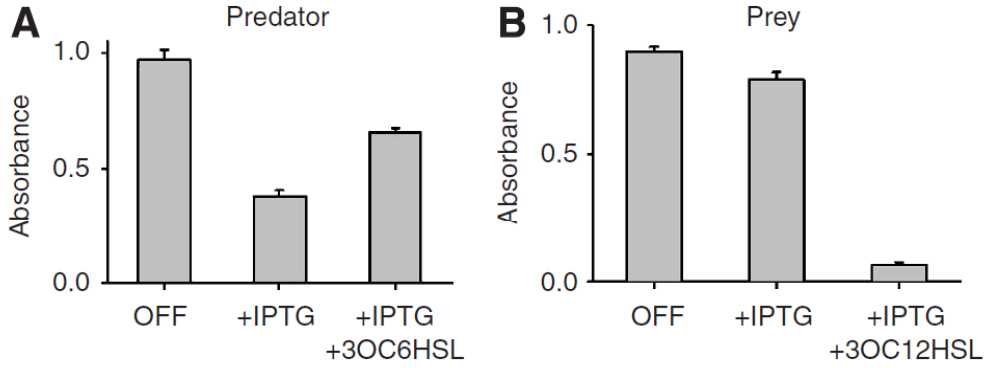
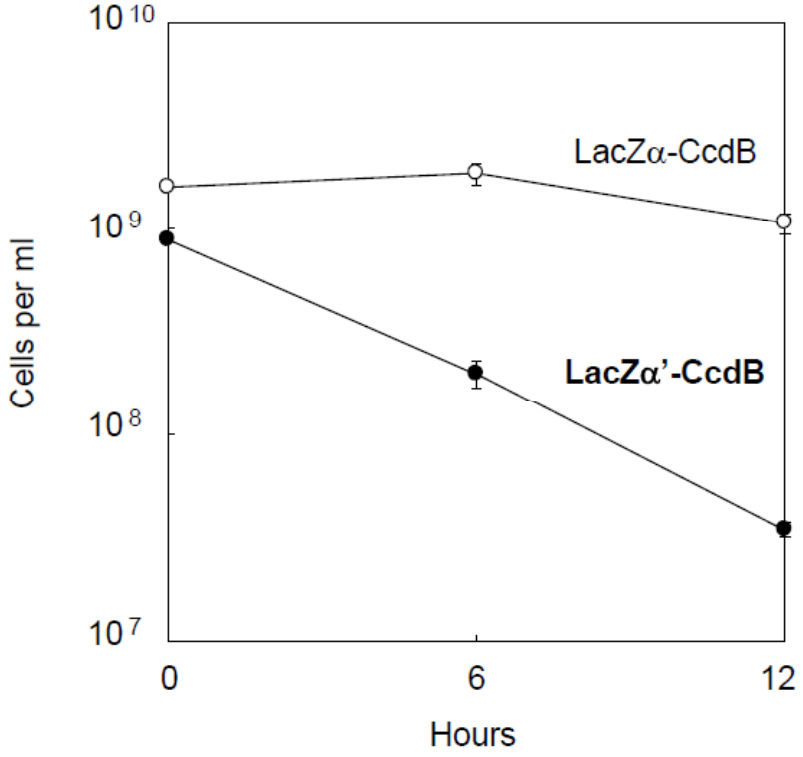


Figure 1 Individual growth behaviors (without interactions) of (**A**) predator and (**B**) prey cells in liquid media. For each condition, 6 ml LBK medium containing chloramphenicol and kanamycin was inoculated with a single bacterial colony and was divided into three 2 ml cultures: 'OFF' cultures contained no inducers, '+IPTG' cultures contained 1 mM IPTG and '+IPTG+AHL' contained 1 mM IPTG and 100 nM AHL, respectively. After 20 h of incubation (light gray bars), optical densities (ODs) of these cultures were measured with a microplate reader (see Supplementary information). Error bars represent standard deviation of triplicate cultures.



Wiki

- Team project description
- Notebook
  - Meetings
  - Lab Work
  - Sample Naming Sheets
- Logo
- Team
- Other pages

Calendar of Events

IGEM 2009 Calendar of Events [edit]

Note: Dates in grey have not been finalized yet. Make sure to check the calendar periodically for any changes!

19 February	IGEM 2009 registration opens
31 March	IGEM 2008 registration closes; Team registration fee due
13 May	DNA Distribution sent to teams **(target deadline; *subject to change*)**
16/17 May	iGEM Workshop, MIT, USA
1 June	Visa invitation letter requests due
20/21 June	iGEM Workshop, Europe
27/28 June	iGEM Workshop, Asia
15 June	Preliminary team rosters due
1 August	Team project descriptions due
18 September	Jamboree attendance fee due
TBD	Request for variance due (notice and description of any use of non-standard parts or devices schemes due)
	Track selection due
	Project abstracts due
18 September	Team rosters due
TBD	Project and part documentation due, including documentation for all medal criteria
	BioBrick Part DNA needs to be received by the Registry
30 Oct - 2 Nov	iGEM Competition Jamboree, MIT, USA

Thank You