

**E.ADEM v0.0.5.2**

**2009.8.22**

2009.5.3

110 days

2009.8.22

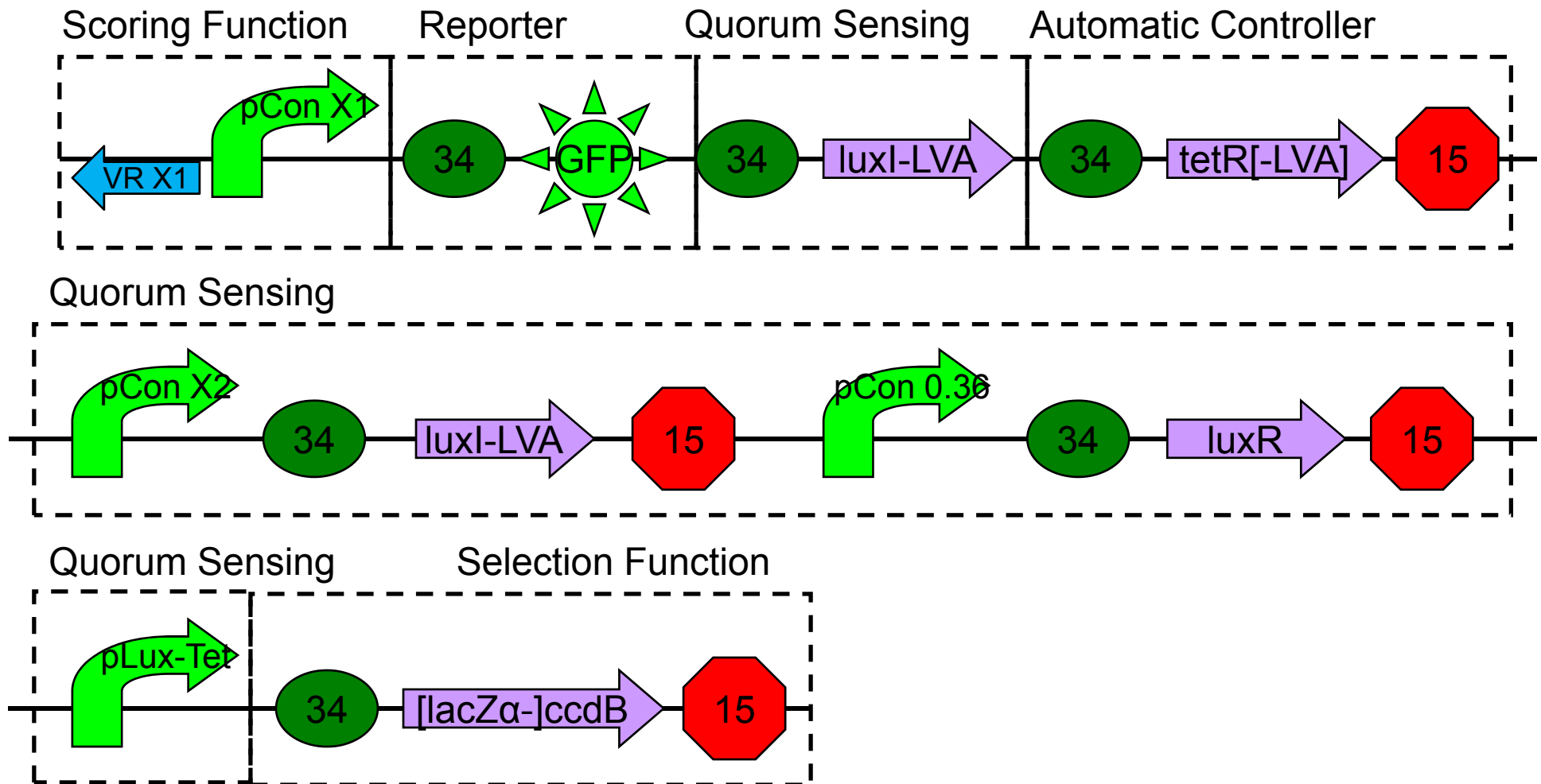
68 days

2009.10.30

# Outline

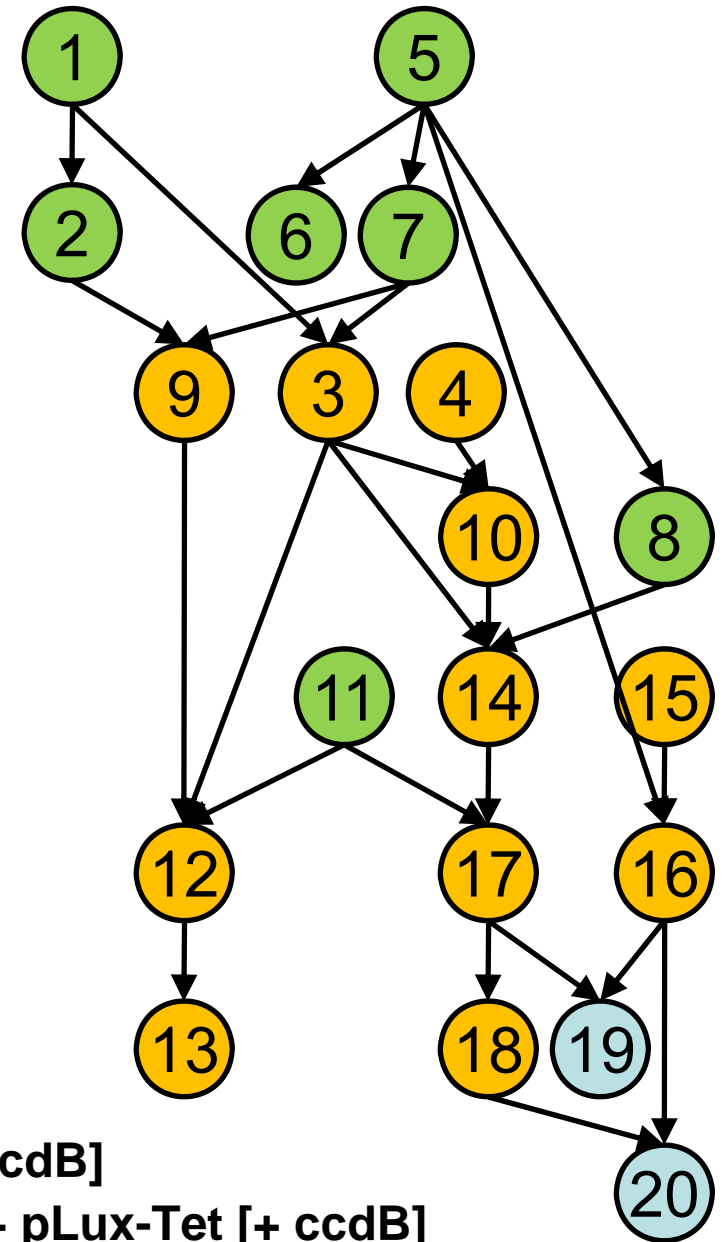
- Assembly
- Measurement
  - General Conditions
  - GFP
  - AHL
  - CcdB
  - LacZ $\alpha$
- Wiki
  - Team project description
  - Notebook
- Instructional Videos

# Assembly

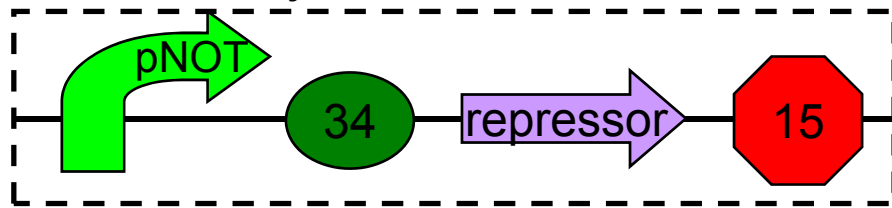


1. pLux-Tet
2. pLux-Tet + GFP
3. pCon + luxR × 4 + pLux-Tet
4. ccdB × 4
5. pCon × 8
6. pCon × 8 + GFP
7. pCon + luxR
8. pCon × 7 + luxI (AHL detection by 9 or GC-MS ?)
9. pCon + luxR × 4 + pLux-Tet + GFP (AHL)
10. pCon + luxR × 4 + pLux-Tet + ccdB × 4 (AHL)
11. tetR × 2
12. tetR × 2 + pCon × 2 + luxR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

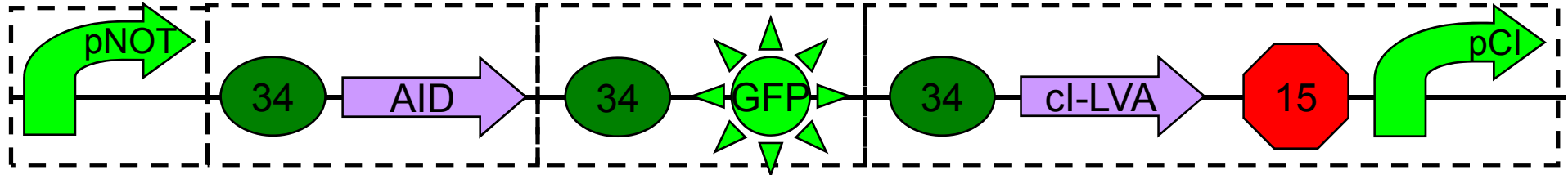
Waiting  
Working  
Done  
Sequenced



### Evolution Object



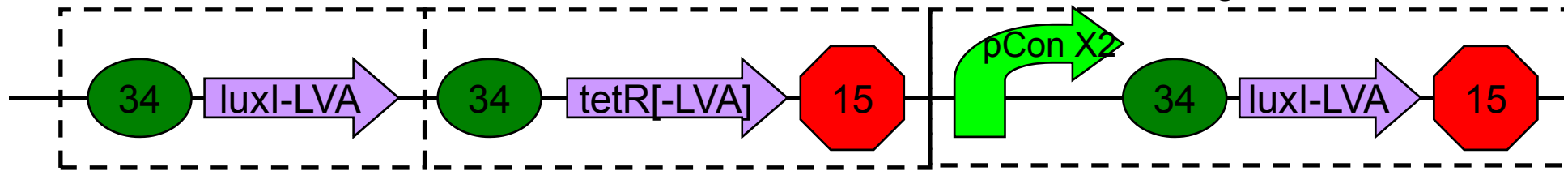
### Scoring Function



### Quorum Sensing

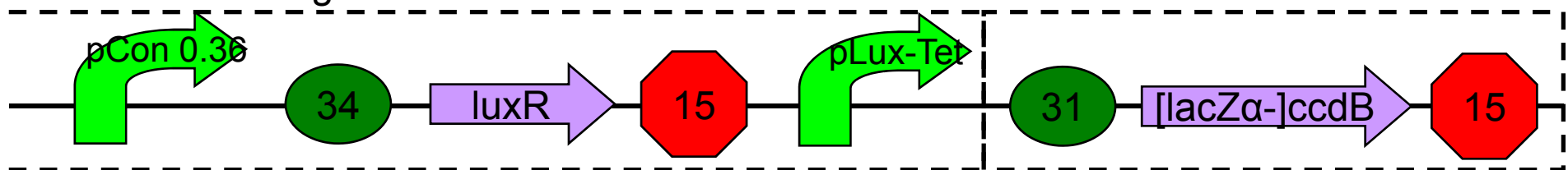
### Automatic Controller

### Quorum Sensing

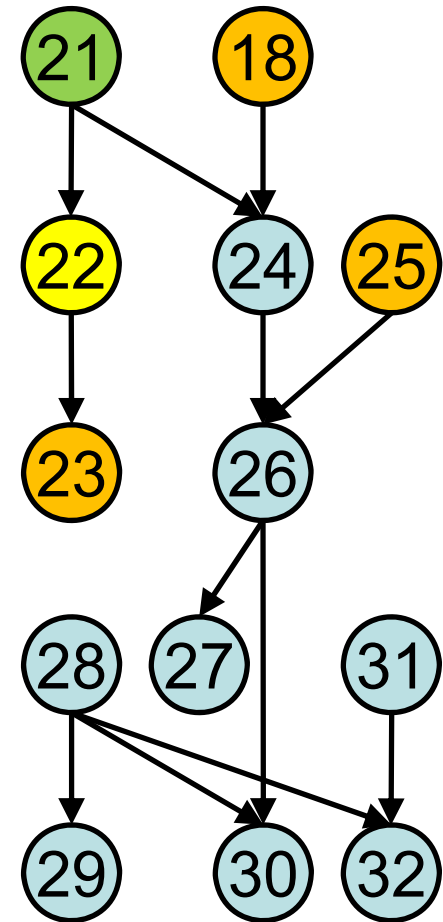


### Quorum Sensing

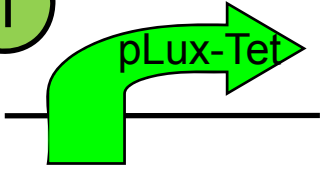
### Selection Function



- 21. *cl* + p*Cl*
- 22. *cl* + p*Cl* + GFP
- 23. (VR + pCon) × 7 + *cl* + p*Cl* + GFP
- 24. *cl* + p*Cl* + *luxI* + *tetR* + pCon + *luxI* + pCon + *luxR* + pLux-Tet [+ *ccdB*]
- 25. AID
- 26. [AID +] GFP + *cl* + p*Cl* + *luxI* + *tetR* + pCon + *luxI* + pCon + *luxR* + pLux-Tet [+ *ccdB*]
- 27. (VR + pCon) × 7 + GFP + *cl* + p*Cl* + *luxI* + *tetR* + pCon + *luxI* + pCon + *luxR* + pLux-Tet [+ *ccdB*]
- 28. pNOT
- 29. pNOT + GFP
- 30. pNOT + [AID +] GFP + *cl* + p*Cl* + *luxI* + *tetR* + pCon + *luxI* + pCon + *luxR* + pLux-Tet [+ *ccdB*]
- 31. repressor
- 32. pNOT + repressor



1



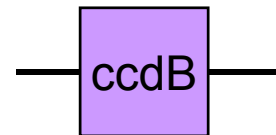
K176000 X+P with nicks  
Sequence OK



G00100  
Synthesis OK



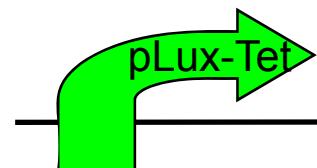
G00101  
Synthesis OK



P1010 in pSB1A3 in DB 3.1  
Length OK



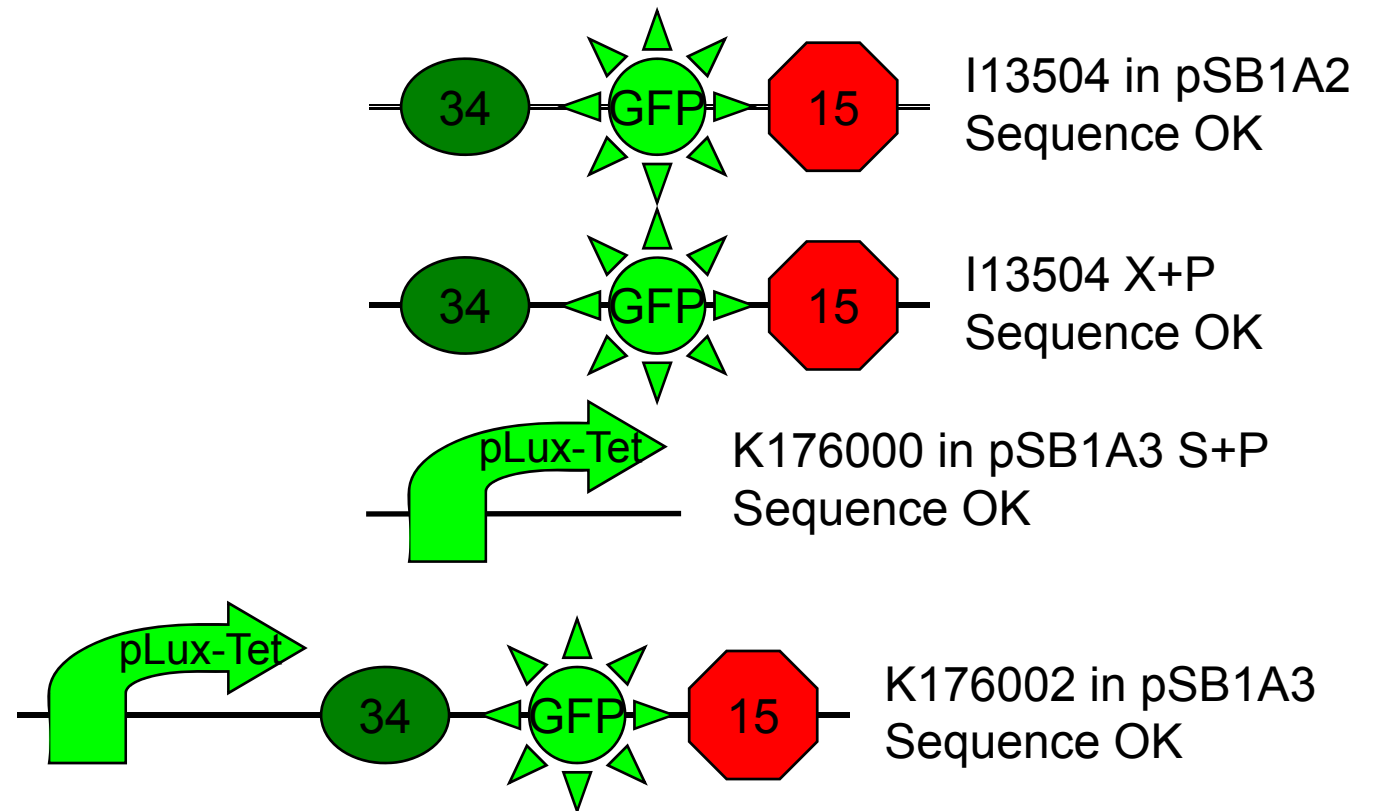
pSB1A3 X+P  
Length OK



K176000 in pSB1A3  
Sequence OK



2

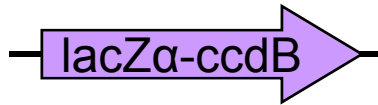


4



K145151 in pSB1A2 in DB3.1

Sequence OK



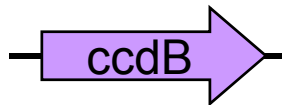
K176003 Fragment Length OK



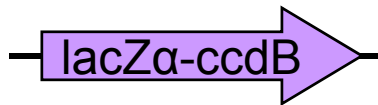
B0034 in pSB1A2 Sequence OK



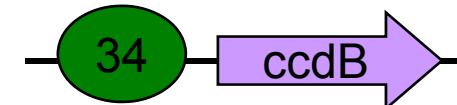
B0034 in pSB1A2 S+P Length OK



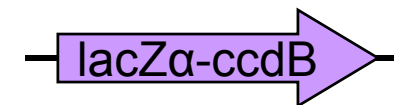
K145151 X+P Length OK



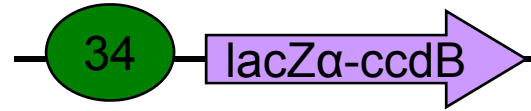
K176003 X+P Length OK



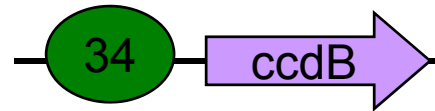
K176010 in pSB1A2 in DB3.1 Ligation



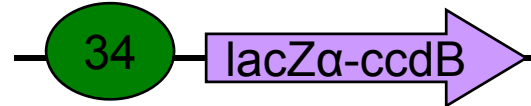
K176003 in pSB1A3 in DB3.1 Sequence OK



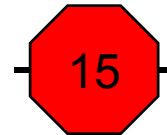
K176027 in pSB1A2 in DB3.1 Sequence OK



K176010 E+S Length OK



K176027 E+S Length OK



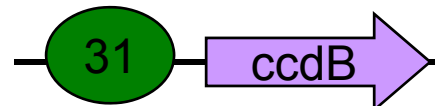
B0015 in pSB1AK3 Sequence OK



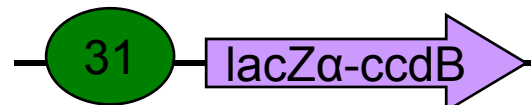
B0031 in pSB1A2 Length OK



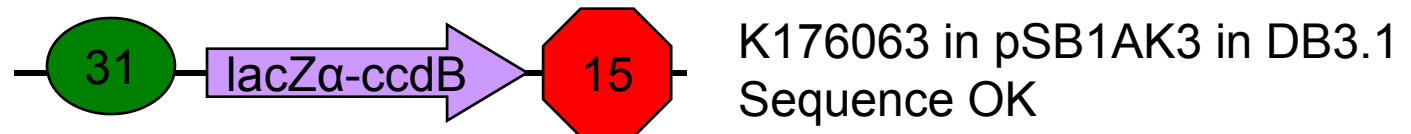
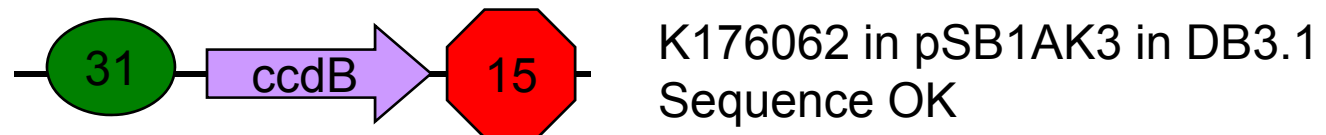
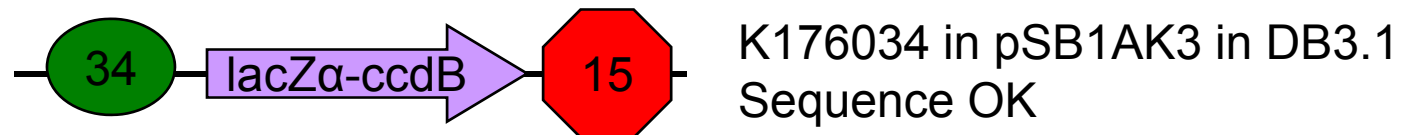
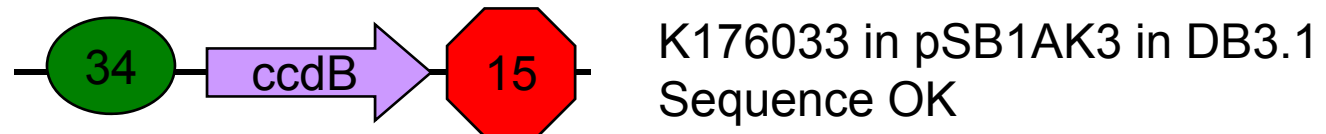
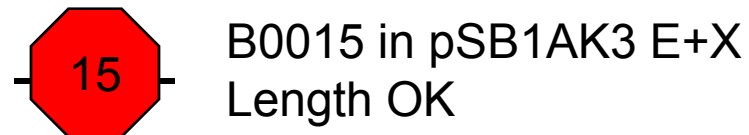
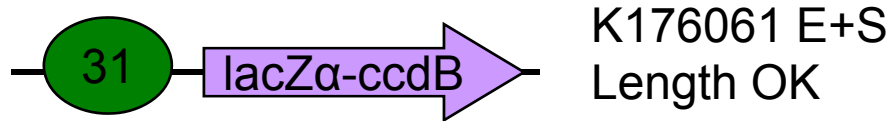
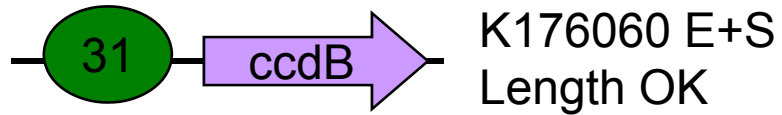
B0031 S+P Length OK



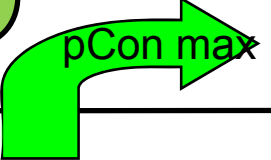
K176060 in pSB1A2 in DB3.1 Sequence OK

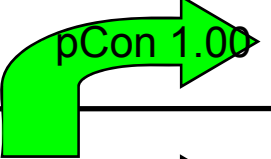


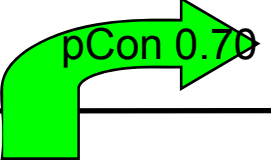
K176061 in pSB1A2 in DB3.1 Sequence OK

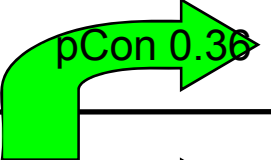


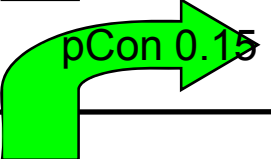
5

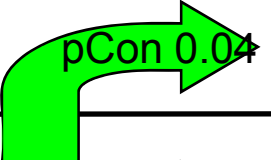
 pCon max  
J23119 in pSB1A2  
Sequence OK

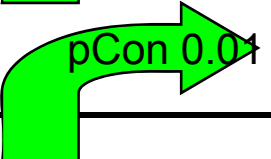
 pCon 1.00  
J23100 in J61002  
Sequence ?

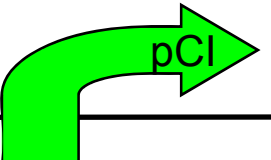
 pCon 0.70  
J23101 in J61002  
Sequence OK

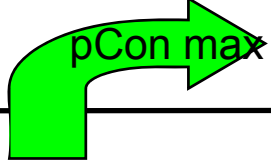
 pCon 0.36  
K176009 in J61002  
Sequence OK

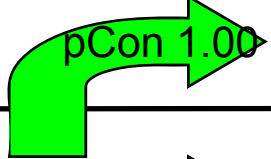
 pCon 0.15  
K176008 in J61002  
Sequence OK

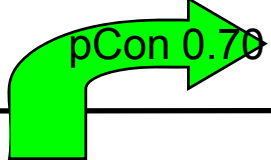
 pCon 0.04  
J23109 in J61002  
Sequence OK

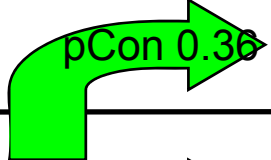
 pCon 0.04  
J23103 in J61002  
Sequence OK

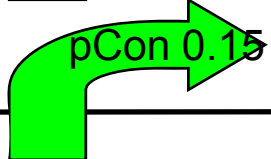
 pCl  
R0051 in pSB1A2  
Sequence OK

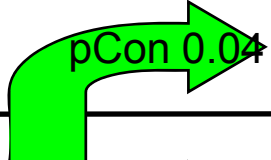
 pCon max  
J23119 X+P  
Sequence OK

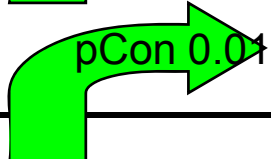
 pCon 1.00  
J23100-J61002SF X+P  
Sequence ?

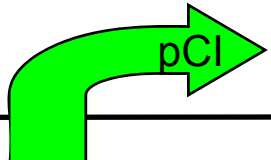
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J23101-J61002SF X+P  
Sequence OK

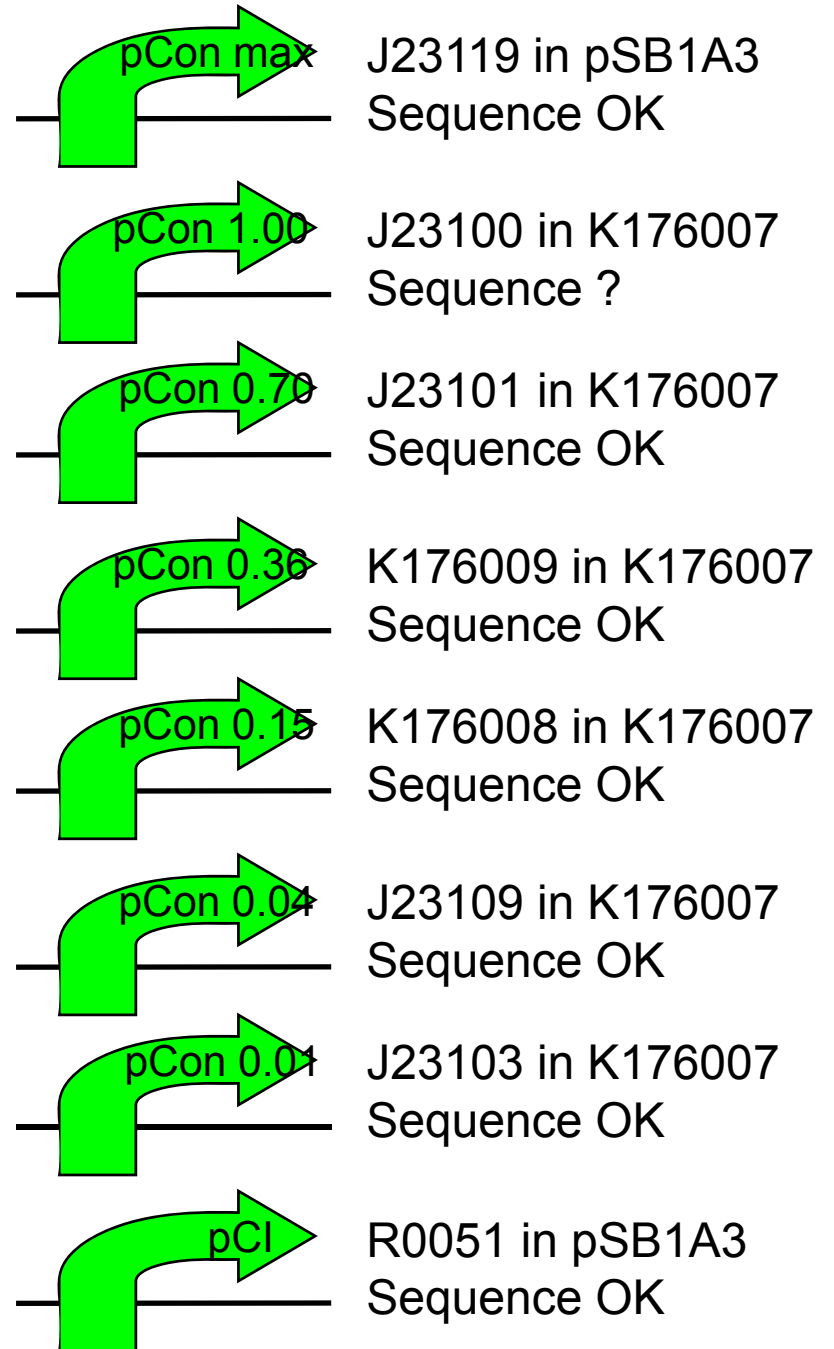
 pCon 0.36  
K176009-J61002SF X+P  
Sequence OK

 pCon 0.15  
K176008-J61002SF X+P  
Sequence OK

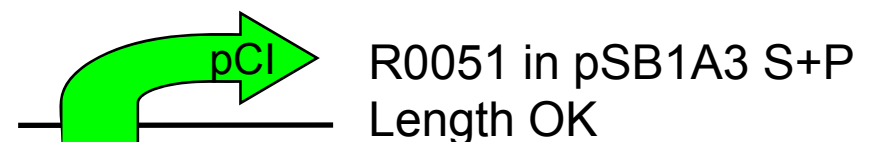
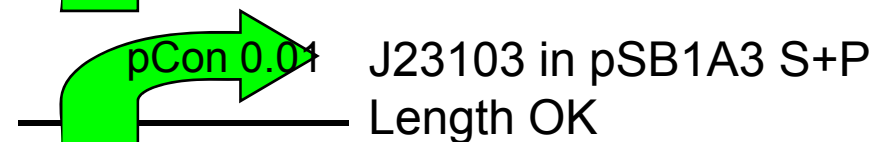
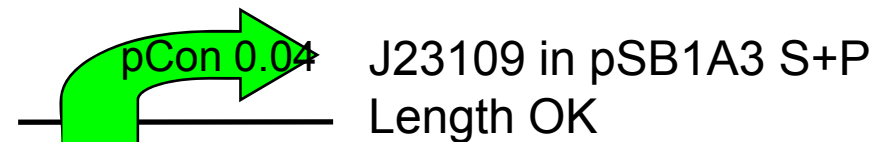
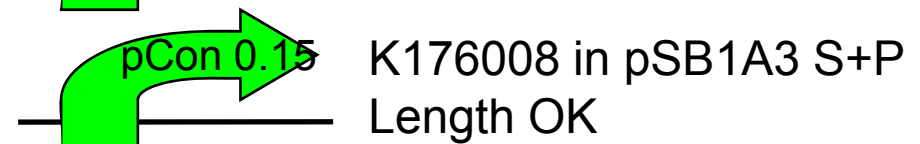
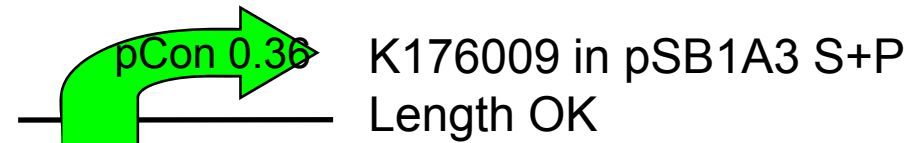
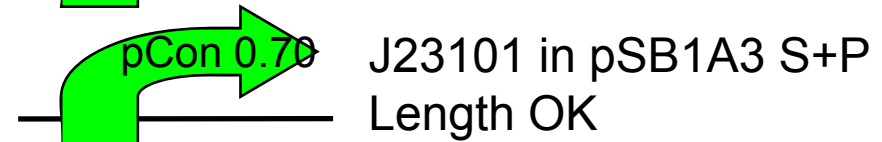
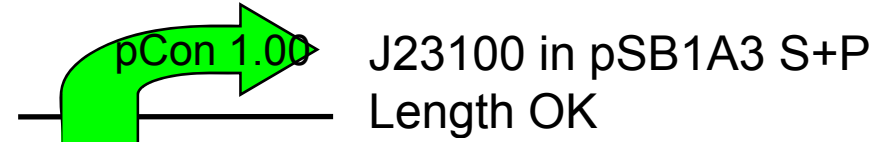
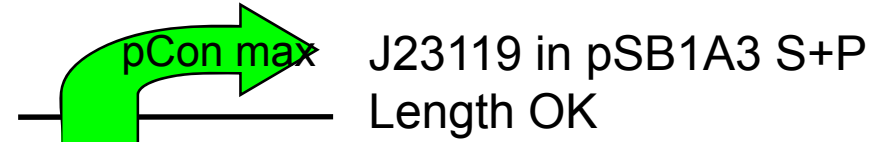
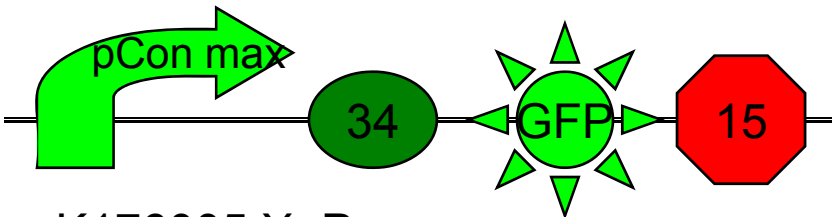
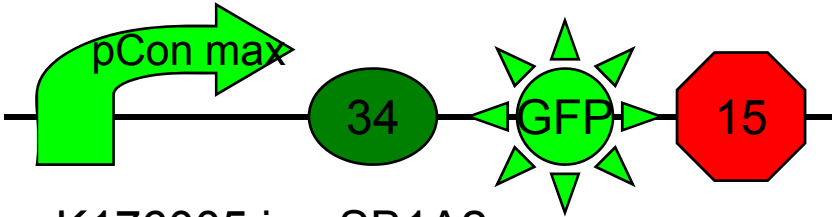
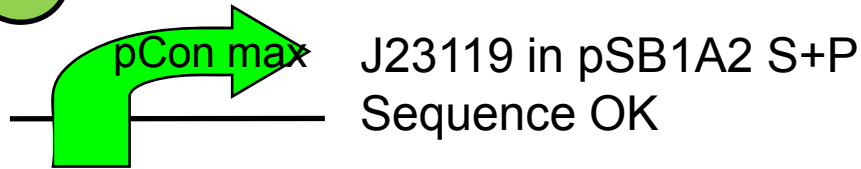
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J23109-J61002SF X+P  
Sequence OK

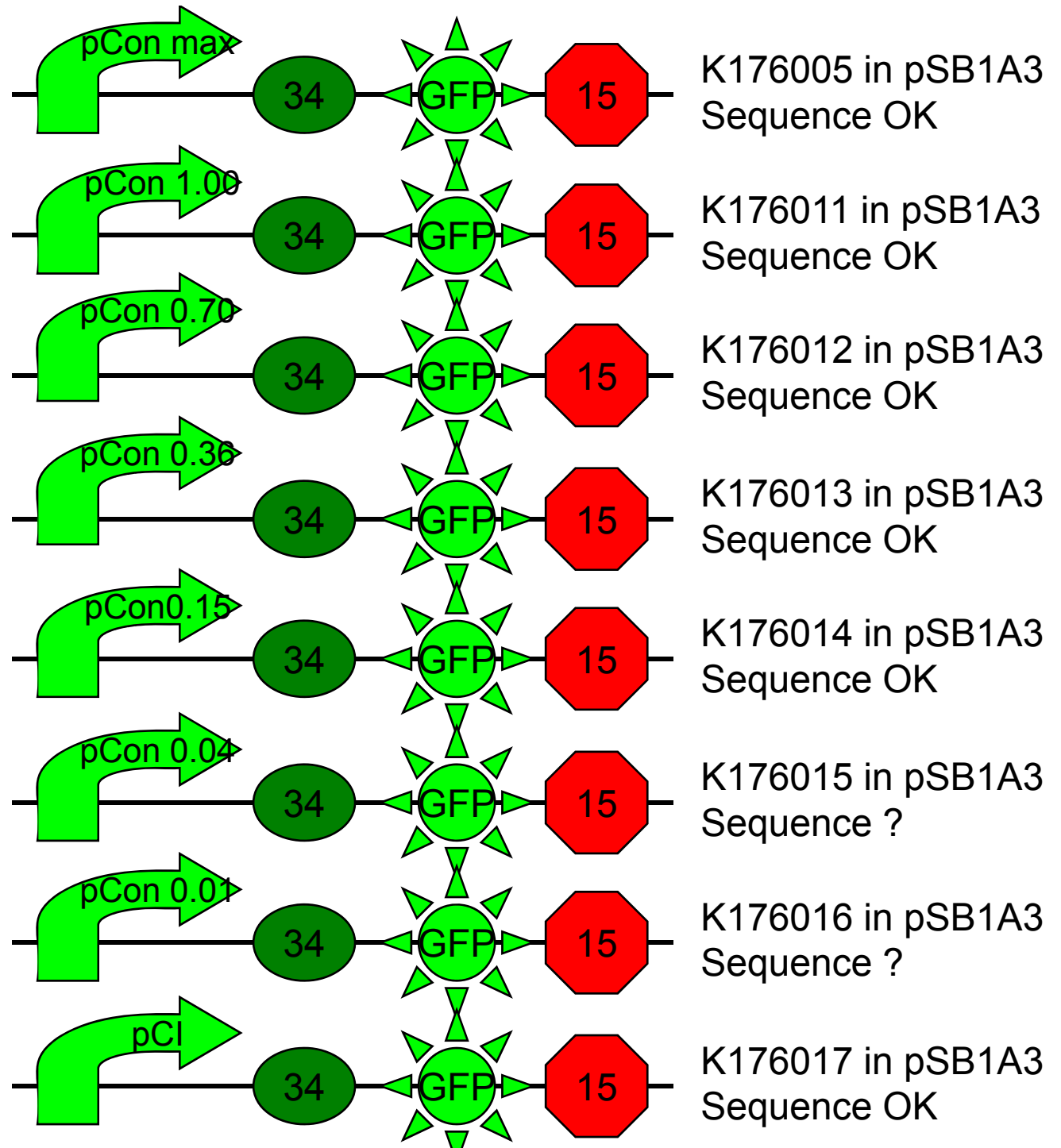
 pCon 0.04  
J23103-J61002SF X+P  
Sequence OK

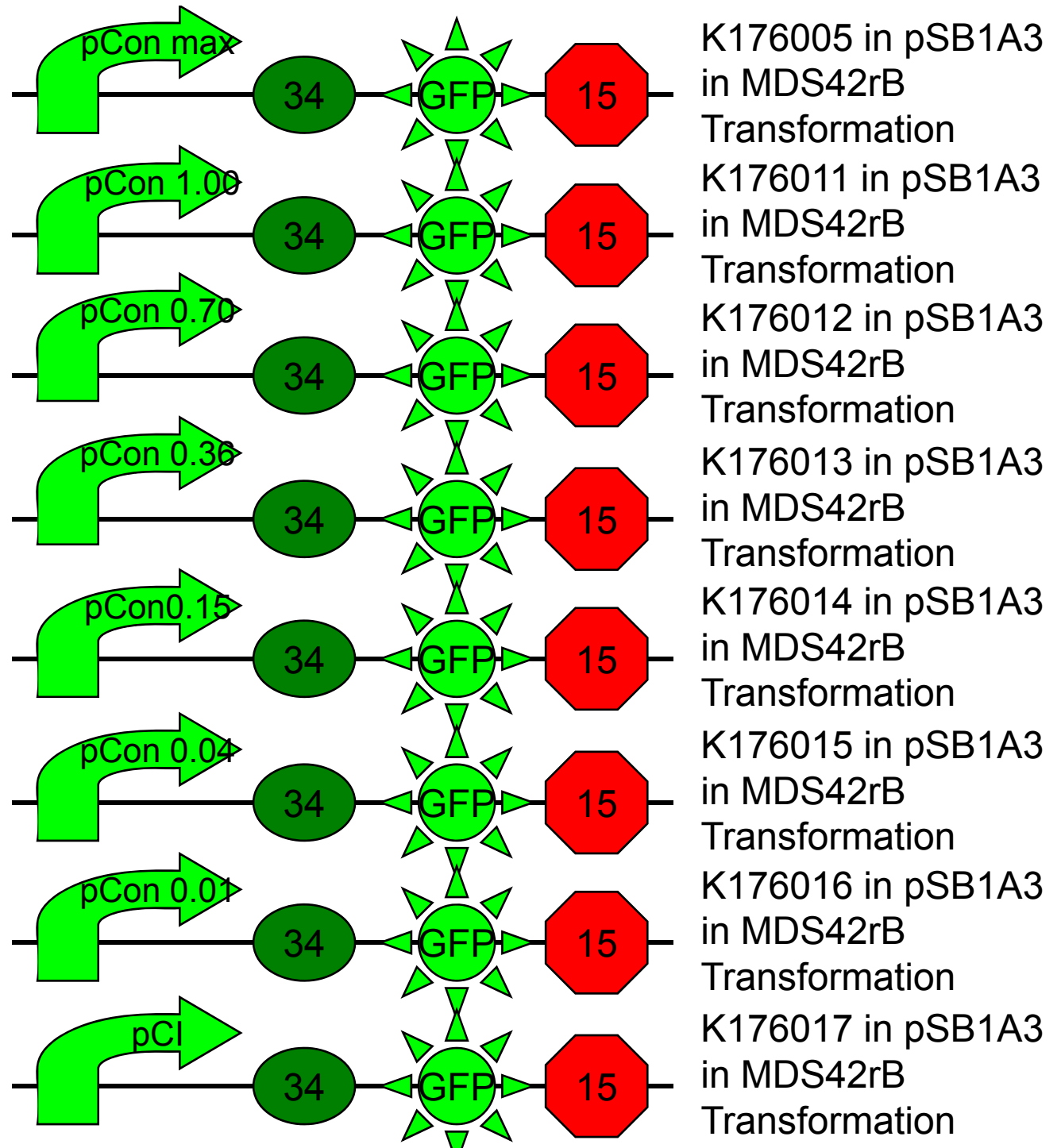
 pCl  
R0051 X+P  
Length OK



6

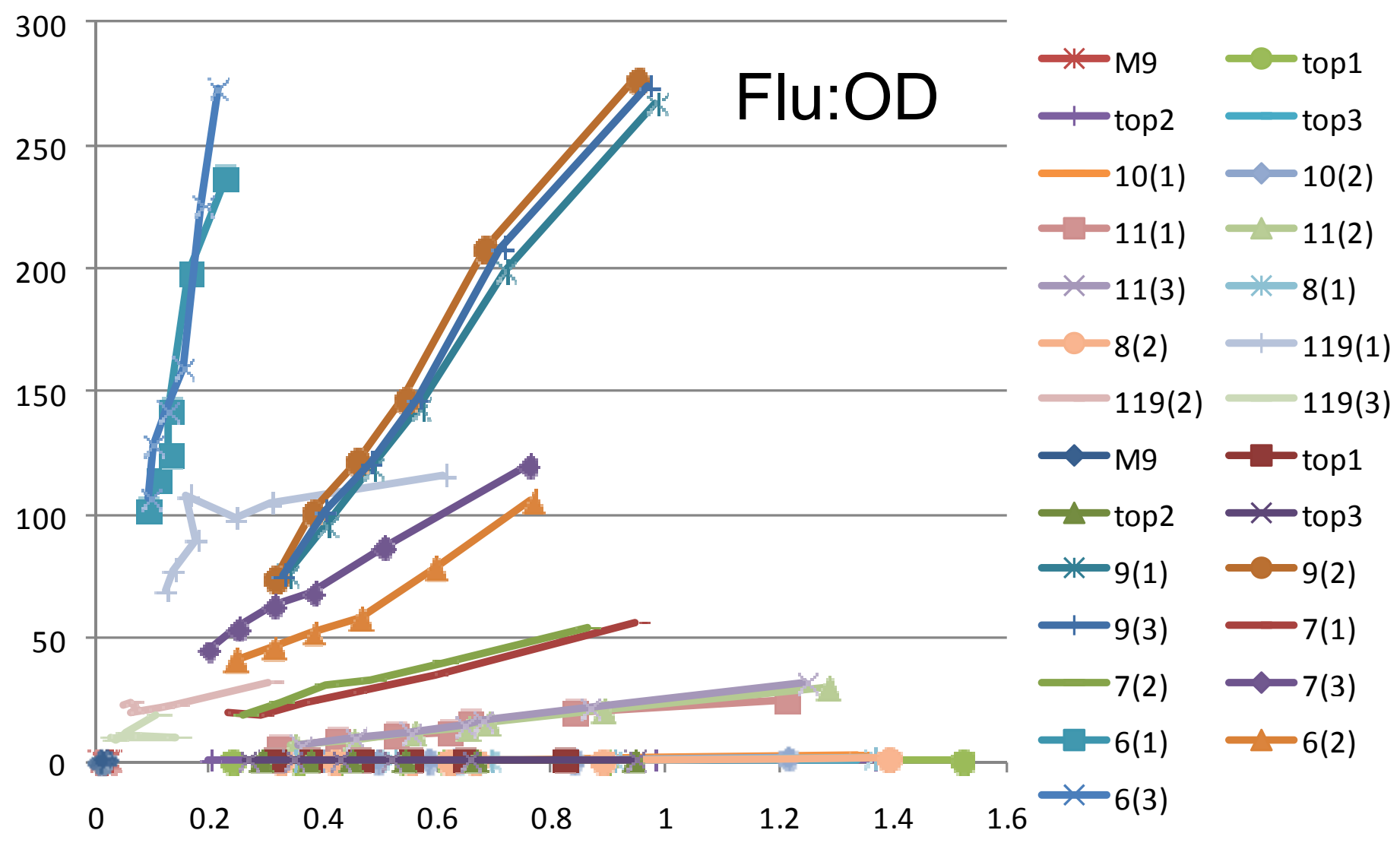




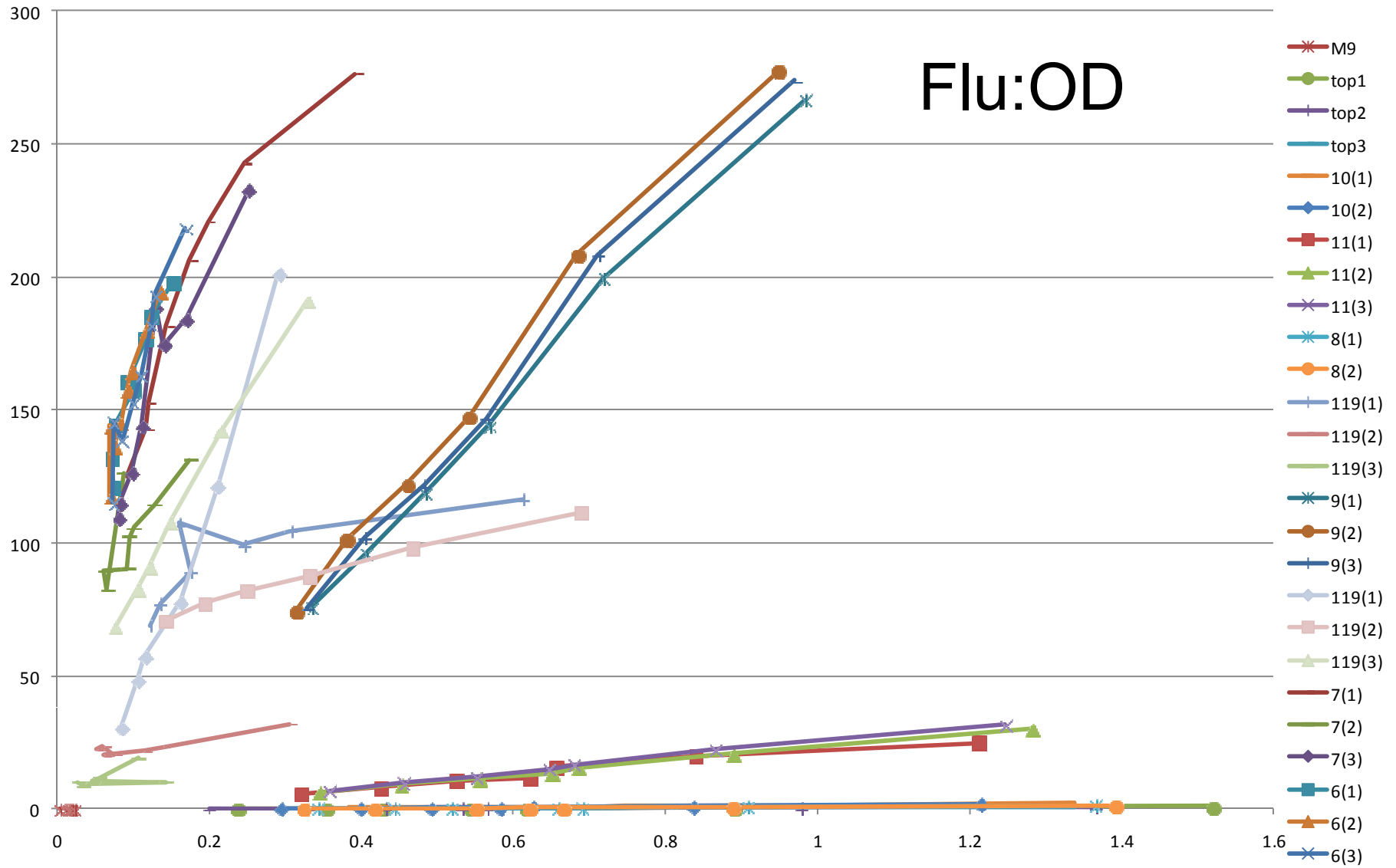




# Flu:OD

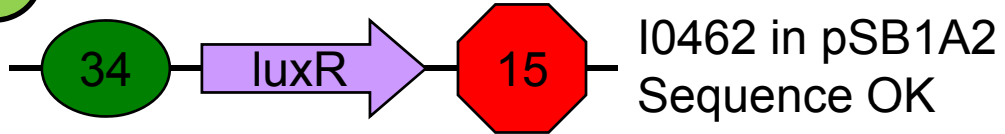


# Flu:OD

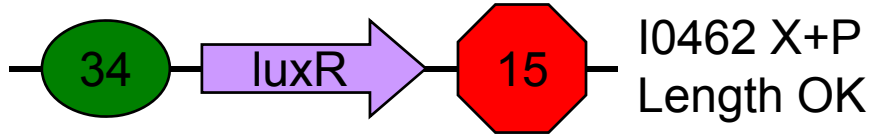




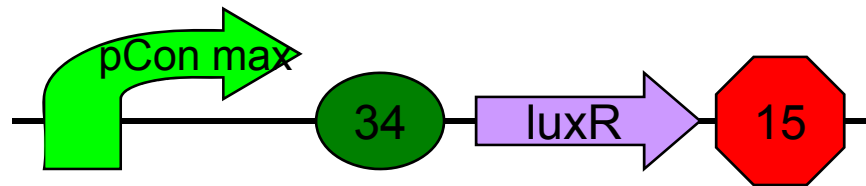
7



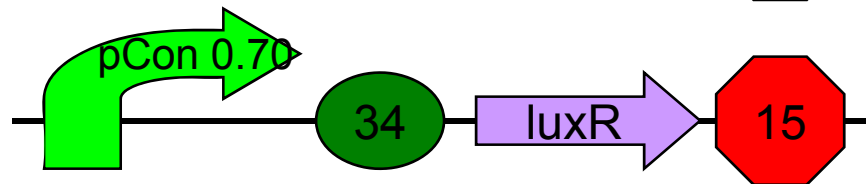
I0462 in pSB1A2  
Sequence OK



I0462 X+P  
Length OK

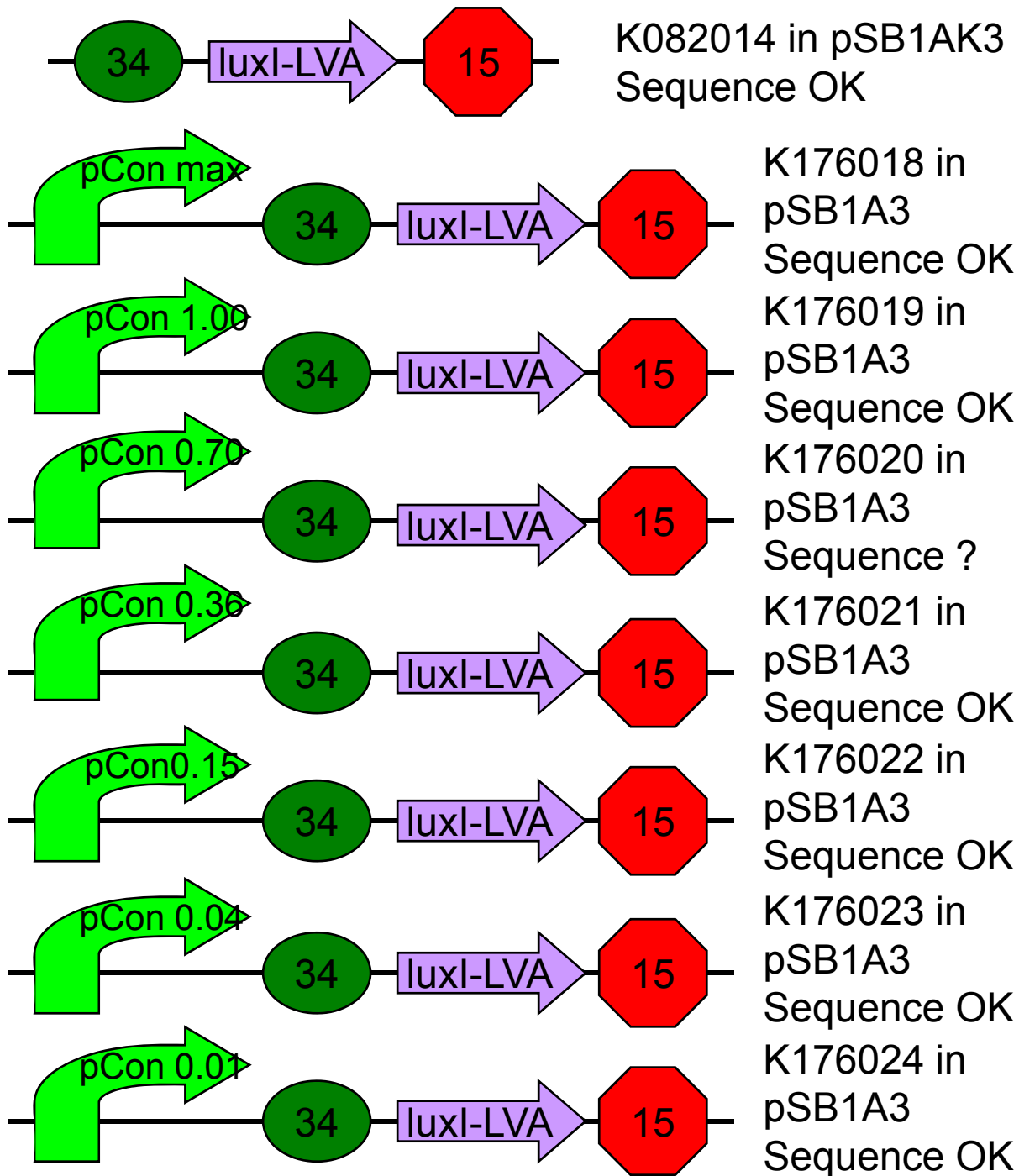


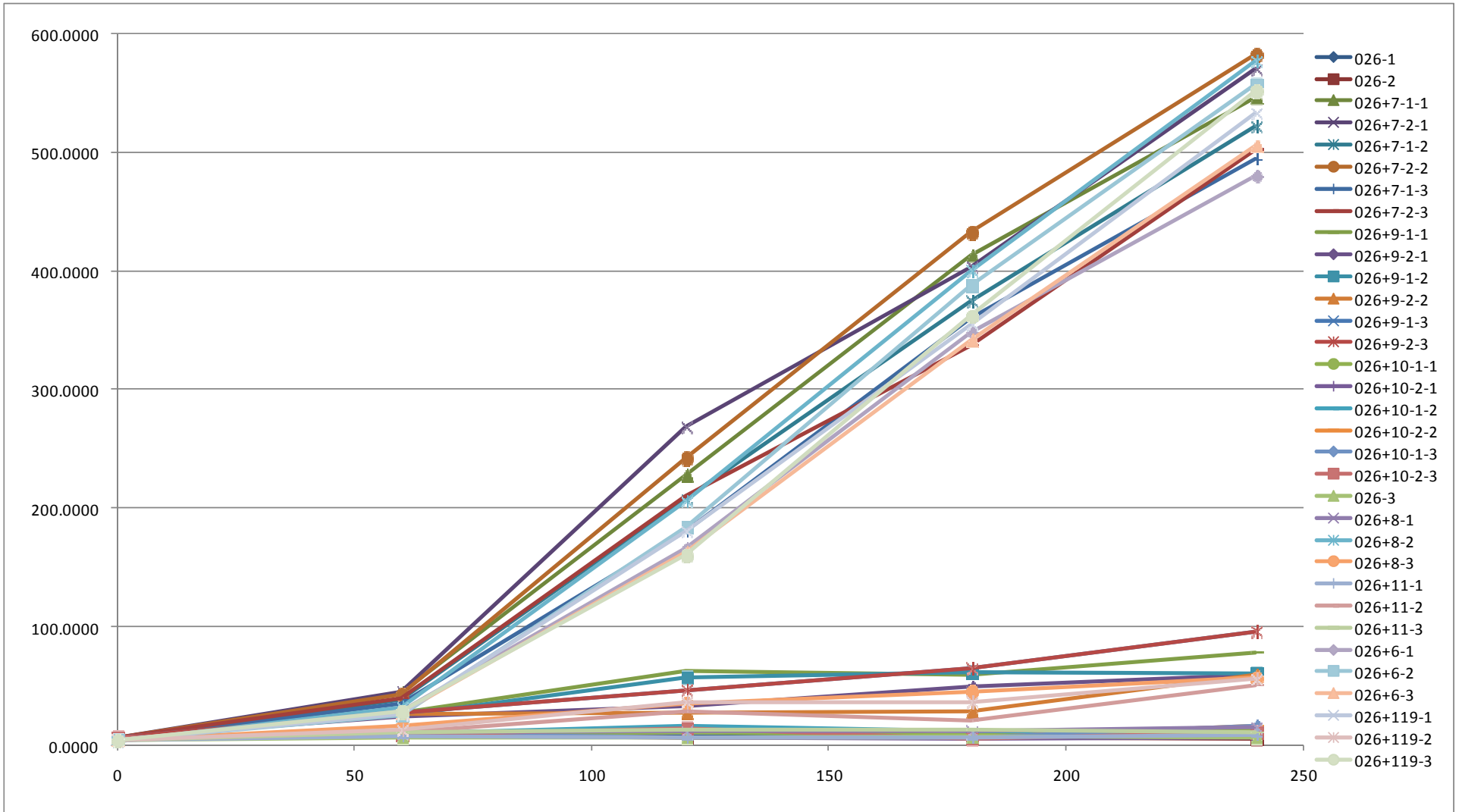
K176004 in pSB1A2  
Sequence OK  
Length ?

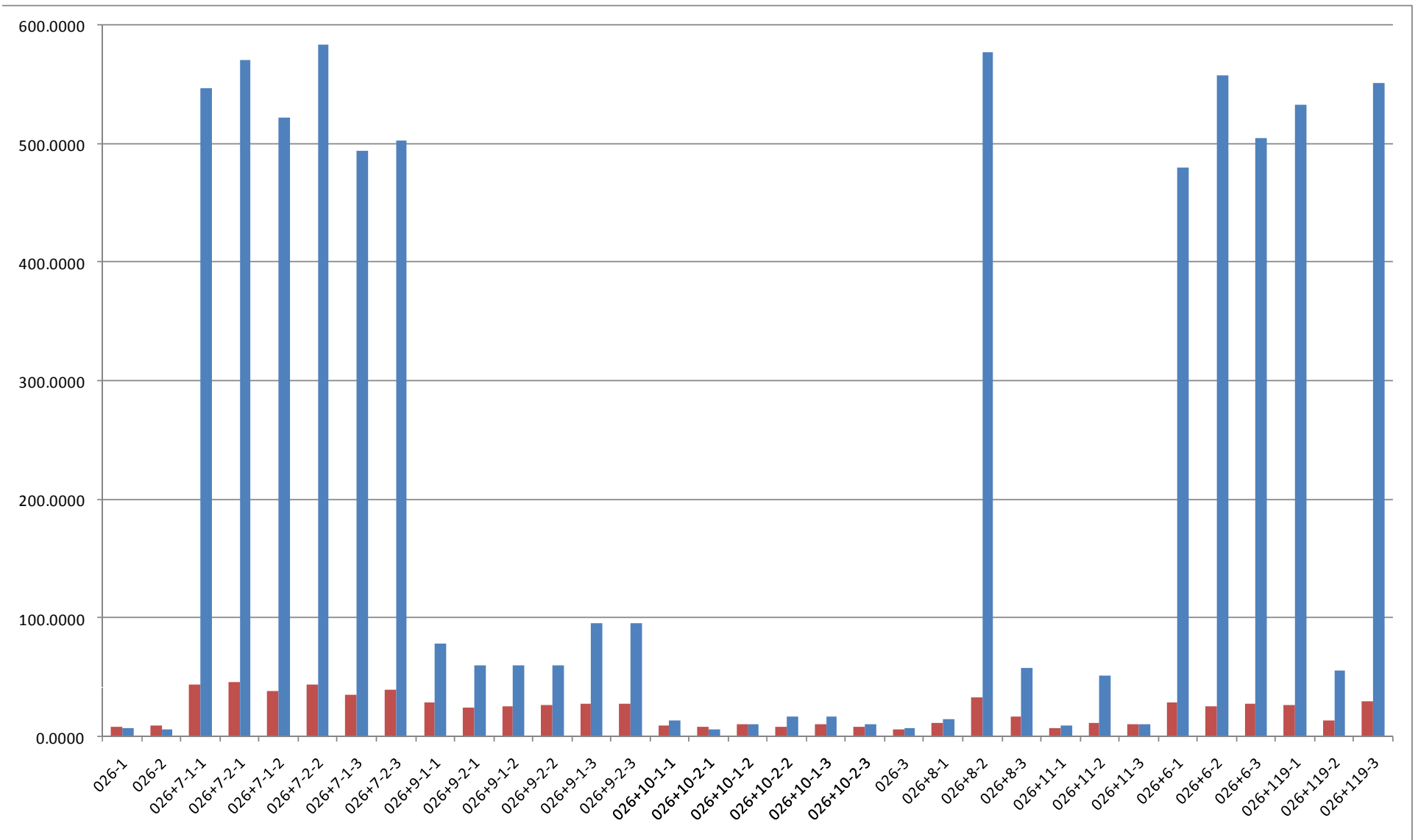


K176025 in pSB1A3  
Sequence ?

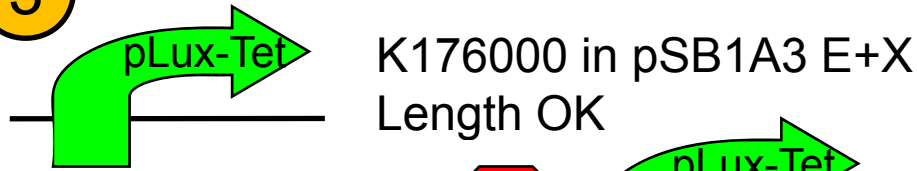
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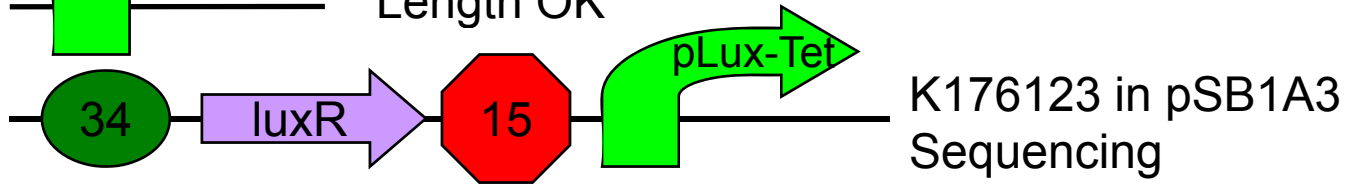




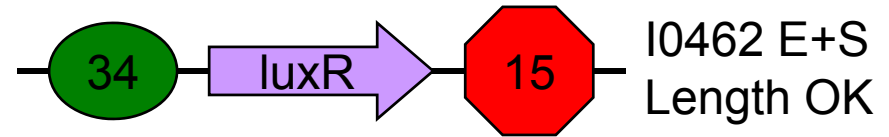
3



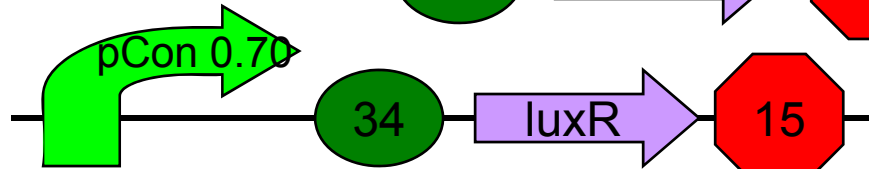
K176000 in pSB1A3 E+X  
Length OK



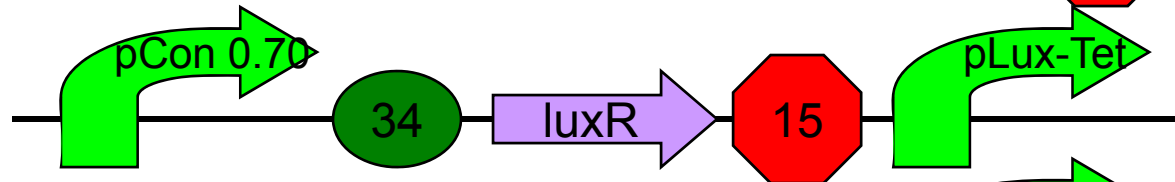
K176123 in pSB1A3  
Sequencing



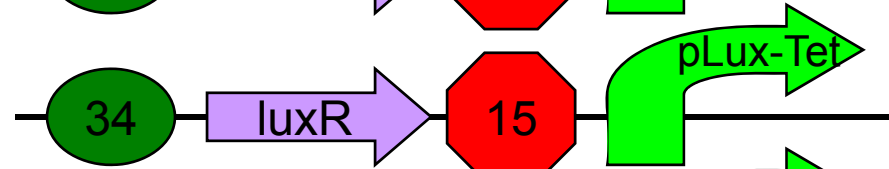
I0462 E+S  
Length OK



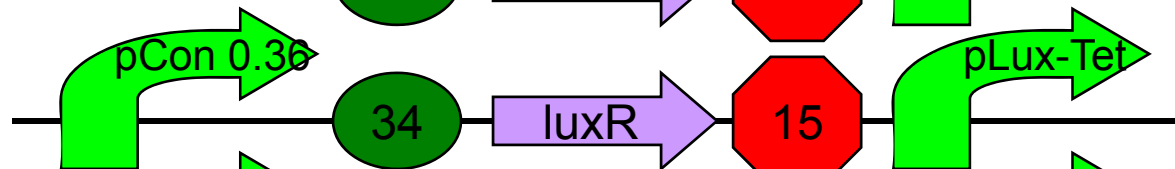
K176025 E+S  
Length OK



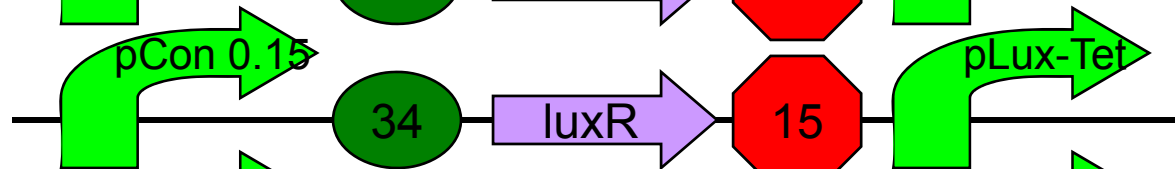
K176028 in pSB1A3  
Sequence ?



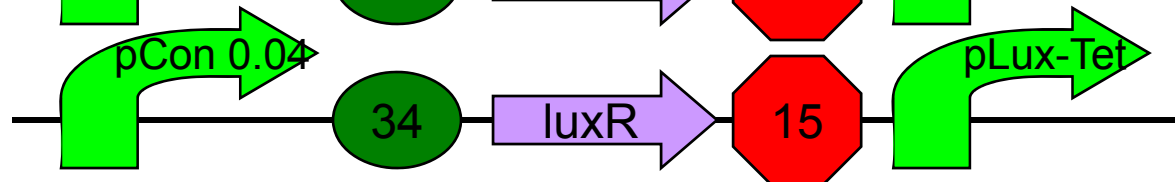
K176123 X+P  
Length OK



K176125 in pSB1A3  
Sequencing



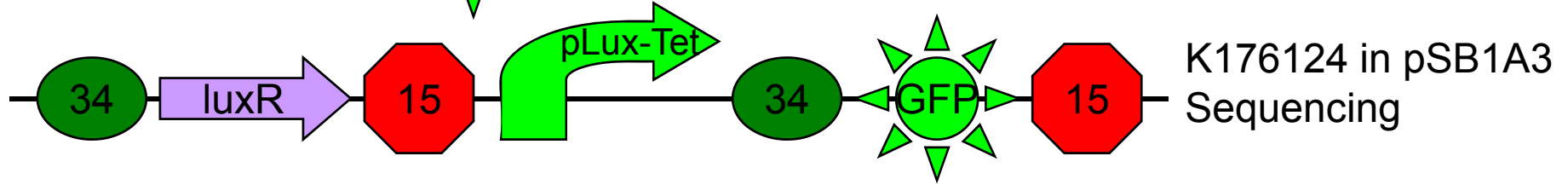
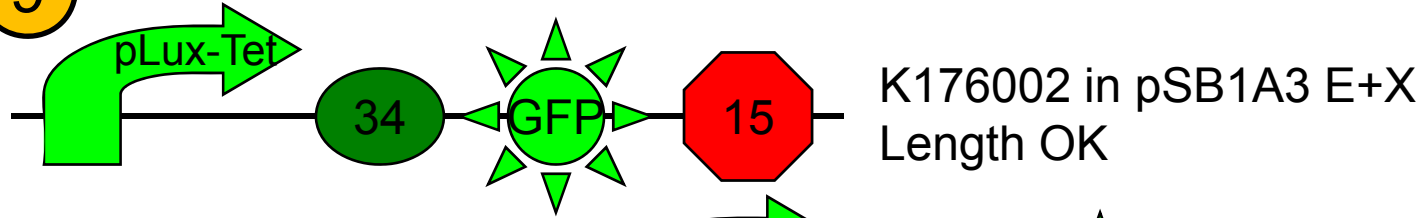
K176127 in pSB1A3  
Sequencing



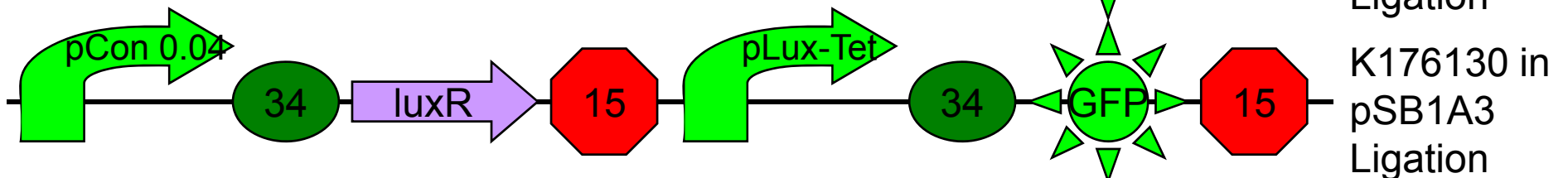
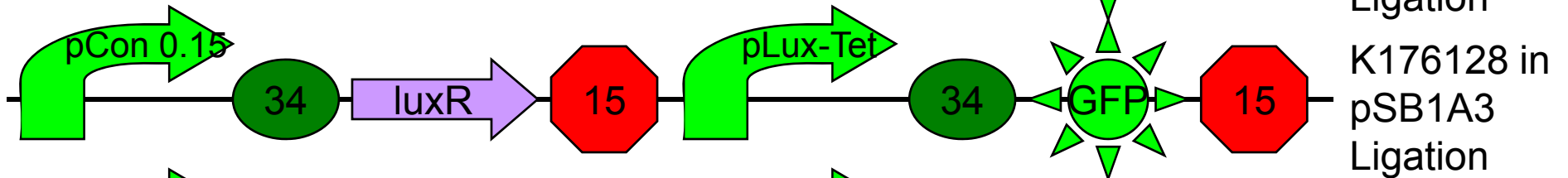
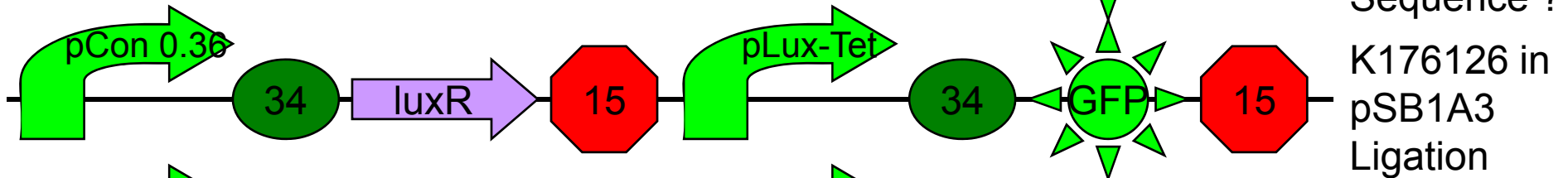
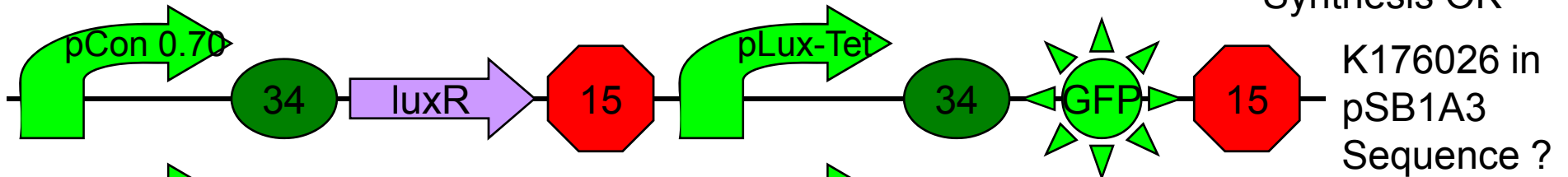
K176129 in pSB1A3  
Ligation



9



VRGFP ←  
G00201  
Synthesis OK

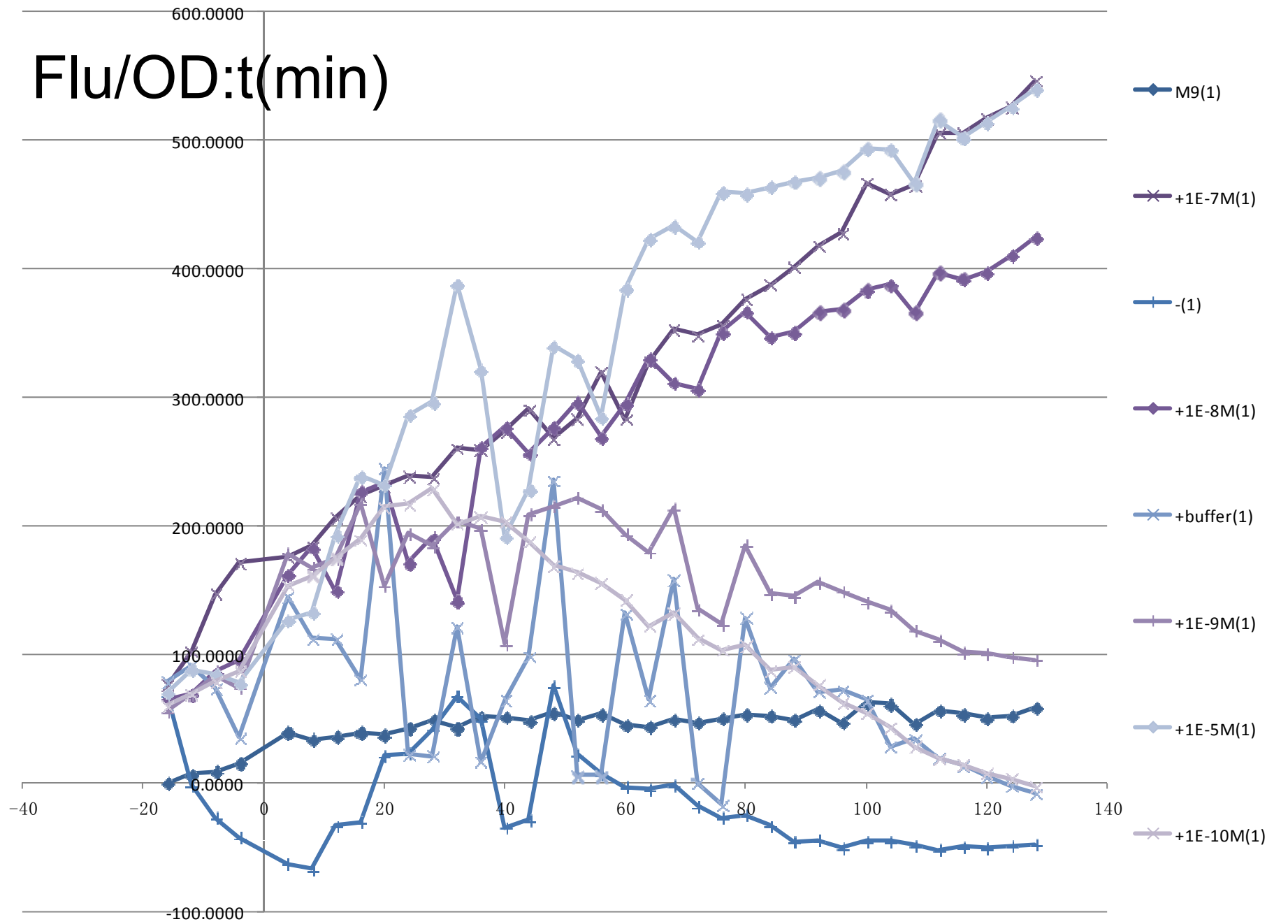


# Methods

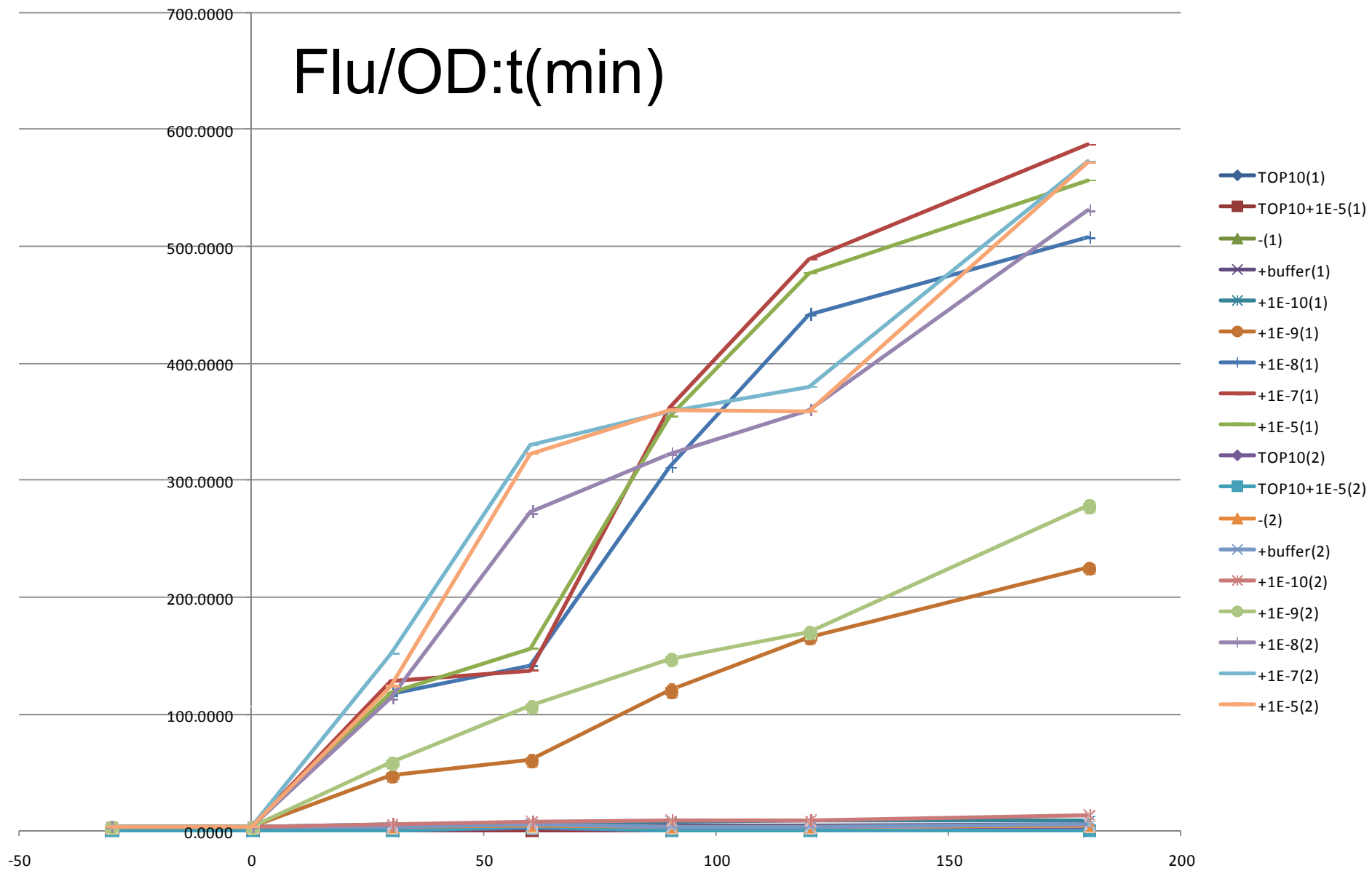
- Plate Reader
- Spectrophotometer



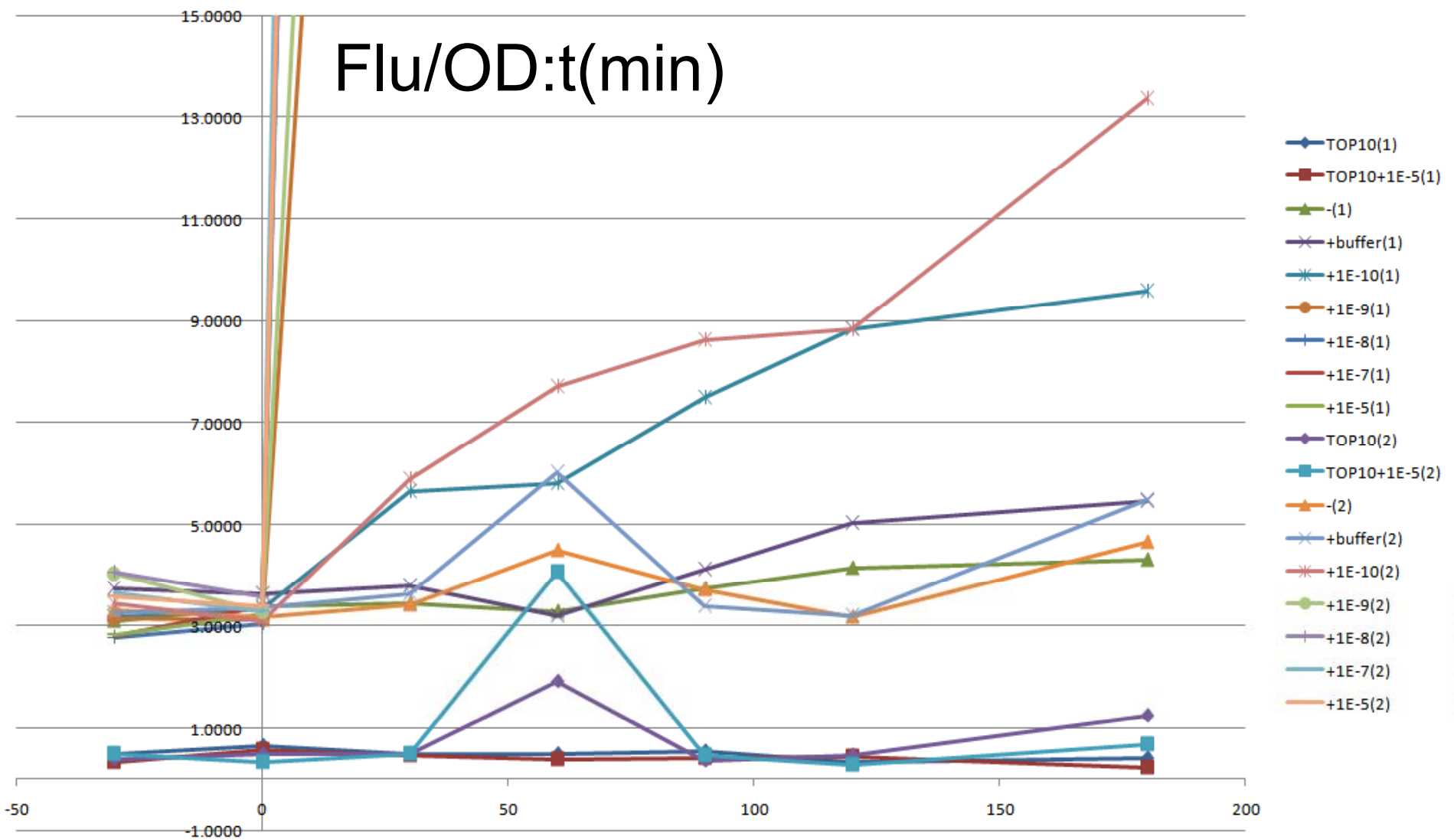
# Flu/OD:t(min)

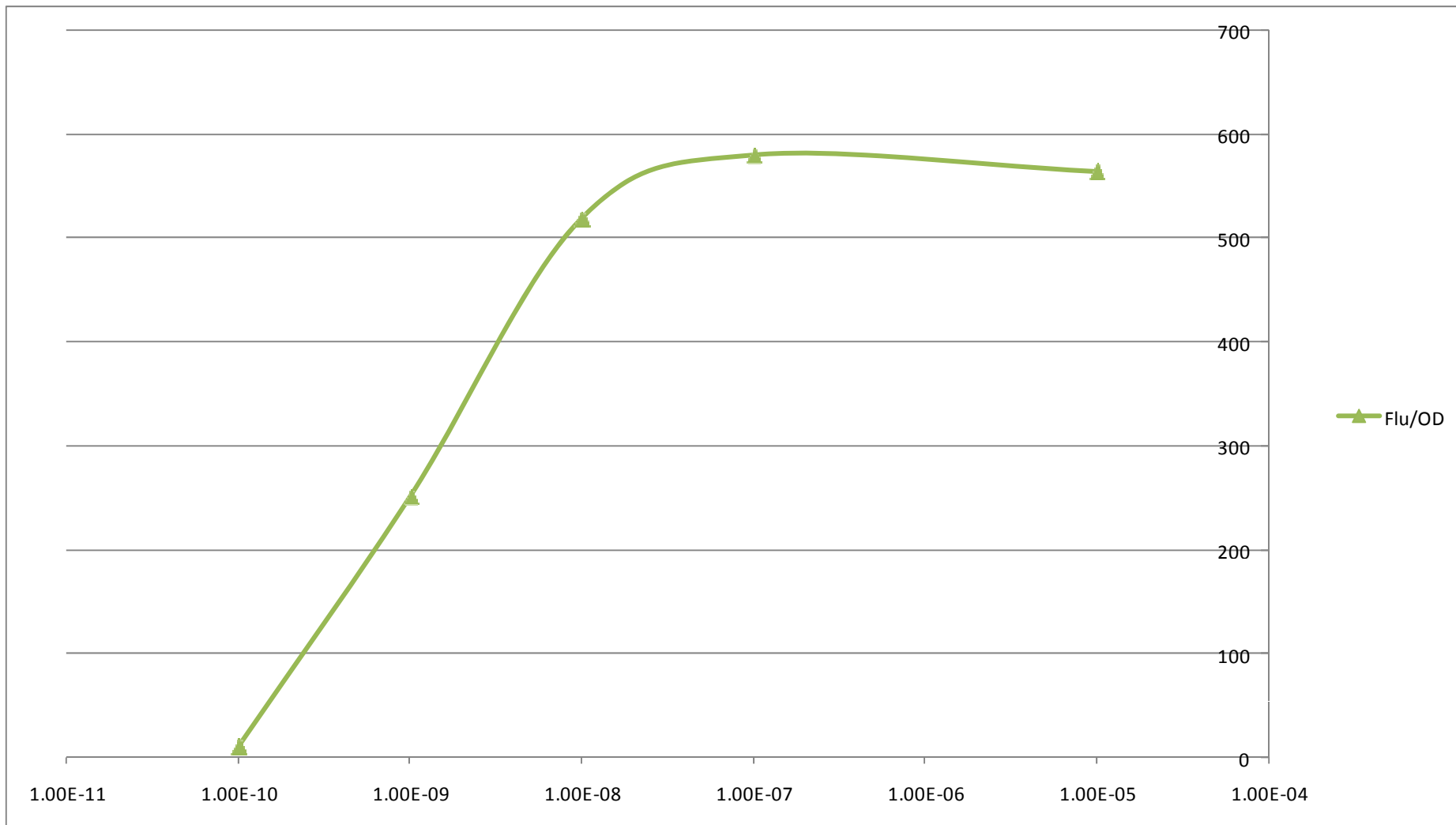


# Flu/OD:t(min)

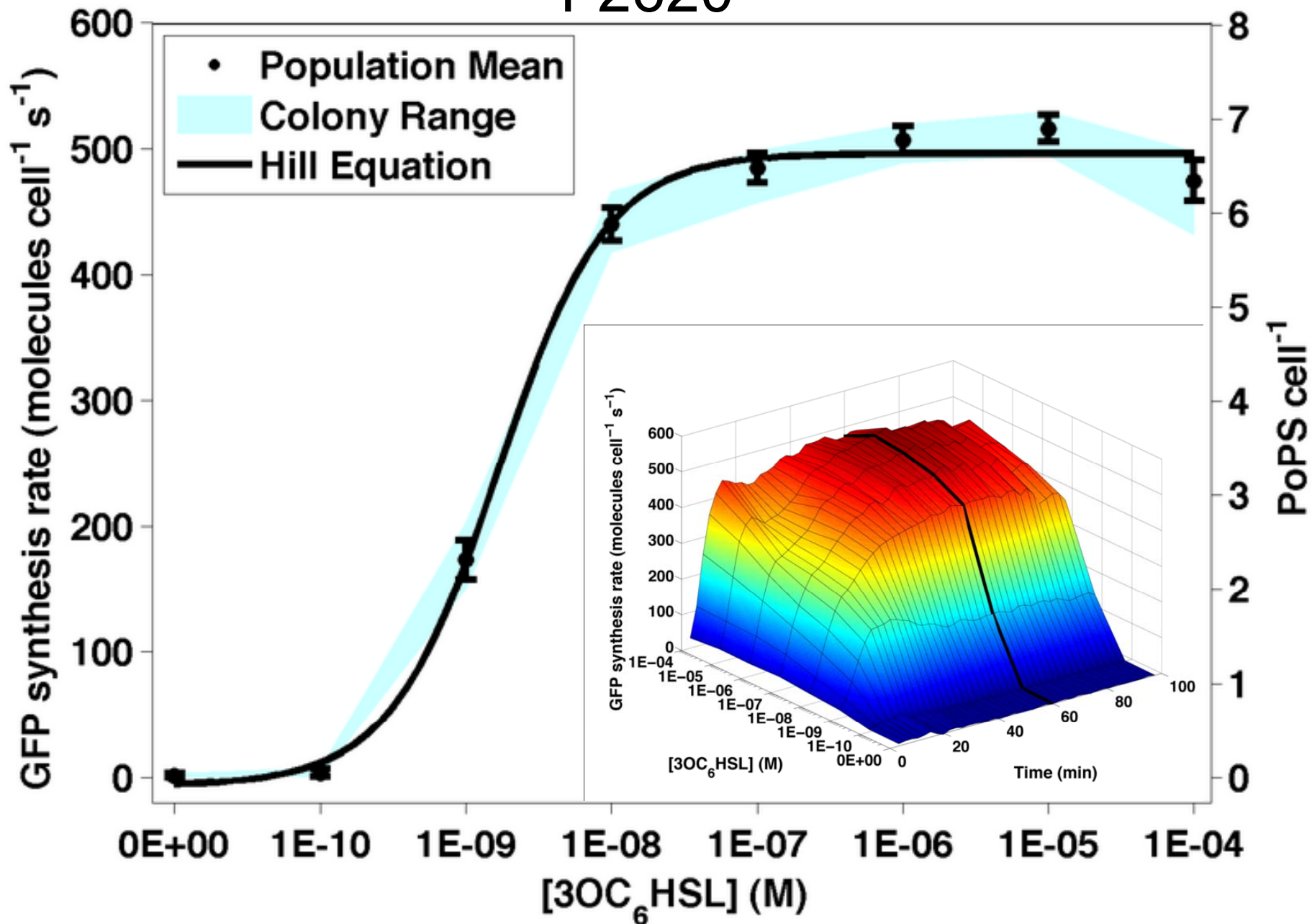


# Flu/OD:t(min)

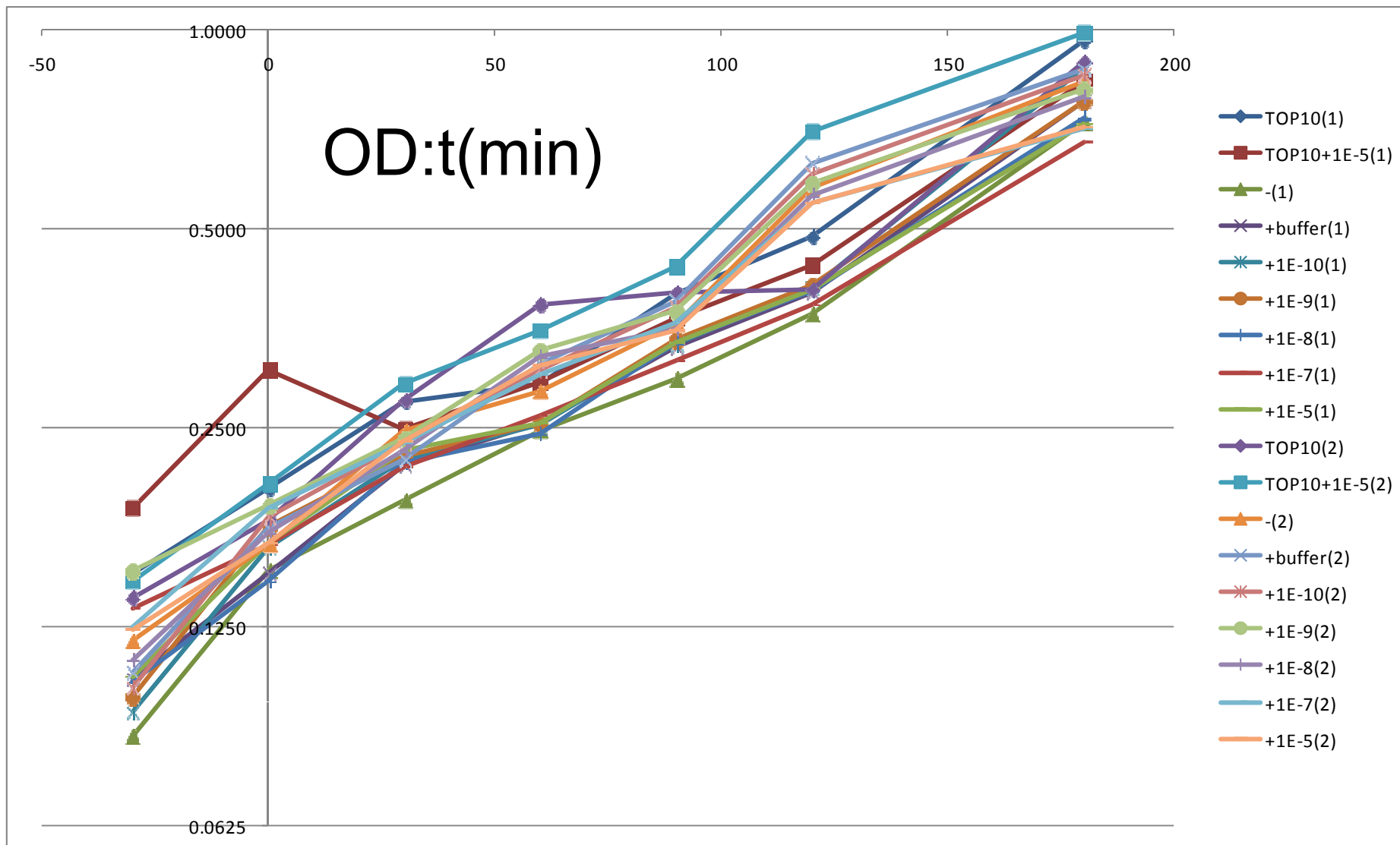




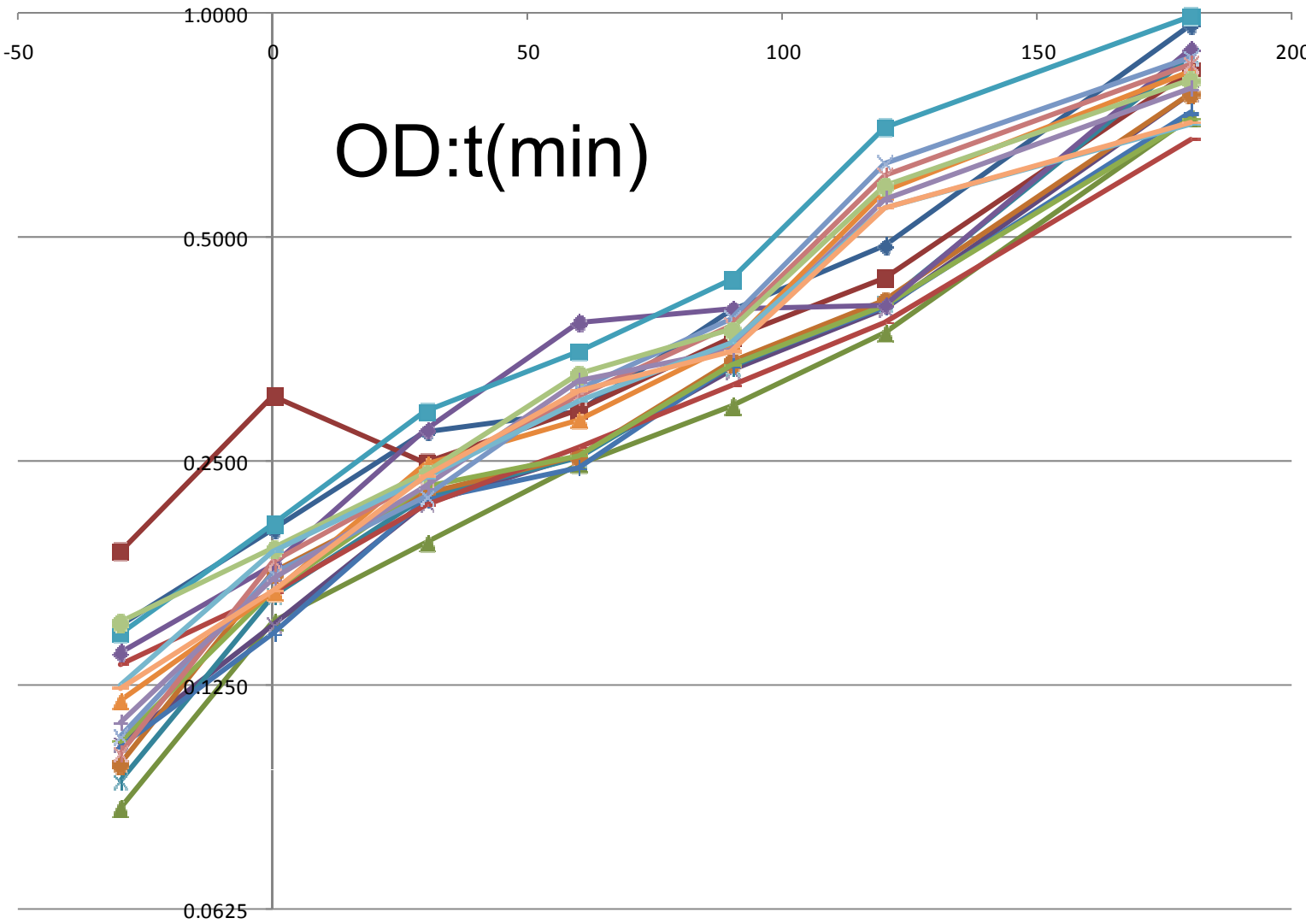
# F2620

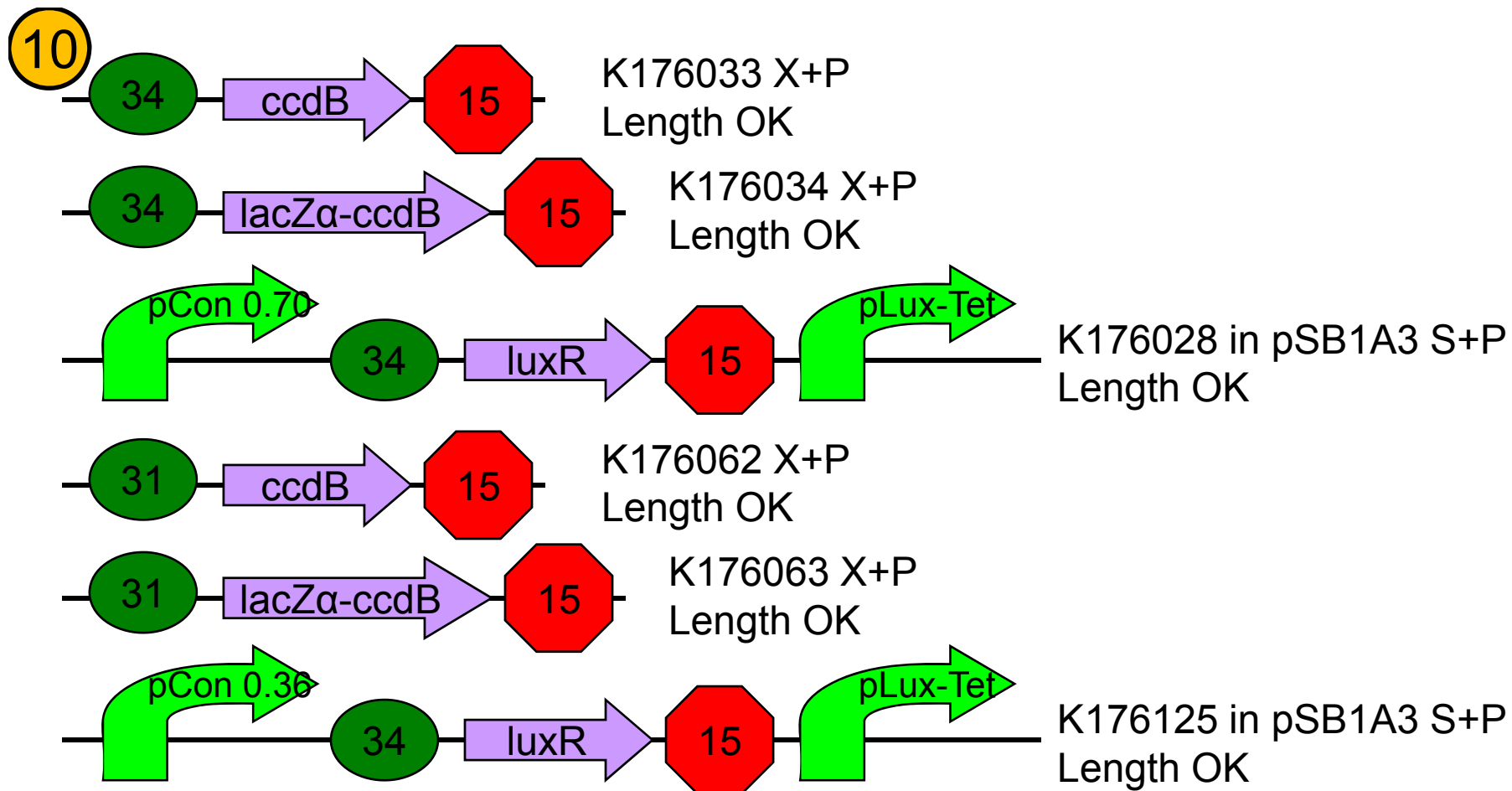






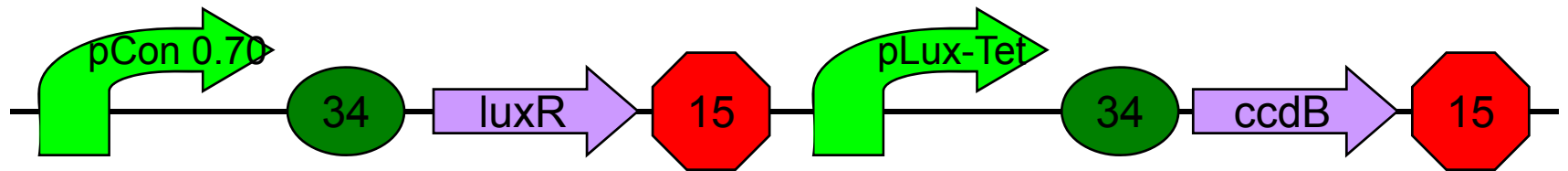
OD:t(min)



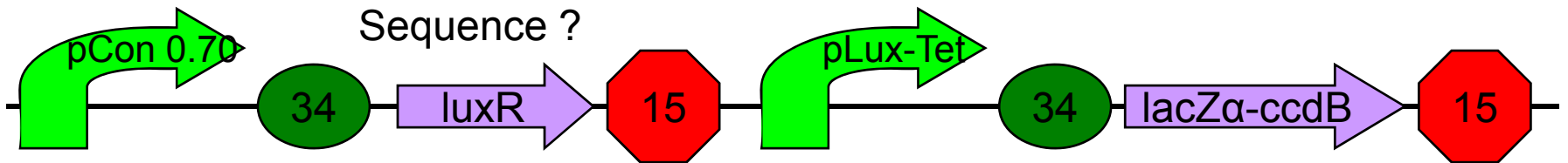


← VRccdB K176058  
Synthesis OK

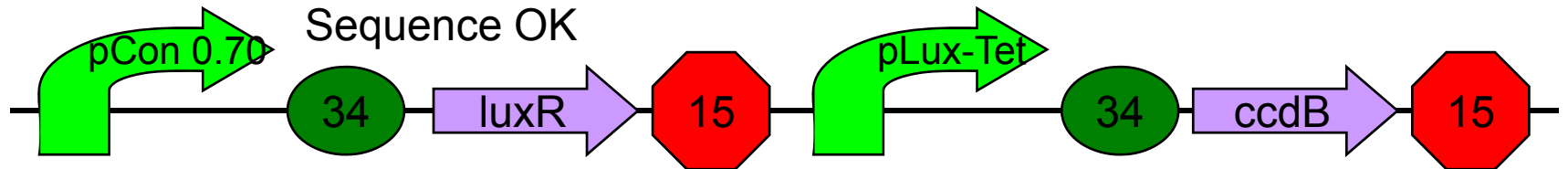
← M13-47 K176059  
Synthesis OK



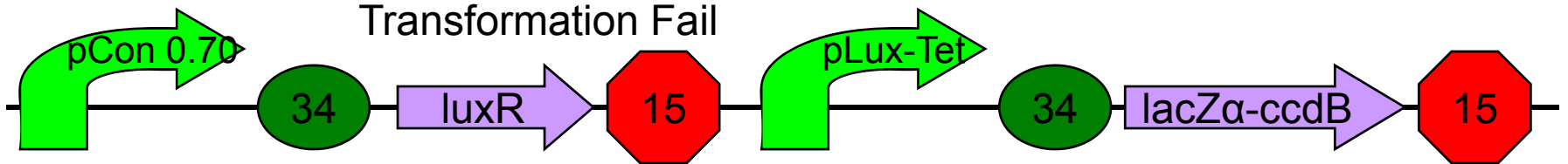
K176035 in pSB1A3 in DB3.1  
Sequence ?



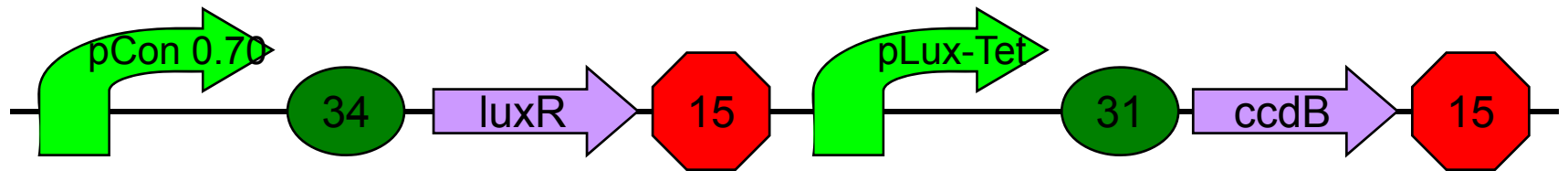
K176036 in pSB1A3 in DB3.1  
Sequence OK



K176035 in pSB1A3 in TOP10  
Transformation Fail

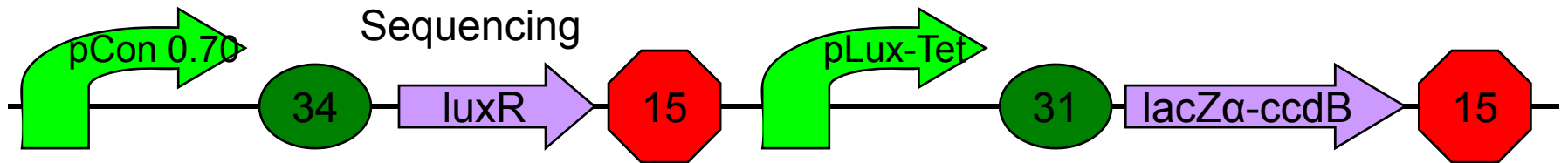


K176036 in pSB1A3 in TOP10  
Transformation Fail



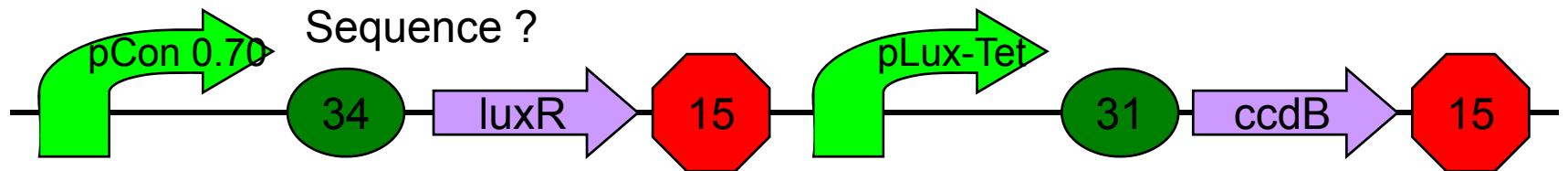
K176089 in pSB1A3 in DB3.1

Sequencing



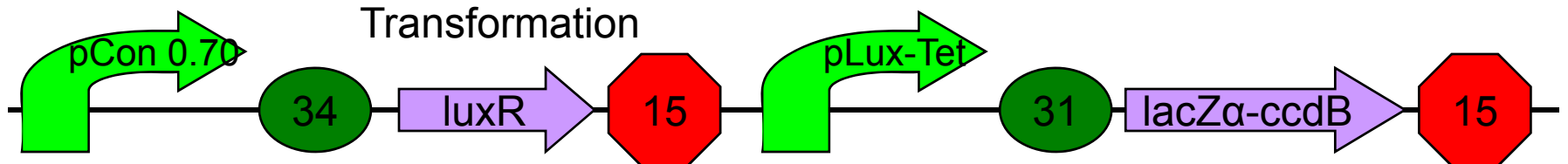
K176090 in pSB1A3 in DB3.1

Sequence ?



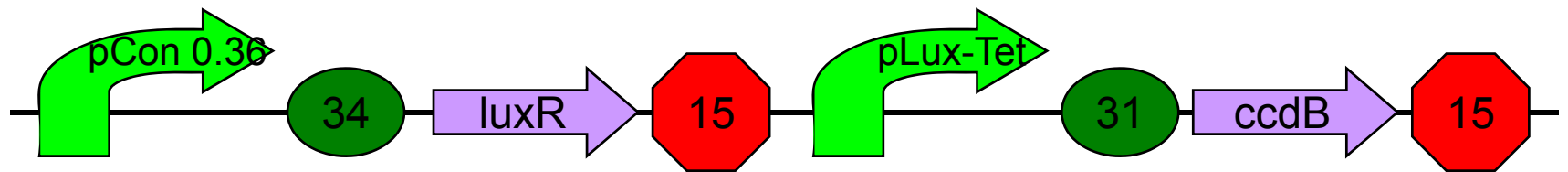
K176089 in pSB1A3 in TOP10

Transformation



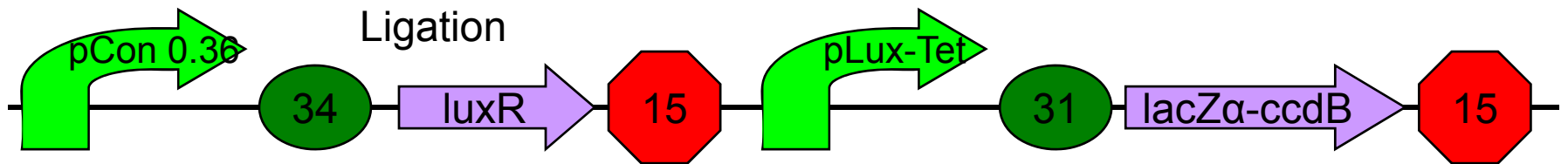
K176090 in pSB1A3 in TOP10

Transformation



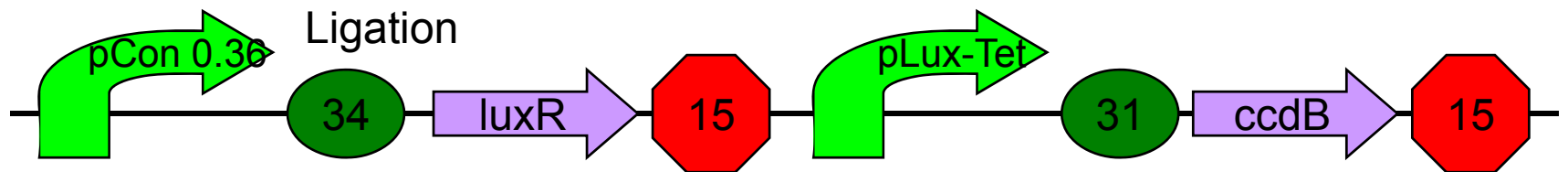
K176131 in pSB1A3 in DB3.1

Ligation



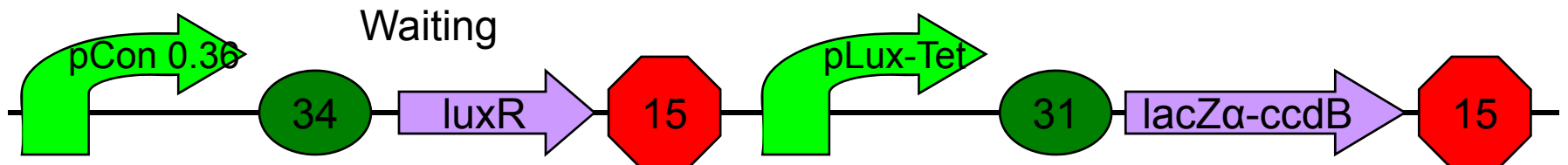
K176132 in pSB1A3 in DB3.1

Ligation



K176131 in pSB1A3 in TOP10

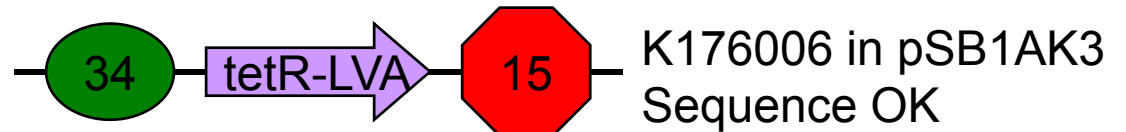
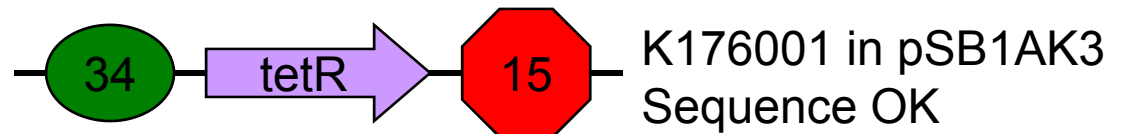
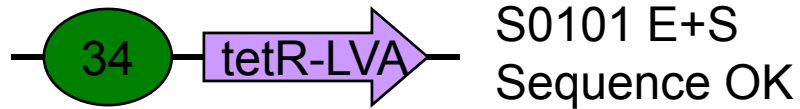
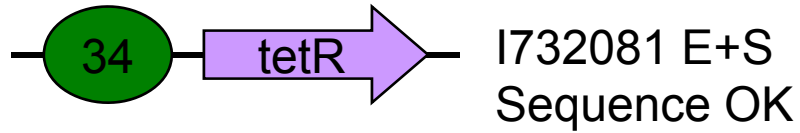
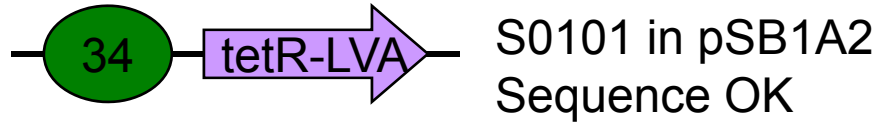
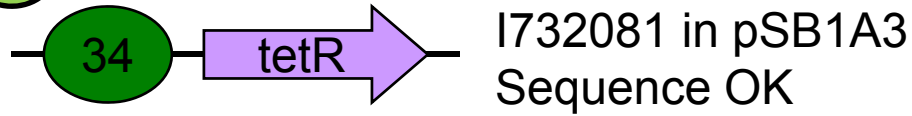
Waiting



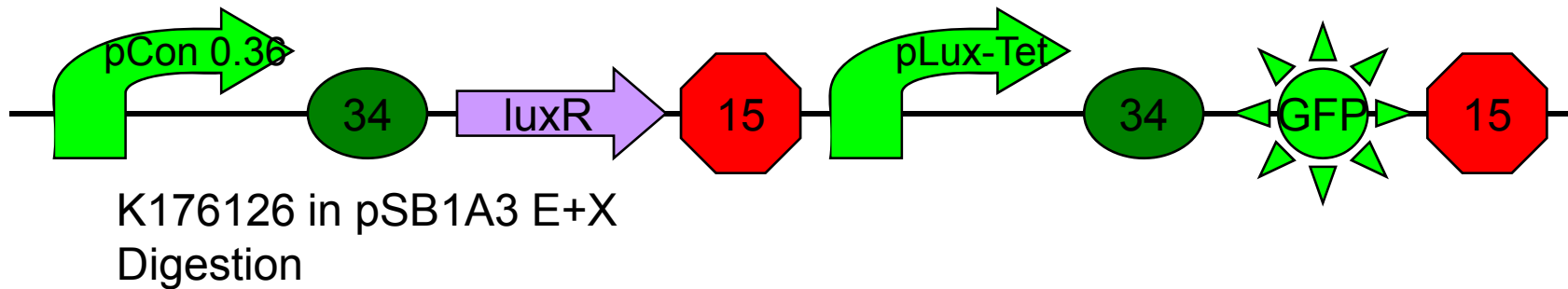
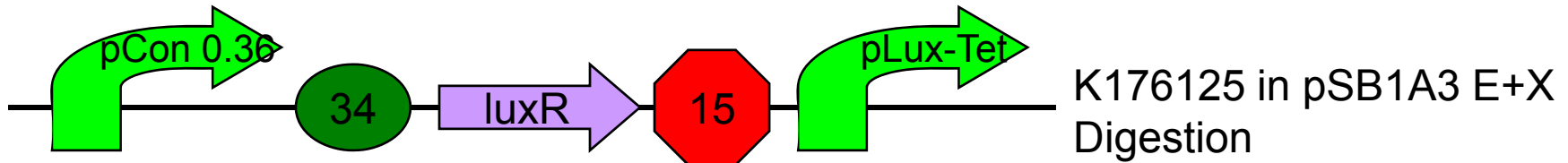
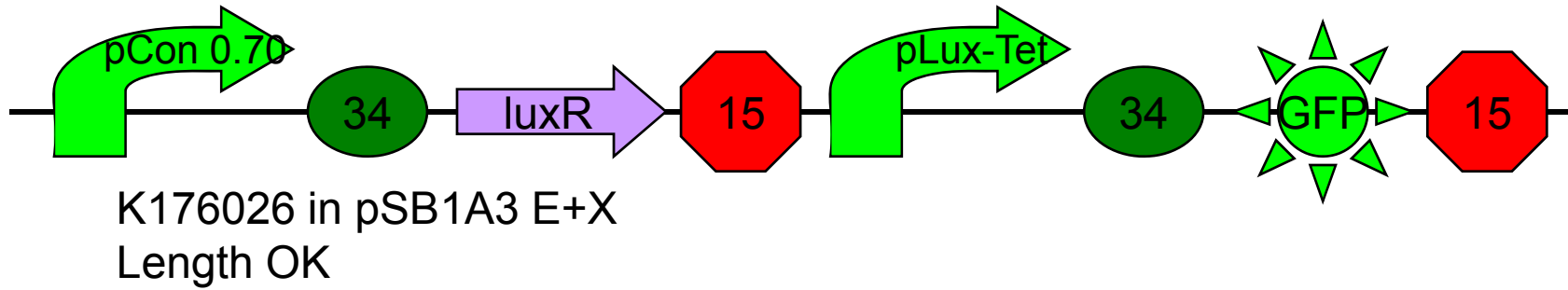
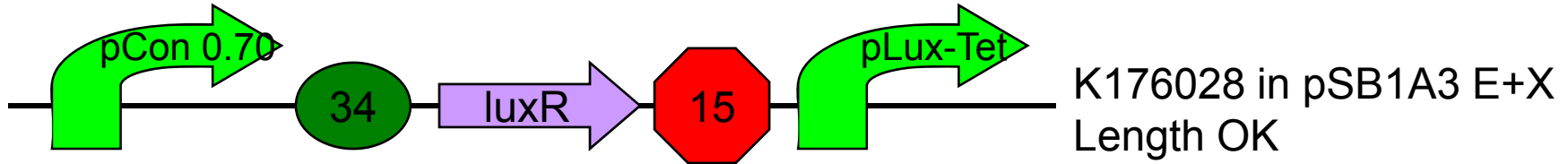
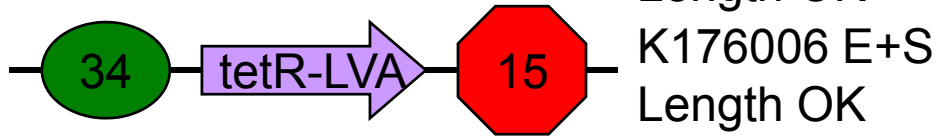
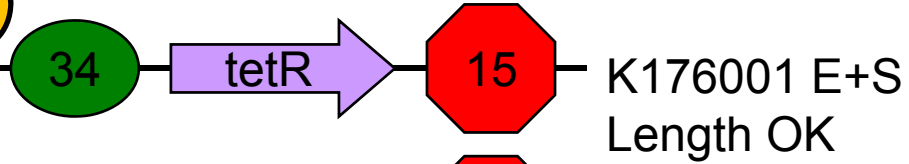
K176132 in pSB1A3 in TOP10

Waiting

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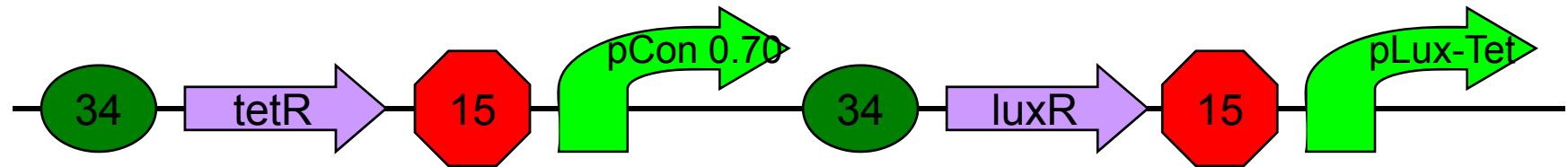


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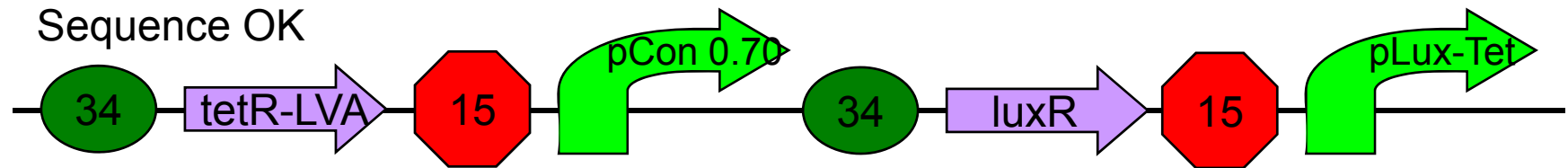




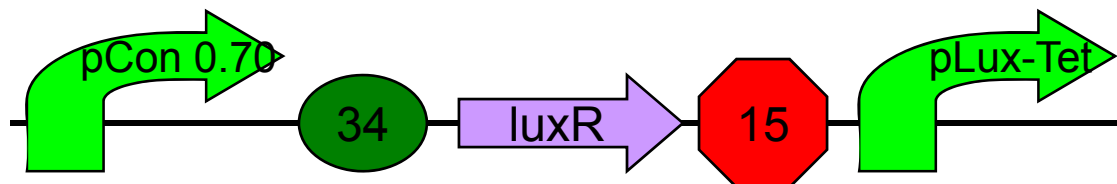
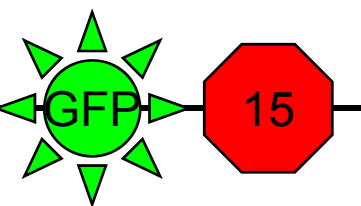
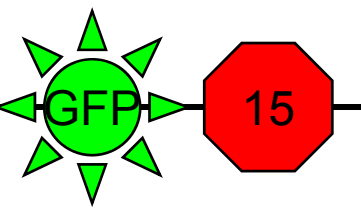
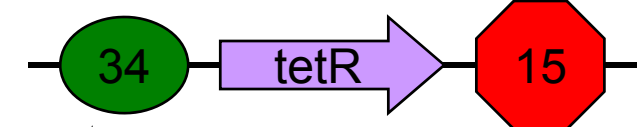
K176057  
Synthesis OK



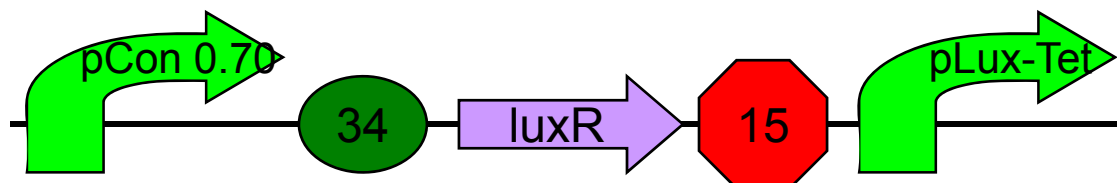
K176031 in pSB1A3  
Sequence OK



K176032 in pSB1A3  
Sequence OK

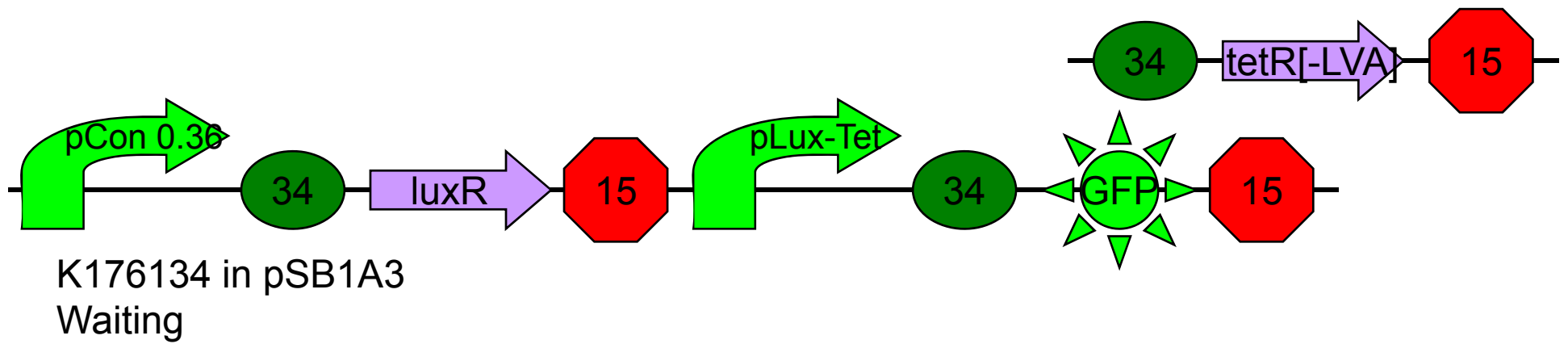
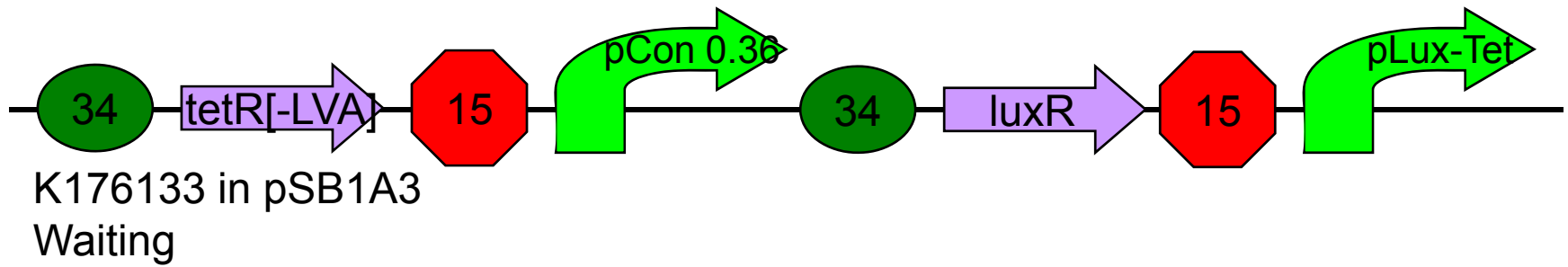


K176029 in pSB1A3  
Sequence ?

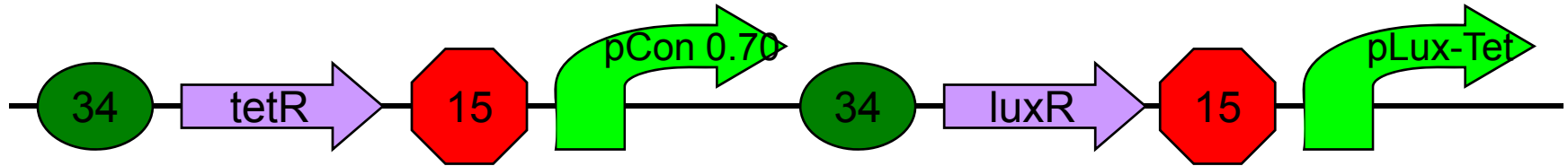


K176030 in pSB1A3  
Sequence ?

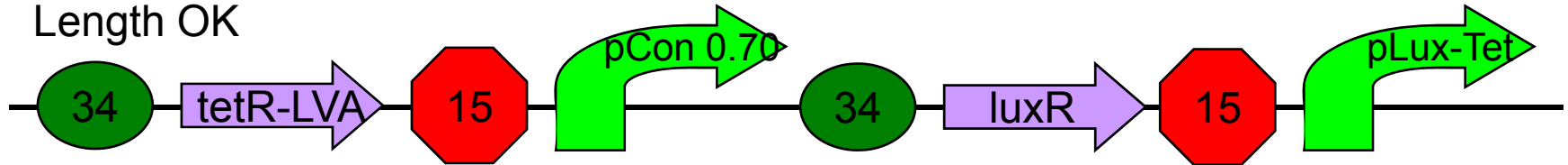




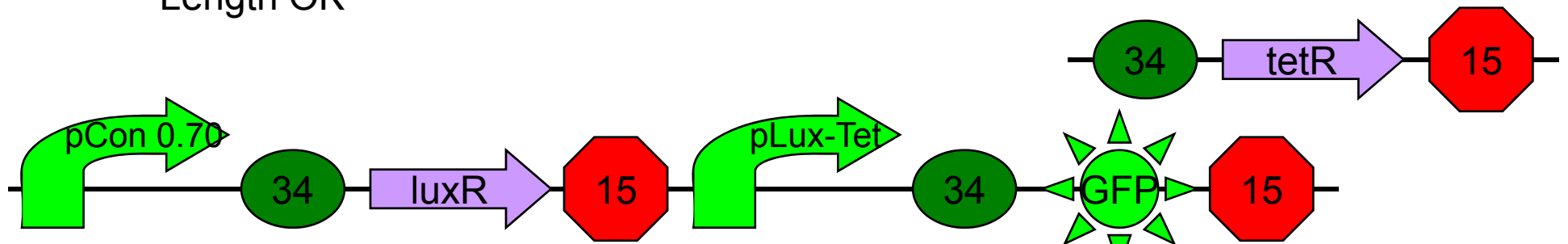
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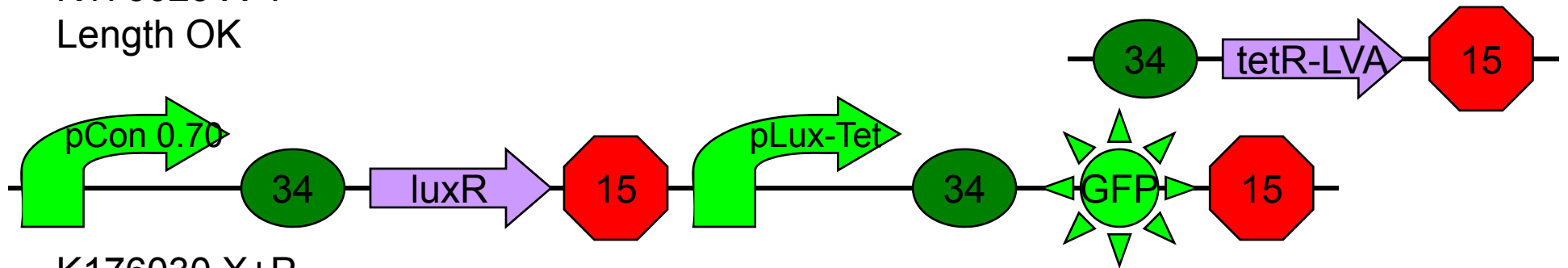
K176031 X+P  
Length OK



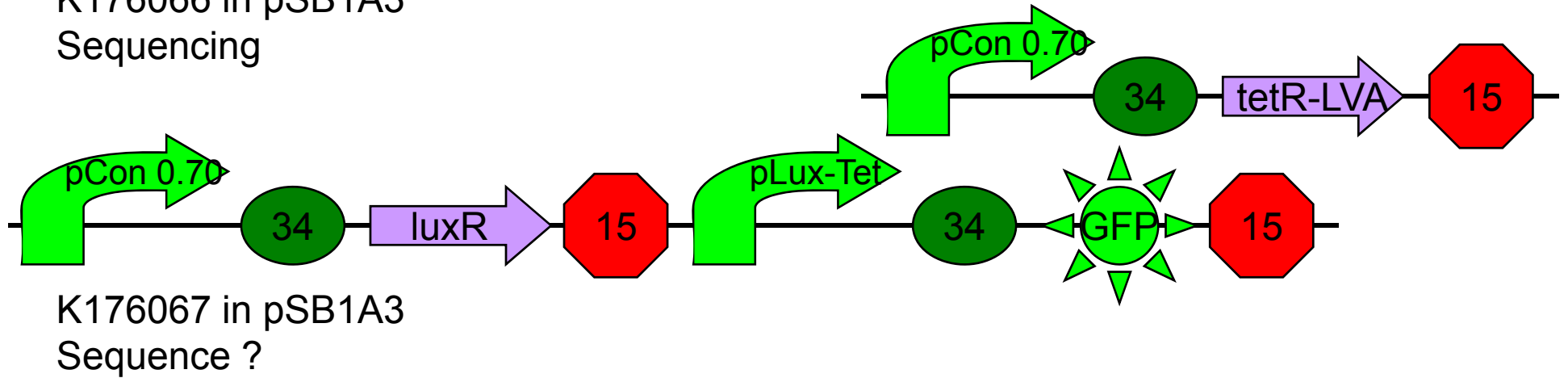
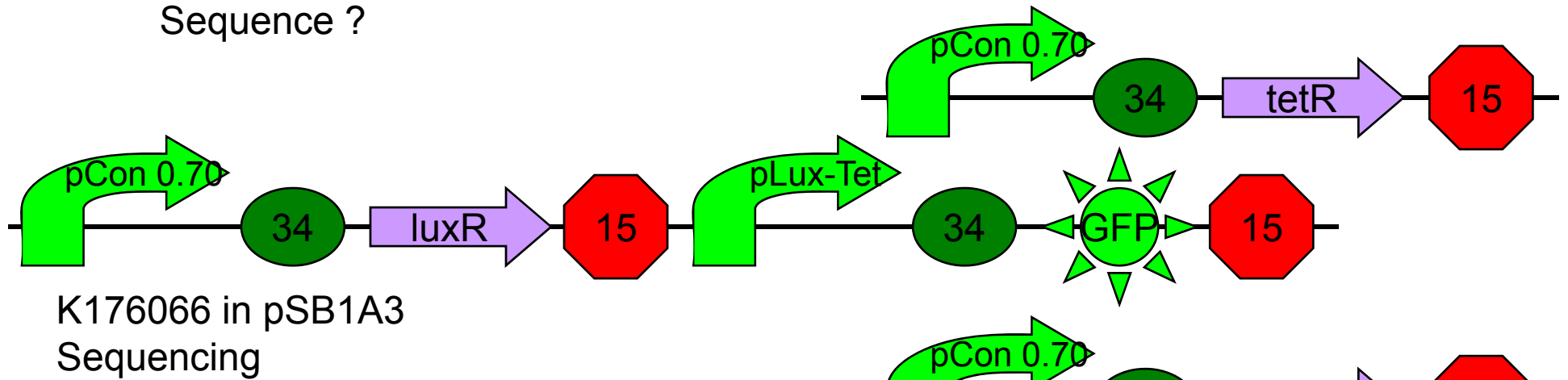
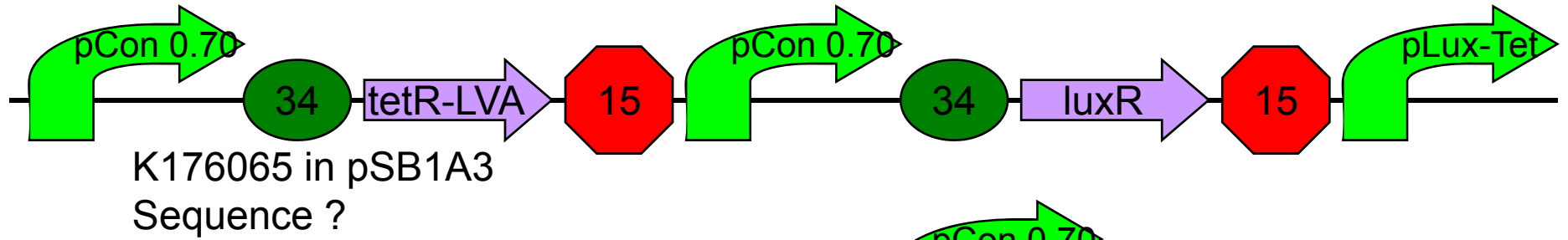
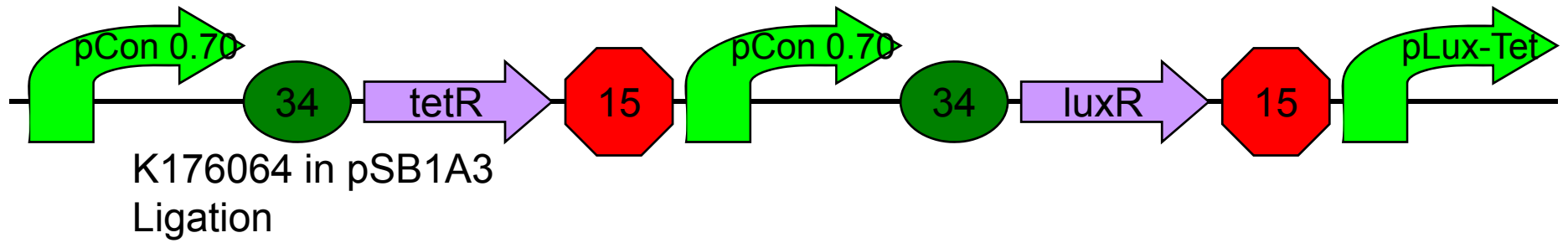
K176032 X+P  
Length OK

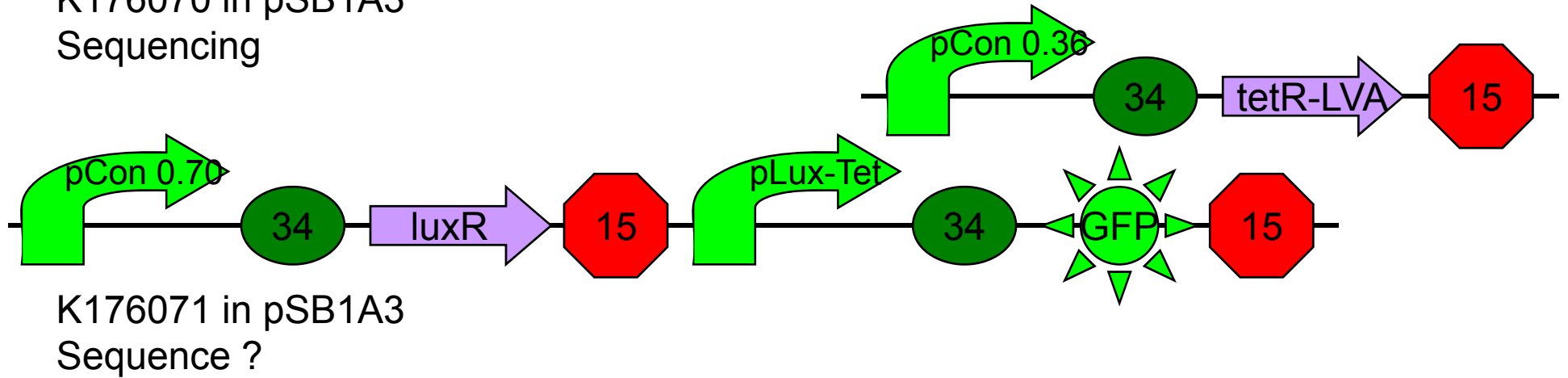
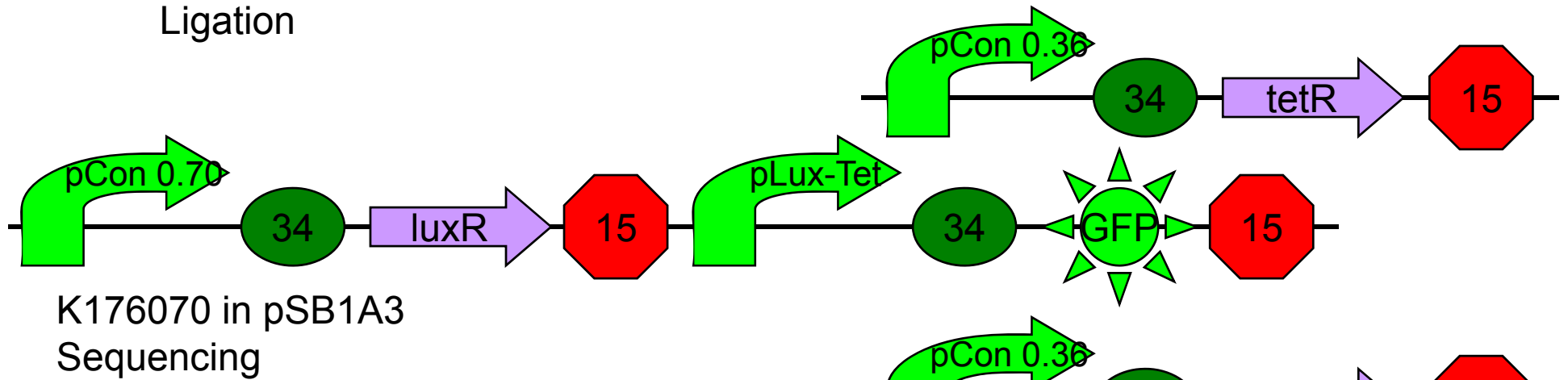
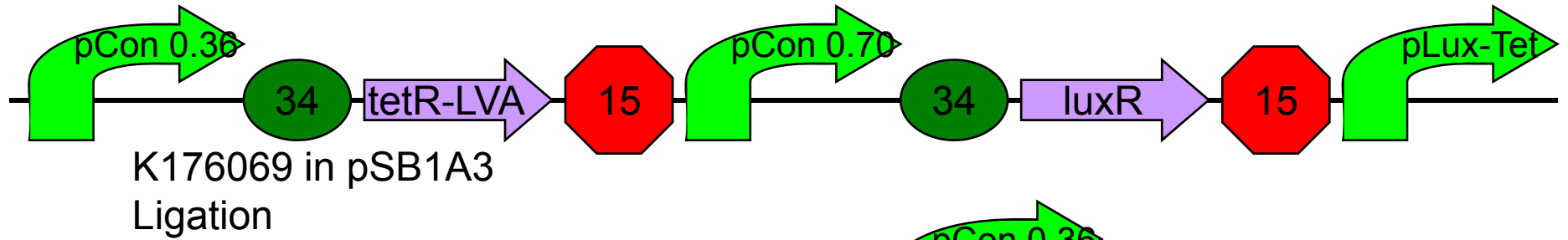
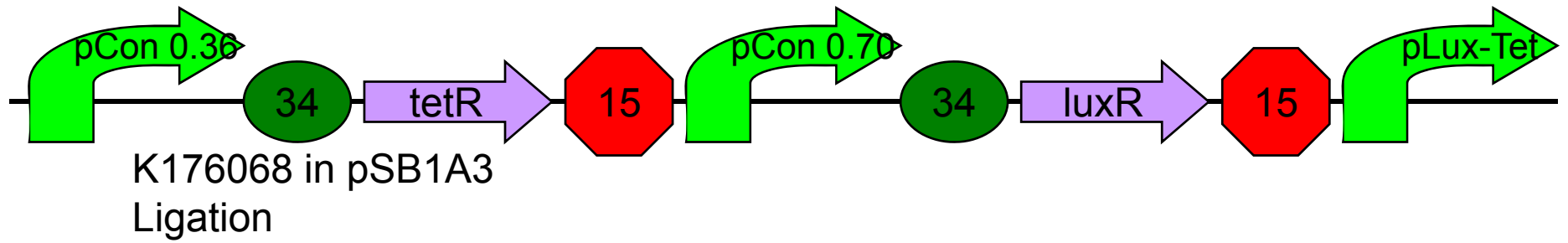


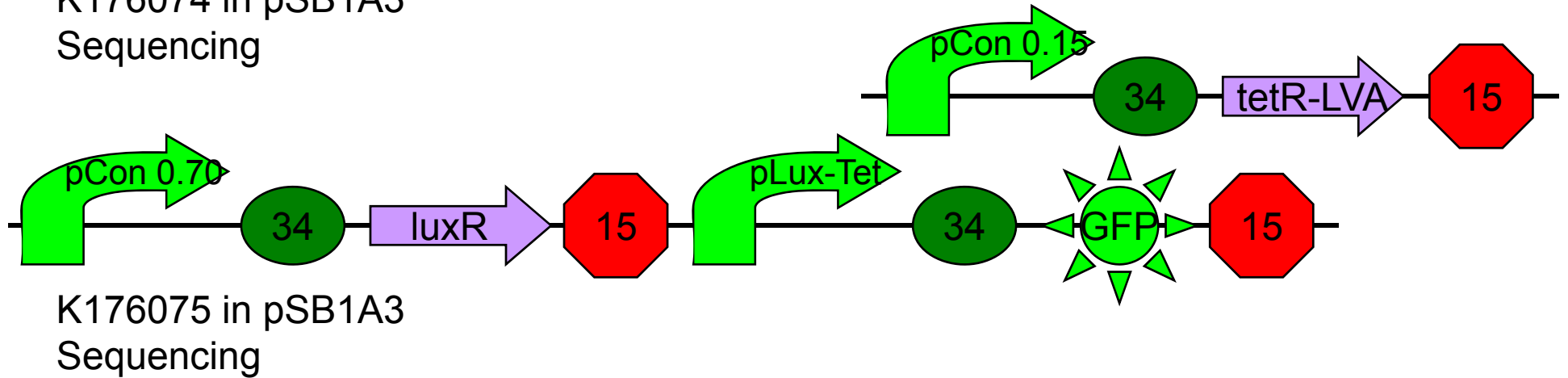
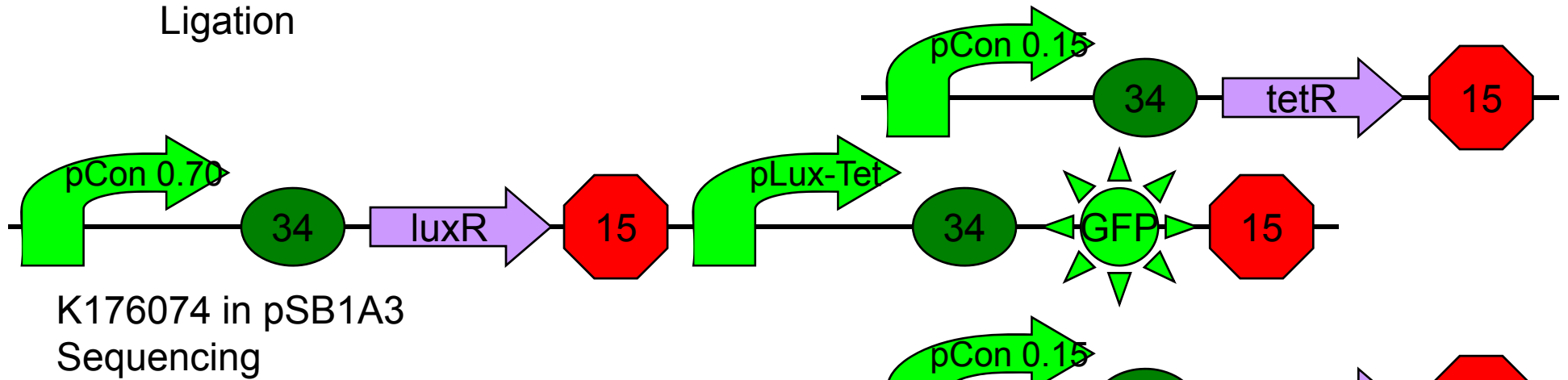
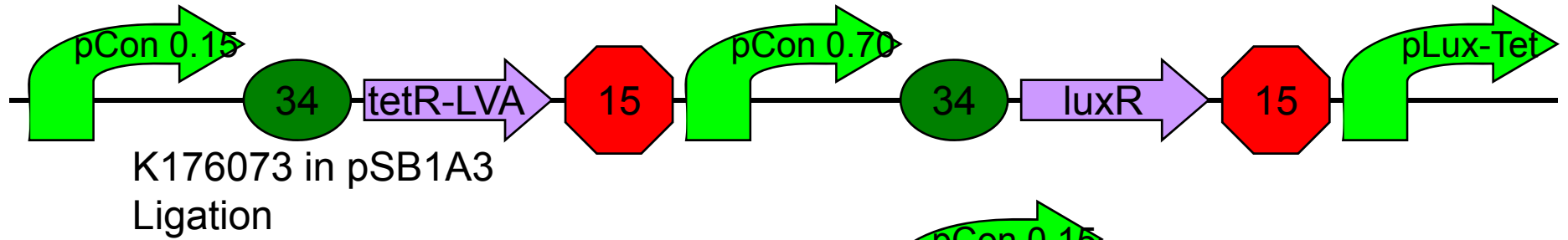
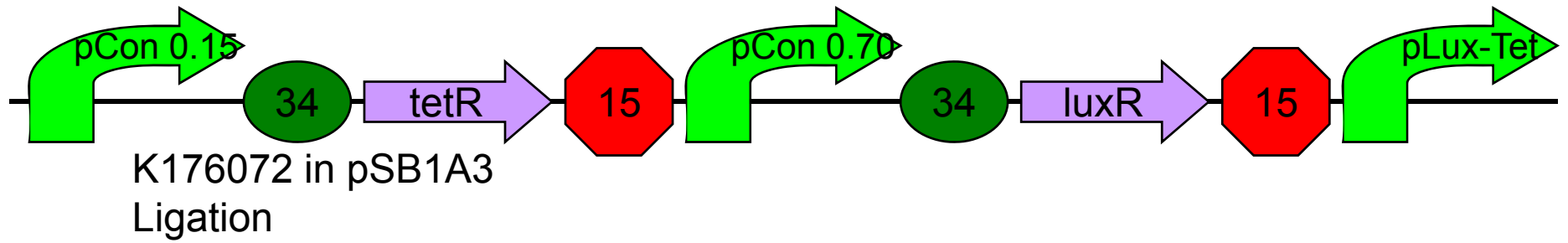
K176029 X+P  
Length OK

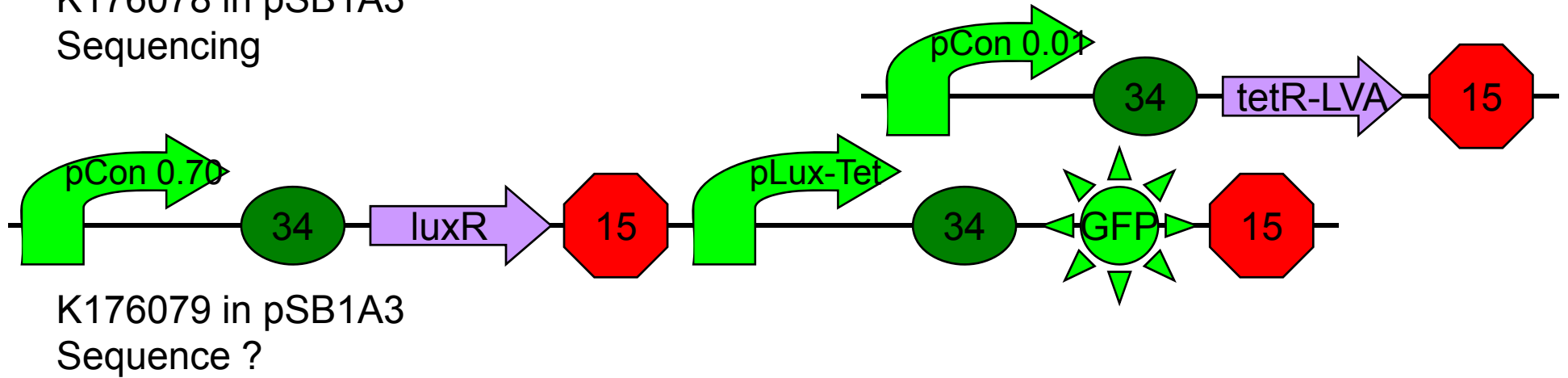
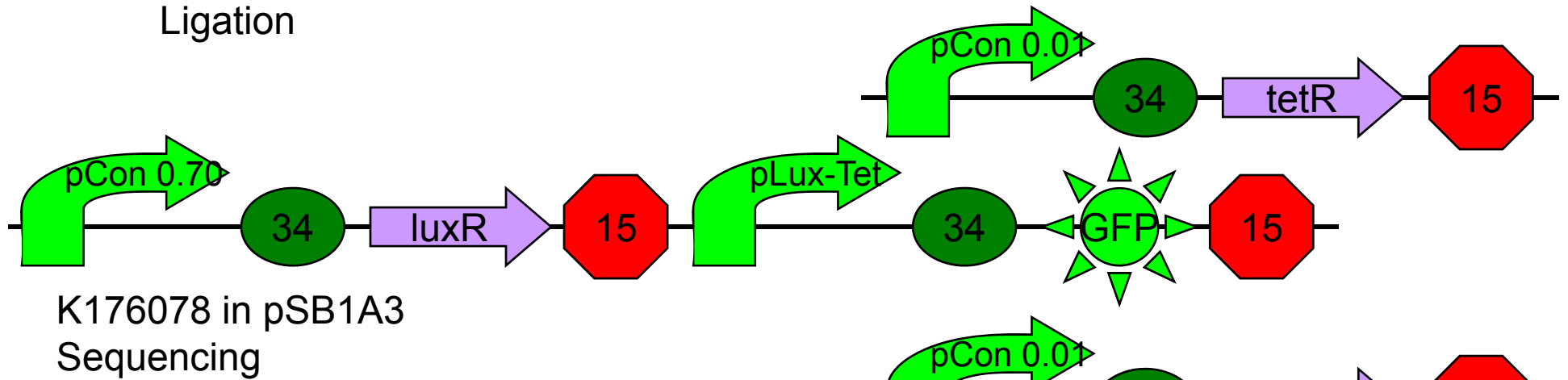
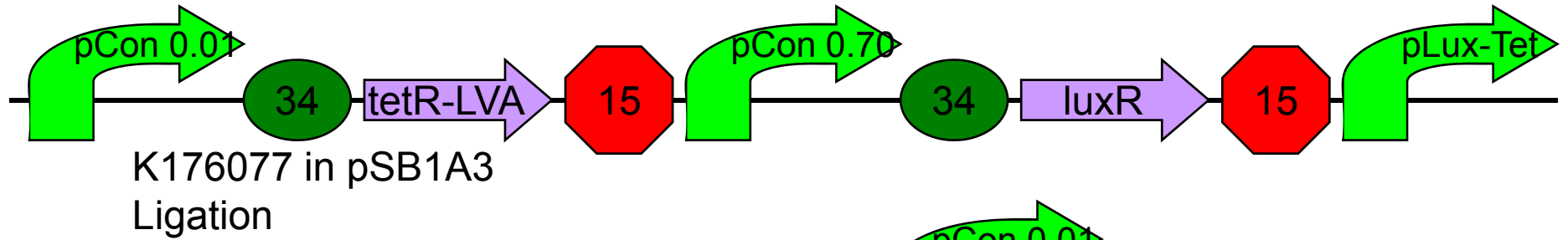
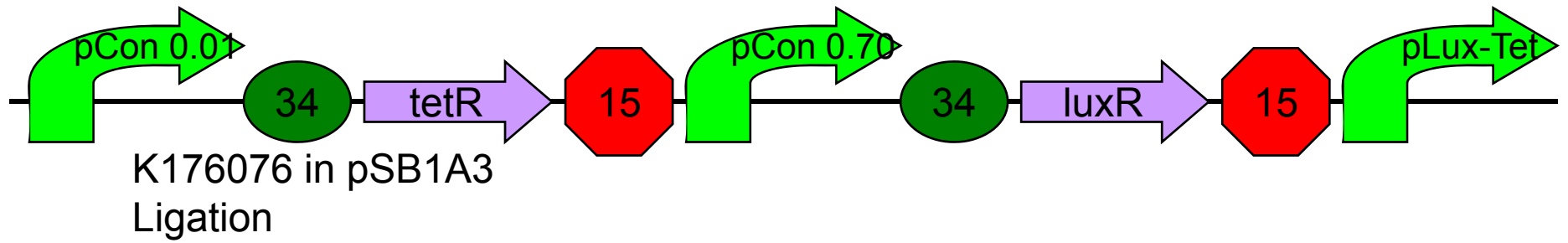


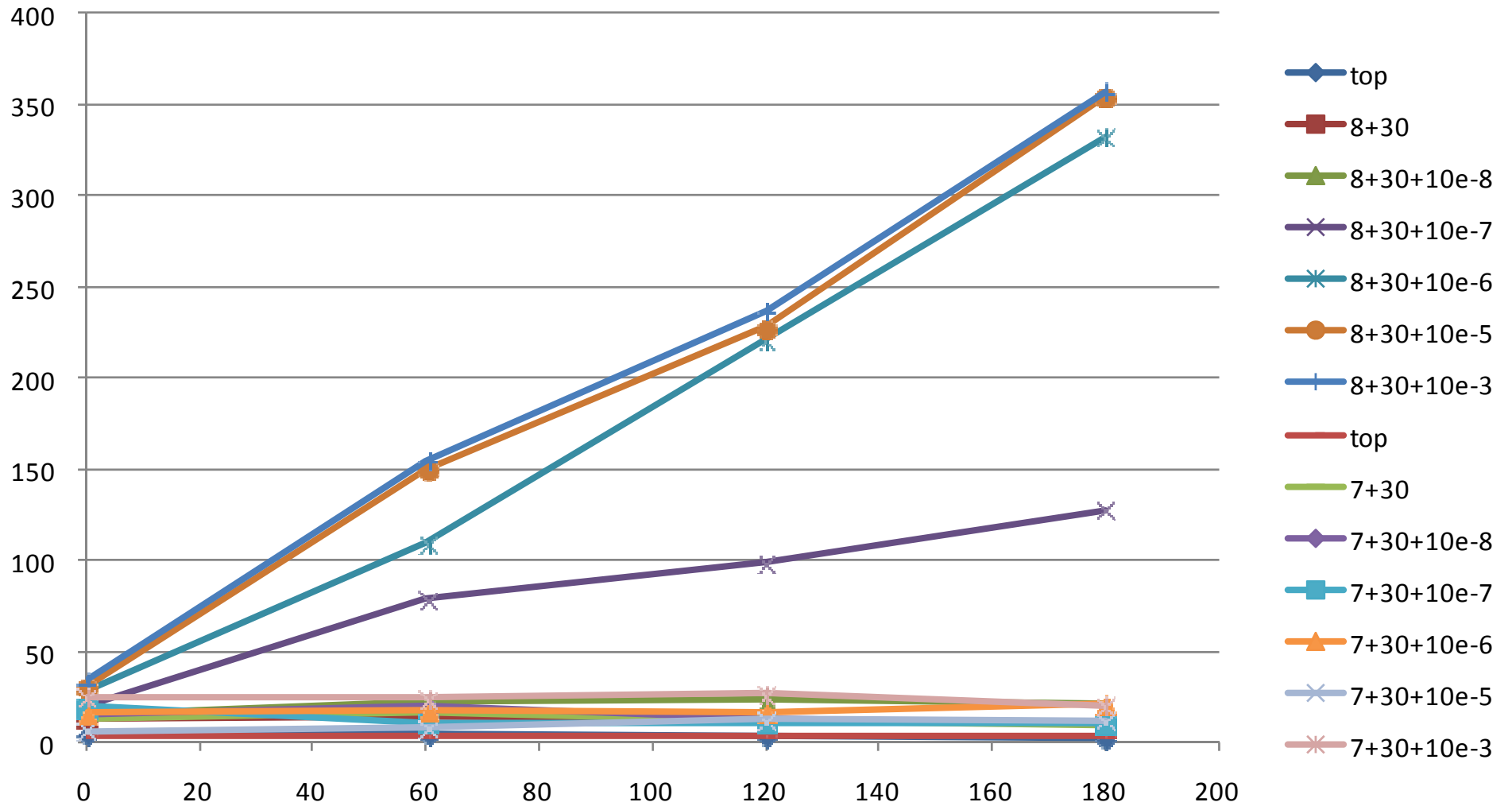
K176030 X+P  
Length OK



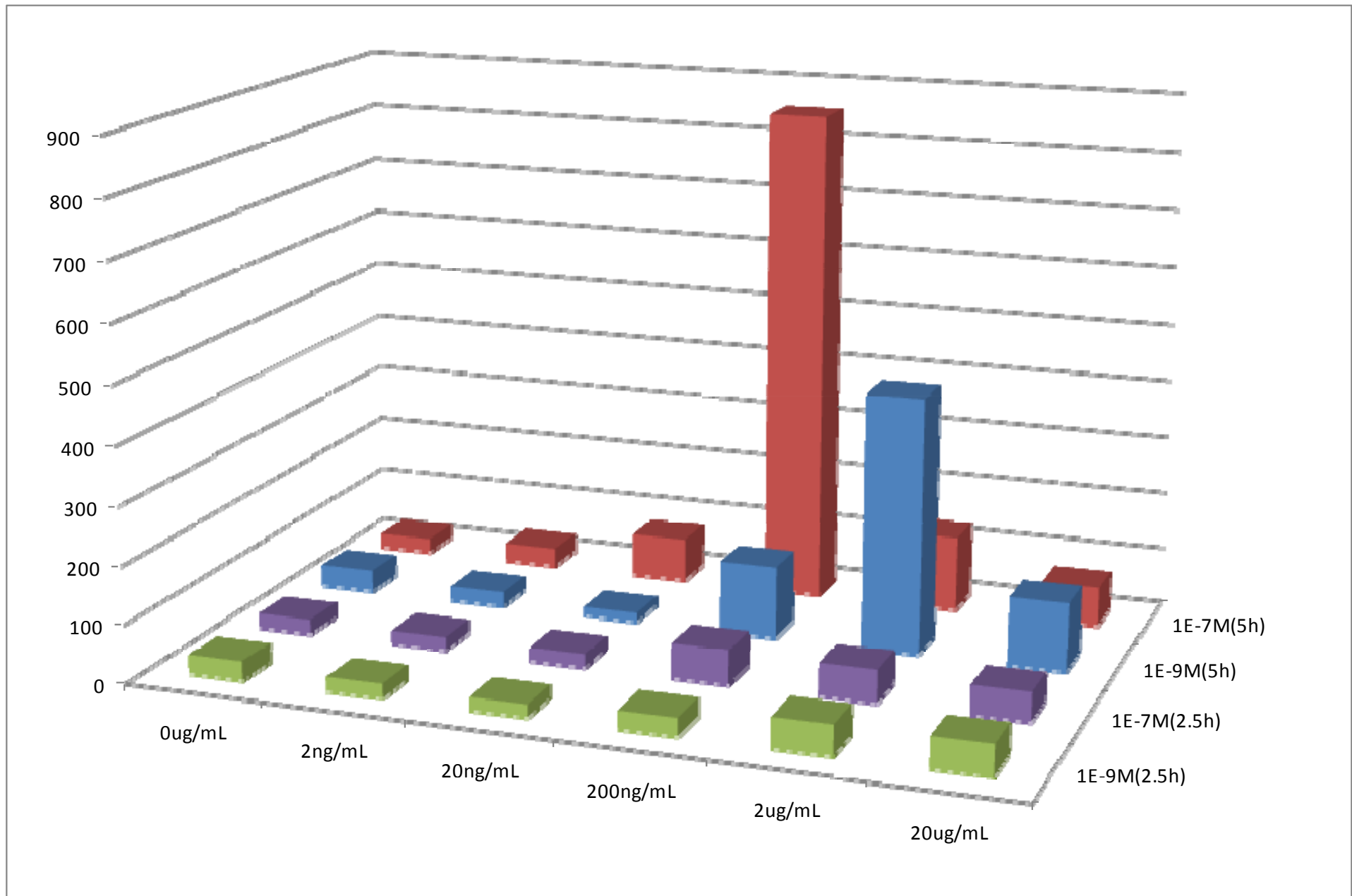






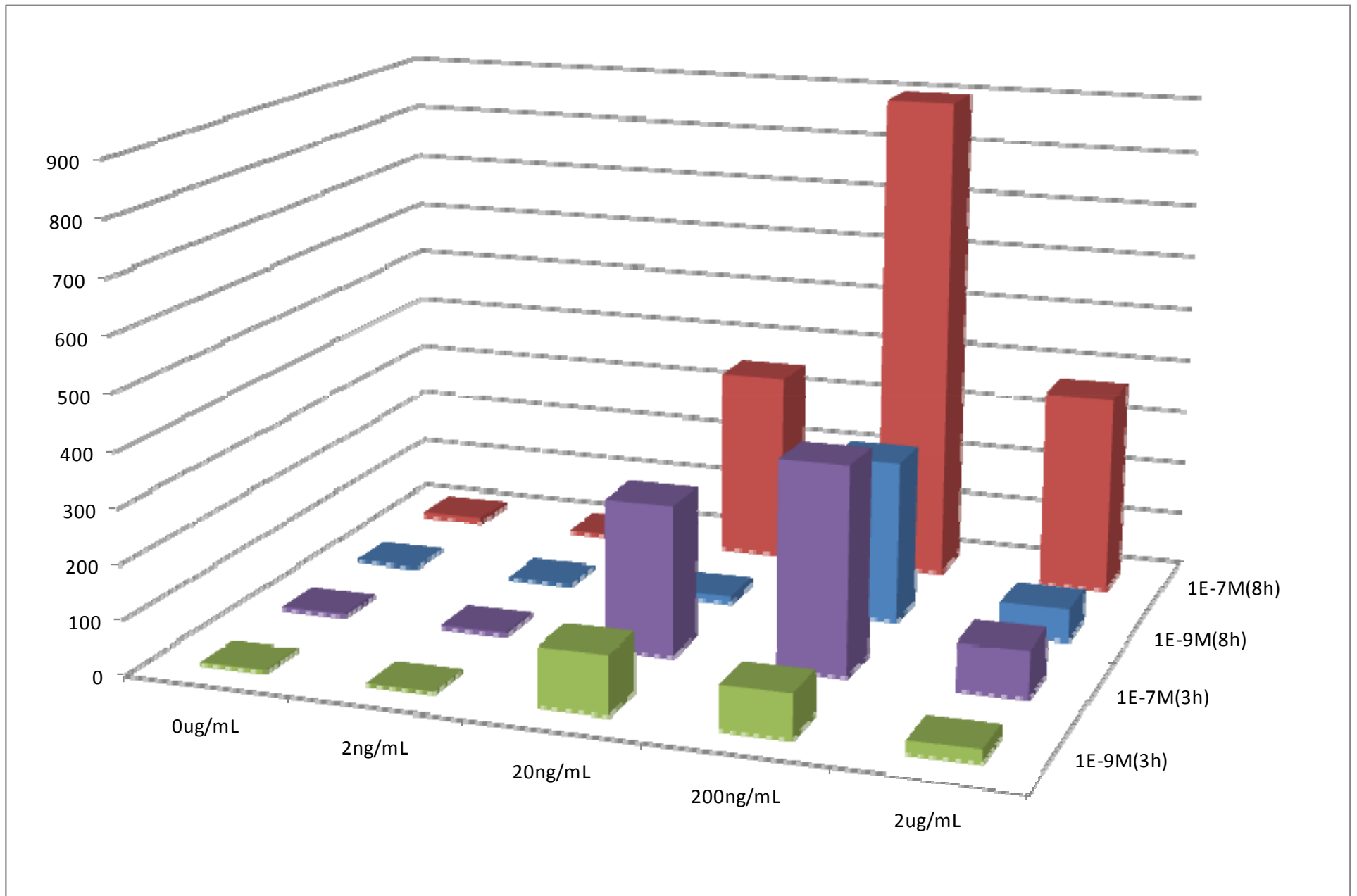


# K176067 (7+30)

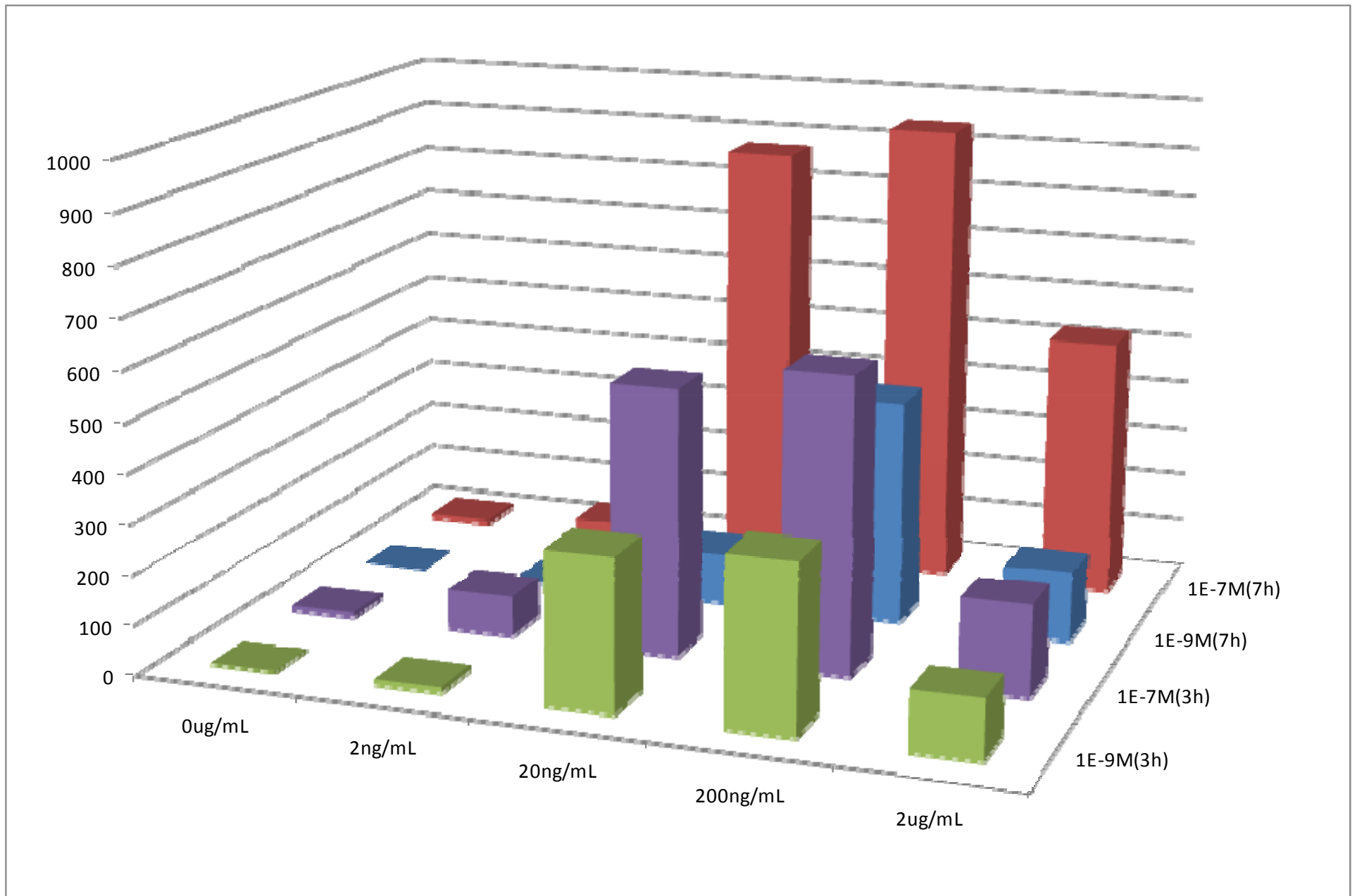


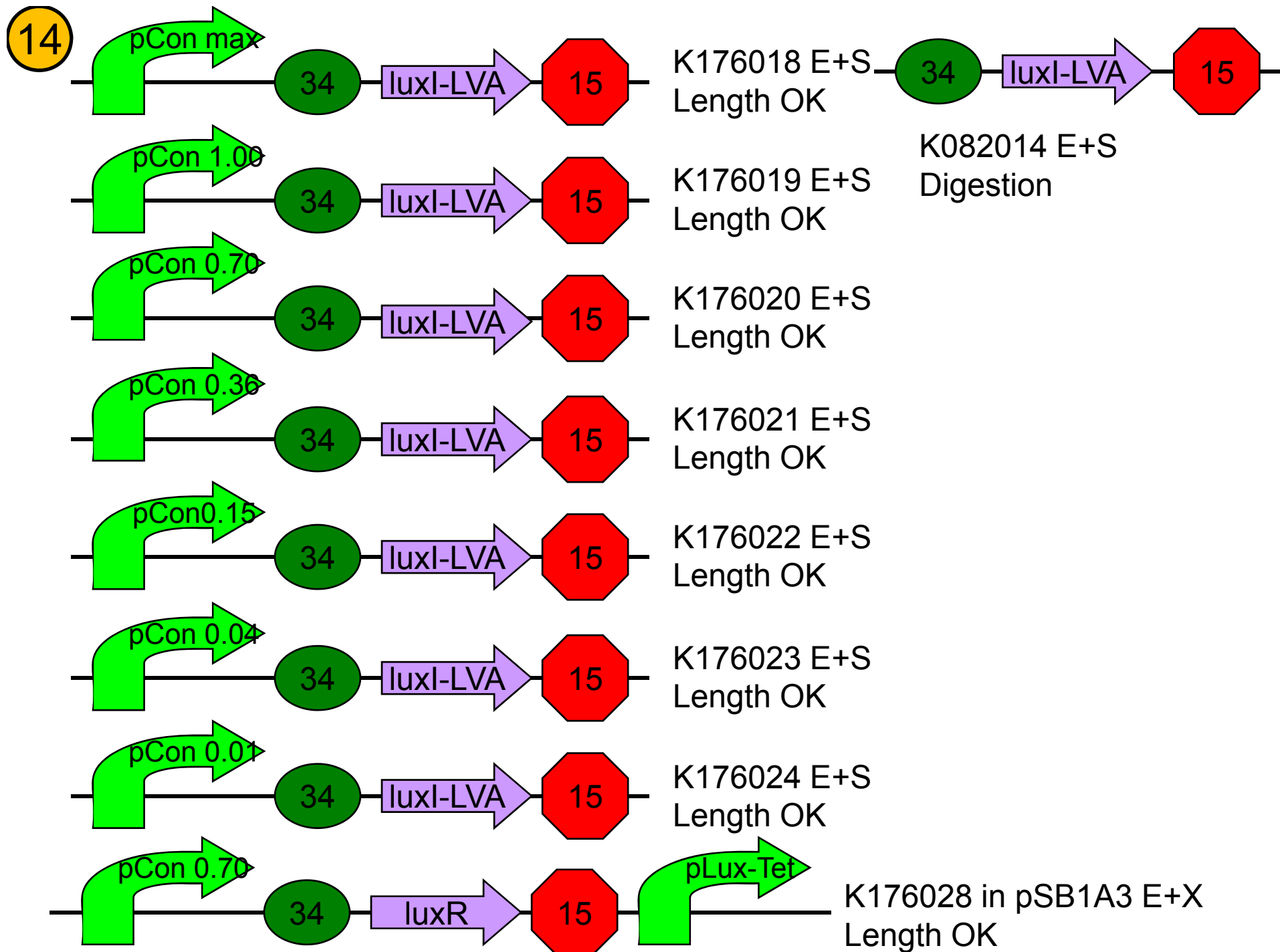


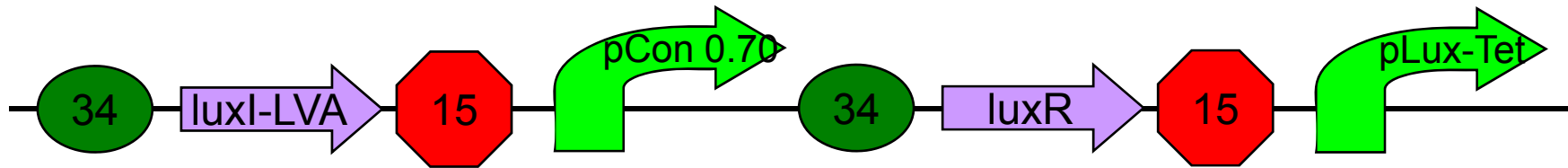
# K176070 (9+29)



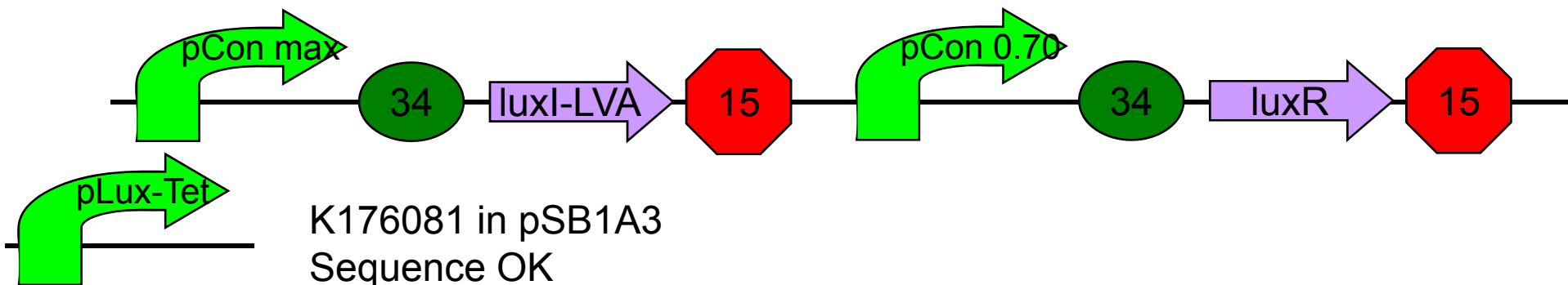
# K176071 (9+30)



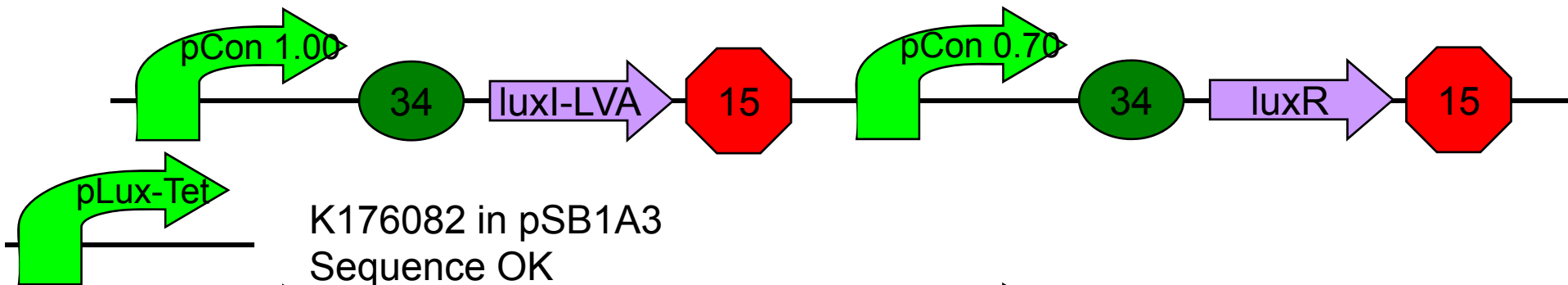




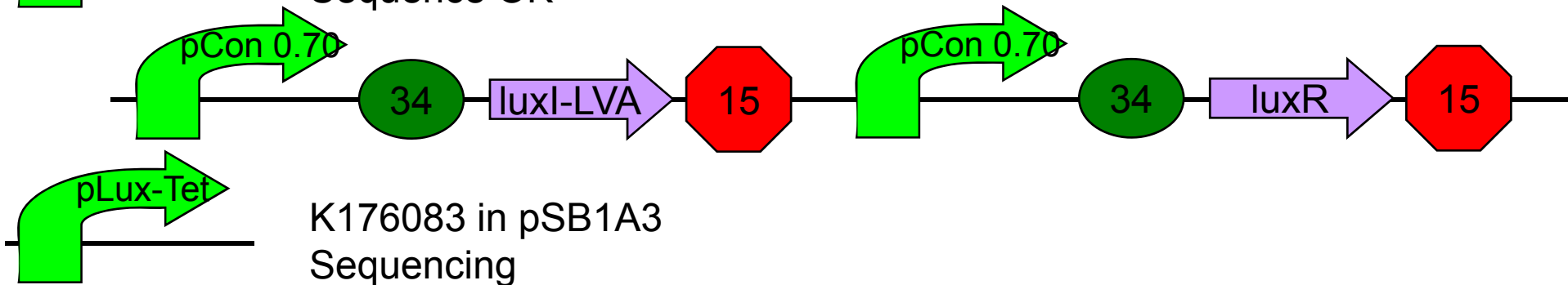
K176080 in pSB1A3  
Waiting



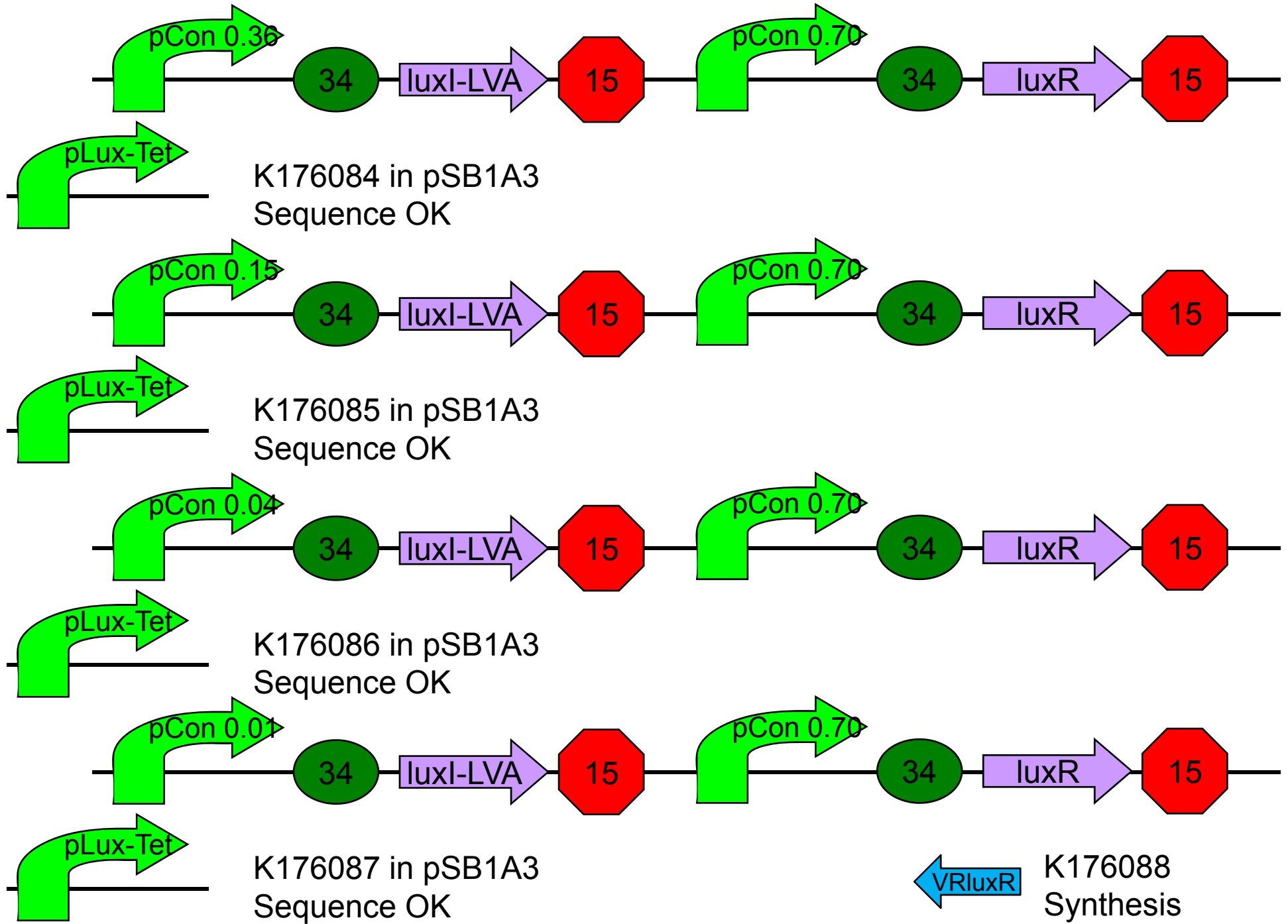
K176081 in pSB1A3  
Sequence OK

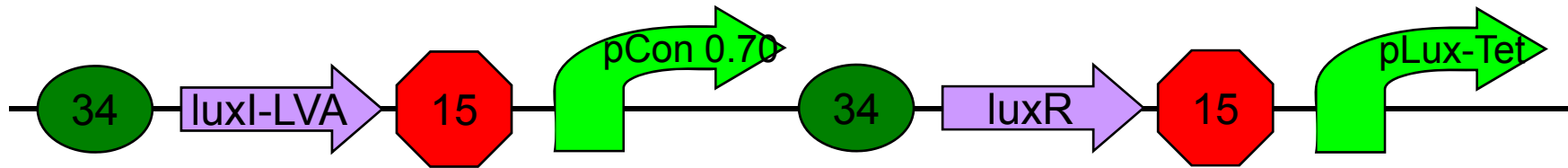


K176082 in pSB1A3  
Sequence OK

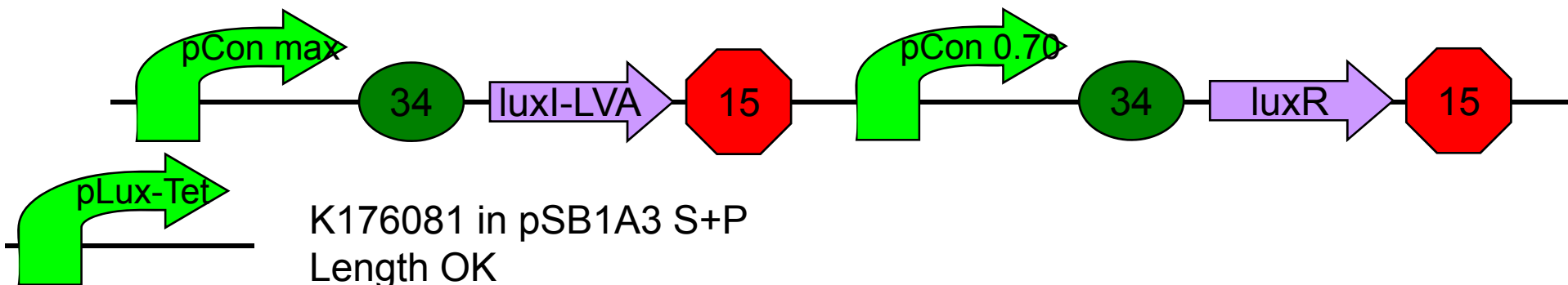


K176083 in pSB1A3  
Sequencing

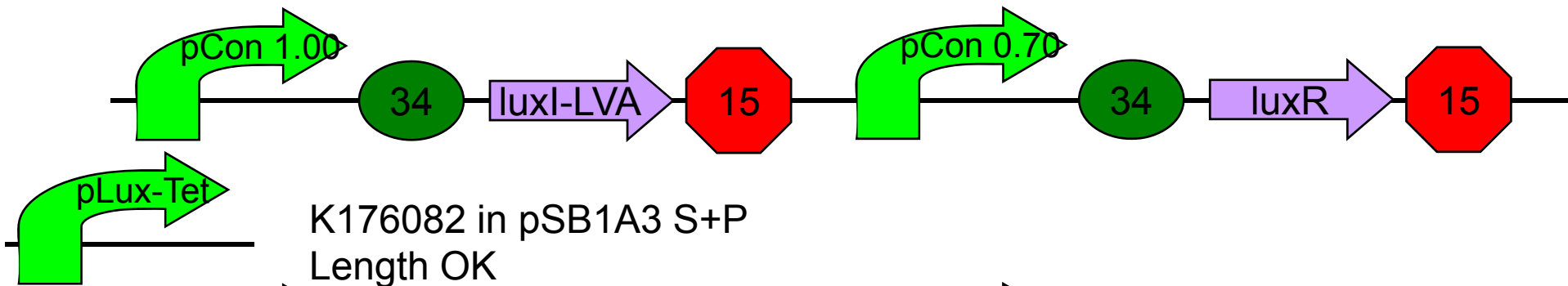




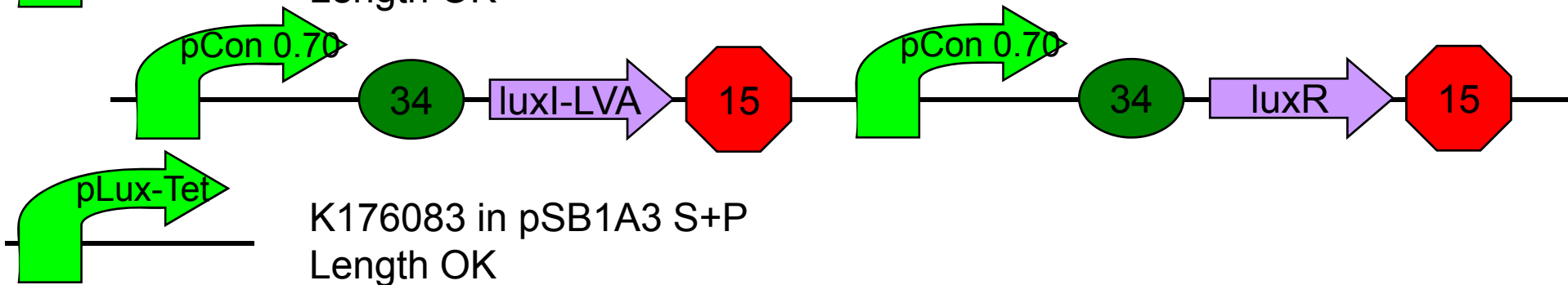
K176080 in pSB1A3 S+P  
Waiting



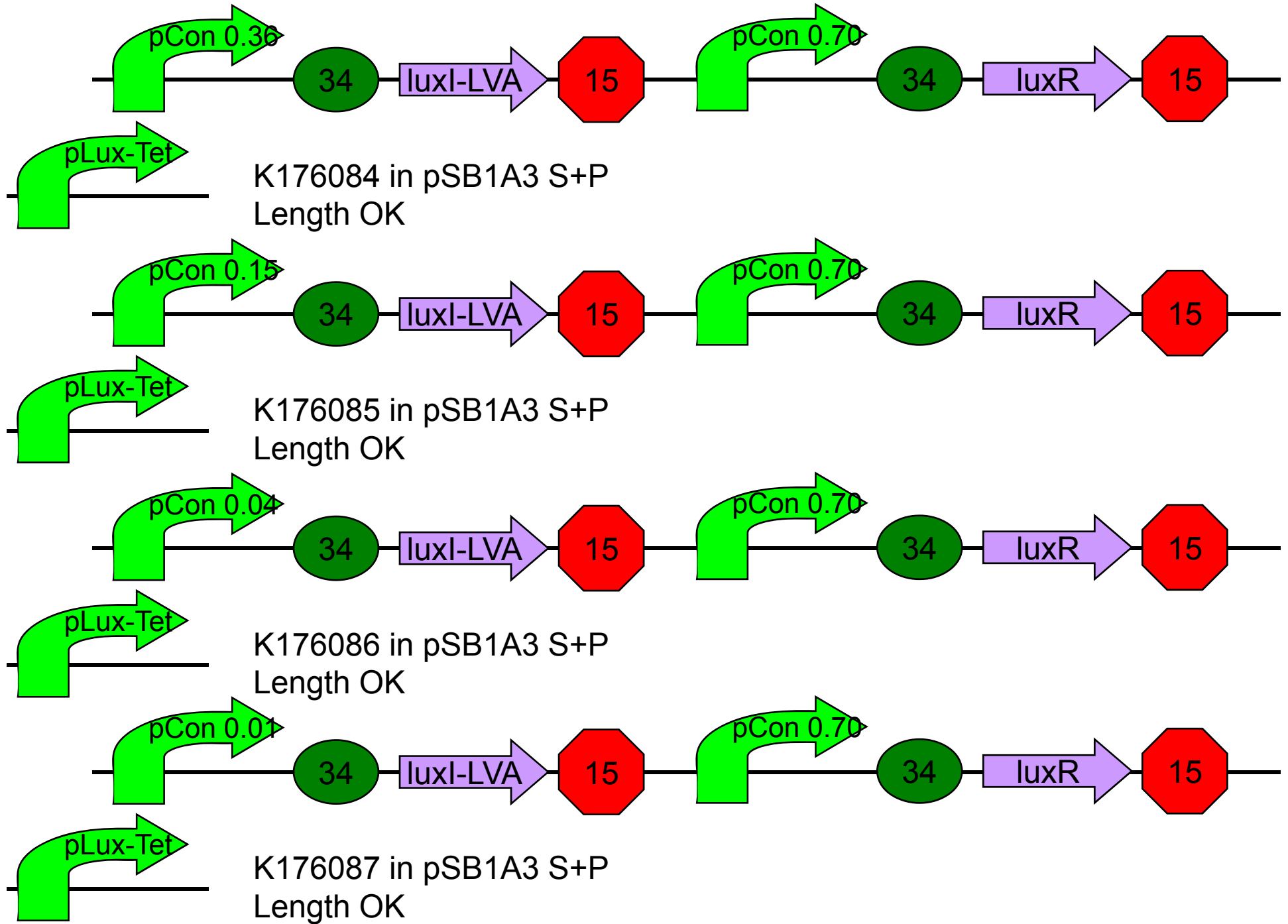
K176081 in pSB1A3 S+P  
Length OK

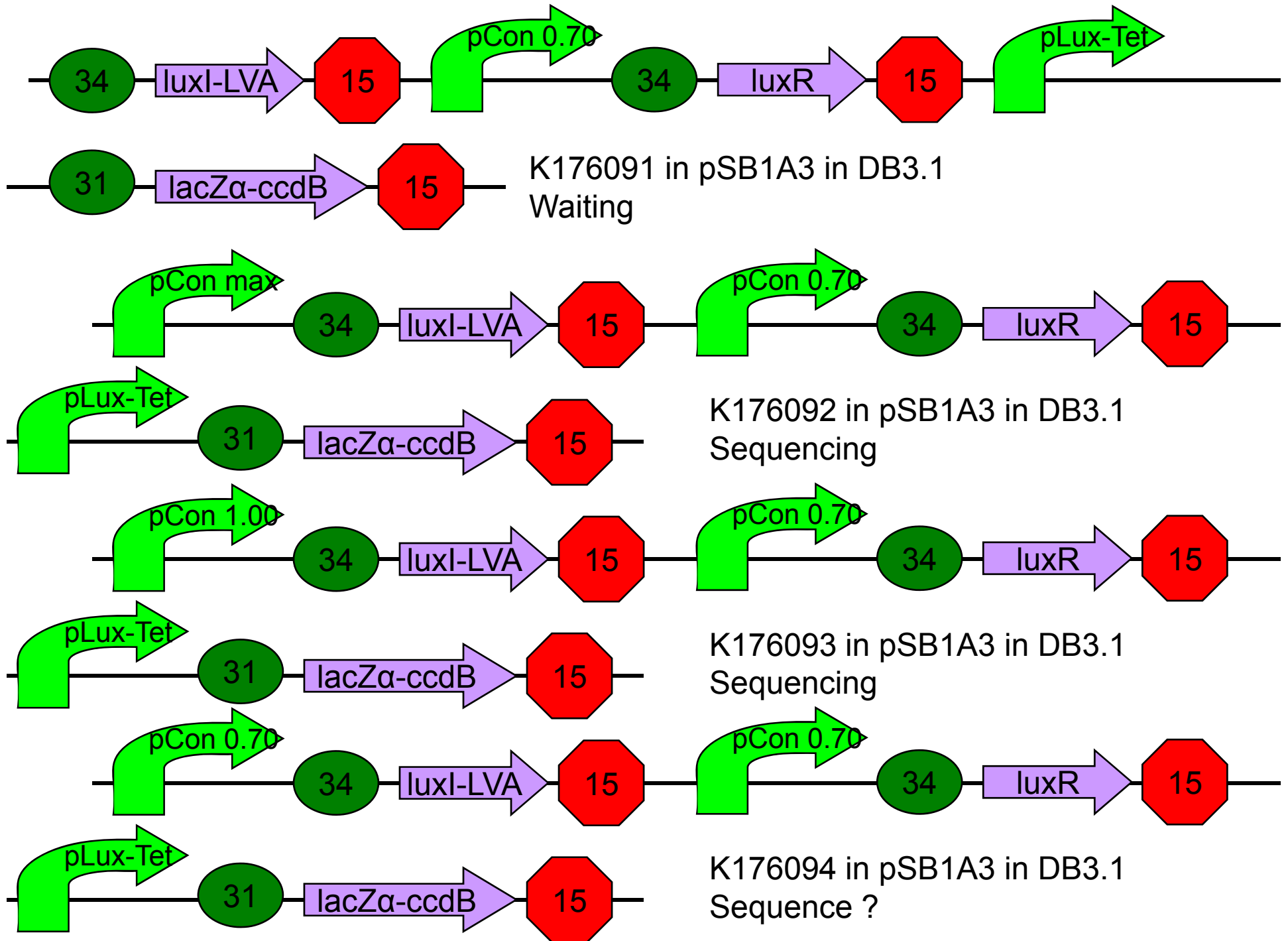


K176082 in pSB1A3 S+P  
Length OK

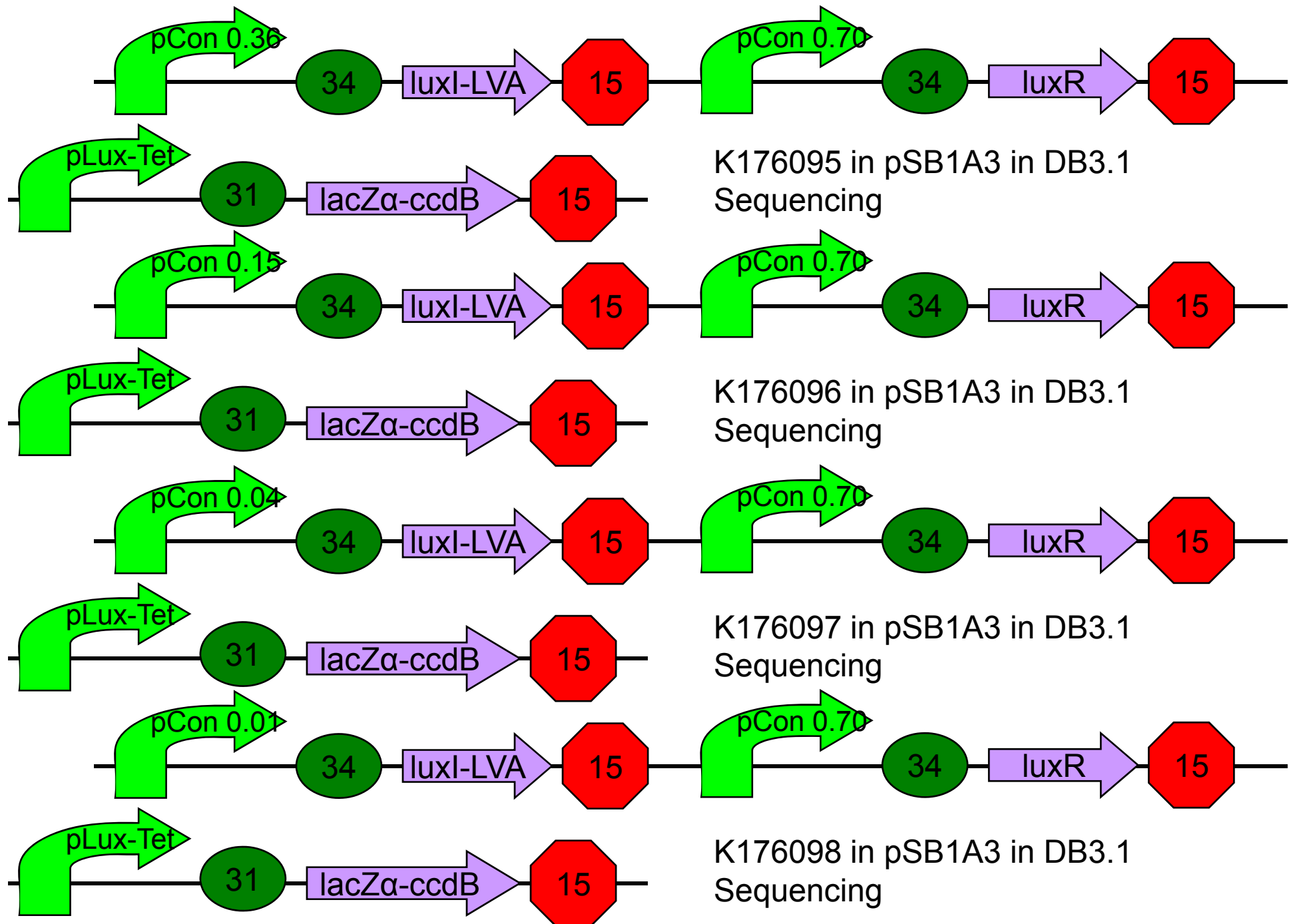


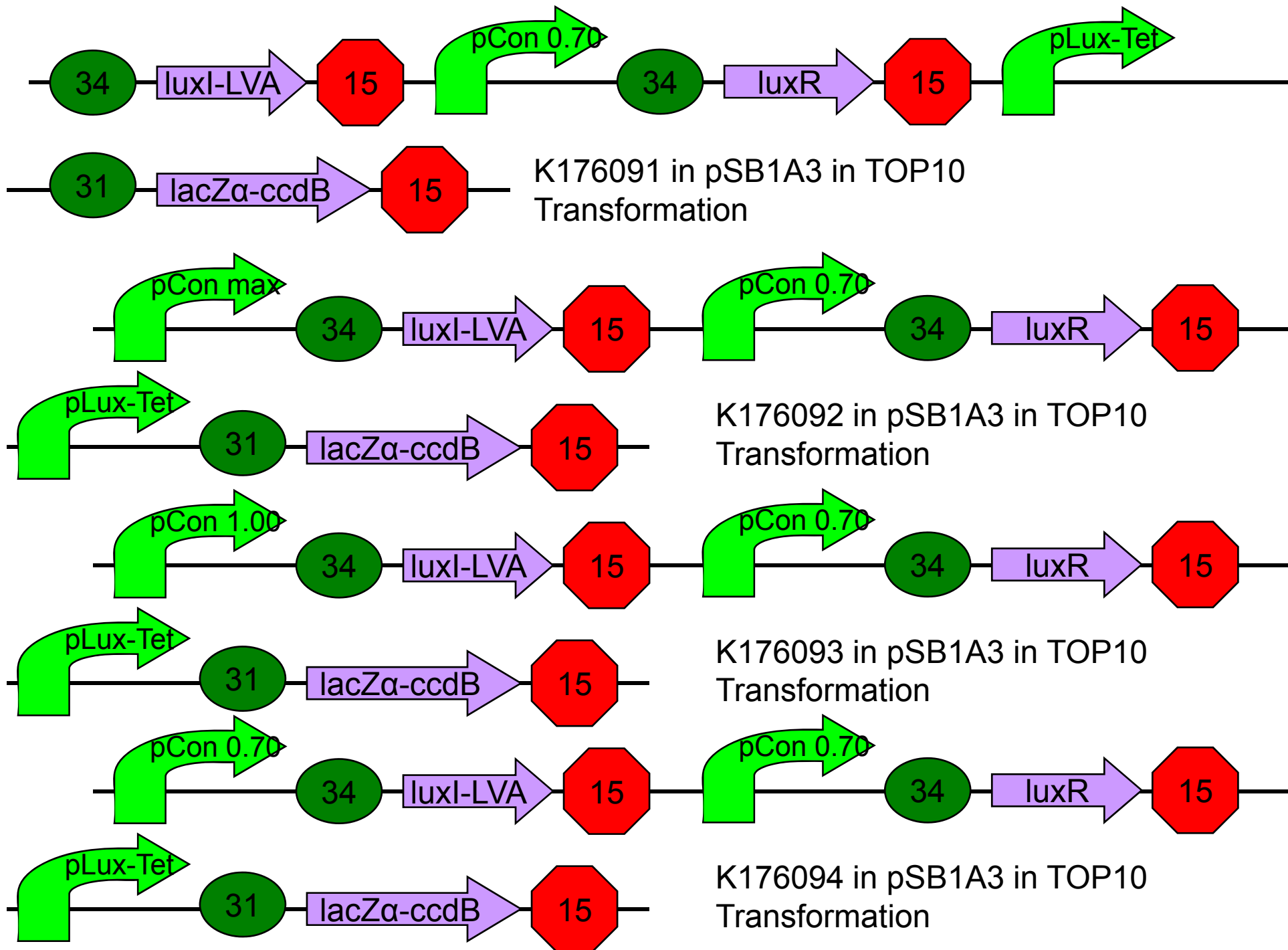
K176083 in pSB1A3 S+P  
Length OK

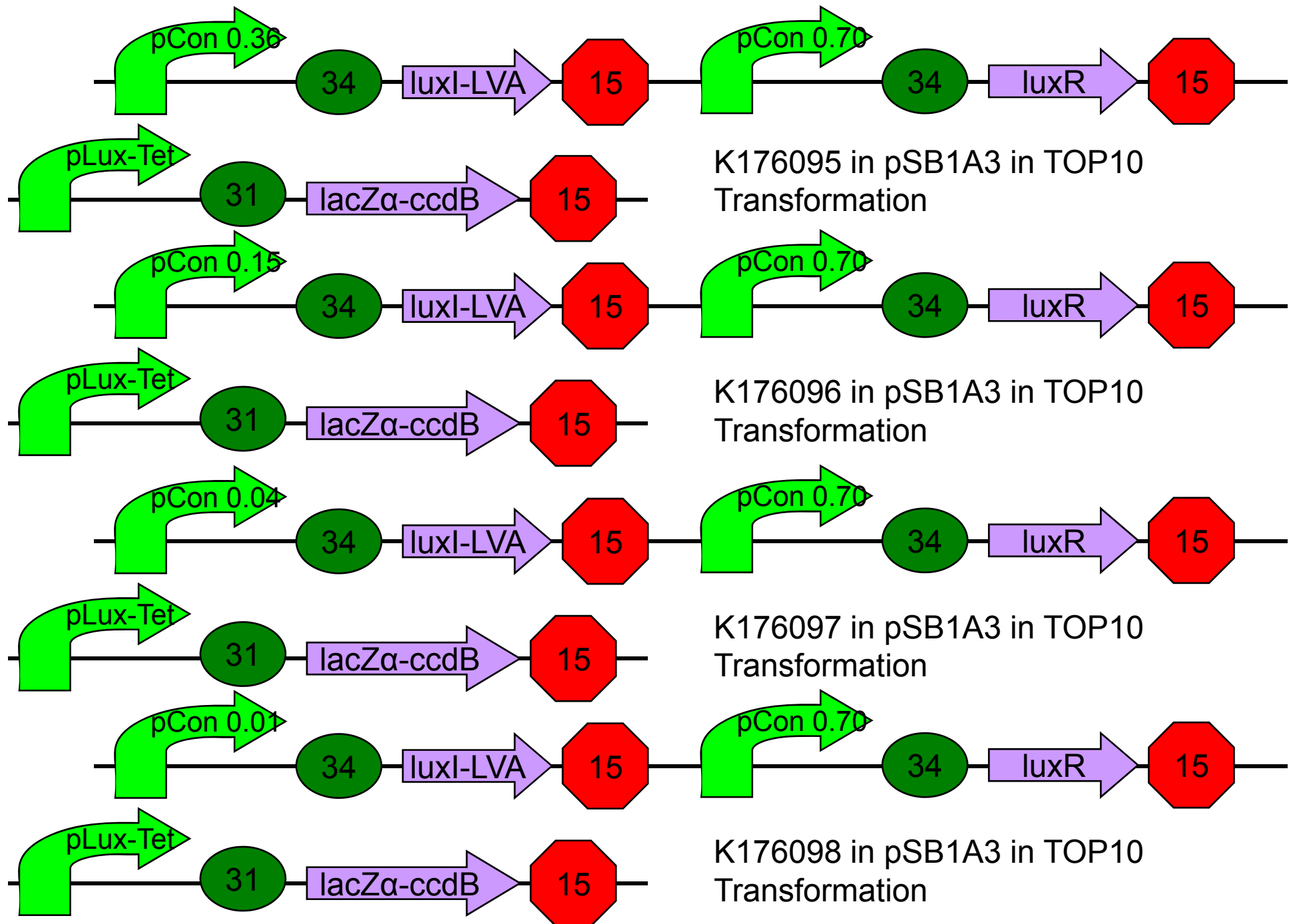


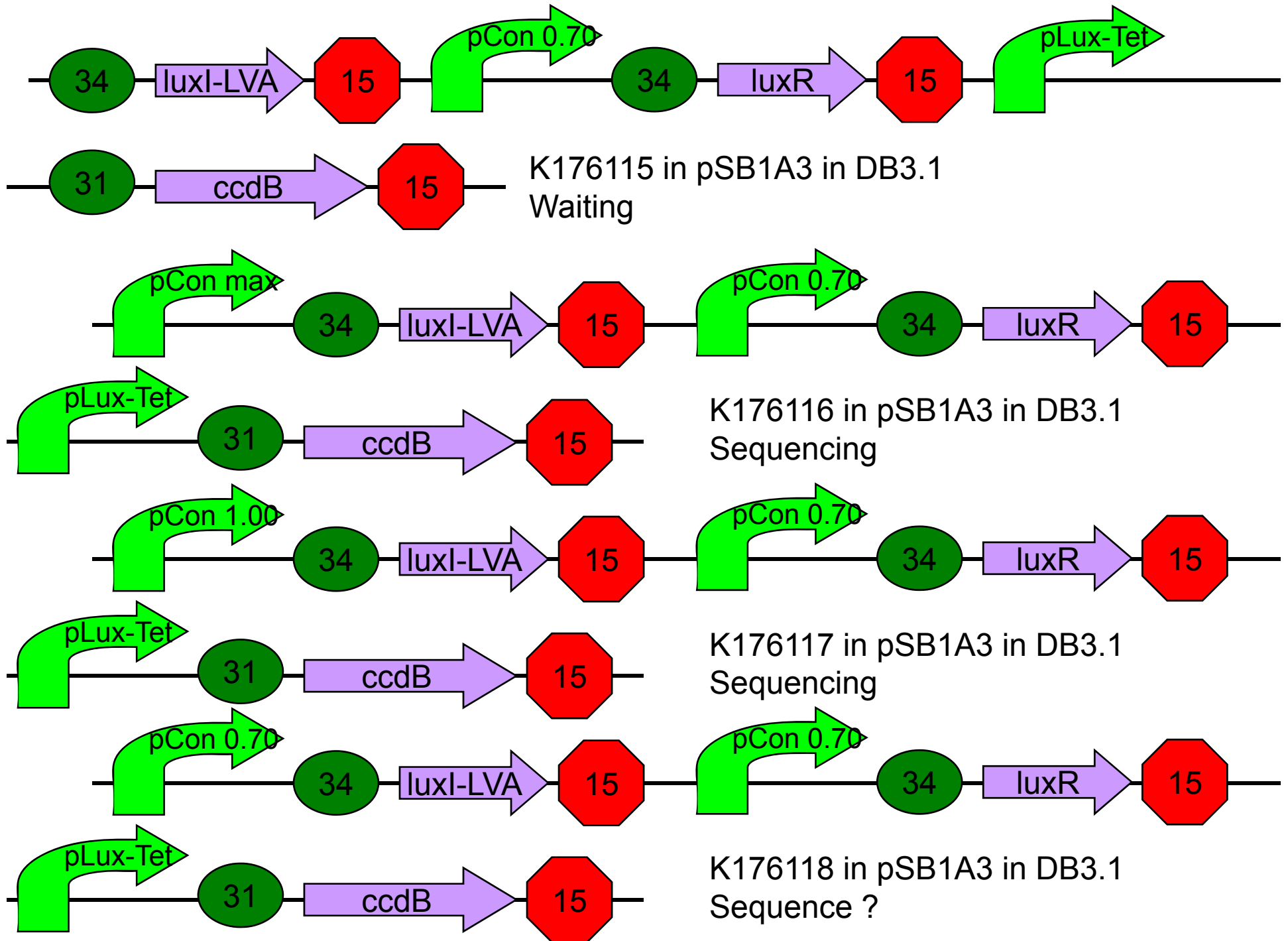


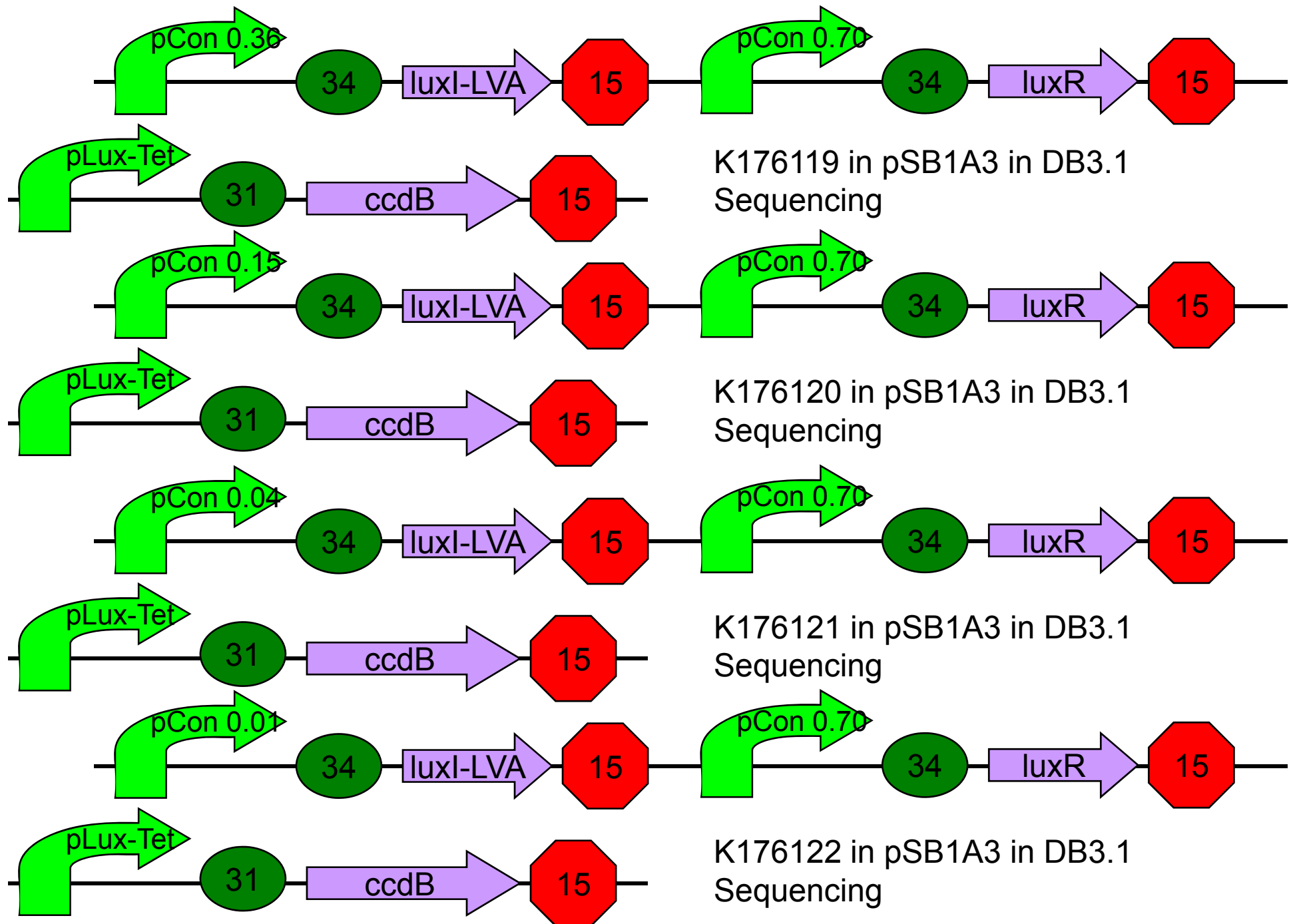


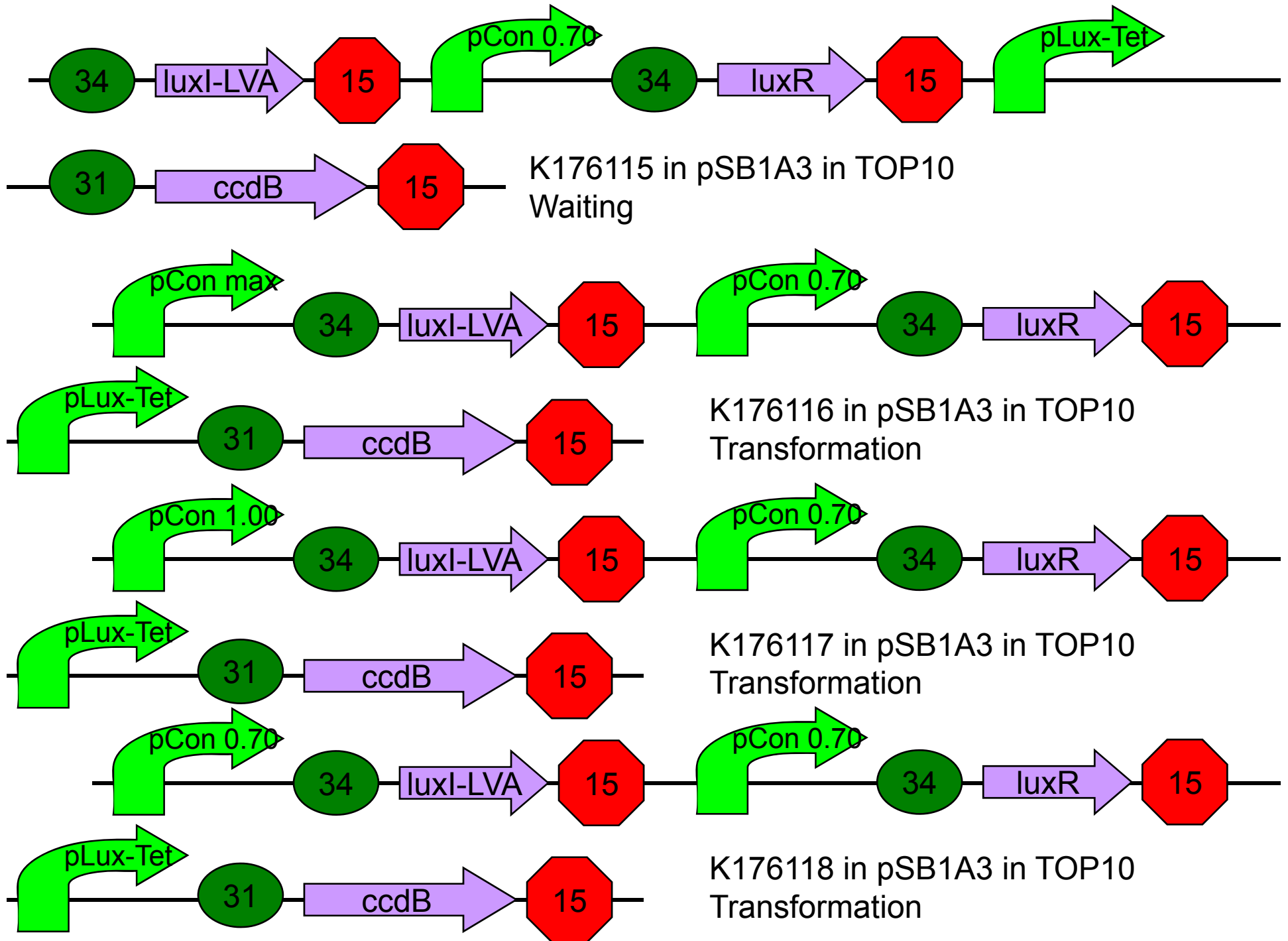


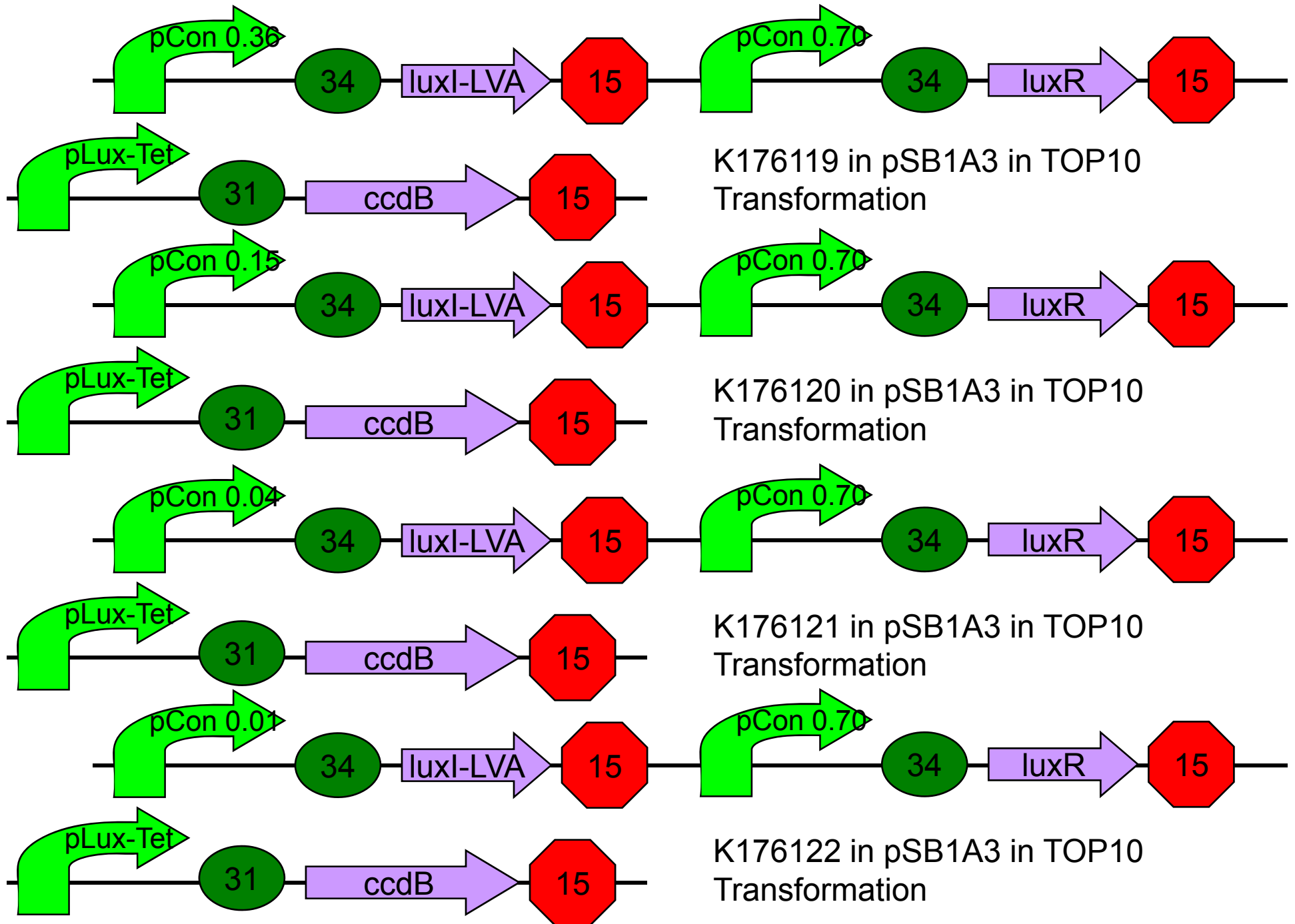




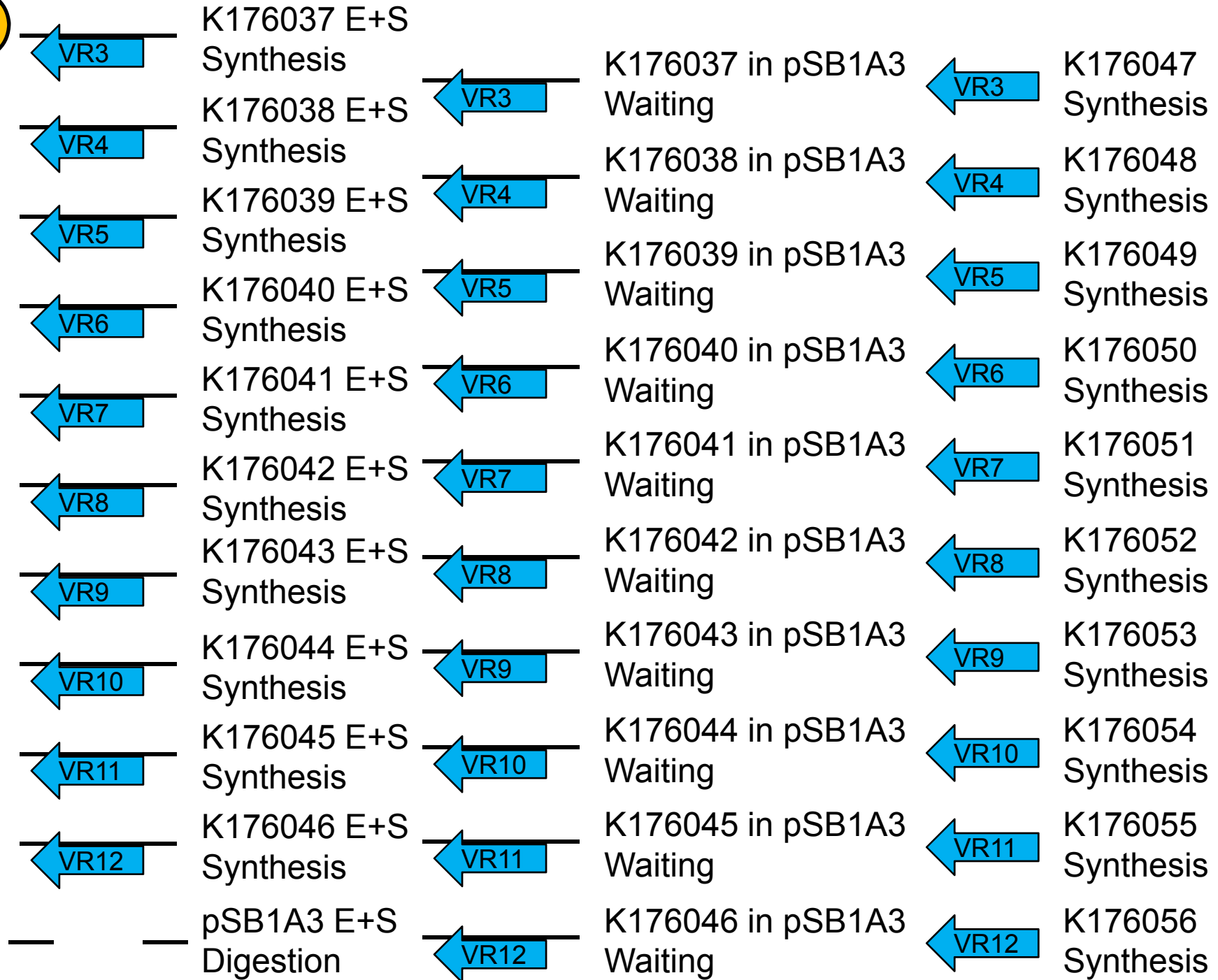






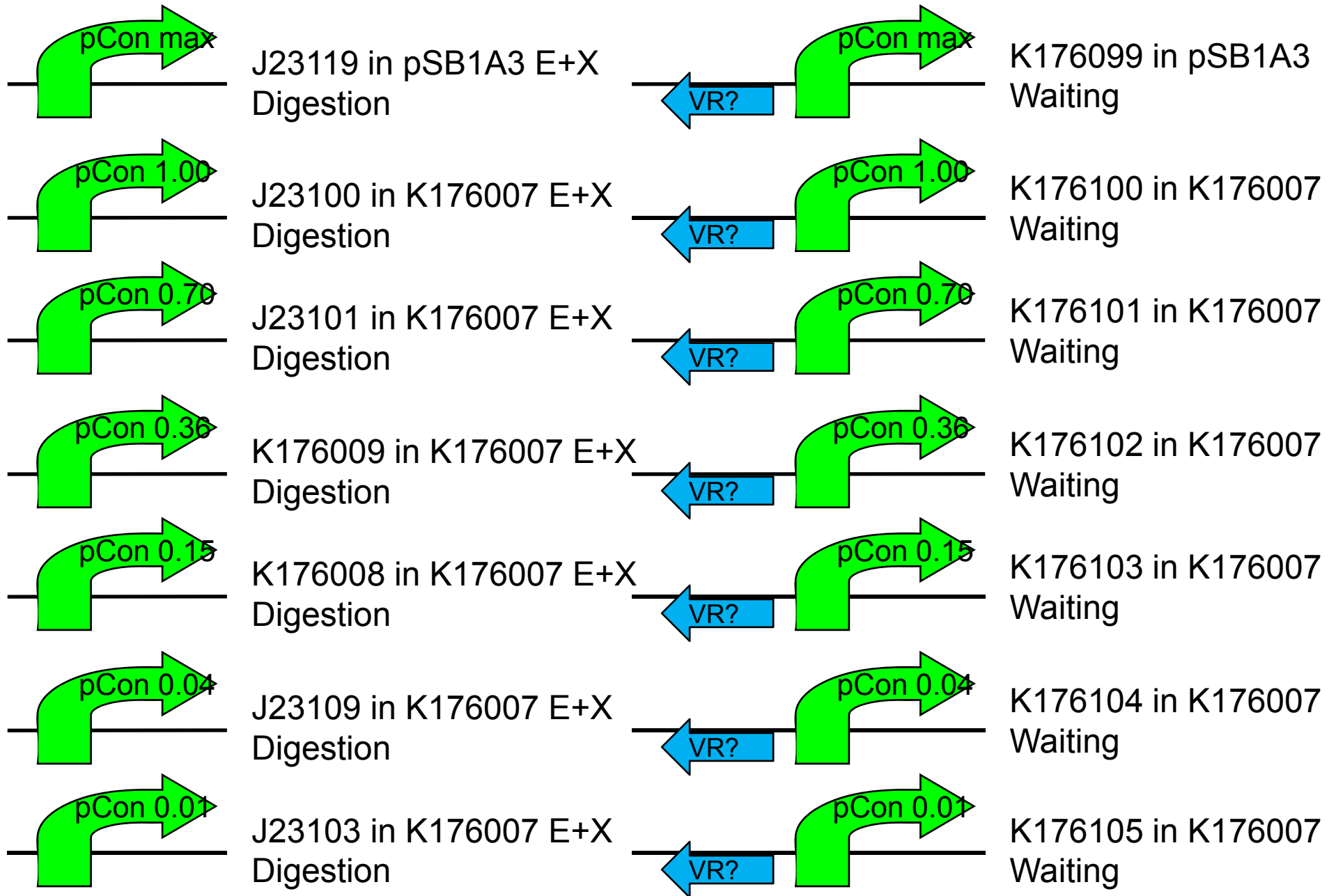


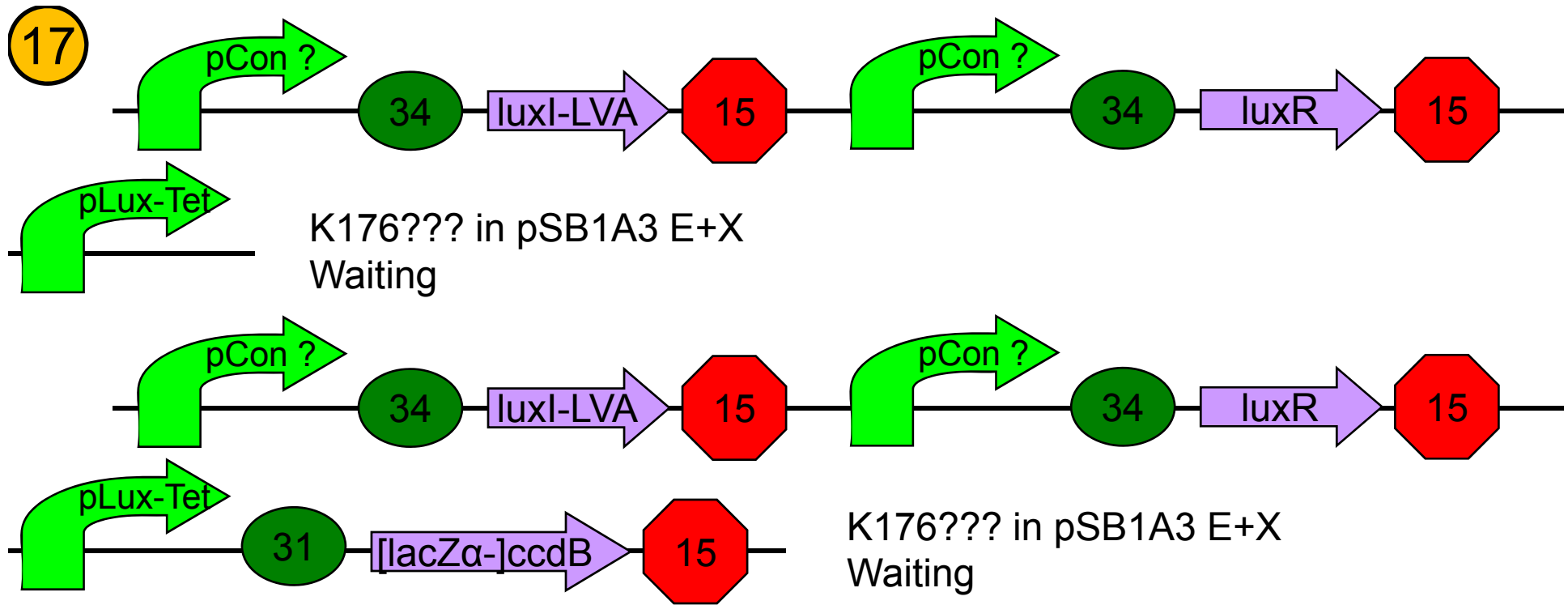
15

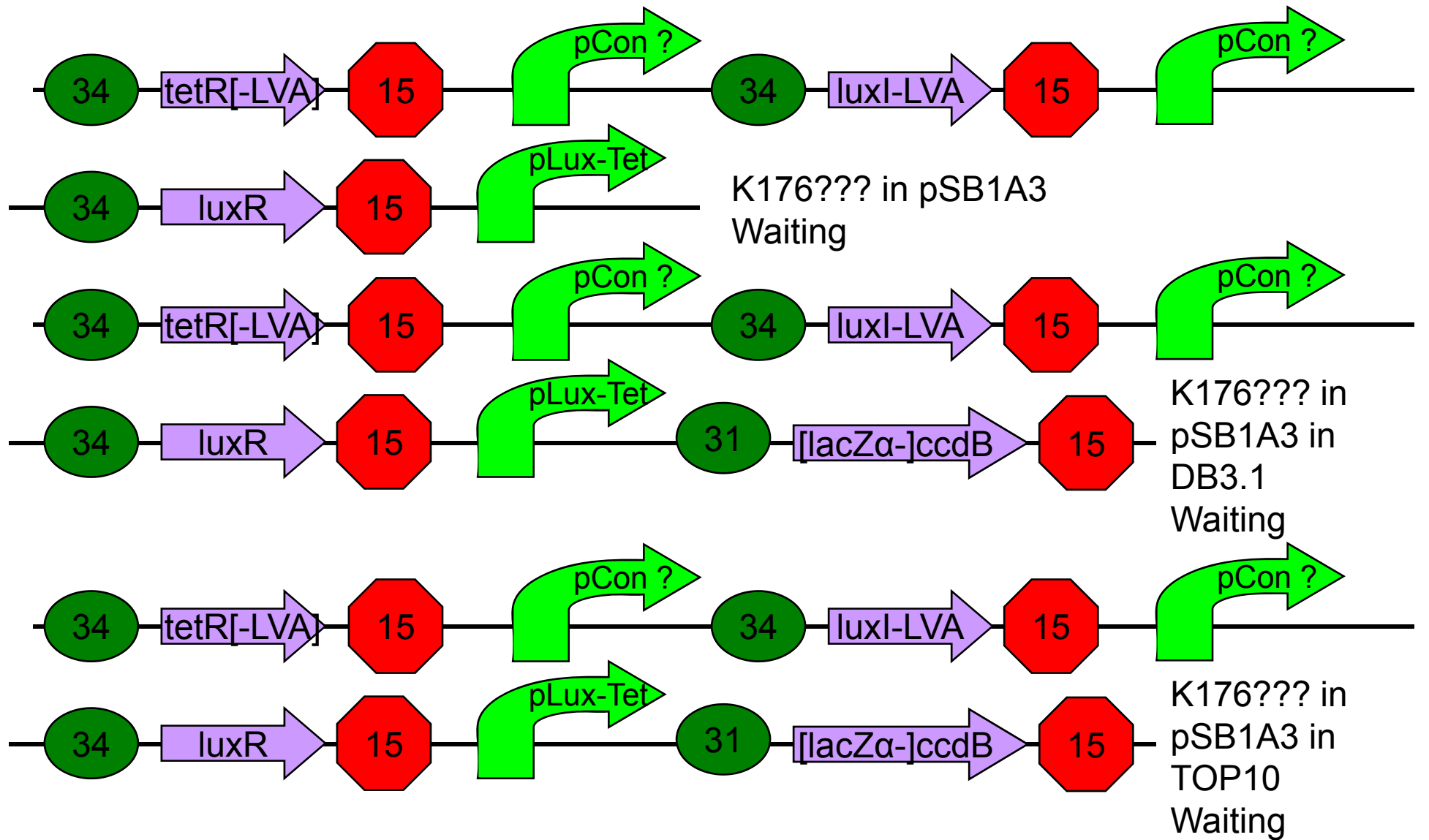




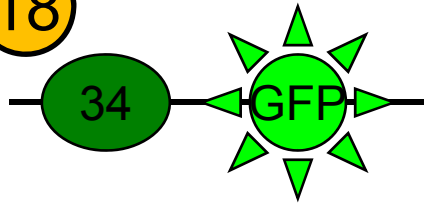
16



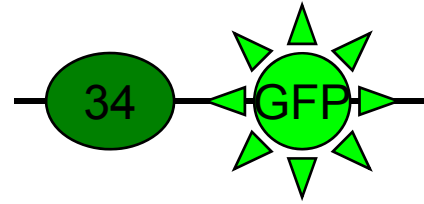




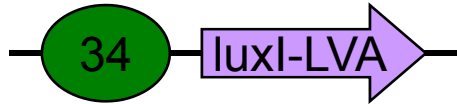
18



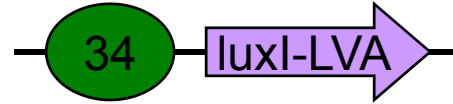
I13500 in pSB1A2  
Sequence OK



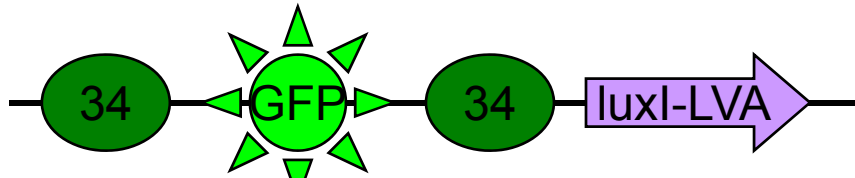
I13500 in pSB1A2 S+P  
Length OK



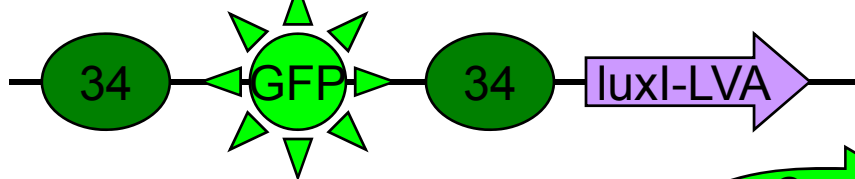
C0261 in pSB1A2  
Sequence OK



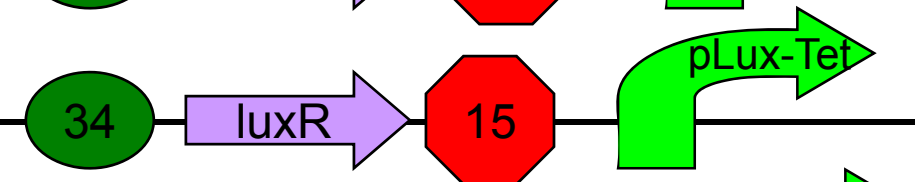
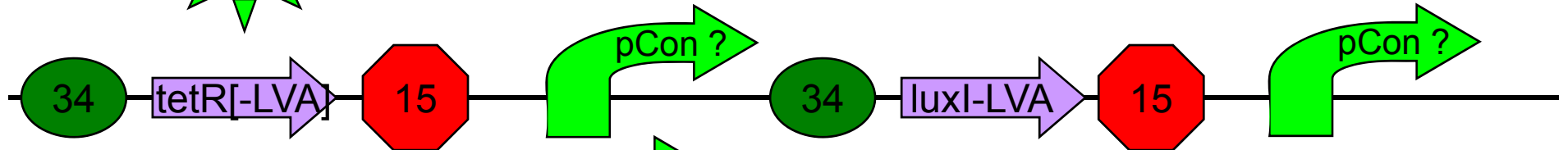
C0261 X+P  
Length OK



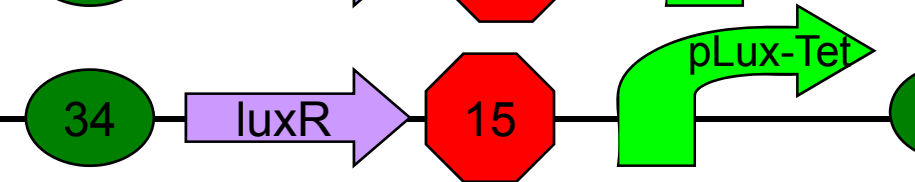
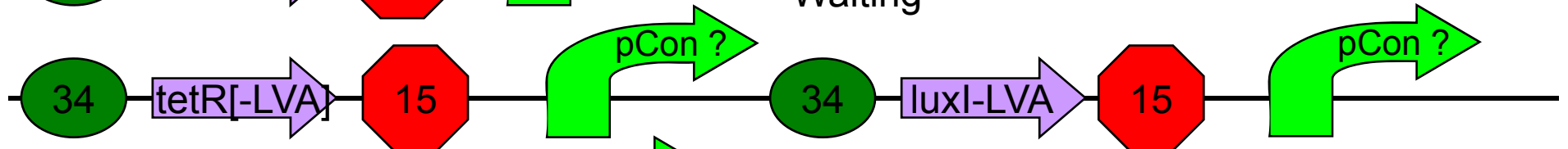
K176106 in pSB1A2  
Sequence OK



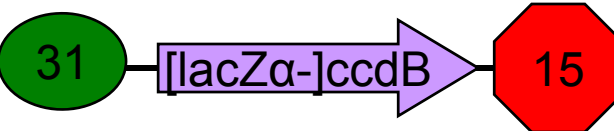
K176106 E+S  
Digestion

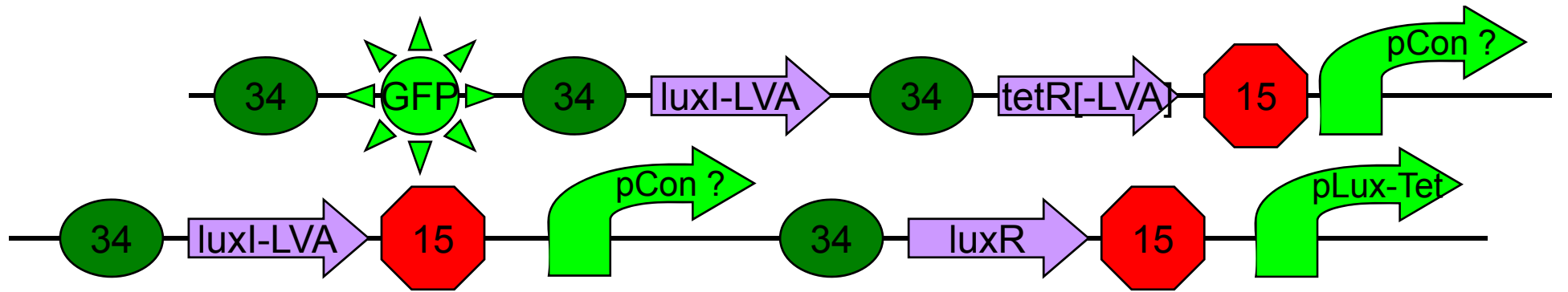


K176??? in pSB1A3 S+P  
Waiting

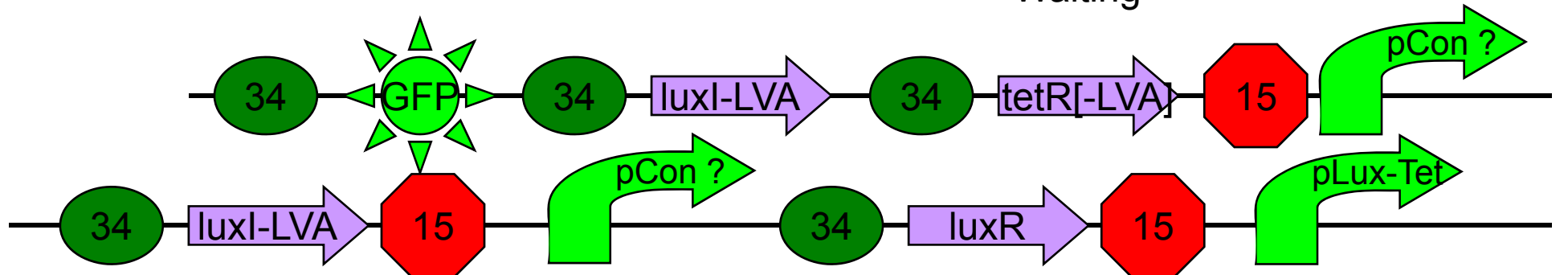


K176??? in pSB1A3 S+P  
Waiting

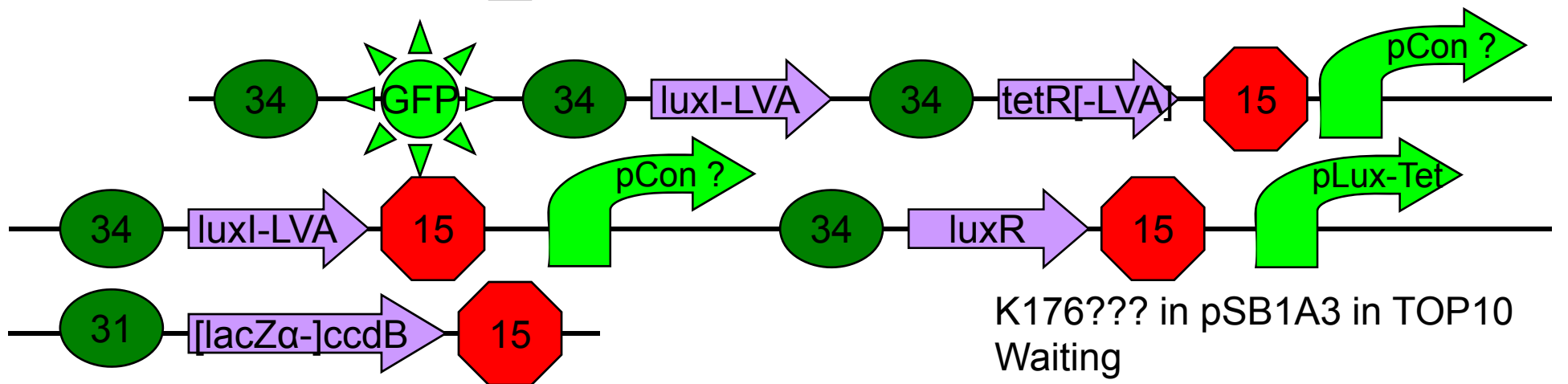




K176??? in pSB1A3  
Waiting



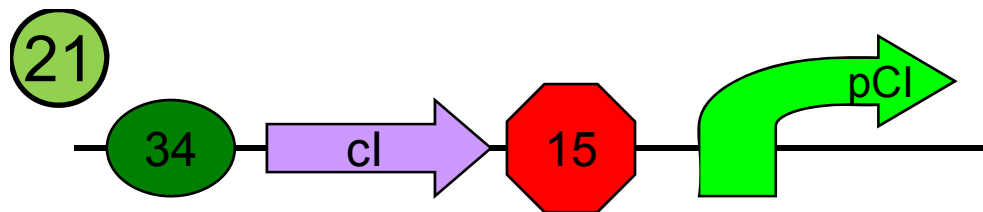
K176??? in pSB1A3 in DB3.1  
Waiting



K176??? in pSB1A3 in TOP10  
Waiting



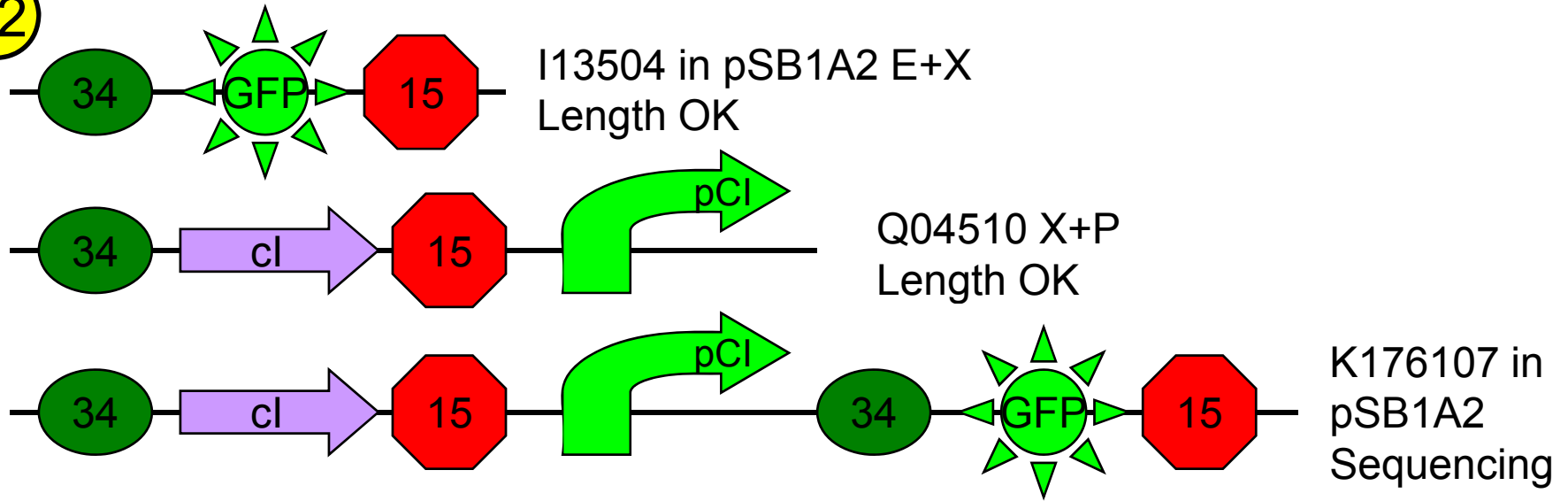




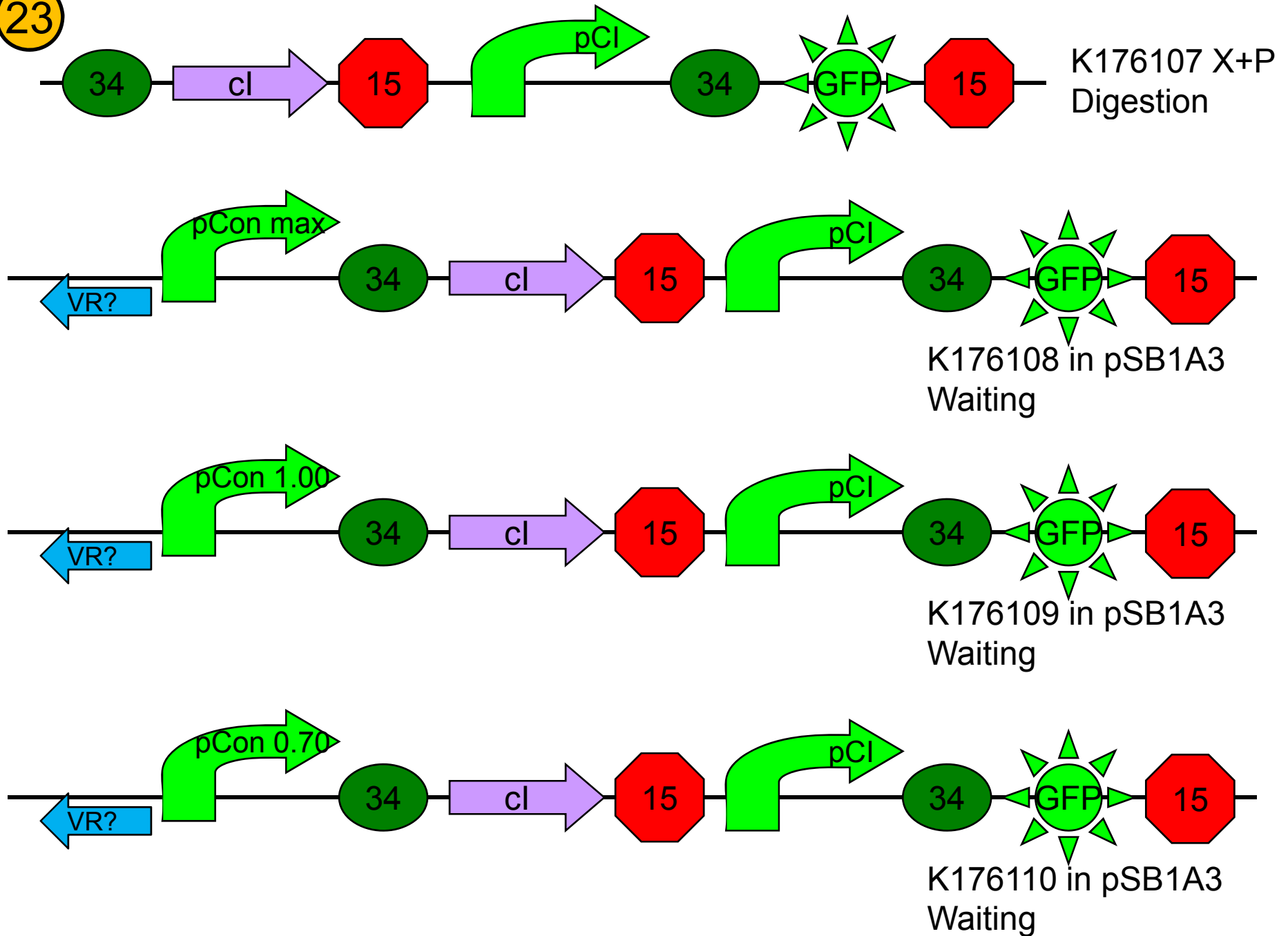
Q04510 in pSB2K3  
Sequence OK

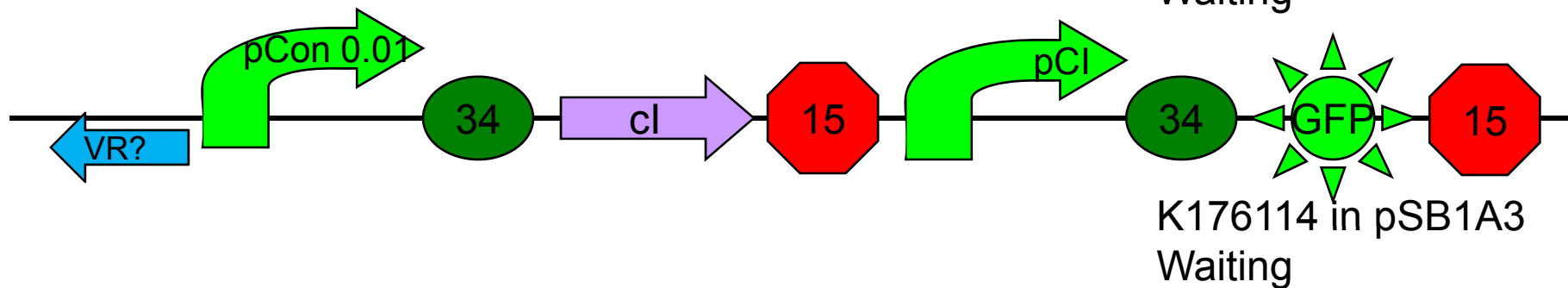
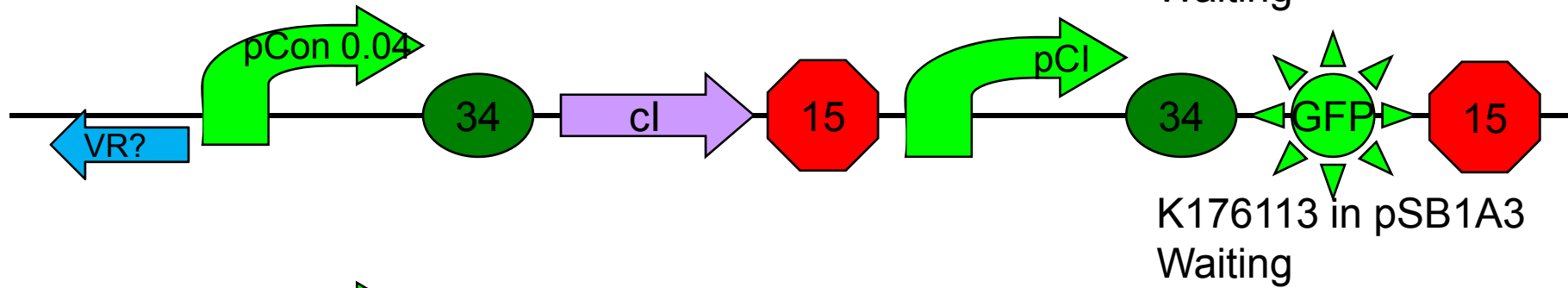
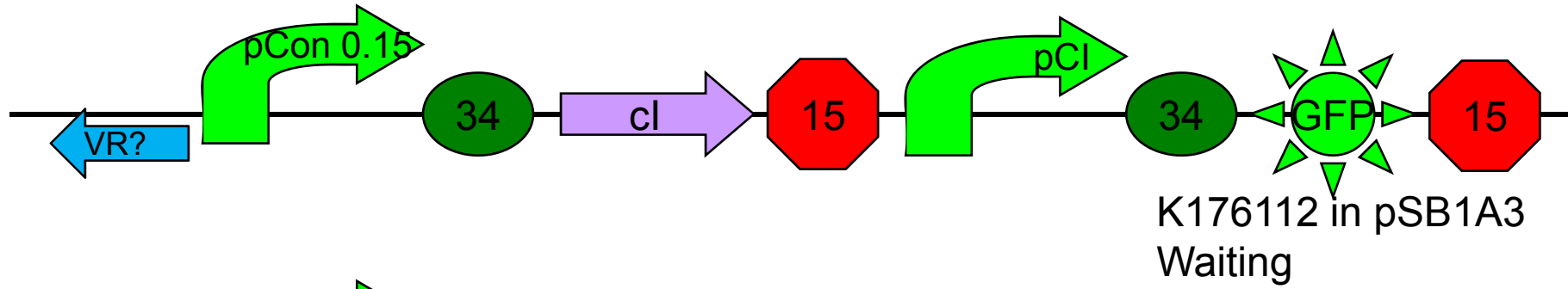
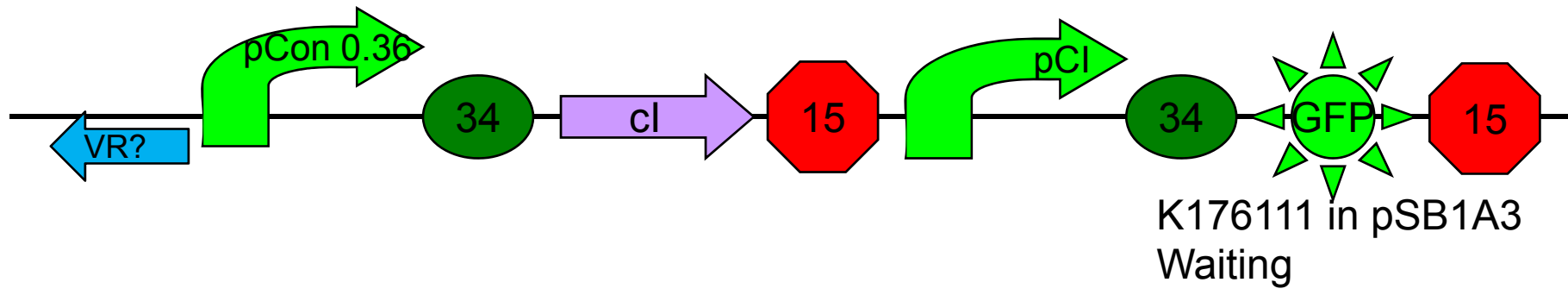


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# Measurement

- Strain
  - TOP10
  - DH5 $\alpha$
  - MG1655
  - MDS 42 recA Blue
- Plasmid
- Medium
  - LB
  - M9
    - Minimal
    - Supplemented
  - EZ Rich Define
  - pH-buffered TBK
  - pH-buffered LBK
- pH
- Temperature
  - 37°C
  - 30°C
  - 34°C
- Pre-warm
- Shake
- Dilution
- Wash

# Wiki

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

Thank You