E.ADEM v0.0.4.1
Work Progress

2009.6.20

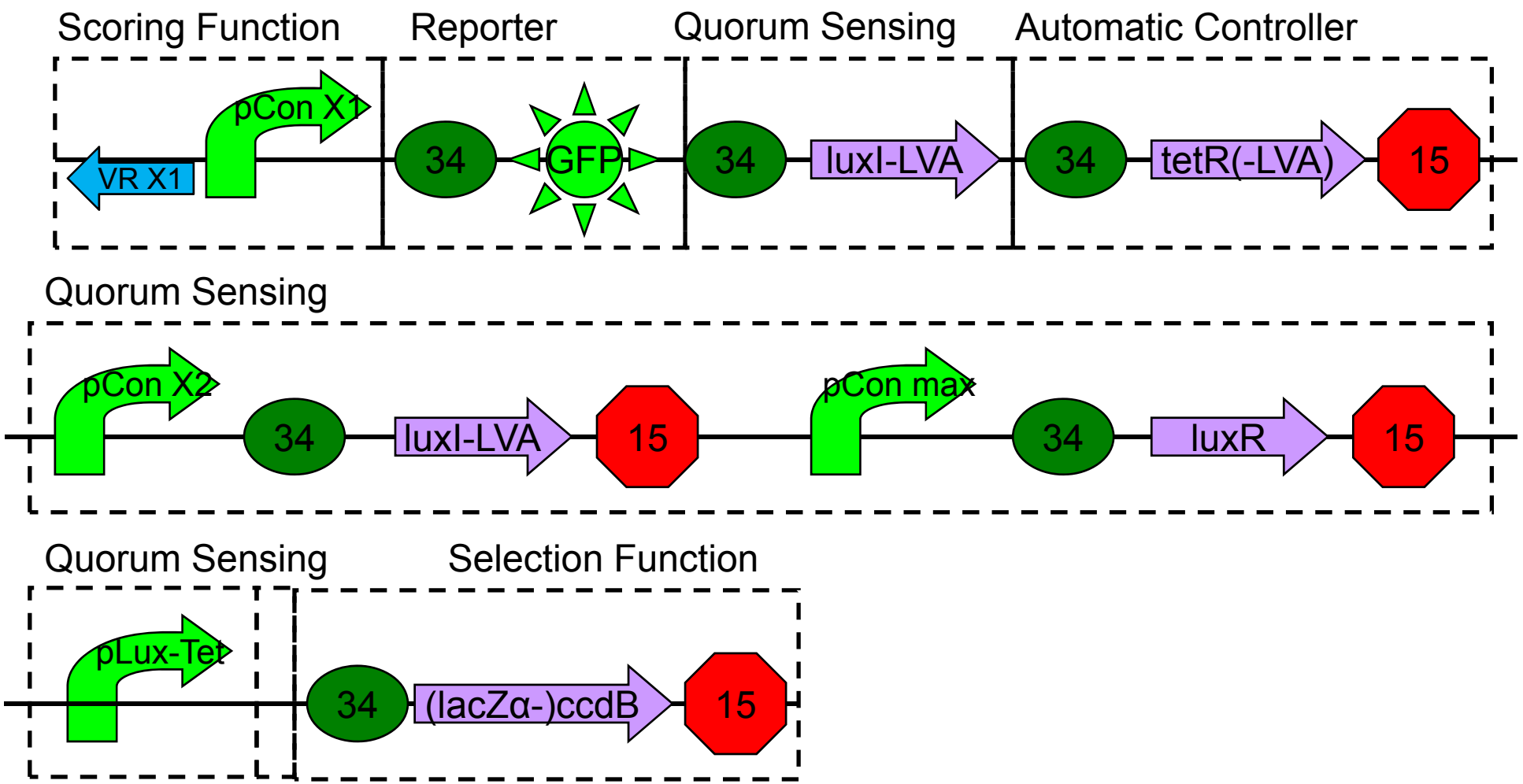
2009.5.3

47 days

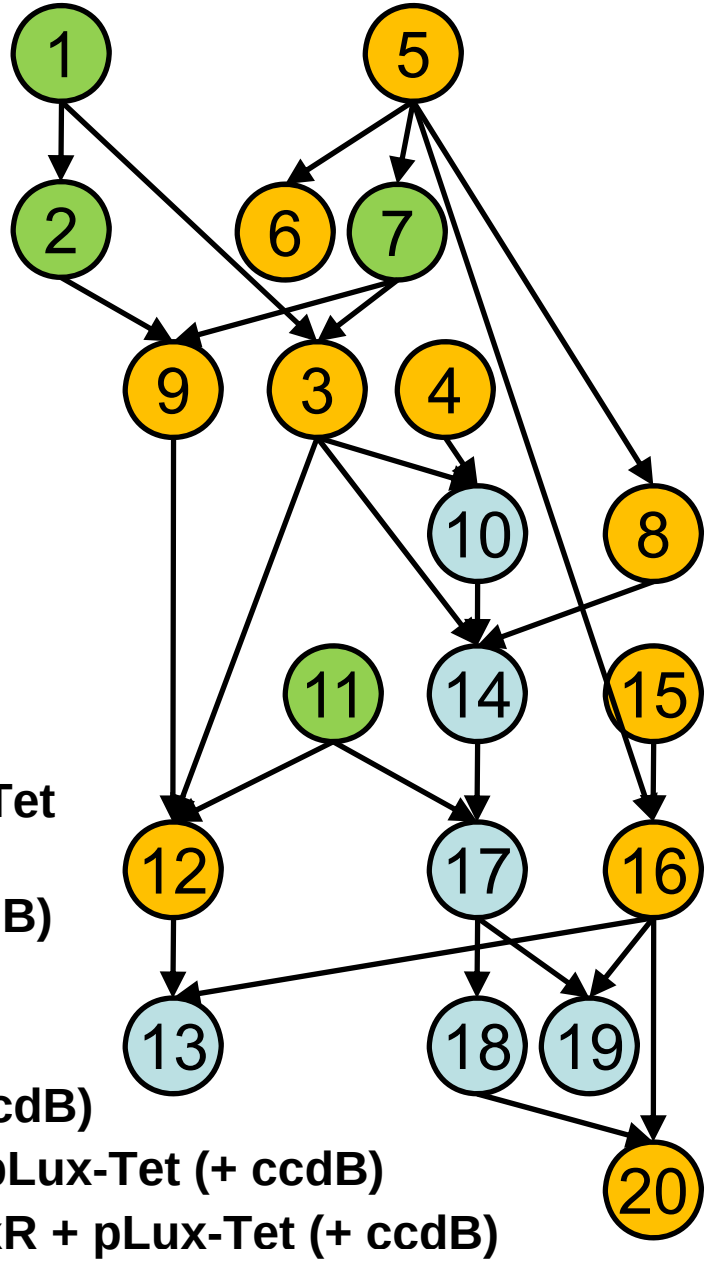
2009.6.13

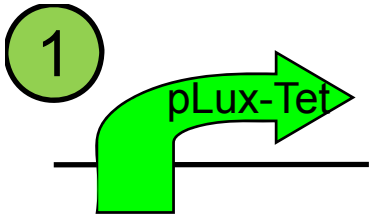
131 days

2009.10.30

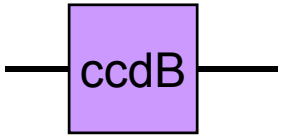

Scoring Function
Reporter
Quorum Sensing
Automatic Controller
pCon X1
VR X1
34
GFP
34
luxI-LVA
34
tetR(-LVA)
15
Quorum Sensing
pCon X2
34
luxI-LVA
15
pCon max
34
luxR
15
Quorum Sensing
Selection Function
pLux-Tet
34
(lacZα-)ccdB
15

1. pLux-Tet
2. pLux-Tet + GFP
3. pCon + luxR + pLux-Tet
4. ccdB×2
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×2 (AHL)
11. tetR×2
12. tetR×2 + pCon + luxR + pLux-Tet (+ GFP)
13. (VR + pCon)×8 + tetR×2 + pCon + luxR + pLux-Tet (+ GFP) (AHL/aTc)
14. (pCon×7 +) luxI + pCon + luxR + pLux-Tet (+ ccdB)
15. VR×8
16. (VR + pCon)×8
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)



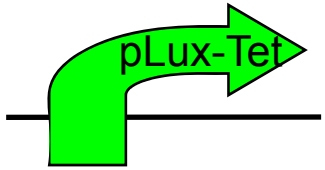


K176000 X+P with nicks
Sequence OK



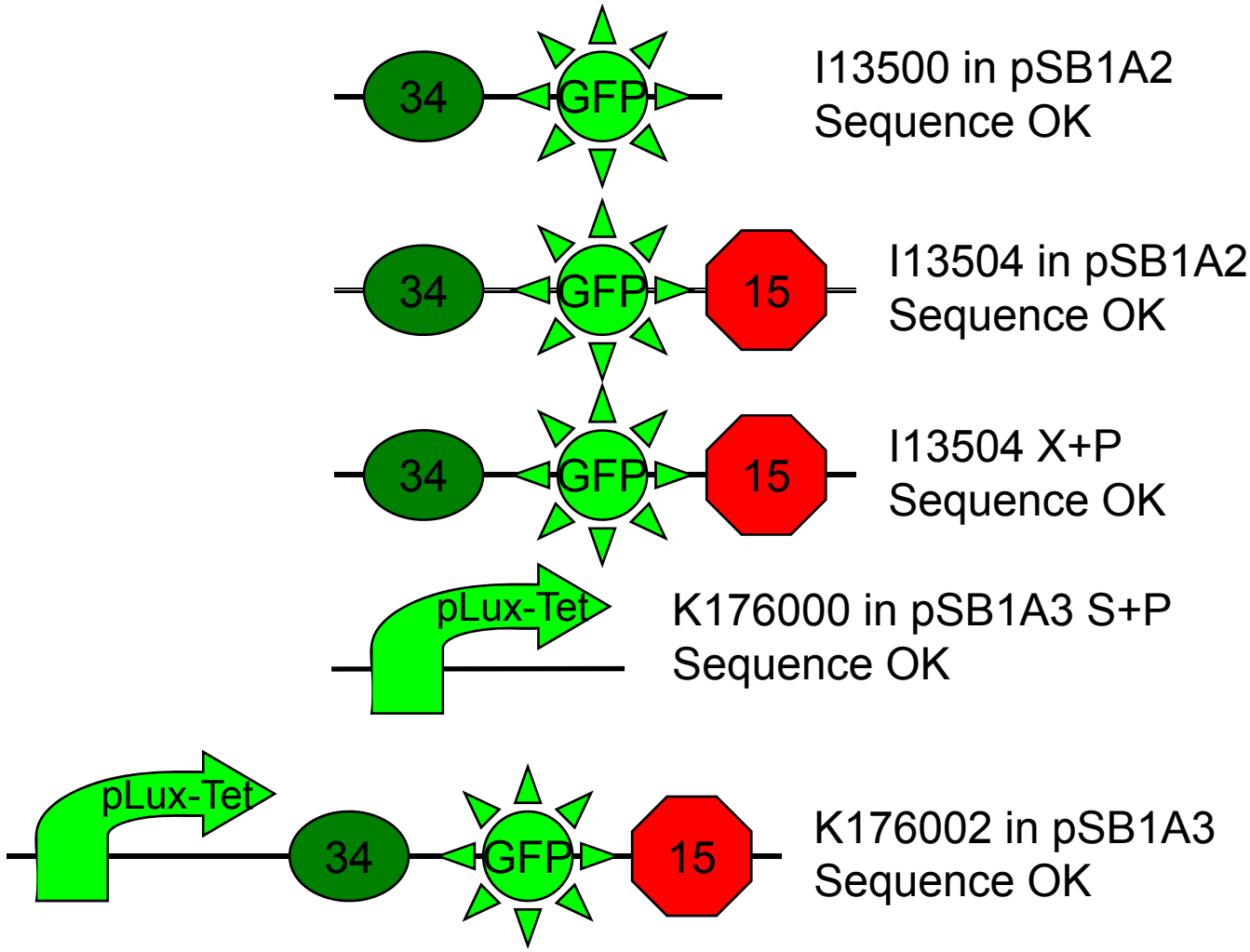
P1010 in pSB1A3 in DB 3.1
Length OK

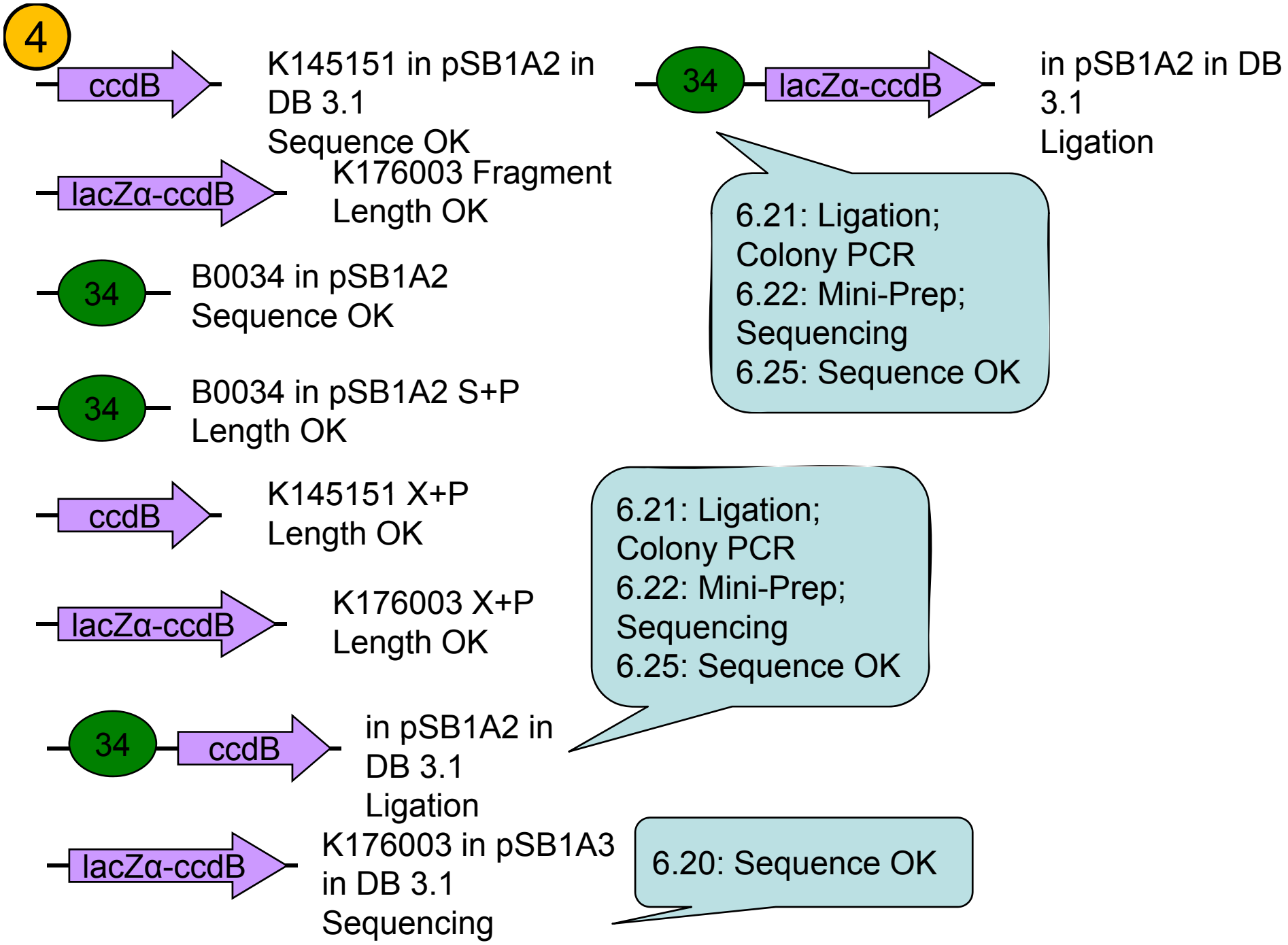
pSB1A3 X+P
Length OK

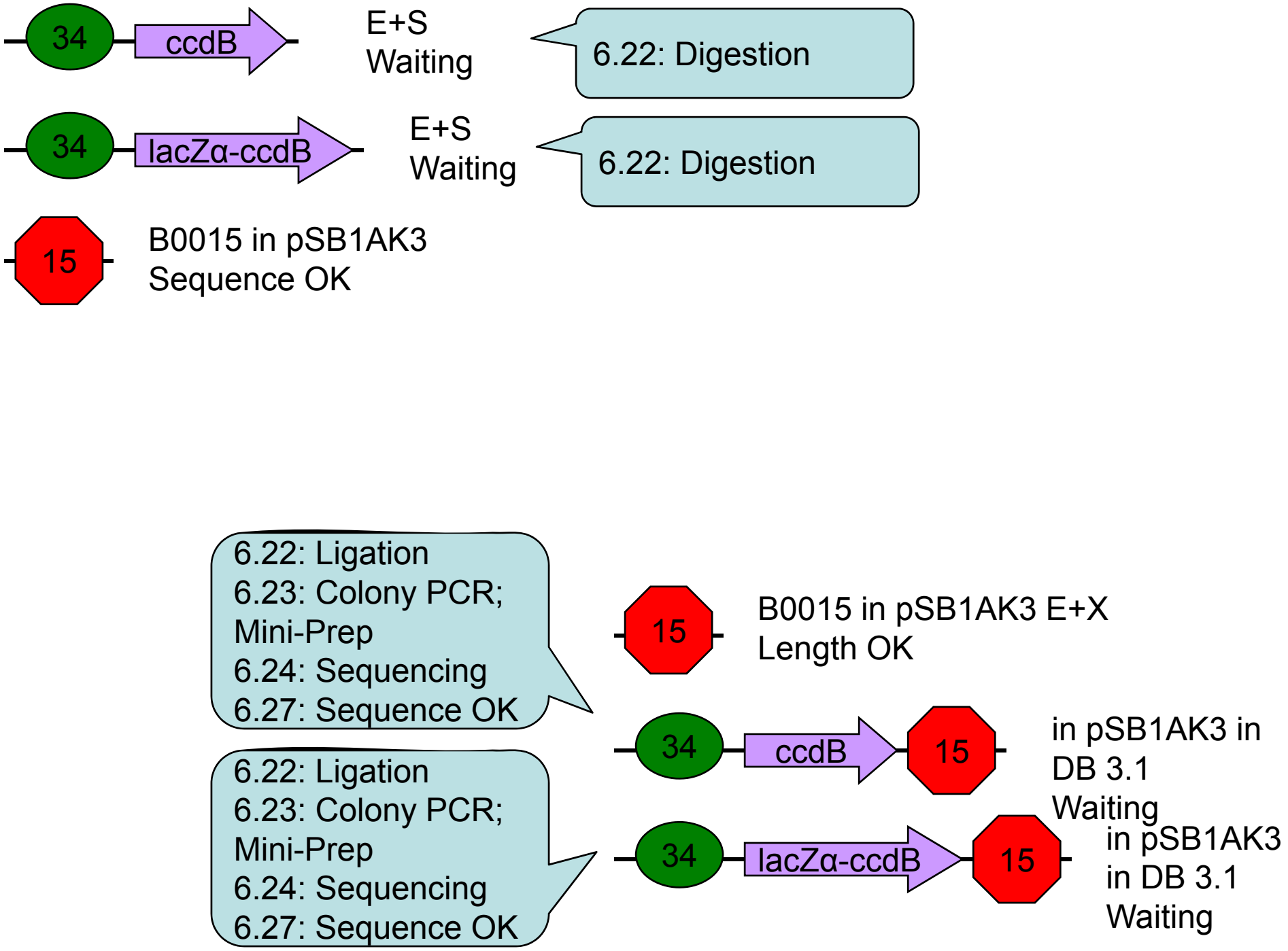


K176000 in pSB1A3
Sequence OK

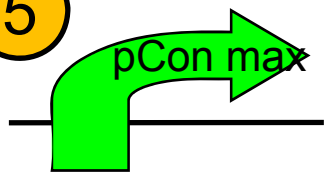
2


34
GFP
I13500 in pSB1A2
Sequence OK
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

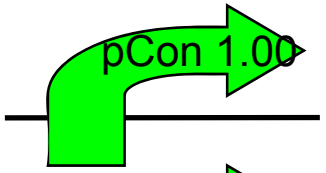

4
ccdB
K145151 in pSB1A2 in DB 3.1
Sequence OK
lacZα-ccdB
K176003 Fragment
Length OK
34
B0034 in pSB1A2
Sequence OK
34
B0034 in pSB1A2 S+P
Length OK
ccdB
K145151 X+P
Length OK
lacZα-ccdB
K176003 X+P
Length OK
34
ccdB
in pSB1A2 in DB 3.1
Ligation
lacZα-ccdB
K176003 in pSB1A3 in DB 3.1
Sequencing
34
lacZα-ccdB
in pSB1A2 in DB 3.1
Ligation
6.21: Ligation;
Colony PCR
6.22: Mini-Prep;
Sequencing
6.25: Sequence OK
6.21: Ligation;
Colony PCR
6.22: Mini-Prep;
Sequencing
6.25: Sequence OK
6.20: Sequence OK


34
ccdB
E+S
Waiting
6.22: Digestion
34
lacZα-ccdB
E+S
Waiting
6.22: Digestion
15
B0015 in pSB1AK3
Sequence OK
6.22: Ligation
6.23: Colony PCR;
Mini-Prep
6.24: Sequencing
6.27: Sequence OK
6.22: Ligation
6.23: Colony PCR;
Mini-Prep
6.24: Sequencing
6.27: Sequence OK
15
B0015 in pSB1AK3 E+X
Length OK
34
ccdB
15
in pSB1AK3 in
DB 3.1
Waiting
34
lacZα-ccdB
15
in pSB1AK3
in DB 3.1
Waiting

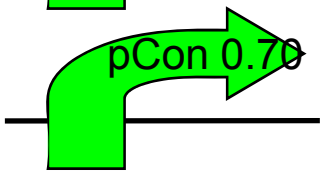
5



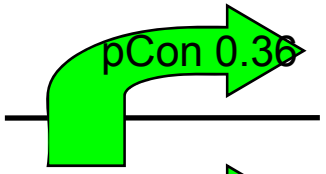
J23119 in pSB1A2
Sequence OK



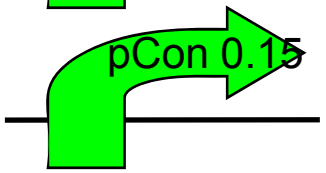
J23100 in J61002
Length OK



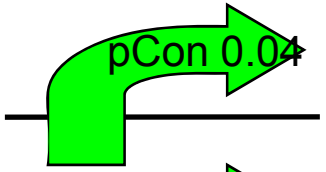
J23101 in J61002
Sequence OK



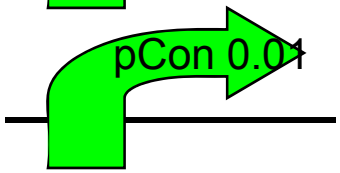
K176009 in J61002
Sequence OK



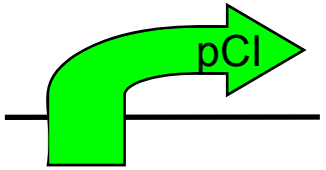
K176008 in J61002
Sequence OK



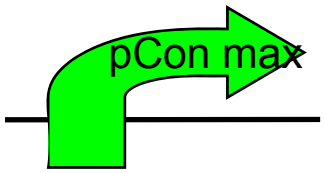
J23109 in J61002
Sequence OK



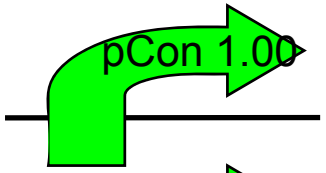
J23103 in J61002
Sequence OK



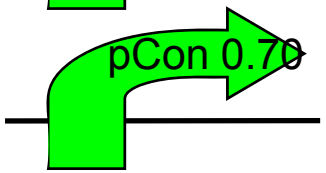
R0051 in pSB1A2
Sequence ?



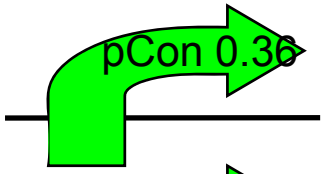
J23119 X+P
Sequence OK



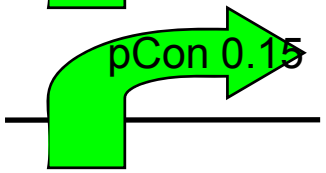
J23100-J61002SF X+P
Length OK



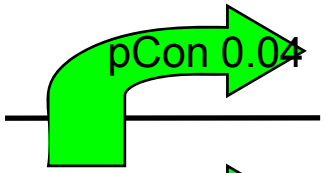
J23101-J61002SF X+P
Sequence OK



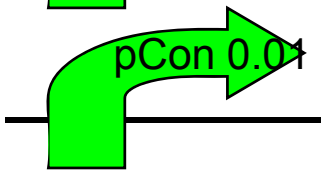
K176009-J61002SF X+P
Sequence OK



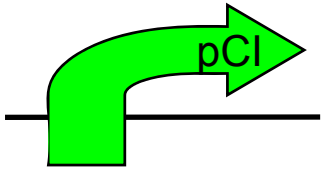
K176008-J61002SF X+P
Sequence OK



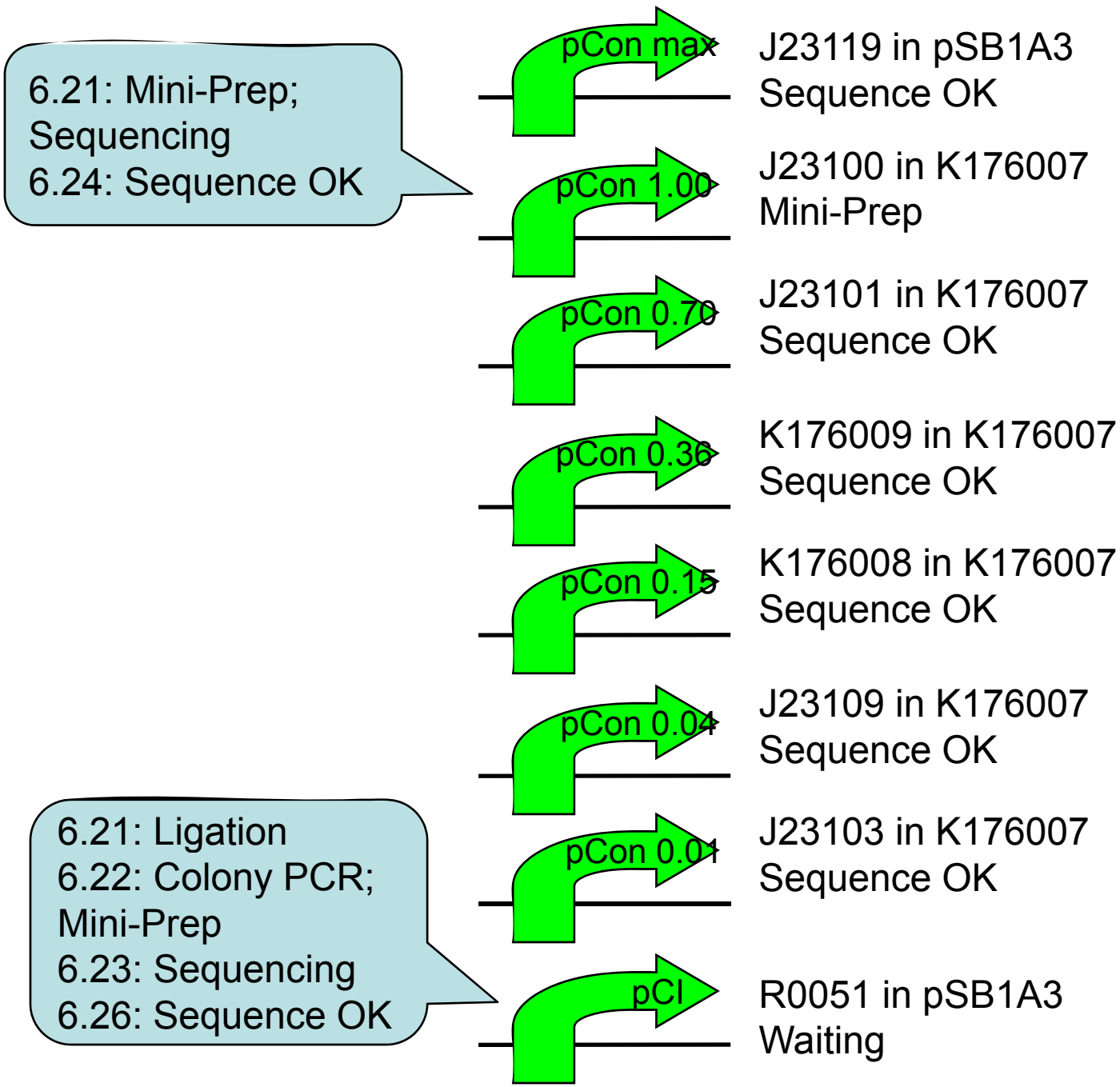
J23109-J61002SF X+P
Sequence OK



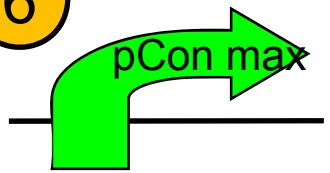
J23103-J61002SF X+P
Sequence OK



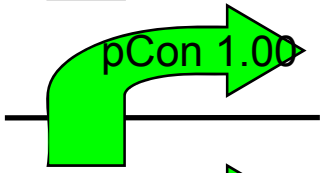
R0051 X+P
Digestion


6.21: Mini-Prep;
Sequencing
6.24: Sequence OK
pCon max
J23119 in pSB1A3
Sequence OK
pCon 1.00
J23100 in K176007
Mini-Prep
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
6.21: Ligation
6.22: Colony PCR;
Mini-Prep
6.23: Sequencing
6.26: Sequence OK
pCI
R0051 in pSB1A3
Waiting

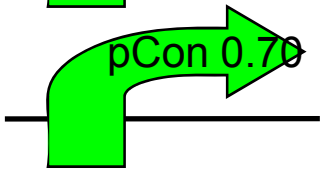
6



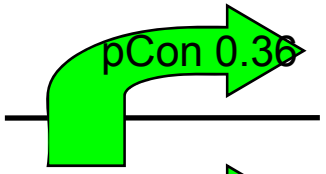
J23119 in pSB1A3 S+P
Length OK



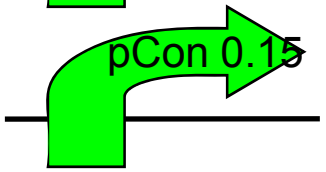
J23100 in K176007 S+P
Waiting



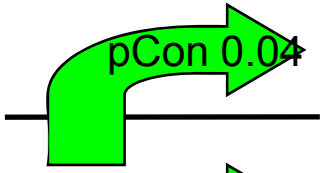
J23101 in K176007 S+P
Length OK



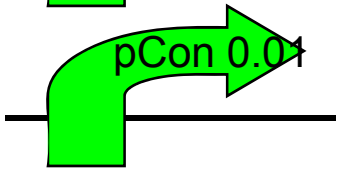
K176009 in K176007 S+P
Length OK



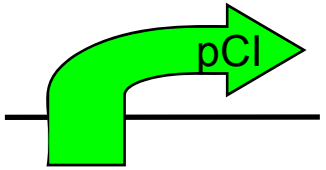
K176008 in K176007 S+P
Length OK



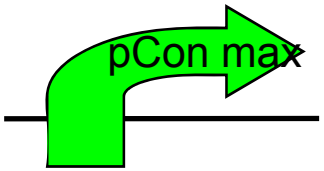
J23109 in K176007 S+P
Length OK



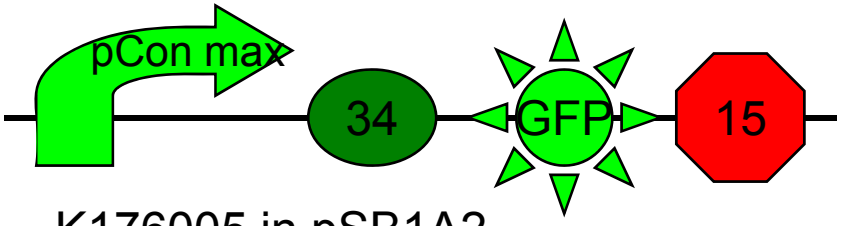
J23103 in K176007 S+P
Length OK



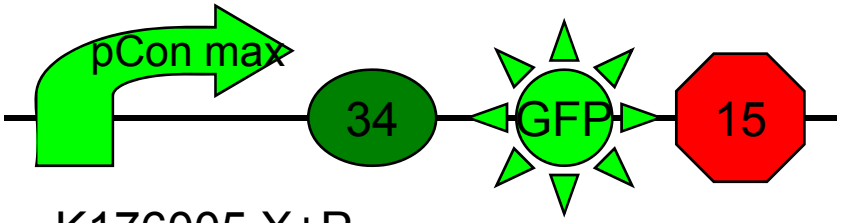
R0051 in pSB1A3 S+P
Waiting



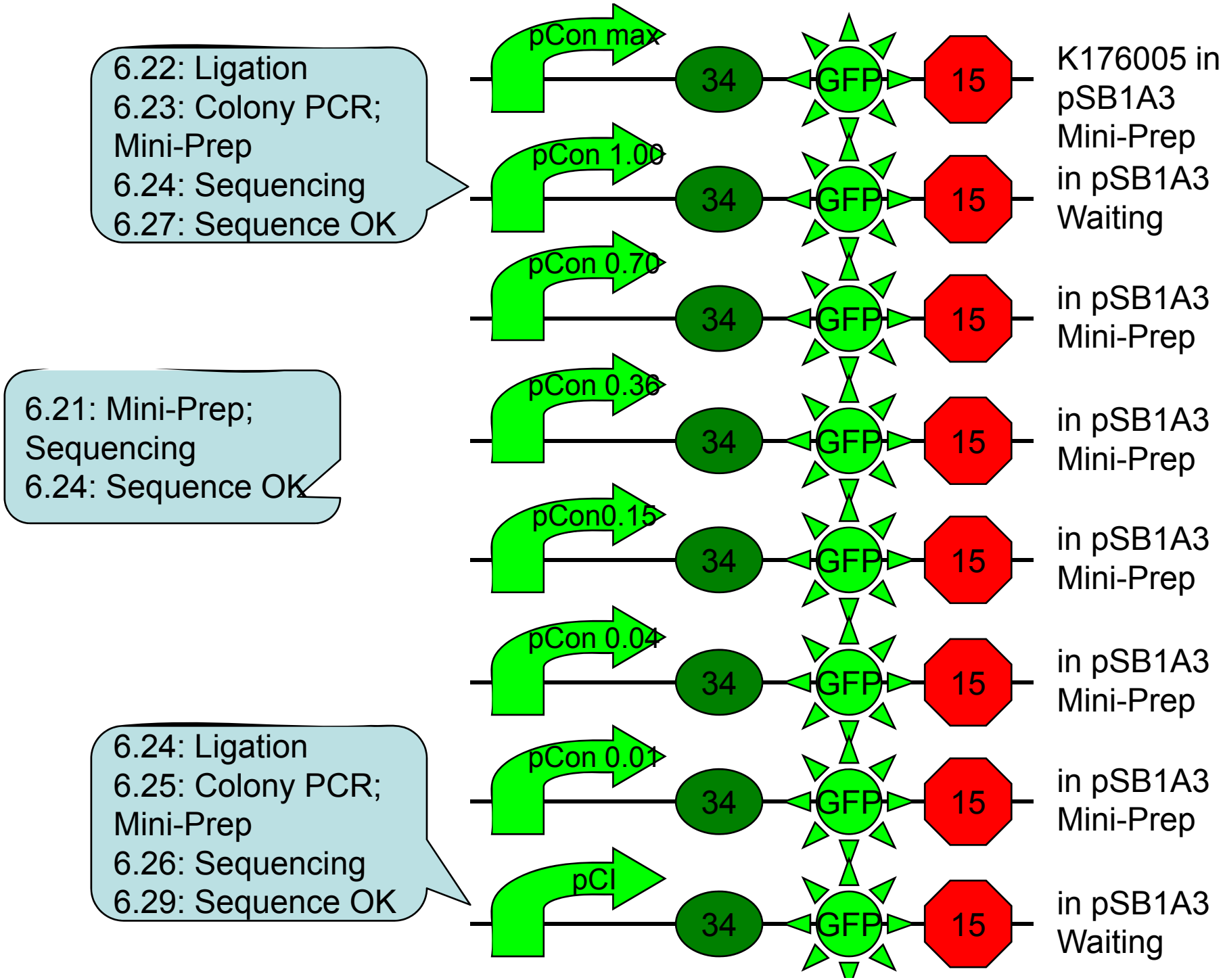
J23119 in pSB1A2 S+P
Sequence OK

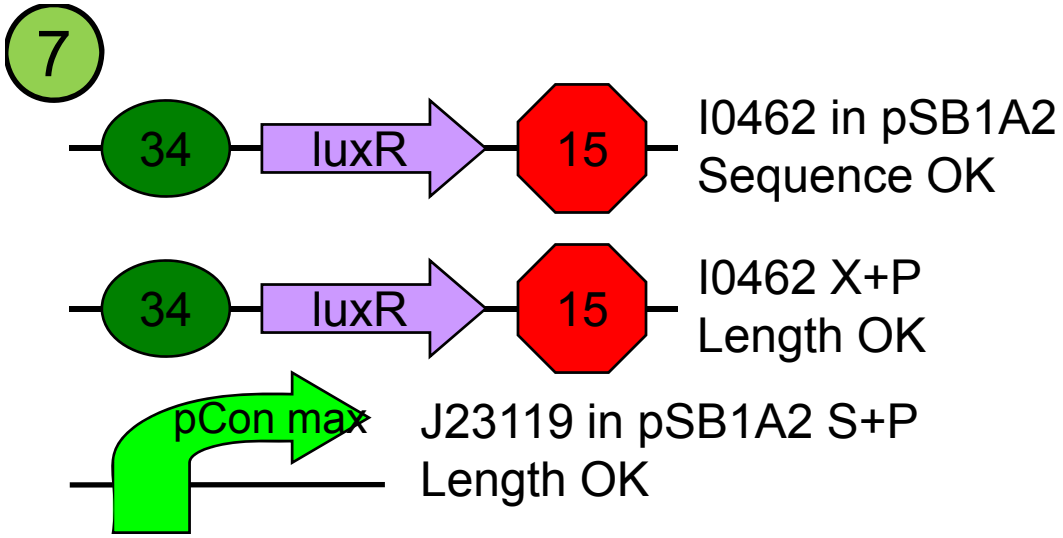


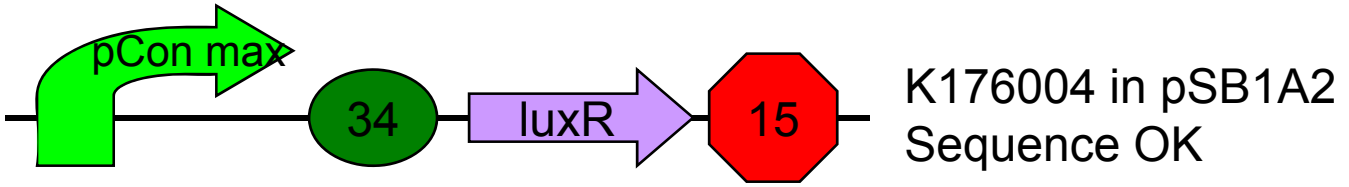
K176005 in pSB1A2
Sequence OK

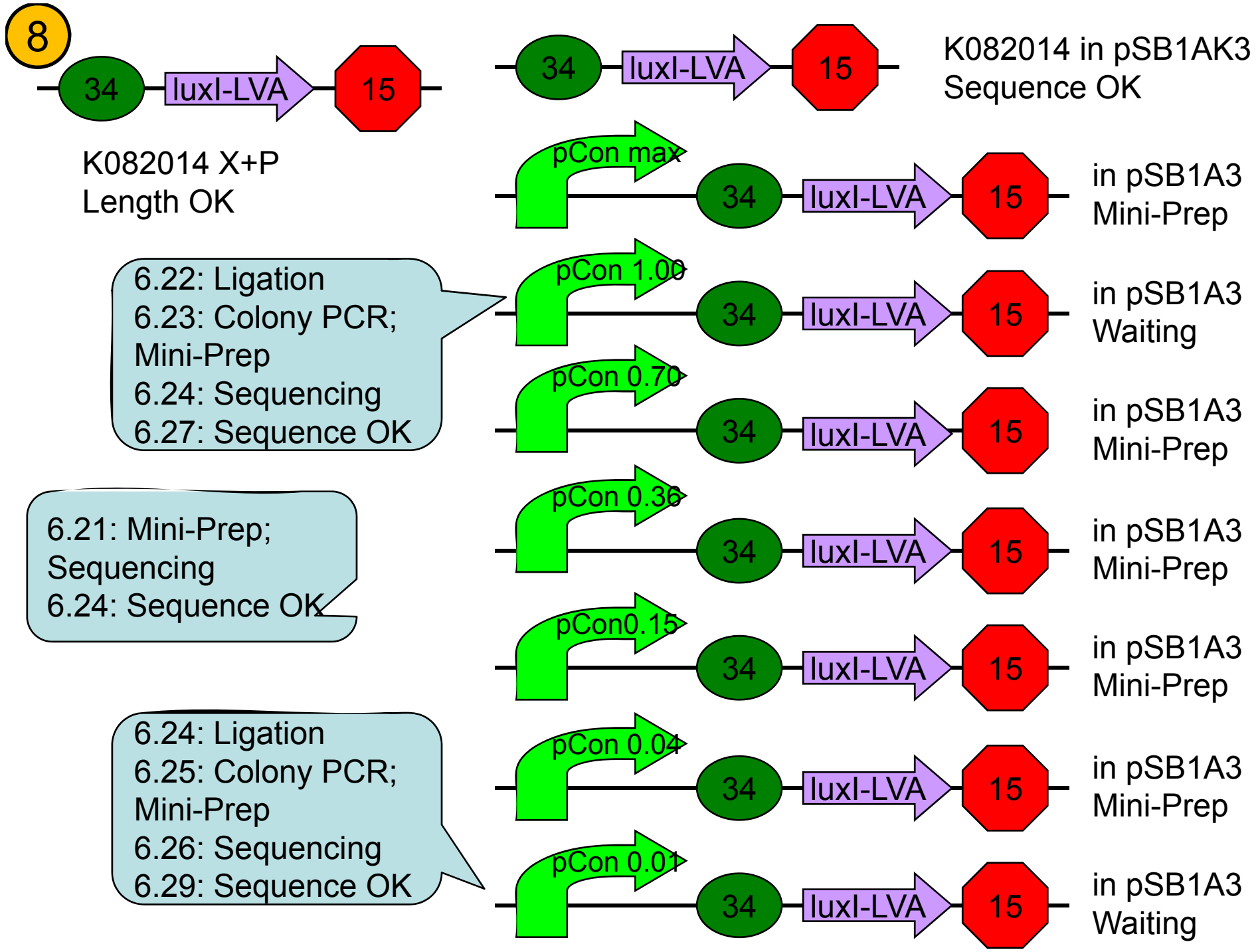


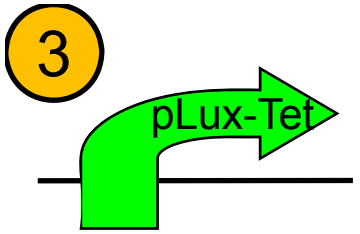
K176005 X+P
Length OK


6.22: Ligation
6.23: Colony PCR;
Mini-Prep
6.24: Sequencing
6.27: Sequence OK
6.21: Mini-Prep;
Sequencing
6.24: Sequence OK
6.24: Ligation
6.25: Colony PCR;
Mini-Prep
6.26: Sequencing
6.29: Sequence OK
pCon max
pCon 1.00
pCon 0.70
pCon 0.36
pCon0.15
pCon 0.04
pCon 0.01
pCI
34
GFP
15
K176005 in
pSB1A3
Mini-Prep
in pSB1A3
Waiting
in pSB1A3
Mini-Prep
in pSB1A3
Mini-Prep
in pSB1A3
Mini-Prep
in pSB1A3
Mini-Prep
in pSB1A3
Mini-Prep
in pSB1A3
Waiting


7
34
luxR
15
I0462 in pSB1A2
Sequence OK
34
luxR
15
I0462 X+P
Length OK
pCon max
J23119 in pSB1A2 S+P
Length OK

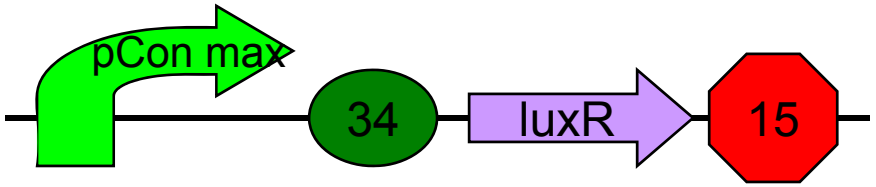

pCon max
34
luxR
15
K176004 in pSB1A2
Sequence OK


8
34
luxI-LVA
15
K082014 X+P
Length OK
34
luxI-LVA
15
K082014 in pSB1AK3
Sequence OK
pCon max
34
luxI-LVA
15
in pSB1A3
Mini-Prep
6.22: Ligation
6.23: Colony PCR;
Mini-Prep
6.24: Sequencing
6.27: Sequence OK
pCon 1.00
34
luxI-LVA
15
in pSB1A3
Waiting
pCon 0.70
34
luxI-LVA
15
in pSB1A3
Mini-Prep
6.21: Mini-Prep;
Sequencing
6.24: Sequence OK
pCon 0.36
34
luxI-LVA
15
in pSB1A3
Mini-Prep
pCon0.15
34
luxI-LVA
15
in pSB1A3
Mini-Prep
6.24: Ligation
6.25: Colony PCR;
Mini-Prep
6.26: Sequencing
6.29: Sequence OK
pCon 0.04
34
luxI-LVA
15
in pSB1A3
Mini-Prep
pCon 0.01
34
luxI-LVA
15
in pSB1A3
Waiting

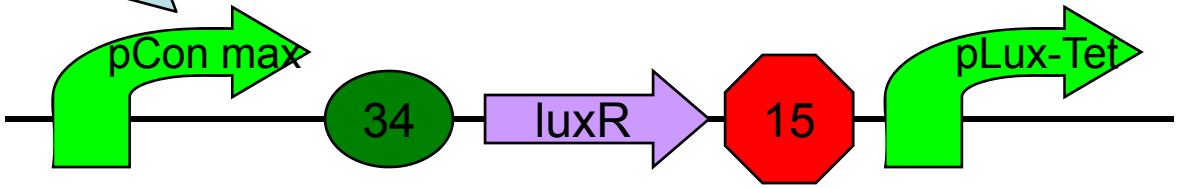


K176000 in pSB1A3 E+X
Length OK

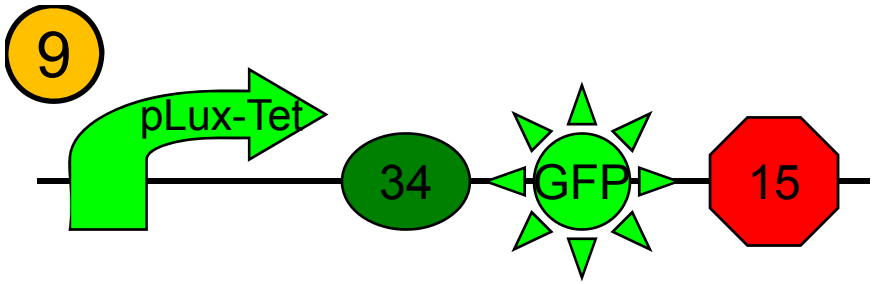
6.21: Ligation;
Colony PCR
6.22: Mini-Prep;
Sequencing
6.25: Sequence OK



K176004 E+S
Length OK

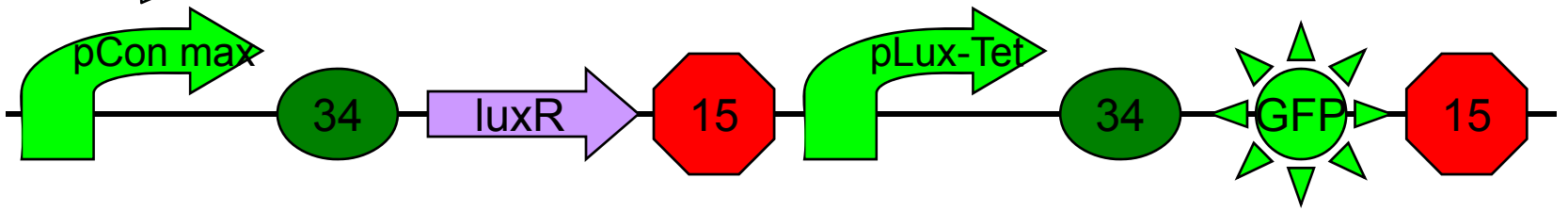


in pSB1A3
Ligation

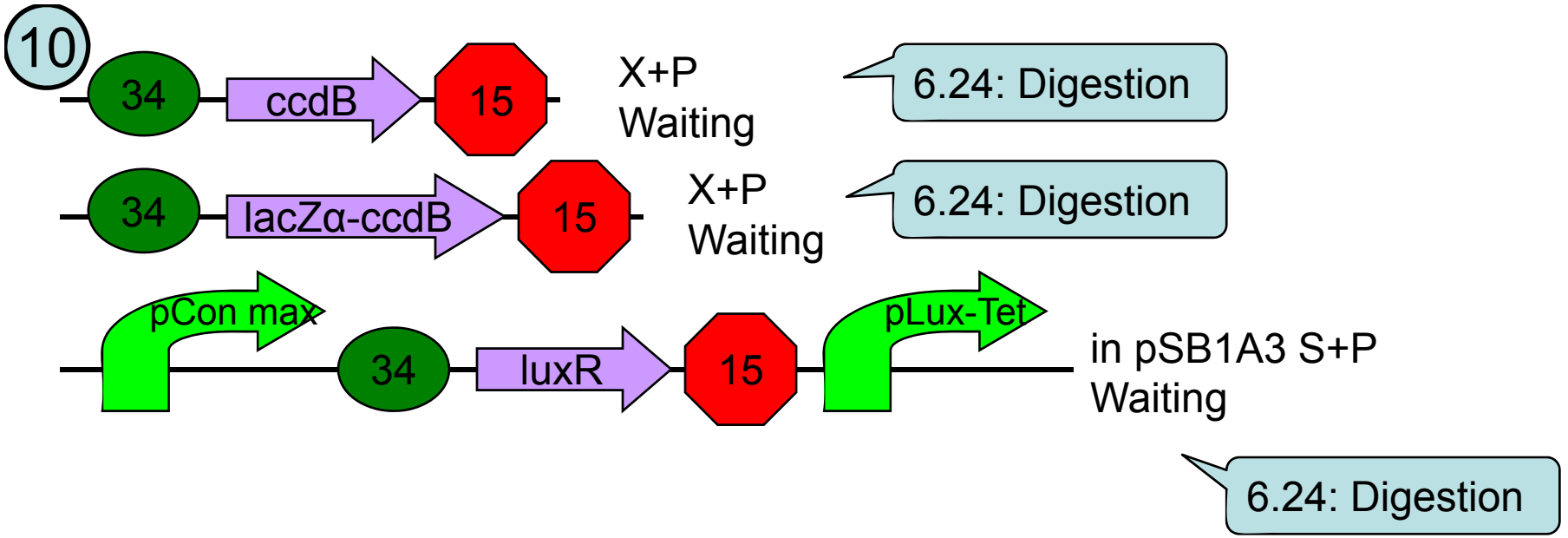


in pSB1A3 E+X
Length OK

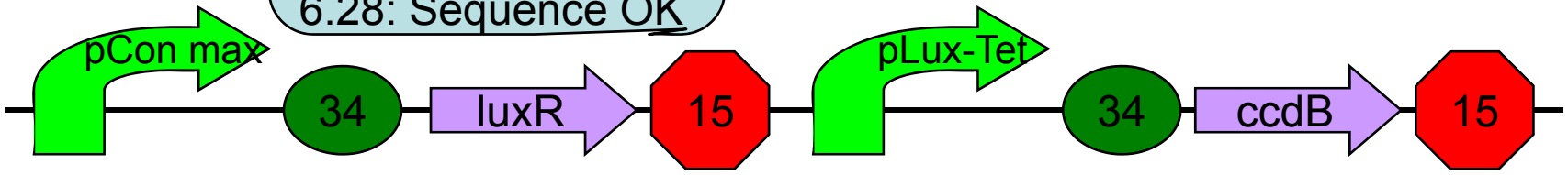
6.21: Ligation;
Colony PCR
6.22: Mini-Prep;
Sequencing
Primer Design;
Sequencing
6.25: Sequence OK



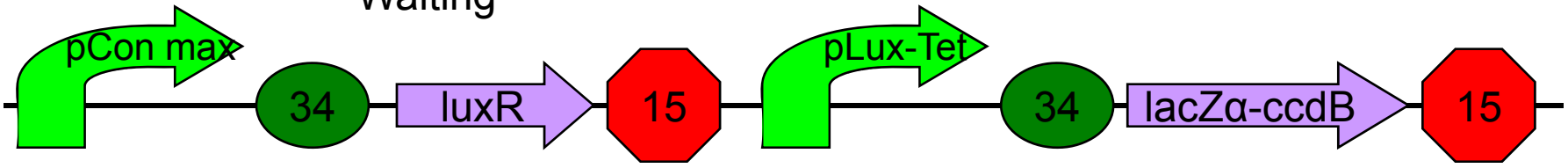
in pSB1A3
Ligation


10
34
ccdB
15
X+P
Waiting
6.24: Digestion
34
lacZα-ccdB
15
X+P
Waiting
6.24: Digestion
pCon max
34
luxR
15
pLux-Tet
in pSB1A3 S+P
Waiting
6.24: Digestion

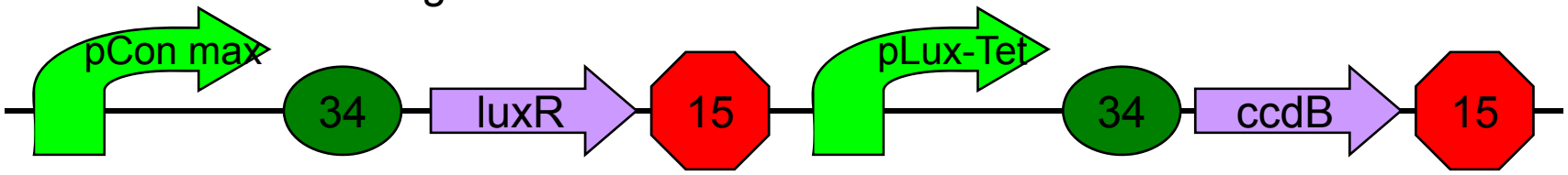
6.24: Ligation
6.25: Colony PCR; Mini-Prep
6.26: Sequencing
6.28: Sequence OK



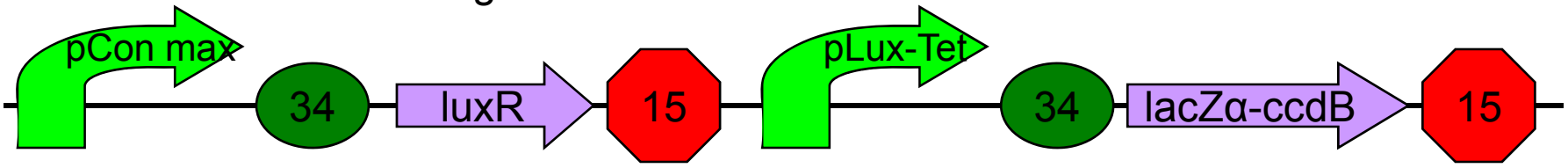
in pSB1A3 in DB 3.1
Waiting



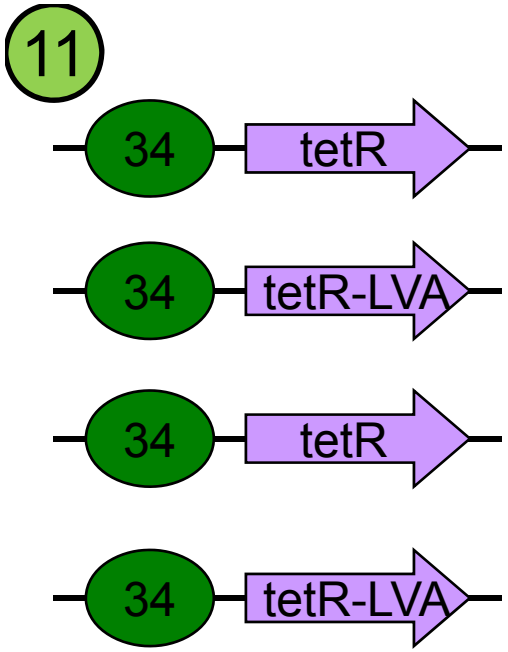
in pSB1A3 in DB 3.1
Waiting



in pSB1A3 in TOP10
Waiting



in pSB1A3 in TOP10
Waiting

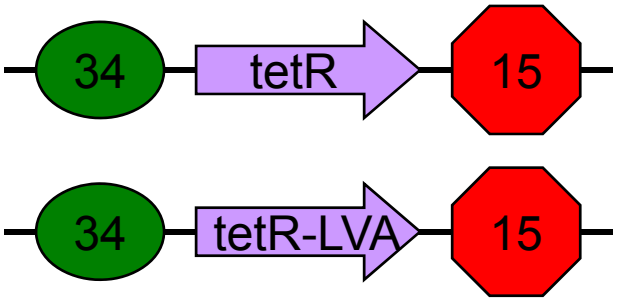


I732081 in pSB1A3
Sequence OK

S0101 in pSB1A2
Sequence OK

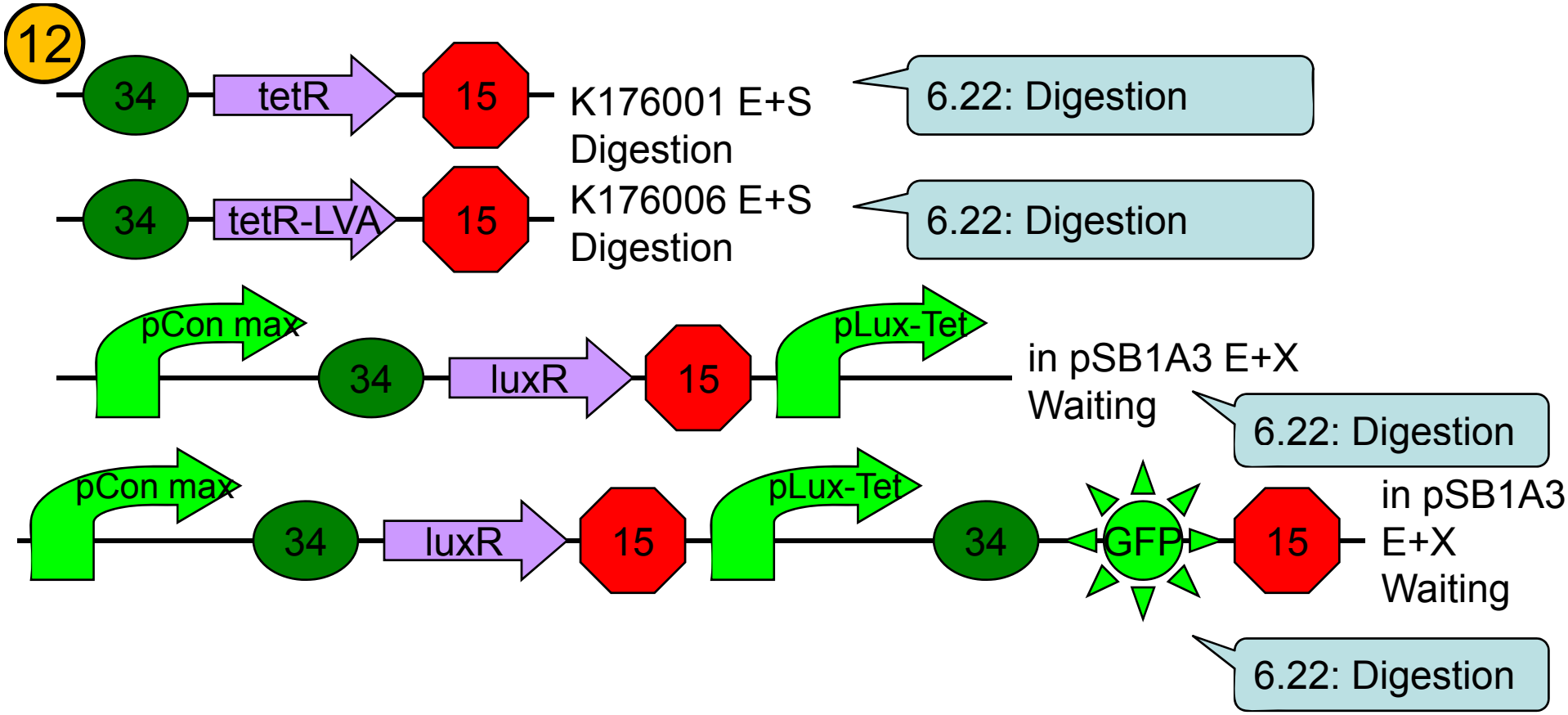
I732081 E+S
Sequence OK

S0101 E+S
Sequence OK

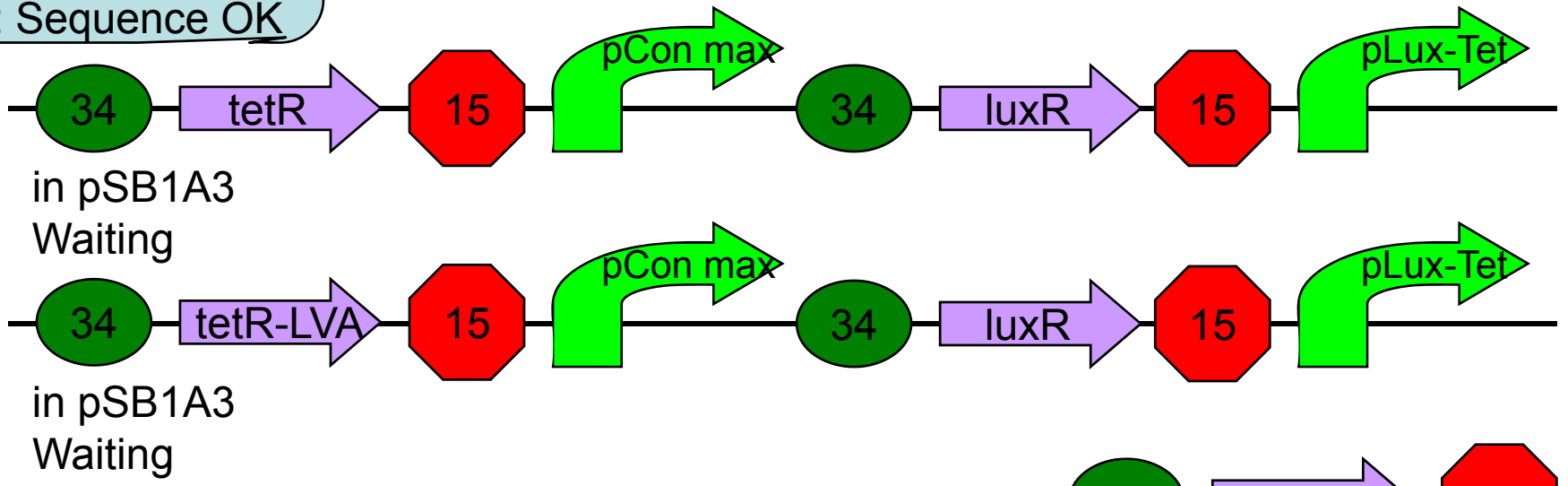


K176001 in pSB1AK3
Sequence OK

K176006 in pSB1AK3
Sequence OK

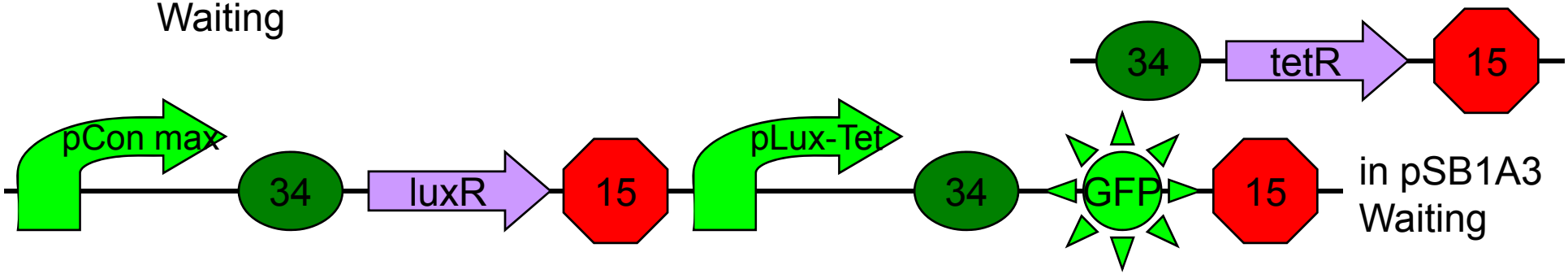

12
34
tetR
15
K176001 E+S
Digestion
6.22: Digestion
34
tetR-LVA
15
K176006 E+S
Digestion
6.22: Digestion
pCon max
34
luxR
15
pLux-Tet
in pSB1A3 E+X
Waiting
6.22: Digestion
pCon max
34
luxR
15
pLux-Tet
34
GFP
15
in pSB1A3
E+X
Waiting
6.22: Digestion

6.22: Ligation
6.23: Colony PCR; Mini-Prep
6.24: Sequencing
6.27: Sequence OK

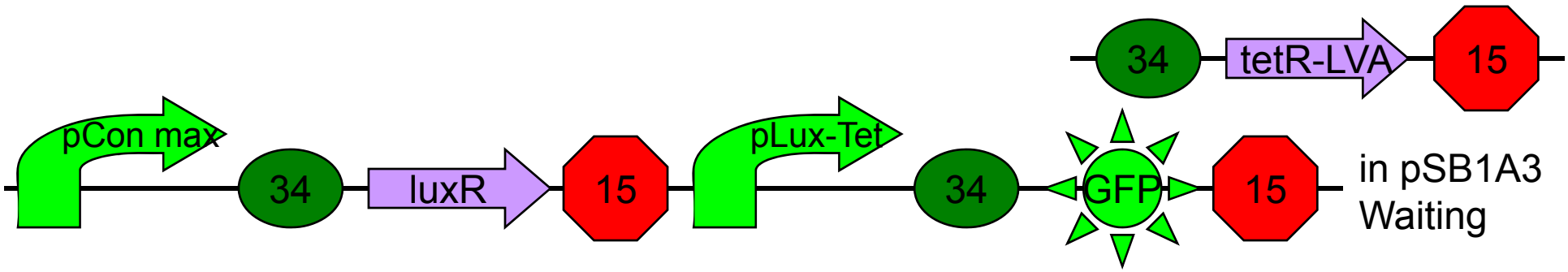


in pSB1A3
Waiting

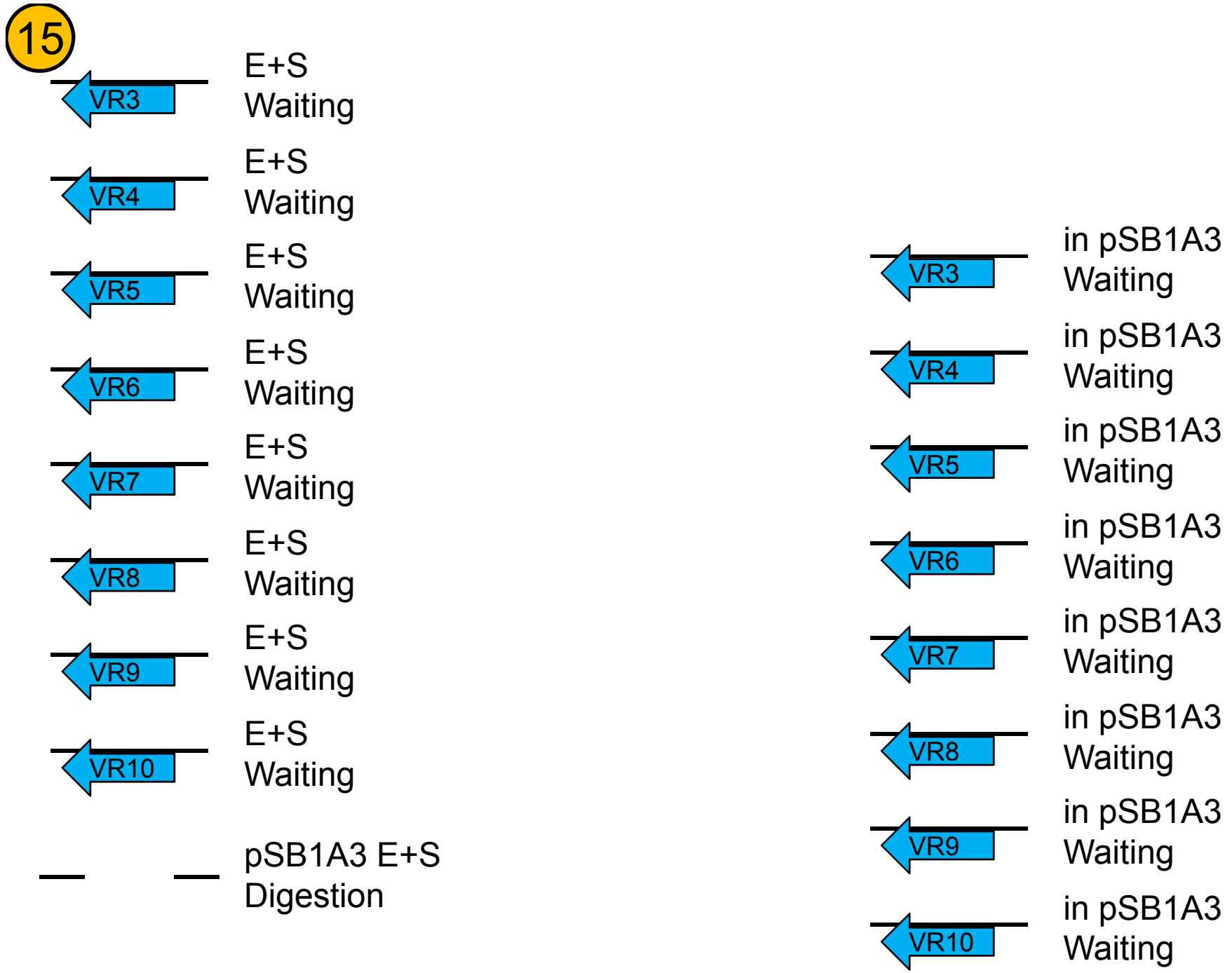
in pSB1A3
Waiting

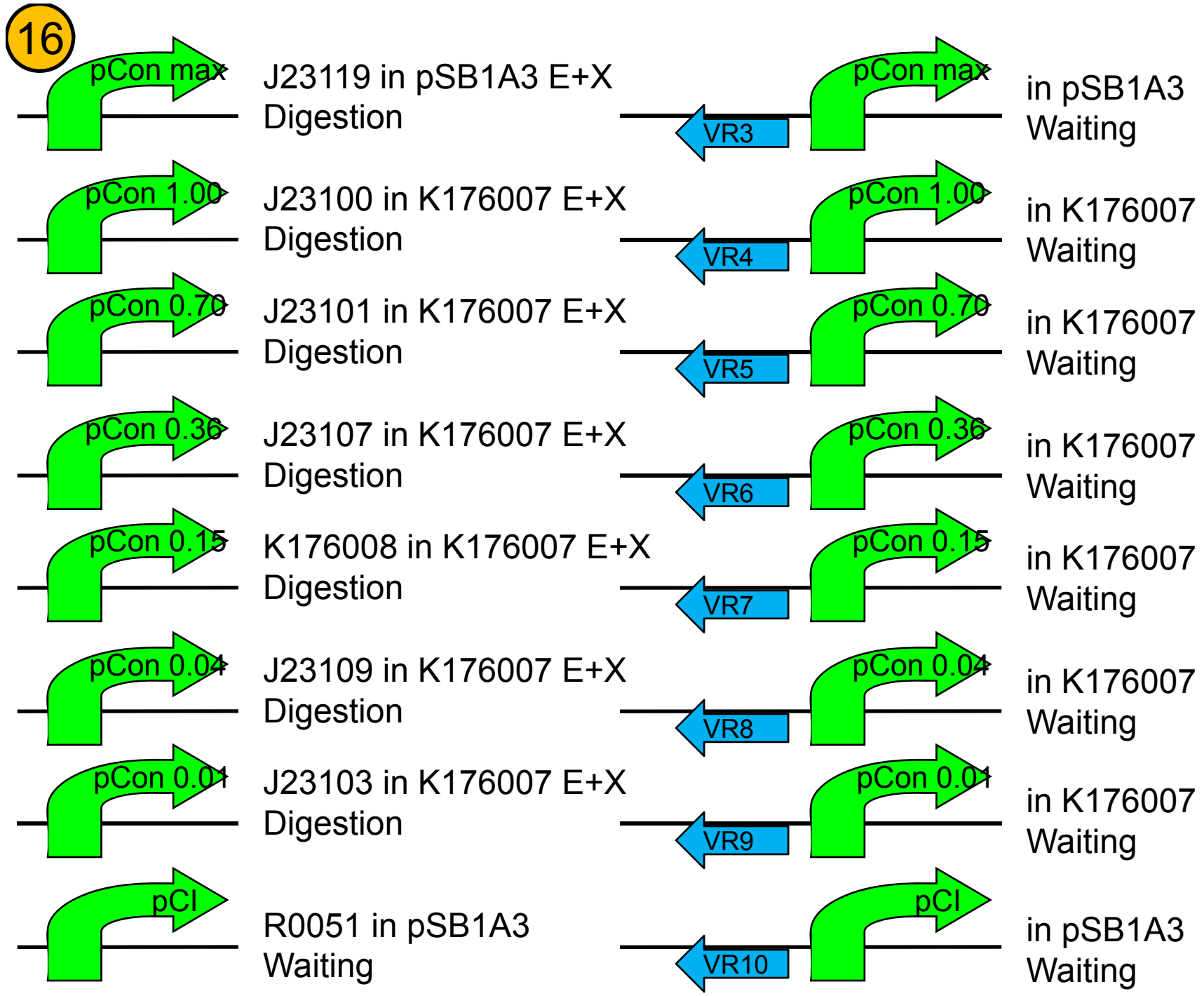


in pSB1A3
Waiting



in pSB1A3
Waiting


15
VR3
E+S
Waiting
VR4
E+S
Waiting
VR5
E+S
Waiting
VR6
E+S
Waiting
VR7
E+S
Waiting
VR8
E+S
Waiting
VR9
E+S
Waiting
VR10
E+S
Waiting
pSB1A3 E+S
Digestion
VR3
in pSB1A3
Waiting
VR4
in pSB1A3
Waiting
VR5
in pSB1A3
Waiting
VR6
in pSB1A3
Waiting
VR7
in pSB1A3
Waiting
VR8
in pSB1A3
Waiting
VR9
in pSB1A3
Waiting
VR10
in pSB1A3
Waiting

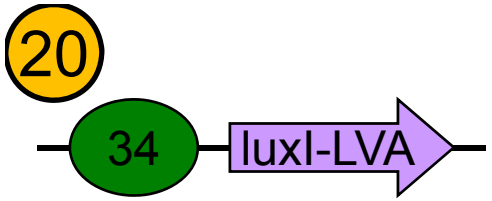

16
pCon max
J23119 in pSB1A3 E+X
Digestion
VR3
pCon max
in pSB1A3
Waiting
pCon 1.00
J23100 in K176007 E+X
Digestion
VR4
pCon 1.00
in K176007
Waiting
pCon 0.70
J23101 in K176007 E+X
Digestion
VR5
pCon 0.70
in K176007
Waiting
pCon 0.36
J23107 in K176007 E+X
Digestion
VR6
pCon 0.36
in K176007
Waiting
pCon 0.15
K176008 in K176007 E+X
Digestion
VR7
pCon 0.15
in K176007
Waiting
pCon 0.04
J23109 in K176007 E+X
Digestion
VR8
pCon 0.04
in K176007
Waiting
pCon 0.01
J23103 in K176007 E+X
Digestion
VR9
pCon 0.01
in K176007
Waiting
pCI
R0051 in pSB1A3
Waiting
VR10
pCI
in pSB1A3
Waiting

13

14

17

18



C0261 in pSB1A2
Sequence OK