

1401 bp

Send samples for sequencing

6175767

21.05

1. 72 (1B8) ? ✓
2. 76 (1A9) ✓
3. 83 (1A1) ✓
4. 52 (1B2)? ✓
5. 75 (1A6) ✓
6. 61 (1A7) ✓
7. 91 (1A5) ✓
8. 58 (1A5) ?? ✓
9. 54 (1A9)? ✓
10. 66 (1A6) ✓

Primers: Amp-Rev

Galle-Rev

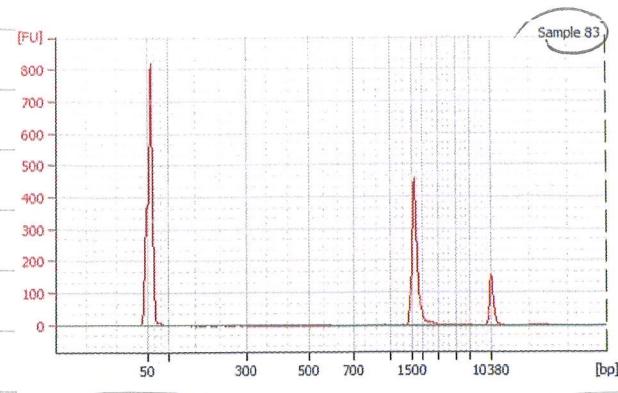
Sequence with GSP

6176004

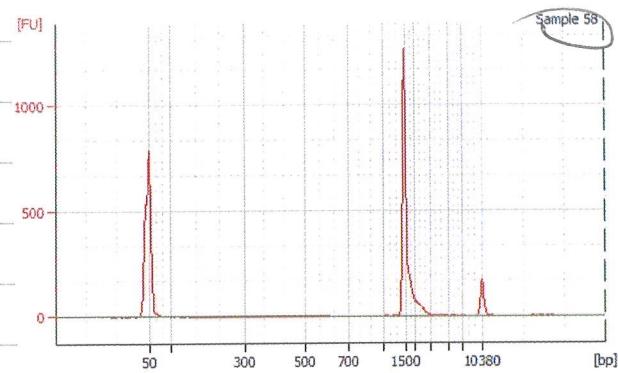
22.05

981. ✓ 1. 72 | 138-2 ✓ 11780 bp ✓ (1783)
991. ✓ 2. 76 | 1A9-2 ✓ 1410 bp ✓ (1428)
- 98 ✓ 3. 83 | 1A1-* ✓ 1633 bp ✓ (1630)
971. ✓ 4. 52 | 1B2-2 ✓ 1355 bp ✓ (1313)
981. ✓ 5. 75 | 1A4-R ✓ 1411 bp ✓
- 99,61 ✓ 6. 61 | 1A4-2 ✓ 1401 bp ✓
671. ✓ 7. 91 | 1A4-R/NAS ✓ 1453 bp ✓
661. ✓ 8. 58 | 1A4-R/NAS ✓ 1474 bp ✓
951. ✓ 9. 54 | 1A4-2 ✓ 1401 bp ✓
571. ✓ 10. 66 | 1A4-R ✓ 1618 bp ✓

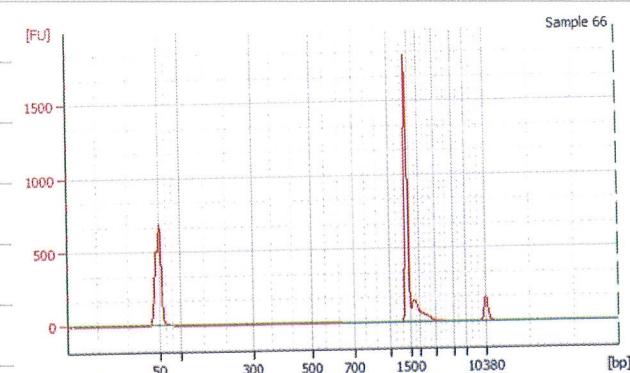
10μM → 3.2 μM
6.4 μL stock
to 13.6 μL



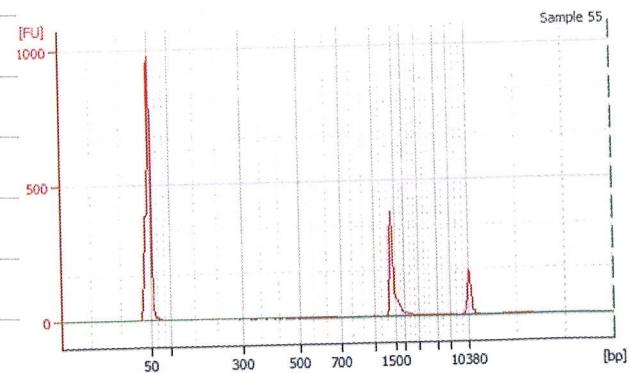
1633 bp (IA1) ✓



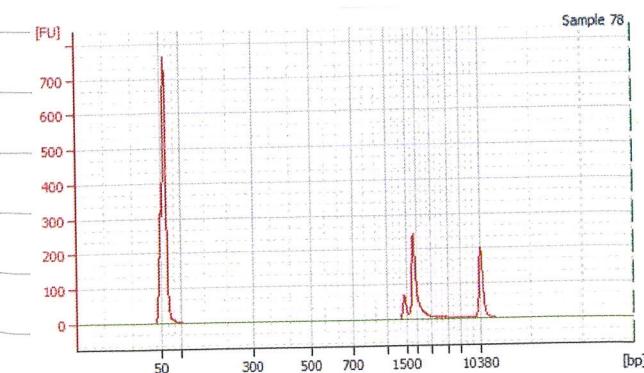
1474 bp



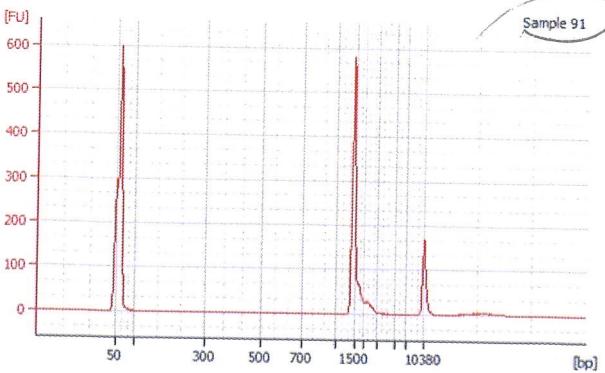
1618 bp



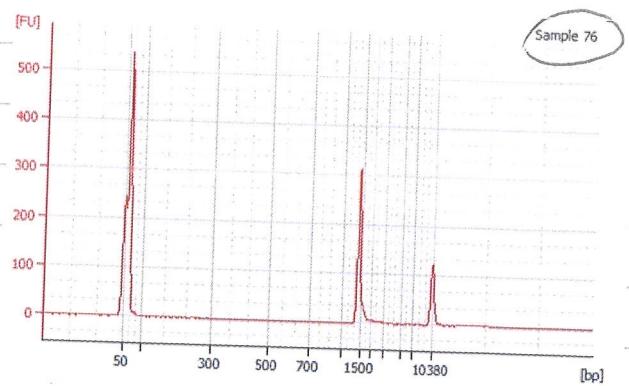
1403 bp



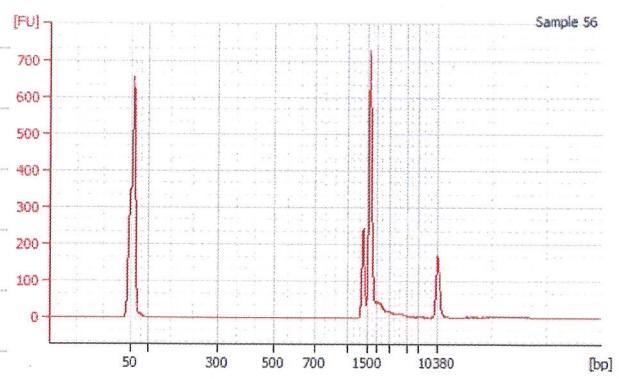
1855 bp



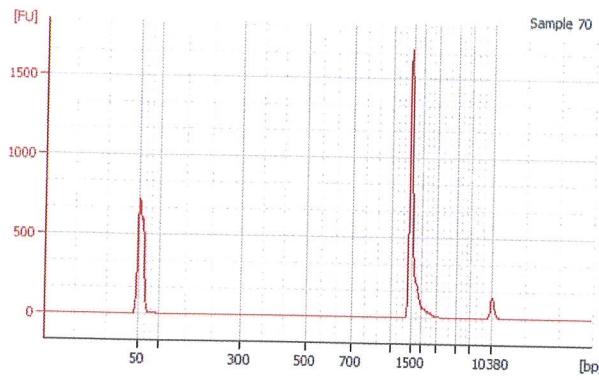
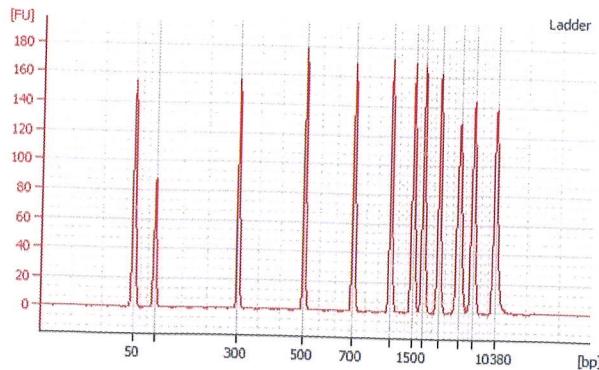
1453 bp



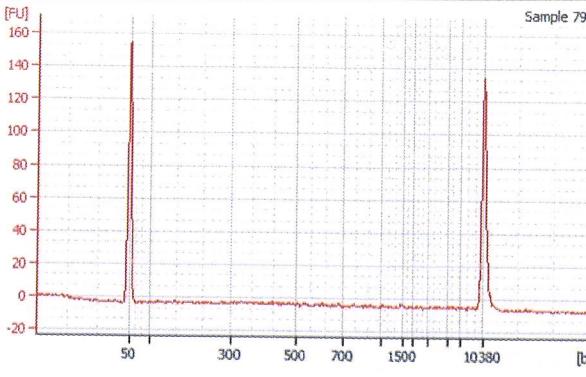
1410 bp (18%) ✓



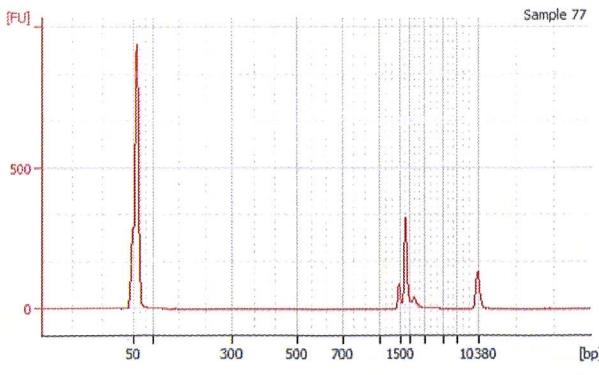
1660 bp (18%)



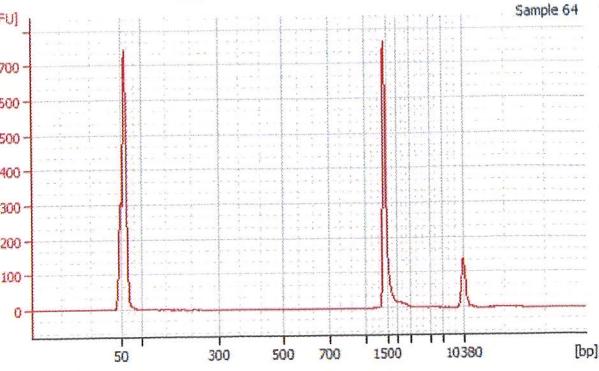
1469 bp



empty!



1782 bp (138)



1441 bp

PCR of KM-RegE1 new transcription lines

MK

5 x HiFi GC Buffer Spe	150 µl	} ⇒ 25 µl each
dNTP mix	0,75 µl	
Amp-Rw (200 µM)	0,75 µl	
Galc-Rw (200 µM)	0,75 µl	
plasmid DNA	1 µl	
KAPA HiFi Polym.	0,5 µl	
MQ	16,25 µl	15,5 µl
	25 µl	



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Program

Transf. ident.

Samples

1. 70	13. 84
repeat!	
2. 79	Leaving well!
3. 77	14. 83
	15. 58
4. 64	16. 66
5. 82	17. 55
6. 88	18. 78
7. 72	19. 52
8. 73	20. 75
9. 51	21. 80
10. 91	22. 71
11. 70	23. 61
12. 56	24. 54



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ins.	in vector
1A7	714
1B2	617
1B4	1042
1B7	790
1B8	912

● TECAN.

ins:	in vector
1A1	1363 1ep1 (CG1W1+8)
1A2	1358 1ep2 (CG8164+)
1A4	1393 1ep3 (CG1W164+)
1A5	1352 1ep3 (CG14164+)
1A6	1067 1ep4 (CG6434)

● TECAN.

PCR products have been purified with QIAquick PCR Purif. Kit

gDNA prep. from new transgenic lines

- Grind flies in 200 µl Buffer A
- Add additional 200 µl Buffer A and grind.
- 65°C | 30 min
- Add 800 µl lysis / KAC sol. → Invert several times
- Ice | 10 min
- Spin 15 min | max speed | RT
- Transfer the supernatant into a 2ml tube.
- Add 600 µl of isopropanol, mix, Spin | 30 min | max speed | RT
- Add 500 µl of 70% EtOH Spin max speed | 10 min | RT
- Discard supernatant → Spin | 1 min
- Discard supernatant → air dry pellet.
- Resuspend in 150 µl of Tris-HCl pH 8.0

Samples

- | | |
|------|------|
| 1. | 13. |
| 2. | 14. |
| 3. | 15. |
| 4. | 16. |
| 5. | 17. |
| 6. | 18. |
| 7. | 19. |
| 8. | 20. |
| 9. | 21. |
| 10. | 22. |
| 11. | 23. |
| 12 - | 24 - |

27. 06

Digestion of gDNA | MspI

20 μl gDNA (2100 ng/re)

5 μl Buffer 4

5 μl MspI

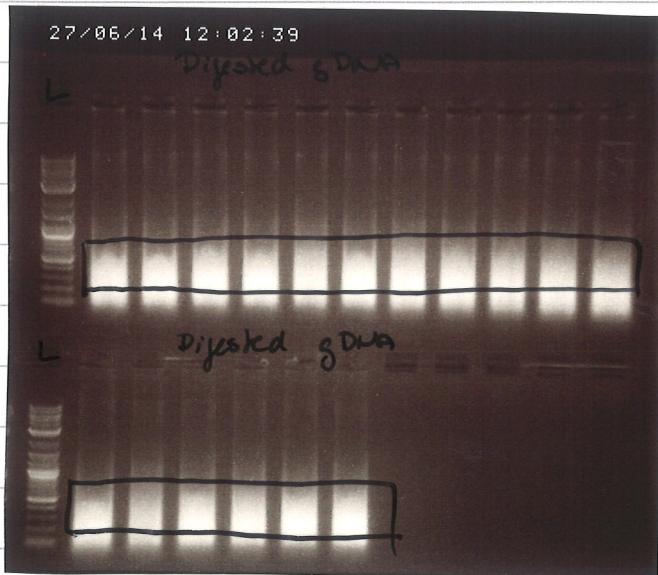
20 μl Nuclease free w.

50 μl

37°C / 90min → Heat inact. 80°C / 10min



Clean up from gel.



30/06

PCR for KU-ReGeL new transformant lines

		MK	
5x HiFi GC Buffer	5 µl	150 µl	
dNTP mix	0.75 µl	22.5 µl	
Amp-Rev (200 µM)	0.75 µl	22.5 µl	
Galc-Rev (200 µM)	0.75 µl	22.5 µl	
genomic DNA	0.5 µl		
KAPA HiFi Polym.	0.5 µl	11 µl	
nuc. free w.	16.75 µl	50.25 µl	
		48.75 µl	
		25 µl	

25 µl each

Program: Transf. ident annealing T: 67°C

Samples

1. 1.	1660 bp (14)	13. 19	— Ladder
2. 3.	1667 bp	14. 21	1635 bp
3. 4	(93 bp)	15. 22	1625 bp
4. 5	(93 bp)	16. 24	1493 bp / 1845 bp
5. 6	(91 bp)	17. 26	1574 bp
6. 9	—	18. 27	1473 bp
7. 10	(93 bp)	19. 28	1815 bp
8. 13	(91 bp)	20. 29	1781 bp
9. 14	1488 bp	21. 30	1406 bp
10. 15	1756 bp	22. 31	1648 bp
11. 17	1480 bp	23. 32	1685 bp
12. 18	1683 bp	24. 33	1425 bp

Clean up PCR-products. → Send to sequencing!

→ Amp-Rev

→ Gal4-Rev

01.07.

Sequencing pYoh366G clones

order no. A183309

Samples

1. pYoh366G-1	Amp_Fw OK	ccdB failed	Cel6r_Rw OK
2. pYoh366G-2	Amp_Fw OK	ccdB failed	Cel6r_Rw OK
3. pYoh366G-3	Amp_Fw OK	ccdB failed	Cel6r_Rw OK
4. pYoh366G-4	Amp_Fw OK	ccdB failed	Cel6r_Rw OK
5. pYoh366G-5	Amp_Fw OK	ccdB_Fw OK ✓	Cel6r_Rw ✓ OK
6. pYoh366G-6	Amp_Fw OK	ccdB failed	Cel6r_Rw OK
7. pYoh366G-7	Amp_Fw OK	ccdB failed	Cel6r_Rw OK
8. pYoh366G-8	Amp_Fw OK	ccdB failed	Cel6r_Rw OK
9. pYoh366G-9	Amp_Fw OK	ccdB failed	Cel6r_Rw OK
10. pYoh366G-10	Amp_Fw OK	ccdB failed	Cel6r_Rw OK
11. pYoh366G-11	Amp_Fw OK	ccdB failed	Cel6r_Rw OK
12. pYoh366G-12	Amp_Fw OK	ccdB failed	Cel6r_Rw OK

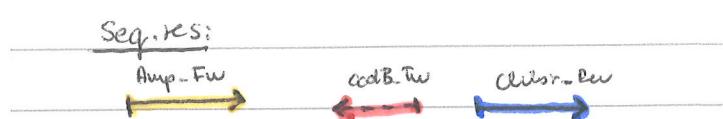
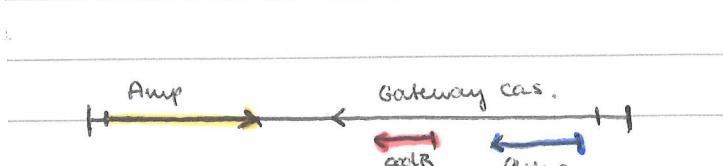
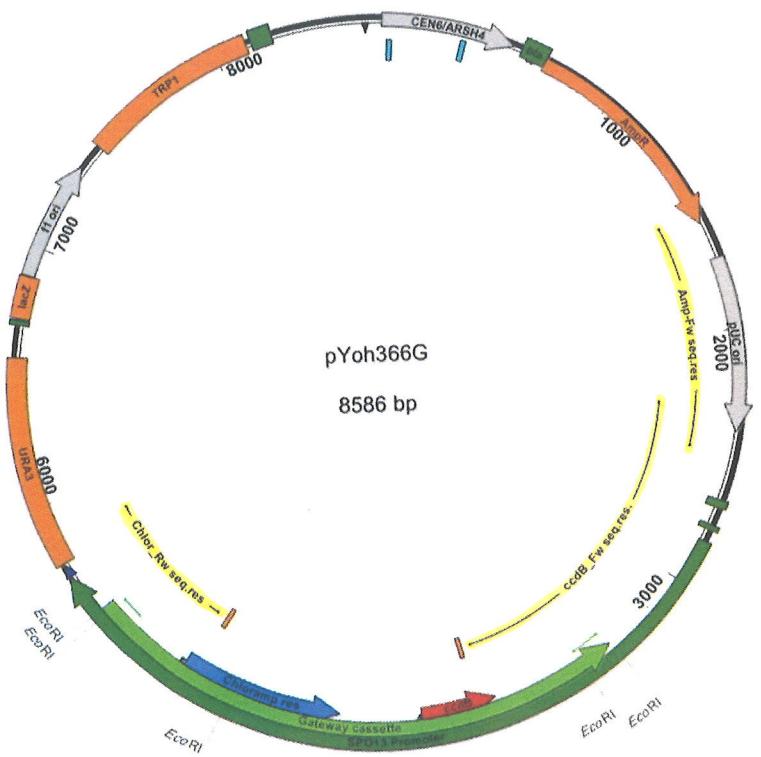
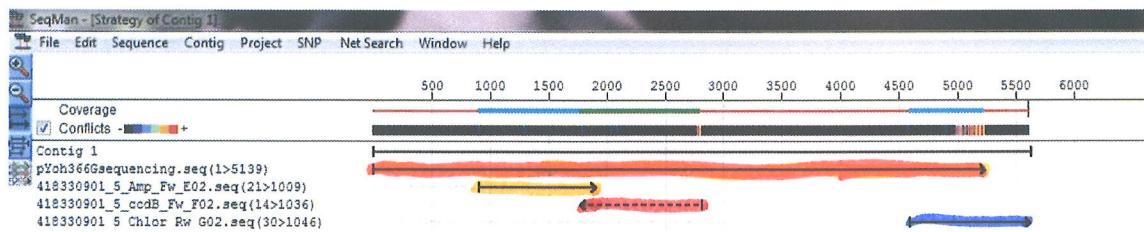
→ Grow up!

primers:

Amp_Fw

ccdB_Fw

Cel6r_Rw



02/07

Linearization of Xba-I/Bgl-II clones

18 μl plasmid DNA

1 μl Buffer 4 (NEB)

1 μl XbaI / BglII

20 μl

37°C / 30 min

→ Heat inactivate 65°C / 15 min

Samples

a

1. 2A2

2. 2A3

3. 2A4

4. 2A6

5. 2A11

6. 2A12 | BglII

7. 2B7

8. 2B8

9. 2B9

10. 2B10

11. 2B11

12. 2C7

13. 2C9 | BglII

14. 2C12

15. 2D1

16. 2D2