Genotyping sgRNA flies with pCFD4 and pCFD3 inserts

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1. Crude genomic DNA preparation:

SB buffer:
10mM Tris-HCl pH8
1mM EDTA
25mM NaCl

Proteinase K:
Stock conc. (100X): 20mg/ml
Working conc.: 200ug/ml (actually 20ug/ml works fine)
add Proteinase K to SB buffer just before experiment

- **a.** place a single fly in an Eppendorf tube
- **b.** add 50ul SB buffer
- **c.** homogenize
- **d.** incubate at 37°C · 30min
- **e.** spin: 16,000g · 2 min
- **f.** transfer the supernatant to PCR tube
- **g.** incubate 95°C · 3min
- **h.** store at 4°C (for longer term, store in -20°C freezer)

2. Genotyping PCR:

Use these primers for pCFD4 to amplify a band of 731bp
pCFD4-F: 5'-GACACAGCGCGTACGTCCTTCG-3'
pCFD4-R: 5'-ACTCTCAGGCTCCAGGTAGG-3'

Use these primers for pCFD3 to amplify a band of ~500bp
pCFD3-F: 5'-ACGTTTTATAACTTATGCCCCTAAG-3'
pCFD3-R: 5'-GCCGAGCACAATTGTCTAGAATGC-3'

a. PCR rxn

2X GoTaq Green	15 ul
H_2O	11.25
FW primer (10uM)	0.625
RV primer (10uM)	0.625
Genomic DNA	2.5 ul

b. PCR program for pCFD3

Step1:	95°C, 2 min
Step2(35 x):	95°C, 30 sec
	50°C, 30 sec

72°C, 1 min
Step3: 72°C, 10 min
Step4: 4°C, ∞

c. <u>PCR program for pCFD4</u>
Step1: 95°C, 2 min
Step2(35 x): 95°C, 30 sec
55°C, 30 sec
72°C, 1 min
Step3: 72°C, 10 min
Step4: 4°C, ∞

3. **Run gel:**
   a. run 4 ul PCR product on agarose gel
   b. check if PCR produces the right size of band and enough DNA for doing PCR purification

4. **PCR purification:**
   a. use QIAquick PCR Purification Kit
   b. elute in ~50 ul H2O
   c. purified DNA should be 10~20ng/ul

5. **Sequence purified PCR product:**
   a. For pCFD3 use pCFD3-F primer
   b. For pCFD4 use pCFD4-F primer

6. **Sequencing data analysis:**
   a. for pCFD3 the sequence is: GTC**<u>G</u>** + <u>sgRNA</u> + GTTTTAGAGC
   b. for pCFD4 the sequence is: AACTTC + <u>sgRNA1</u> + 496bp + <u>sgRNA2</u> + GTTTT