

TAIL PCR for detection of P insertion

Primers (sitting in the P3 of pUAST):

for 1st PCR T1BUAS: GCAGAAGCTTTGCGTACTCGC

for 2nd PCR T2D: ATTCAAACCCACGGACATG

for 3rd PCR (this primer I haven't tried yet since I directly sequenced the 2nd PCR)

T2En: AATCATATCGCTGTCTCACTCA

AD1-AD3 primers (as published in the Plant Journal):

AD1: NTCGASTWTSGWGTT

AD2: NGTCGASWGANAAGAA

AD3: WGTGNAGWANCANAGA

1st PCR: using T1BUAS and one of the three AD primers (AD3, the most degenerate primer, worked best for me)

1.6 μ l dNTPs (2.5mM each)

1.45 μ l MgCl₂ (25 mM)

2 μ l 10Xbuffer

0.25 μ l TAQ

0.4 μ l T1BUAS*

AD* primers are used in different amounts: 4 μ l AD1, 6 μ l AD2 or 8 μ l AD3

1 μ l template (single fly genomic prep, see below)

H₂O to 20 μ l

2nd PCR: using T2D and the same AD primer that has been used for 1st PCR

1.6 μ l dNTPs

1.45 μ l MgCl₂

2 μ l 10Xbuffer

0.25 μ l TAQ

0.4 μ l T2D*

AD* primer: 3 μ l AD1, 4 μ l AD2 or 4 μ l AD3

1 μ l template (directly use 1/50 dilution of 1st PCR)

H₂O to 20 μ l

* primer stock concentration: 10 pmol/ μ l (= 10 μ M)

2nd PCR is checked on gel (you will see bands for almost all your PCRs but some might be too short to sequence far enough) and subjected to EXOSAP-IT (from USB, cat# 78200) before sequencing:

9 μ l from 2nd PCR reaction + 2 μ l EXOSAP (the provider's protocol says to use much more but 2 μ l turned out to be enough)

37°C for 20'; 80°C for 15' (kills enzymes)

after incubations add 60 μ l H₂O =this dilution can be directly sequenced (I used T2D, T2En obviously also should work)