

PCR confirmation of *C. elegans* single-copy insertions

Reagents needed

- LongAmp PCR kit (New England Biolabs)
- OG967 (AGGCAGAATGTGAACAAGACTCG)
- OG970 (ATCGGGAGGCGAACCTAACTG)
- Purified genomic DNA (100ng / reaction) from single-copy insertions candidates at the ttTi5605 locus (other insertions sites require different primers)

PCR

1 reaction:

Component	1 reaction:	X reactions:
5x buffer	5 μ l	
dNTPs	0.75 μ l	
OG967	0.5 μ l	
OG970	0.5 μ l	
LongAmp Taq	1.0 μ l	
Genomic DNA	~100ng per reaction	
water	To 25 μ l	

1. Run the PCR under the following conditions in the MJ thermocycler (named "s.copy"):
 - a. 95° - 30 seconds
 - b. 94° - 10 seconds
 - c. 60° - 50 seconds
 - d. 65° - 1 min / kb
 - e. 65° - 10 min
 - f. 4° - forever

} 35 times
2. Negative control is N2 gDNA = 3Kb
3. As another control, can also include EG4887 = 8.5Kb
4. Run 5 μ l of reaction on a 0.7% gel
5. If the appropriate sized product is observed, digest the band with enzymes that cut in your promoter and/or CDS to validate that the insert is present in correct orientation and does not contain deletions/insertions.