

## YFP-tagging of genes, with TTPCR-method

### 1) First PCR

Product	Primers	Template	DNA polymerase
p1-p2	p1, p2	Colombia genomic DNA (0,3 ul)	ExTaq (Takara
p3-p4	p3, p4	Colombia genomic DNA (0,3 ul)	ExTaq RR001A)
cYFP	YFPleft,YFPright	YFP#3 plasmid	Pfu ( )

PRC reaction: 50 ng DNA as template  
1x buffer  
0.2 mM dNTP  
0.2 uM (or 1 pmol) of each primer  
0.025 U/ul ExTaq or Pfu DNA polymerase  
Reaction volume: 40ul

PCR program: 1 cycle 94'C 3 min  
(cold start) 30-35 cycles 94'C 30 sec  
Ta 1 min (60oC)  
68'C x min (1 min/kb)  
1 cycle 68'C 2x min

# Ta varies with primers, Ta= the lowest Tm -5'C of the 2 primers, (overhang not annealing to the template is not included in Tm)

# The x min varies with the length of your PCR product. x= 2 min+length (kb) of PCR product.

# cYFP product is made with Pfu polymerase, and is therefor not Pfu-polished, go directly to step 4)

2) Purify PCR-products using GFX PCR purification from Amersham (To remove dNTPs, primers and enzyme) Final volume 50 ul (or less volume, more concentrated).

3) Pfu-polish products (to get rid of A overhang put on by Ex-Taq) using Pfu polymerase.

PCR program: 72'C, 30 min, no cycling  
PRC reaction: 25 ul template (TTPCR product from 2) above)  
(cold start) 1x Pfu buffer  
0.2 mM dNTP  
0.025 U/ul Pfu polymerase  
(no primers!)

Reaction volume: 50 ul

4) Run product on 1% agarose gel, stain with methylene blue and cut out the fragment, gel purify using GFX gel band purification kit from Amersham (same kit as in 2) above, but different protocol) Final volume 50 ul (or less volume, more concentrated).

### 5) Second PCR = TTPCR

Product:	Primers:	Template	DNA polymerase
TTPCR product	p1-p2, p3-p4	YFP	ExTaq (TaKaRa)

PRC reaction: 3 ul P1P2 + 1 ul P3P4 + 1 ul cYFP (from 4) above)  
1x buffer  
0.2 mM dNTP  
0.2 uM (or 1 pmol) of each primer  
0.025 U/ul ExTaq

Reaction volume: 20ul

PCR program:           94'C   3 min  
(cold start)       94'C   30 sec           X 25-30  
                  68'C   x min

x would vary from 6-10 min.

6) Purify PCR-products using GFX PCR purification from Amersham (To remove dNTPs, primers and enzyme) Final volume 50 ul in dH<sub>2</sub>O (or less volume, more concentrated).

7) Pfu-polish products (to get rid of A overhang put on by Ex-Taq) using Pfu polymerase.

PRC reaction:       25 ul template (TTPCR product from 6) above)  
                  1x Pfu buffer  
                  0.2 mM dNTP  
                  0.025 U/ul Pfu polymerase  
                  (no primers!)

Reaction volume: 50 ul

PCR program:       72'C, 30 min, no cycling

8) Run product on 0.7% agarose gel, stain with methylene blue and cut out the fragment, gel purify using GFX gel band purification kit from Amersham (same kit as in 2) above, but different protocol) Final volume 50 ul in dH<sub>2</sub>O (or less volume, more concentrated).