Protocol for Making 3500 bp dsDNA Tethers – R Brau / Mo Khalil

This protocol presents a procedure for making DNA tethers via PCR. In particular, it will describe how to make a 3500bp dsDNA tether containing a biotin molecule on one of its 5' ends and a digoxegenin molecule on the other. These motifs can be changed readily to yield many experimental configurations.

Reagents

TE buffer
- 10 mM Tris (pH 7.5) (Sigma T1503)
- 1 mM EDTA (Acros 118432500)
- filter through 0.2 μm pore diameter filter

Taq DNA Polymerase Kit (Invitrogen, 10342020)
- Taq DNA Polymerase
- 10X PCR buffer
- 50mM $MgCl_2$

dNTPs (2.5mM)
- 6 μL 100mM dATP (Invitrogen, 10216018)
- 6 μL 100mM dCTP (Invitrogen, 10217016)
- 6 μL 100mM dGTP (Invitrogen, 10218014)
- 6 μL 100mM dTTP (Invitrogen, 10219012)
- 216 μL ddH_2O

M13mp18 plasmid (Bayou Biolabs, P-105)
- 5 μg/mL in TE buffer

Oligo Primers (IDT)
- 20 μM in TE buffer

  Forward: 5'-BIO-AAT CCG CTT TGC TTC TGA CT-3'
  Reverse: 5'-DIG-TTG AAA TAC CGA CCG TGT GA-3'

QiaQuick Purification Kit (Qiagen, 27104)

Procedure

1. Dilute all reagents to the appropriate concentrations as specified above.

2. Combine 10 μL of the DNA plasmid and 2 μL for each of the appropriate forward and reverse primers in a PCR tube.

3. Prepare the following reagent cocktail for as many PCR tubes as needed:

Reagents	Volume (µL)
ddH_2O	61
10X buffer	10
dNTP's	10
$MgCl_2$	3
Taq Polymerase	2
Total	86

Note that the polymerase is usually the last reagent added to the cocktail and that, since it is dissolved in glycerol, pipetting up and down several times is required to obtain uniform mixing.

4. Add the appropriate cocktail amount (86 µL) to each PCR tube. The volume in each tube should equal 100 µL. Mix well and place in PCR machine.

5. Run PCR program for amplification as follows:

Stage	Temp (°C)	Time (min)	Cycles
Supermelt	94	3	1
Melt	94	1	30
Anneal	48	1	
Elongate	72	7	
Final Extension	72	14	1

6. Clean up the PCR products with the QiaQuick purification kit. Note that 2 tubes can be pooled together if they contain the same PCR products.

7. Check the quality of the PCR products by running them in an electrophoresis gel.