DNA_13v2

PCR AMPLIFICATION, OF FISSION YEAST TELOMERES

25-10-2016, Thomas van Emden; modified from Azzalin Lab protocol

Use:
Amplification of telomeres to check their length.

Buffers:
10x OPA buffer:

- 100 mM Tris base, pH 7.5, 100 mM magnesium acetate, and 500 mM potassium acetate

Weigh 5.36 g. of magnesium acetate tetrahydrate, 3.03 g. of Tris base and 12.27 g. of potassium acetate. Add to approximately 200 mL of water. Adjust to pH 7.5 with glacial acetic acid. Top up to 250 mL.

10x buffer $^{w}/_{o}$ Mg^{2+}:

- 750 mM Tris-HCl pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1 % Tween-20

Starting Material:
DNA isolated using the yeast DNA extraction kit

Precautions:
When resuspending gDNA avoid EDTA which inhibits TdT reaction

Procedure
Denaturation of gDNA

1. In a 0.5 µL tube or PCR strip, add 8 µL of OPA 1X buffer and 1 µL of gDNA (around 100 to 200 ng).
2. Boil 5 min at 96°C (10 min at 96°C if done from a yeast colony), and cool rapidly at 4°C.

Tailing 3'-OH ends

3. To the 9 µL reaction add 1 µL of tailing mix (1U TdT (NEB #M0315S), 1X OPA, 1mM dCTP). Tail 30 min at 37°C and heat-inactivate the enzyme 10 min at 65°C and denature 5 min at 94°C. Proceed immediately with the PCR reaction.

PCR Amplification of 3'-OH tailed telomeres

4. To the 10 µL tailing reaction add 30 µL of PCR mix:

0.3 µL	Sp 494-FOR (Sg2708) at 100 µM
0.3 µL	dG18-BamHIof (Sg2709) at 100 µM
0.8 µL	dNTP at 10 mM
4.0 µL	10x buffer $^{w}/_{o}$ Mg^{2+}
0.5 µL	Phusion polymerase at 5U/µL
H_2O up to 30 µL	

Amplification of 3' ends was performed as follow:

94°C for 3 min	
94°C for 20 sec	
65°C for 20 sec	
72°C for 30 sec	30 cycles
72°C for 5 min	
4°C	

5. Run PCR products on a …% gel to check telomere length.

Solutions and reagents

- 10x OPA (One-Phor-All) buffer: previously available from Amersham Pharmacia Biotech

- Oligonucleotides:

Oligo N° Azzalin Lab	Name	Sequence
N°229	M13REVlong	aacagctatgaccatgattacgca
N°230	M13FORlong	gttttcccagtcacgacgttgt
N°368	BAM136	GTGTGGAATTGAGTATGGTGAA
N°389	Sp 494-FOR	TGAGTGTGCTGGAGTACGTT
N°525	dG18-BamHI	CGGGATCCGGGGGGGGGGGGGGGG
N°989	NMT_prm_1	cattctcactttctgacttatagtcgc