3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

YαN-21 Sequencing Primer

Catalog Number **P8959**
Store Temperature –20 °C

Product Description
Nucleotide sequence:
5′-OH-AGC-ACA-AAT-AAC-GGG-TTA-TTG-3-OH′

The YαN-21 sequencing primer is a chemically synthesized, 21 base, single strand oligodeoxyribonucleotide designed for double strand DNA sequencing of FLAG® fusion junctions corresponding to the N-terminus of FLAG fusion proteins expressed by the YEpFLAG-1™ yeast expression vector.

The primer is complementary to map position 1388-1408 base pairs of negative, non-coding DNA strand within the α factor coding sequence of the YEpFLAG-1 expression vector.

The YαN-21 sequencing primer is supplied at an initial concentration of 5 μM in 0.1× TE buffer (1 mM Tris, pH 8.0, 0.1 mM EDTA).

Reagents Required but Not Provided

- 1× TE buffer (10 mM Tris, pH 8.0, 1.0 mM EDTA)
- 5 M NaOH
- 3 M potassium/5 M acetate: To 60 ml of 5 M potassium acetate add 11.5 ml of glacial acetic acid and 28.5 ml of water. The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate.
- Isopropanol

Procedure

A. Preparation of Denatured YEpFLAG-1 DNA Template
The following protocol is designed to allow sufficient template for two sets of 4 sequencing reactions (2G, 2A, 2T, and 2C reactions).

1. Denature 7 micrograms of highly purified YEpFLAG-1 supercoiled DNA in 70 μl of 1× TE by adding 3 μl (1/25 volume) of 5 M NaOH and incubating at 37 °C for 5 minutes.

2. Precipitate the denatured YEpFLAG-1 supercoiled DNA for 30 minutes at room temperature by adding 150 μl (2 volumes) of 3 M potassium/5 M acetate: isopropanol (1:3 ratio mixture).

3. Collect the precipitated and denatured YEpFLAG-1 DNA by centrifugation at 10,000 × *g* for 5 minutes.

4. Wash the YEpFLAG-1 DNA pellet with 1 ml of ethanol and dry.

5. Resuspend the YEpFLAG-1 DNA in 20 μl of 1× TE. The final concentration should be 0.35 μg/μl.

The irreversibly denatured YEpFLAG-1 DNA template can be stored at –20 °C at this point if desired.

B. Priming YEpFLAG-1 DNA Template with YαN-21 Sequencing Primer
The following protocol uses 10 μl or half of the preceding preparation of irreversibly denatured YEpFLAG-1 DNA template. This is sufficient for one set of 4 sequencing reactions (G, A, T, and C).

1. Dilute a 3 μl aliquot of the YαN-21 sequencing primer with 6 μl of 1× TE to make a final concentration of 1.67 pmol/μl.

2. Add 2-3 μl (3.5-5 pmoles) of YαN-21 sequencing primer to 10 μl (1 pmole) of denatured YEpFLAG-1 DNA template.

3. To 12-13 μL of YαN-21 primer/YEpFLAG-1 DNA template add an appropriate volume of sequencing buffer to make the buffer concentration 1×. Heat at 70 °C in a wet temperature block for 2 minutes.

4. Slowly cool the mixture to 45 °C by placing the temperature block at room temperature for about 20 minutes.

5. Distribute the YαN-21 primer/YEpFLAG-1 DNA template to 4 tubes to be used in the G, A, T, and C DNA sequencing reactions.

   The YαN-21 primer/YEpFLAG-1 DNA template is now ready for supercoil sequencing of the DNA sequence corresponding to the N-terminal FLAG fusion junction.

   The DNA sequence corresponding to the fusion junction will be ≥85 bases away from the 3′ end of the YαN-21 sequencing primer reading from the bottom of an autoradiogram.

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