A Typical DNA Tailing Reaction

Overview

Protocol

1. Mix:
   a. 5.0 µl (10X) TdT Buffer
   b. 5.0 µl (2.5 mM) $CoCl_2$ solution provided
   c. 5.0 pmols DNA (330 ng for 100 bp, 1 µg for 300 bp, 10 pmols DNA ends)*
   d. 0.5 µl 10 mM dNTP (alpha-32_P dATP may also be used)
   e. 0.5 µl Terminal Transferase (20 units/µl)
   f. deionized water to a final volume of 50 µl.

   *To determine approximate amount of DNA (ng/pmol), multiply the number of base pairs by 0.66. Example: 300 bp x 0.66 = 198 ng/pmol. For 5.0 pmols multiply by 5, resulting in 990 ng/5 pmol.

   The table below can be used as a guide (values are approximate and are given for a 30 minutes incubation at 37°C in the recommended buffer).

   The rate of addition of dNTP's and thus the length of the tail is a function of the ratio of 3´ DNA ends: dNTP concentration, and also which dNTP is used.

   DNA Tailing Guide:

pmols 3' ends pmols dNTP	**Tail Length**			
	dA	dC	dG	dT
1:100	1-5	1-3	1-3	1-5
1:1,000	10-20	10-20	5-10	10-20
1:5,000	100-300	50-200	10-25	200-300

2. Incubate at 37°C for 30 minutes.
3. Stop the reaction by heating to 70°C for 10 minutes or by adding 10 µl of 0.2 M EDTA (pH 8.0).