Stopping the DNA polymerase activity at a specific site with a dideoxynucleotide: selective labelling of single stranded circular DNA

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Recently a method for the generation of high density labelled M13 single stranded DNA probes has been described by Macedo et al. (1). It is based on the extension of a primer downstream of the insert sequence (2) under controlled conditions so that the second strand does not reach the insert sequence. The insert sequence will remain single stranded and can be used like any single stranded probe. The reaction is controlled by using limiting concentration of a nucleotide, suboptimal temperature and an accurate amount of Klenow polymerase. The drawback of this protocol is the necessity to exactly control the polymerase reaction to avoid both too far elongation of the labelled strand (reading through the insert) and too short elongation (decreasing label density).

To circumvent this, we have developed a method to stop the DNA polymerase activity at any desired specific site. In our system, which can be used for any single stranded circular DNA, the labelling reaction is stopped by an oligodeoxynucleotide termed as "stopper". This is an oligonucleotide modified at the 3' end by addition of a 2',3'-dideoxynucleotide with terminal deoxynucleotidyl transferase. The hereby generated 3' deoxy-terminus stops the strand elongation reaction.

When using the M13 hybridization primer as a primer and an M13 universal sequencing primer modified with the appropriate dideoxynucleotide as a stopper, all ssDNA based on M13 and pUC derivatives can be labelled independently of the insert.

Method for dideoxy-modification of an oligonucleotide:

50pmol (= 1uM) M13 -40 sequencing primer (Pharmacia) are reacted in 50ul of 140mM K-cacodylate, 1mM CoCl₂, 1.2mM mercaptoethanol, 100uM ddGTP with 50U terminal deoxynucleotidyl transferase (Boehringer Mannheim) at 30°C for 1 hour. After phenol/chloroform extraction the modified oligo is desalted over a NAP-column (Pharmacia) and dried in a vacuum centrifuge.

Labelling reaction:

200ng ss M13 DNA are annealed to 5ng "stopper" and 5ng hybridization primer (tenfold excess each) and subsequently reacted with 2U Klenow polymerase in the presence of 33uM biotinyl dUTP and each 33uM dATP, dCTP and dGTP at room temperature for 1 hour. As the reaction is carried out to completion, the reaction conditions are not as crucial for optimal results as they are in the previously described protocols.

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References:

1. Macedo, A.M., Medeiros, A.C. and Pena, S.D.J. (1989) *Nucleic Acids Res.* **17**, 4414
2. Hu, N. and Messing, J. (1982) *Gene* **17**, 271-276