Protocols

High-Resolution Mapping on Pachytene Chromosomes and Extended DNA Fibres by Fluorescence *in-situ* Hybridisation

Xiao-bo Zhong, Paul F. Fransz, Jannie Wennekes-van Eden, Pim Zabel, Ab van Kammen, and J. Hans de Jong

E-mail: Hans.dejong@celgen.el.wau.nl
(X-bZ, PZ, AvK) Department of Molecular Biology; (PFF, JW-vE, JHdJ) Department of Genetics, Dreijenlaan 2, 6703 HA Wageningen, Wageningen Agricultural University, The Netherlands

Key Words: extended DNA fibres, FISH, fluorescence *in-situ* hybridisation, *Lycopersicon esculentum*, pachytene chromosomes, tomato

Abstract: This article describes two protocols for high-resolution physical mapping of DNA sequences in tomato using fluorescence *in situ* hybridisation (FISH). The first technique involves FISH to spread chromosomes from pollen mother cells at pachytene and proves to be an excellent method for assigning DNA sequences to chromosome regions at a resolution of up to a few hundred kilobase. An even higher resolution was obtained for extended DNA fibre, prepared from interphase nuclei and used as hybridising component. This technique permits strong enhancement of physical map resolution to values of a few kilobase. The power of both methods simultaneously applied for the same material was demonstrated with the combination of the telomeric repeat and the tomato specific telomere-associated repeat TGR1 as example.

Multicolour fluorescence *in-situ* hybridisation (FISH) has been developed as one of the major cytogenetic tools for constructing accurate physical maps of single-copy markers and repeti-

1Author for correspondence.

Abbreviations: DAPI, diamidino-phenyl-indole; EDTA, ethylene diamine tetraacetate; FISH, fluorescence *in-situ* hybridisation; FITC, fluorescein isothiocyanate; SDS, sodium dodecyl sulphate; SSC, standard saline citrate; TGR, tomato genomic repeat.
tive sequences along chromosomes (reviewed by Jiang and Gill, 1994; Joos et al., 1994). Its resolution for human mitotic metaphase chromosomes, usually in the range of 1 to 3 Mb (Lawrence et al., 1990; Lichter et al., 1990), is limited by the high degree of chromatin condensation. Similar results with FISH mapping with metaphase chromosomes of plants, especially in the case of species with relatively small genomes, such as Arabidopsis, rice, tomato and sorghum, stimulated the development of protocols for enhanced resolution FISH with interphase and prophase chromosomes or decondensed chromatin.

Nuclei at different mitotic and meiotic stages, including meiotic prophase I, have been compared for their suitability for high-resolution mapping. Especially good results have been obtained with chromosomes of pollen mother cells at late pachytene, containing well-spread bivalents generally ten to fifteen times longer than their metaphase counterparts, exhibiting clearly differentiated patterns of light euchromatin and dark heterochromatin blocks, and thin, weakly stained centromeres. As one of the best cytogenetic model plant species, tomato shows a distinct pachytene karyotype with chromomere patterns unique for each chromosome. This provides cytological maps with a differentiation level close to that of mammalian G-banding, allowing identification of all twelve chromosome pairs in the complement (Ramanna and Prakken, 1967) and providing an outstanding diagnostic tool for high-resolution FISH mapping in this species (Zhong et al., 1996).

Improvements in resolution have been achieved with FISH applied to mammalian interphase nuclei (Lawrence et al., 1990; Trask et al., 1991) and free chromatin or extended DNA fibres (Heng et al., 1992; Haaf and Ward, 1994; Wiegant et al., 1992; Parra and Windle 1993; Heiskanen et al., 1994; Florijn et al., 1995). Hybridisation of probes to extended DNA fibres allows the delineation and ordering of contiguous sequences at a resolution of a few kilobase or less (Parra and Windle, 1993; Florijn et al., 1995). Recently, this powerful technique has been applied in higher plants in order to visualise and map cosmids, λ and plasmid clones on extended DNA fibres from Arabidopsis thaliana and tomato (Fransz et al., 1996).

In this report we present comprehensive FISH protocols for both pachytene chromosomes and extended DNA fibres of Lycopersicon esculentum. Probes of the telomeric repeat [TTTAGGG] from Arabidopsis thaliana and TGR1, the tomato-specific, telomere-associated repeat, serve as an example in two-colour FISH experiments for demonstrating the enhancement of mapping resolution of both techniques.