Scientific article

Development of a 10-plex microsatellite system for dog typing

Ulricke Olschyna, Cornelia Jaeger, Margaretha König, and Holger Engel

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

Abstract: A novel method was developed for dog typing using a 10-plex microsatellite marker system. Ten primer pairs, generating PCR products of unique size for individual detection, were selected from published canine microsatellite markers. The Type-it® Microsatellite PCR Kit was used for 10-plex amplification without the need for optimization of PCR conditions. The kit buffer promotes stable and efficient annealing of the different primers in the multiplex reaction, while preoptimized concentrations of HotStarTaq® *Plus* DNA Polymerase ensure high specificity and sensitivity in the multiplex reaction. Primers were labeled with fluorescent dyes. Following amplification with the Type-it Microsatellite PCR Kit, products were analyzed using capillary electrophoresis. Use of 10 primer pairs and two different fluorescent labels enabled successful typing of three dogs

Introduction

Multiplex microsatellite PCR enables the identification of a subject and the determination of differences between individuals. Short tandem repeats (STRs) and variable number tandem repeats (VNTRs) are analyzed within a genome. The number of times the STRs or VNTRs are repeated at a specific locus varies between individuals. As these tandem repeats follow the rules of inheritance, an individual will have an allele match from each parent. This match will diminish with decreasing relatedness. PCR of the repeats produces sufficient material to determine the size of the tandem repeats. The size distribution pattern over a number of loci not only distinguishes between individuals within a species, but can also indicate how closely related different species are.

Amplification of the tandem repeats from multiple sites requires multiple pairs of primers. Suitable markers need to be identified and validated, which is usually time consuming and error prone. Simultaneous use of the marker-relevant multiple primer sets usually requires time-consuming optimization of parameters, including annealing and enzyme and buffer conditions, to ensure comparable amplification efficiency. Differences in hybridization kinetics of the different primer pairs can affect hybridization efficiency, which may result in poor yields of some of the PCR products. If the associated peaks are missing, false positive or negative typing results may be obtained.

QIAGEN's Type-it Microsatellite PCR Kit overcomes this need for optimization. The Type-it Microsatellite PCR Buffer contains a unique synthetic additive, Factor MP, which promotes stable and efficient annealing of all the primers to the nucleic acid template in a multiplex reaction. The increased hybridization efficiency and primer stability provide high and comparable product yields for all targets.

The Type-it Microsatellite PCR Kit was used with a 10-plex microsatellite marker system based on previously identified microsatellite markers (1) to distinguish between 3 different dogs.

Experimental protocol

Initially, 20 primer pairs were selected from a list of published canine markers (1), ensuring that each PCR product would be of a unique size and could, therefore, be detected by the same fluorophore without overlap. Each fluorescently labeled primer pair was tested individually. Primers enabling unambiguous separation of STRs on the capillary electrophoresis instrument (ABI PRISM® 3730 Genetic Analyzer; Life Technologies) were selected for the main experiment. Using the 10 chosen primers (Table 1) divided into 2 different dye-labeled groups without any allelic overlap, 10-plex amplification was carried out with the Type-it Microsatellite PCR Kit, according to the instructions in the kit handbook. Optimization of PCR conditions was not required. Analysis of PCR products was performed using two dye channels (FAM™ and NED™) on the capillary electrophoresis instrument.

Results

After initial individual testing of the 20 primer pairs, 10 pairs were selected for the 10-plex amplification. Of the 10 microsatellites that were simultaneously amplified, 6 were identified in the FAM channel and 4 in the NED channel, ranging from 106 to 305 bp for the animal shown (Figure 1). The peaks were easily distinguishable by size, which enabled successful typing of the 3 different dogs.

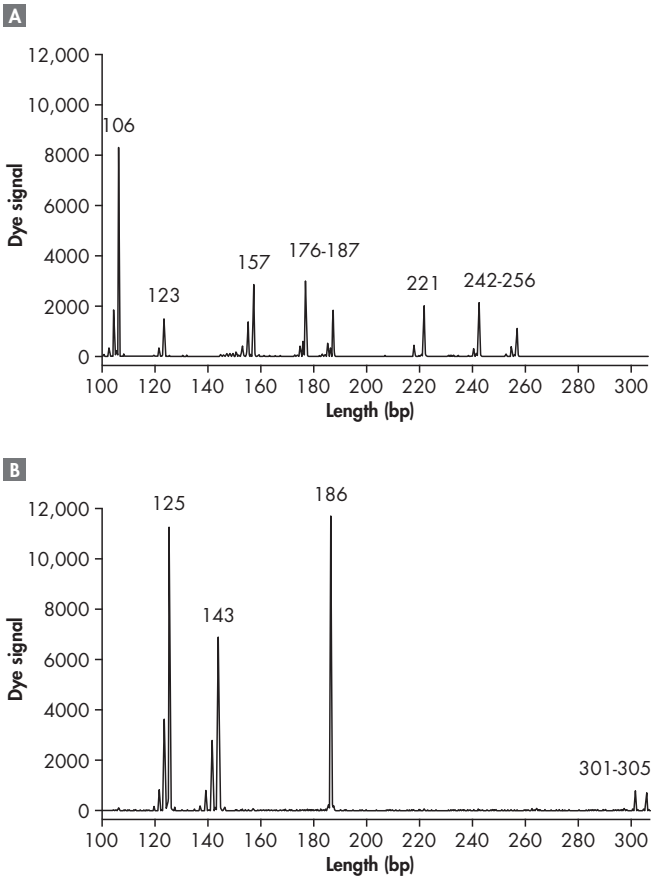

Figure 1. Successful dog typing without the need for optimization.
A 10-plex microsatellite analysis was performed. Two channels were used. A FAM: 6 microsatellites detected; B NED: 4 microsatellites detected.

Conclusions

The 10 chosen canine primer pairs in combination with the Type-it Microsatellite PCR Kit enabled fast and easy typing of 3 different dogs, without the need for optimization. Preoptimized kit conditions ensure high specificity and the specialized buffer system enables amplification of all marker sets in parallel.

The new multiplex microsatellite assay can be easily established based on the published primer sequences. Even primers described for singleplex PCR can be combined in multiplex assays for successful typing. The unique features of the Type-it Microsatellite PCR Kit makes this kit highly suitable for all potential microsatellite, minisatellite, or STR amplification systems, without tedious and time-consuming optimization.

Reference

1. Richman, M., Mellersh, C.S., André, C., Galibert, F., and Ostrander E.A. (2001) Characterization of a minimal screening set of 172 microsatellite markers for genome-wide screens of the canine genome. J. Biochem. Biophys. Methods **47**, 137.

Table 1. Primers used for 10-plex microsatellite analysis of canine DNA selected from reference (1)

Label/channel	Marker	Fragment size (lit)	Fragment size (detected)	Repeat	Sequence
FAM	C08.410	112	109–117	di	5': GAGGAAAACCAAGTGATTTTGG 3': ACCTGCAAGTGACCCTCTCT
	C01.246	124	123–128	di	5': GACTTCATTCATCCATTCATGA 3': GGATCGAGTCCAACGTGG
	CXX.672	156	150–156	di	5': AGGTCAGGGACAATATACCGT 3': CCTGAGCCCAAGGCAGAC
	C01.424	188	176–193	di	5': AGCTTAGCTTACTGCCCTGG 3': TCCTTTGGTTTTTAGCAGGG
	FH2060	223	217–229	tetra	5': GTTTTGAGGAAGCCTTGCTG 3': GAAGGAAGGGGCCAGTATTC
	C26.733	257	256–264	di	5': CCCTCTACTTATGTCTCGGCC 3': GAGAGGAGAAACAACCAACACC
NED	CXX.279	124–126	113–129	di	5': TGCTCAATGAAATAAGCCAGG 3': GGCGACCTTCATTCTCTGAC
	CXX873	140–144	136–144	di	5': CTGGCAGATTACAGGTAGC 3': GTTCTCCAAAGCACTCAT
	FH2516	188	182–188	tetra	5': AATGGATGGAACTTAGGGCA 3': CTGCATCTGGTAACCATCGA
	FH2016	285–309	281–318	tetra	5': CATTTTTAAGGATGGAGACAGC 3': AACAGTGTCCCATGGCCTAC

Ordering Information

Product	Contents	Cat. no.
Type-it Microsatellite PCR Kit (70)	For 70 x 25 µl reactions: Type-it Multiplex PCR Master Mix, Q-Solution®, RNase-Free water	206241
Type-it Microsatellite PCR Kit (200)	For 200 x 25 µl reactions: Type-it Multiplex PCR Master Mix, Q-Solution, RNase-Free water	206243
Type-it Microsatellite PCR Kit (2000)	For 2000 x 25 µl reactions: Type-it Multiplex PCR Master Mix, Q-Solution, RNase-Free water	206246

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

For more information on multiplex PCR-based microsatellite analysis, see www.qiagen.com/microsatellite-dog-typing

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