Cloning and Characterisation of Novel Single Locus Probes for Forensic Purposes

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INTRODUCTION

From the analysis of multi-locus probes (Jeffreys et al, 1986; Fowler et al, 1988) it has previously been demonstrated that there are numerous hypervariable loci in the human genome. Many hypervariable loci have been fortuitously identified (Wyman and White, 1980; Bell et al, 1982; Higgs et al, 1981; Capon et al, 1983). A cloning strategy, used specifically to isolate hypervariable DNA was developed by Wong et al (1986, 1987) who used lambda libraries of human DNA; the insert consisting of size selected high molecular weight (5-15kB) DNA fragments. This procedure enriched the library for tandemly repeated minisatellites. Recently, Armour et al (1990) introduced a more successful and simpler method for cloning hypervariable DNA by constructing Charomid libraries (Saito and Stark, 1986). Charomids are cosmid derived vectors which do not have the same size constraints of lambda vectors, hence the cloning of rather unstable minisatellite fragments, which tend to lose repeats, is much improved.

Libraries of size selected DNA (4-9kB) have been constructed in Charomid 9-36. A new hypervariable locus (B6.7) has been isolated by screening the library with the multilocus probe 3'HVR alpha globin (Fowler et al, 1988) under low stringency conditions.

CHAROMID CLONING AND CHARACTERISATION OF PROBES

A charomid library was prepared from human genomic DNA pooled from 20 unrelated individuals. DNA was fully digested with SauIIIa and the 6-9kB fragments were purified by electrodialysis. DNA from this fraction was ligated into the BamHI site of Charomid 9-36, packaged using Gigapack Gold (Stratagene) and transfected into E. coli NM554 cells (recA mcrA, mcrB). Charomid clones were selected on Luria Bertoni agar (LUA) containing ampicillin (50µg/ml), grown overnight, and replica plated onto Hybond N filters (Amersham). The DNA on the filters was subjected to denaturation and microwave fixation.

Probe 3'HVR alpha globin was labelled with ^{32}P by random oligonucleotide priming (Fienberg et al, 1984). Filters were incubated overnight at 61°C in hybridisation solution (Church and Gilbert, 1984) containing 0.5ng/ml of labelled probe, without competitor DNA. Filters were washed under low stringency conditions in 1xSSC, 0.1% SDS at 61°C, prior to autoradiography using Amersham-MP film. Colonies which showed signals were picked, replated at low density and re-screened with 3'HVR. Positive colonies were picked and overnight minipreps were grown in Luria Bertoni (LB) medium containing ampicillin (50µg/ml). Charomid DNA was extracted from the overnight culture by the alkaline lysis method (Sambrook et al, 1989), its concentration established by fluorometry (Labarca and Paigen, 1980) and then restricted with SauIIIa (10u/µg DNA) for 4-5 hours. Electrophoresis of the restricted DNA was carried out in 0.8% agarose (Seakem GTG) gels. Insert bands were sized against a lambda HindIII digest and a 1kb DNA ladder (Gibco BRL). Bands sized between 2 and 9kb were recovered by electrodialysis, phenol/ chloroform extracted and ethanol precipitated twice.

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Probes were characterised by hybridising the radiolabelled insert DNA against Southern blots of HinfI restricted human genomic DNA prepared from the blood of a panel of random individuals. Blots were washed twice under high stringency conditions in 0.1xSSC, 0.1% SDS at 65°C and autoradiographs prepared as described previously.

The clones carrying positive insert fragments were categorised according to their hybridisation pattern:

	NUMBER OF CLONES
Satellite*	2
Monomorphic	0
Polymorphic	13 (Some are characterised in Table 1)

* These clones generated a largely monomorphic ladder of hybridising DNA fragments presumed to be derived from large segments of satellite DNA.

TABLE 1. Characterisation of polymorphic clones detected by 3'HVR

Clone No.	No. of Repeat Isolates	No. of Alleles Observed	Allele Size Range (kb)	No. of People Studied	Heterozygosity
1	2	17	1.5 - 8	11	73%
4	0	16	2 - 10	11	73%
8	2	2	4.8 - 5	23	23%
10	0	7	4 - 9	17	53%
17	1	32	1 - 12	28	88%
7[a]	1	24	2 - 20	7	
19[b]	0	16	3 - 15	5	

[a] Complex pattern observed with between 3-6 bands per individual.
[b] Complex pattern observed with between 2 bands per individual.

Clone 17 (B6.7) was employed to rescreen charomid libraries resulting in the detection of approximately 600 positively hybridising clones (2% of all library clones - 4 fold more than detected by 3'HVR). Furthermore, 95% of all clones detected by 3'HVR were also hybridisation positive with clone 17. Polymorphic clones were characterised further (Table 2).

TABLE 2. Characterisation of polymorphic clones detected by clone 17

Clone No.	No. of Repeat Isolates	No. of Alleles Observed	Allele Size Range (kb)	No. of People Studied	Heterozygosity
17/9	2	18	2 - 10	21	91%
17/26	1	13	1 - 4.5	12	77%
17/45	0	9	3.5 - 6.5	10	67%
17/23[a]	0	2	4.8 - 5	23	23%

[a] Repeat isolate of 3'HVR Clone 8.

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CHARACTERISATION OF PROBE B6.7

A population study was carried out using DNA from 24 unrelated white Caucasians. These results revealed at least 29 different alleles and a heterozygosity of 88%. The allele sizes in this population ranged from 1-15kb.

B6.7 shows approximately 70% homology with a sequence found in the rabbit C repeat (Cheng et al, 1984). The C family serves as a template for RNA transcription by RNA polymerase III (Fritsch et al, 1980), and shares structural properties with human Alu repeats, although their sequences are quite different. B6.7 shows no structural homology with either rabbit C or Alu repeats. There is close homology with the 3'HVR alpha-globin sequence and with the core sequence proposed by Jarman et al (1986).

COMPARISON OF B6.7 SEQUENCE WITH 3'HVR ALPHA-GLOBIN

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TCTCTATAGGACATGAGGGTGGACAGTGAGGGGGG B6.7
        ||||  | |||   ||||
        CGACACGGGGGGAAACAG          3'HVR Alpha-globin
              | ||||  ||||
              GNGGGGN-ACAG          Core Sequence (Jarman et al, 1986)
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The new probes described are the subject of patent applications. Probe B6.7 has been deposited with the Centre for Applied Microbiology and Research, Public Health Laboratory Service, Porton Down, Salisbury, Wiltshire, Deposit reference C91031501. Requests for probes should be sent to P Gill (CRSE).

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