# USE OF THE POLYMERASE CHAIN REACTION FOR TYPING Gc VARIANTS

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We have developed a polymerase chain reaction (PCR) system for typing group specific component (Gc). Our Gc DNA typing system has several advantages:

1. The 2, 1F and 1S Gc alleles can be typed unambiguously.
2. An individual's Gc type can be obtained from any biological sample from which DNA can be isolated and amplified (e.g. blood, hair, semen, saliva, bone).
3. The Gc typing can be combined with other genetic marker systems typed by DNA analysis (e.g. HLA DQα) to give a very high power of discrimination.

We made use of two published Gc cDNA sequences to determine which regions of the Gc gene to amplify and study. One Gc cDNA sequence is from a type 2 individual (Yang 1985) and the other cDNA sequence is from a type 1 individual (Cooke 1985). The subtype of the type 1 individual is not known. When these two cDNA sequences are compared, there are 4 regions containing base changes that correspond to amino acid changes. These sequence differences are summarized below.

| CODON | 152 | 310311 | 416 | 420 |
|---|---|---|---|---|
| | *gly* | *valglu* | *asp* | *lys* |
| TYPE 2 | ---GGA---- | GTAGAG---- | GAT---- | AAG |
| TYPE 1 | ---GAA---- | GTGAGA---- | GAG---- | ACG |
| | *glu* | *valarg* | *glu* | *thr* |

At position 152, there is a single base difference. We have not yet studied this region. Within the adjacent codons 310 and 311 there is a 4 base difference that results in a single amino acid change. We made PCR primers to amplify this region and have obtained sequence information from 6 individuals representing the 6 Gc genotypes (2, 1F, 1S,

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2-1F, 2-1S, 1F-1S). We found the sequences to be identical in every case; the sequences matched the published type 2 sequence. The apparent difference between the type 1 and type 2 sequences in this region is due most likely to an error in the published type 1 sequence. Another possibility is the type 1 sequence may have been derived from an individual carrying a rare Gc variant.

The polymorphisms at codons 416 and 420 involve only single base differences between the two types, but they have proved to be the most interesting. The continuous sequence through this region is shown below.

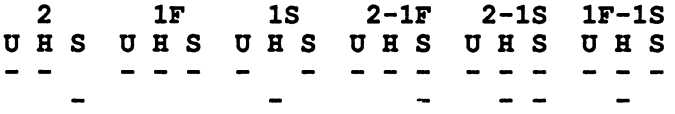
```
                    HaeIII
TYPE 1    ---CCTGAGGCCACACCCACGGAAC---

CODON              416          420

TYPE 2    ---CCTGATGCCACACCCAAGGAAC---
                              StyI
```

The generic type 1 sequence contains GGCC, which is the recognition sequence for the restriction enzyme HaeIII. However, this site is not present in the type 2 DNA because of the base change at codon 416. Similarly, the type 2 sequence contains CCAAGG, which is the recognition sequence for the restriction enzyme StyI. The base change at codon 420 results in the loss of this site in the type 1 sequence.

Because these sites are close together, we were able to make a PCR product containing both of these sites. If the PCR product contains the HaeIII site or the StyI site, then after digestion with the appropriate restriction enzyme there will be two fragments. The difference in size between the undigested PCR product and the digested fragments is easily distinguished on an agarose gel. We amplified DNA from 6 individuals representing the 6 Gc genotypes, incubated the PCR product with either HaeIII or StyI in separate reactions and analyzed the products on agarose gels. Our results of digestion of the 3 Gc homozygotes are summarized in the table below.

| | HaeIII | StyI |
|---|---|---|
| 2 | - | + |
| 1F | - | - |
| 1S | + | - |

If these restriction site combinations are characteristic of each of the three Gc alleles, then the digestion patterns of the 3 Gc heterozygotes can be predicted. The restriction patterns we observed for each of the six genotypes are shown below in a schematic diagram.




**U: undigested PCR product**
**H: incubation with HaeIII**
**S: incubation with StyI**

The patterns for the heterozygotes are those predicted by the restriction enzyme digestion data from the homozygotes. For example, for type 2-1F, neither the 2 allele nor the 1F allele contains a HaeIII site, and there is no digestion by HaeIII. In contrast, the 2 allele contains a StyI site but the 1F allele does not, giving rise to one undigested product and one StyI digested product in that lane of the gel. Each Gc genotype has a distinct restriction pattern, allowing us to type Gc unambiguously. We have analyzed 41 individuals by this method with no deviation from these restriction patterns.

The DNA polymorphisms of the 6 Gc genotypes were confirmed by direct DNA sequence analysis of the PCR products. The sequence differences between the 3 homozygotes are summarized below.

```
2       AATTGCCTGATGCCACACCCAAGGAACTGGCA
1F      ----------------------C----------
1S      ----------G----------C----------
```

These differences in sequence allowed us to identify the amino acid differences between the 3 Gc alleles in this region.

| | aa 416 | aa 420 |
|---|---|---|
| 2 | *asp* | *lys* |
| 1F | *asp* | *thr* |
| 1S | *glu* | *thr* |

We will continue to use this method to look for additional Gc polymorphisms throughout the Gc gene and to look at the sequences of rare variants.

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