Electronic letter

Hair roots as the ideal source of mRNA for genetic testing

Kathy King, Frances A Flinter, Peter M Green

EDITOR—Standard genetic tests usually involve obtaining a blood sample for DNA extraction. Other commonly used sources of DNA are buccal cells¹ (from a cheek scraping) and hair roots,² which are viable alternatives to a blood sample and less invasive to obtain, particularly useful in children. Some genetic tests require full mutation screening (at least of the affected subject in a family) and this can be greatly simplified, particularly for large, multiexon genes, by screening the mRNA as opposed to the DNA.³ The reason for this is simple; the mRNA contains all the relevant sequence in a compact “ready to screen” package rather than the many discrete packages (exons) found in DNA. In addition, mRNA is one step further down the gene-protein pathway and can yield more relevant information on the effect of a mutation (at least with regard to splicing). The problem with using RNA as the primary source for mutation analysis has been that it is rather less stable than DNA and is easily degraded both in vivo and in vitro by endogenous and exogenous ribonucleases. In order to extract it from blood, it is necessary to separate the lymphocytes from the red blood cells before following standard extraction procedures. This, coupled with the fact that the blood must be fresh (ideally no more than 24 hours old), makes the use of RNA extracted from blood a labour intensive and awkward route to mutation detection.

A compounding difficulty in RNA screening is that the gene of interest may only be expressed at very low levels in the tissue analysed, for instance, lymphocytes. This can be circumvented, at least in some cases, by the use of two rounds of PCR (“nested PCR”) to amplify trace quantities of the appropriate message (known as “ectopic transcripts”).⁴ However, we have found this to be unreliable for the *COL4A5* gene, in which mutations are responsible for Alport syndrome.⁵ Consequently, we sought an alternative but easily accessible source of RNA. Hair roots, although widely used for DNA, seem to have been overlooked as a source of RNA. We used off the shelf proprietary RNA extraction kits (“RNA Isolator” from Genosys) to obtain RNA from between 10 and 20 hair roots. The RNA was resuspended in 30 µl of RNase free water and 1 µl used for RT-PCR (using the Access-RT-PCR kit from Promega). As can be seen in fig

Figure 1 Amplification of the *COL4A5* gene from hair root RNA. Lanes 1–4 show the amplification products on an agarose gel from four overlapping sections covering the entire coding sequence of the *COL4A5* gene from nested PCR reactions. The lane marked ‘m’ is molecular weight markers. The sizes of the products are 1347, 1448, 1522, and 1596 bp, respectively.

1, the amplification of four overlapping segments that cover the *COL4A5* gene is clear evidence that the RNA is of good quality (and quantity) to make mutation screening for this large and complex gene much easier. Mutations have already been detected in these RT-PCR products⁶ after screening with fluorescent chemical cleavage of mismatch.⁷ We have also had success amplifying the ectopic transcripts of the factor VIII gene. Thus, at least for these two genes, hair roots serve as a good material for RNA analysis. We do not know whether the reason for improved RT-PCR is that hair roots have wider gene expression than lymphocytes or that they have higher levels of ectopic transcription.

Hair roots are easy (and cheap) to transport nationally or internationally and are not considered to be at high risk of viruses in the same way that blood is. We have successfully extracted RNA from hair roots that had been at room temperature for up to 10 days after plucking, suggesting that the RNA is better protected in the hair root cells than in blood (which does not yield RNA after this length of time). Our experience suggests that it is immaterial whether the hair is grey or artificially dyed, although yield is lower from fine hair than from thick hair. Eyebrow hair and chest

Division of Medical
and Molecular
Genetics, GKT School
of Medicine, Guy's
Hospital, London
SE1 9RT, UK
K King
F A Flinter
P M Green

Correspondence to:
Dr Green,
peter.green@kcl.ac.uk

hair have also yielded good RNA (so baldness is no obstacle!). Plucking individual hairs is virtually painless and so is less distressing for young children. We believe that the ease of obtaining hair roots, the increased stability of the RNA, and the ease of RNA preparation may make this the ideal source for many mutation detection projects.

This work was supported by the National Kidney Research Fund.

- 1 Lench N, Stanier P, Williamson R. Simple non-invasive method to obtain DNA for gene analysis. *Lancet* 1988;i:1356-8.

- 2 Higuchi R, von Beroldingen CH, Sensabaugh GF, Erlich HA. DNA typing from single hairs. *Nature* 1998;332:543-6.
- 3 Roberts RG, Bentley DR, Barby TF, Manners E, Bobrow M. Direct diagnosis of carriers of Duchenne and Becker muscular dystrophy by amplification of lymphocyte RNA. *Lancet* 1990;336:1523-6.
- 4 Naylor JA, Green PM, Montandon AJ, Rizza CR, Giannelli F. Detection of three novel mutations in two haemophilia A patients by rapid screening of whole essential region of factor VIII gene. *Lancet* 1991;337:635-9.
- 5 Barker DF, Hostikka SL, Zhou J, Chow LT, Oliphant AR, Gerken SC, Gregory MC, Skolnick MH, Atkin CL, Trygvason K. Identification of mutations in the COL4A5 collagen gene in Alport syndrome. *Science* 1990;248:1224-7.
- 6 King K, Flinter FA, Green PM. Towards a comprehensive mutation screening strategy for Alport syndrome. *J Med Genet* 2000;37(suppl 1):S77A.
- 7 Rowley G, Saad S, Giannelli F, Green PM. Ultrarapid mutation detection by multiplex solid-phase chemical cleavage. *Genomics* 1995;30:574-82.