LETTER TO THE EDITOR

Still no evidence for heterogeneity in Best's vitelliform macular dystrophy

In the November 1995 issue of the Journal, Mansergh *et al*[1] suggest that there is genetic heterogeneity in the autosomal dominant eye disorder Best's vitelliform macular dystrophy (BMD) previously mapped to 11q13 (MIM 153700).[2-5] They analysed markers from chromosome 11 in two families, BTMD1 of Irish origin and Fam E of German origin. The conclusion was that the gene previously mapped to 11q13 does not cause Best's disease in the German Fam E family. However, all the markers included in the study, except for PYGM, lie on the centromeric side of the BMD gene.[4 5] In table 1 of the paper, the two point lod scores for these markers are shown and Fam E was not analysed for PYGM. In the multipoint analyses, illustrated in fig 3, the data have been calculated assuming four different penetrances but they have failed to include a single marker on the telomeric side of the gene. Not surprisingly then, Fam E show lod scores below -2, the criterion used for exclusion of linkage. The authors thus arrive at the incorrect conclusion of excluding linkage to the BMD region, without including the BMD region in their analyses. In our opinion there is still no evidence of genetic heterogeneity in Best's macular dystrophy and we are looking forward to seeing if the German Fam E shows linkage to the BMD region when more closely located flanking markers are analysed.

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1 Mansergh FC, Kenna PF, Rudolph G, *et al.* Evidence for genetic heterogeneity in Best's vitelliform macular dystrophy. *J Med Genet* 1995;**32**:855–8.
2 Forsman K, Graff C, Nordström S, *et al.* The gene for Best's macular dystrophy is located at 11q13 in a Swedish family. *Clin Genet* 1992; **42**:156–9.
3 Stone EM, Nichols BE, Streb LM, Kimura AE, Sheffield VC. Genetic linkage of vitelliform macular degeneration (Best's disease) to chromosome 11q13. *Nature Genet* 1992;**1**: 246–50.
4 Graff C, Forsman K, Larsson C, *et al.* Fine mapping of Best's macular dystrophy localises the gene in close proximity to but distinct from the D11S480/ROM1 loci. *Genomics* 1994;**24**: 425–34.
5 Weber BH, Walker D, Muller B, Mar L. Best's vitelliform dystrophy (VMD2) maps between D11S903 and PYGM: no evidence for locus heterogeneity. *Genomics* 1995;**20**:267–74.
6 Stohr H, Weber BH. A recombination event excludes the ROM1 locus from the Best's vitelliform macular dystrophy region. *Hum Genet* 1995;**95**:219–22.

This letter was shown to Dr Mansergh *et al*, who reply as follows.

We would like to make a number of comments in reply to the letter submitted by Drs Graff and Wadelius. We would like to highlight that the region of linkage to the BMD gene excluded in our study was based on the map generated by Weber *et al.*[5] In addition we used markers from the Genethon map which mapped to the same region of chromosome 11 as BMD. We note that the map used in the study of Graff *et al*[4] is significantly different from that of Weber *et al*[5] and uses a number of markers which have not been placed on the Genethon map. It seems that genetic distances estimated between markers in this region of chromosome 11 may be greater in the study by Graff *et al*[4] than that estimated from previous studies. For this reason we are currently analysing additional markers from the new Genethon map and also flanking markers in the region of linkage according to the mapping data of Graff *et al.*[4] In this way we will confirm whether or not there is locus heterogeneity in our pedigree.

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BOOK REVIEWS

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YAC Protocols. Methods in Molecular Biology Volume 54. Editor Davis Markie. (Pp 378; $69.50.) Totowa, New Jersey: Humana Press. 1996.

This Methods in Molecular Biology volume is an attempt at bringing together "a coherent collection of protocols" for the construction, manipulation, and use of YACs. For the most part this is a successful attempt with protocols for: creating YAC libraries; analysing YACs; using YACs in mapping, construction of other libraries (cosmids, etc), and cDNA selection; engineering YACs with specific modifications, fragmentation, and recombination to generate longer contiguous pieces; manipulating YACs such as moving them between strains; and finally reintroduction of the YAC inserts back into mammalian cells. However, this volume suffers from a lack of consistency. Some useful methods are missing and much improved versions of some of the methods exist. Although some of this is the result of the inevitable advances made in the time it takes to go to press, some is because of lack of a coherent plan for the volume.

In a collection of protocols where there is a large degree of overlap in media, solutions, and intervening steps of the methods, it would have been helpful if all the common components were gathered in one place or at least reference given to the first usage. There are sporadic attempts at this in this collection, as in chapter 29 which many of the previous chapters refer to, but this is not consistent throughout. There are numerous versions of how to spheroplast yeast cells and each one is slightly different. At least four different enzymes are used for digesting away the yeast cell wall throughout the collection where any one will do for most applications. In other places there are steps such as "1 μg Highly purified YAC DNA" with no method or reference on how to get the DNA.

As a yeast geneticist who has been asked for help and advice from people dealing with YACs, I see several places for improvement. One is simply the language. A YAC containing yeast strain should not simply be called a YAC. The first time I was asked how to make high quality YAC DNA I assumed that the person wanted the YAC molecule isolated away from the yeast genomic DNA in quantities high enough for their particular use. This is much more difficult than just making good quality genomic DNA of the strain containing the YAC, which is what was required in this instance. Another place for improvement is scale. There is little need for 1–4 mg of DNA obtained from 500 ml cultures. The amounts needed for Southern analysis, probe construction, and even "mini" library construction are orders of magnitude less. It's much easier to work with 1–5 ml cultures. This reduction in scale also holds for preparing DNA in agarose plugs for pulsed field gel analysis. A third place for improvement is in the protocols themselves, at least the yeast specific ones. The lithium acetate transformation procedure given for YAC modification is fine but very inefficient. Modifications exist that yield 10^5 to 10^6 transformants per μg of circular test plasmid (rather than the $10–10^3$) and which are in fact easier than the protocol presented.

The editor has gathered protocols from experts in the field who have tried and tested their methods and generally give numerous hints at troubleshooting in the notes section at the end of each protocol. Many of the protocols can be used effectively as presented. However, in some cases these protocols would be difficult to master by the uninitiated. In particular, some of the yeast genetic techniques are not easy to get up and running in a non-yeast laboratory. I imagine a similar statement can be made about the transfection of mammalian cells chapters. Very few non-yeast laboratories will go to the trouble and expense of getting a micromanipulator for tetrad dissection. A more economical and easier method for meiotic manipulation of YACs is random spores in which spores are separated and plated either selectively or screened for appropriate markers afterwards. This is particularly economical with time as AB1380 is a notoriously poor sporulator in many crosses making tetrad dissection difficult even for a yeast geneticist. A protocol for random spores would be useful. Similarly, the twin spot analysis of mitotic recombination events requires several difficult intermediary steps (protoplast fusion, sublethal UV induction of recombination, etc). This method allows for the separation of two YACs in the same strain (a very common problem not directly addressed in this volume). However, a simpler method exists involving meiotic segregation (another protocol missing in this

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