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 suggest that there is genetic heterogeneity in the autosomal dominant eye disorder Best's vitelliform macular dystrophy (BMD) previously mapped to 11q13 (MIM 135700). 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. 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 testing 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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BOOK REVIEWS

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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, insertions, 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 existing techniques. 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 with such 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 grouped together to their functional group. As it is, there are sporadic attempts at this in the collection, as in chapter 29 which many of the previous chapters refer to, but this is not consistent throughout. There are numerous versions of protocols for specific tasks and oftentimes the instructions are slightly different. At least four different enzymes are used for digesting away the yeast cell wall throughout the collection where any protocol for most applications one would be asked to use. 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 a 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 DNA 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 amount needed for Southern analysis, YAC construction, and even “mini” library construction are orders of magnitude less. It is much easier to work with 1–5 ml cultures. This reduction in scale also includes the preparation of 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 for the construction of YAC is fine but very inefficient. Modifications exist that yield 106 to 109 transformants per μg of circular test plasmid (rather than the 10–102) 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. In this respect protocols can be useful. However, in some cases these protocols would be difficult to master by the uninitiated. In particular, some of the 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 microinjection manipulator for tetrad dissection. A more economical and easier method for meiotic manipulation of YACs is random spores in which spores are separated and plated and replication of the target gene on the YAC 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 for at least a year. As a protocol for random spores would be useful. Similarly, the twin spot analysis of mitotic recombination events requires several difficult intermediary steps (derivation of protoplast fusion, selection and 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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