Intelligent linkage analysis using gene density estimates

Sir — In November 1994, Antonarakis suggested in *Nature Genetics*¹ that human genome linkage searches could be made more efficient by targeting gene-rich chromosomal regions. At that time, information about such regions was based largely on CpG island density, GC content and chromosome banding techniques, which provided only indirect evidence and very approximate locations. However, with the publication of the first human gene map², it has become possible to pinpoint such regions more accurately and to relate them directly to the genetic map. By scanning the data available on the world wide web (<http://www.ncbi.nlm.gov/science96>), I have selected two sets of Génethon markers, set A and set B (Table 1), to screen expressed sequence tag (EST)-rich regions of the genome.

within 5 cM of at least 37% of the mapped ESTs. This figure is likely to be a substantial under-estimate, as I did not count ESTs mapping to overlapping intervals which extended further than 5 cM from the markers. Also linkage can often be detected at distances much greater than 5 cM. It is interesting, within my own particular field of research, that set A markers would almost certainly have detected linkage to 10 of the 15 autosomal RP loci³, while set B markers would pick up a further three.

Variation in EST density is perhaps not as great as the 1994 data set reviewed by Antonarakis might have implied, but it is substantial never the less. Several chromosomes are conspicuous by their absence from the above lists. As Antonarakis¹ pointed out, chromosomes

all listed as gene rich¹, are all represented in the lists above.

This analysis is of course far from perfect. Genetic distances vary from map to map, so gene densities derived in this way must be regarded as estimates. Also these estimates use EST density rather than gene density, as the task of determining whether each of the 'unidentified transcripts' duplicates another entry would be immense. In time it may be possible to correlate each marker with the density of Unigene transcription units within a given genetic distance of it, to produce a Gene Density Index or GDI, which could be quoted along with heterozygosity for each marker, and used in the design of 'intelligent' linkage searches. Such searches would start with a list of candidate genes and regions then move on to a list of

appropriately spaced markers ordered by gene density. This strategy would be equally applicable to genome scans for single gene defects or to searches for complex susceptibility or quantitative trait loci.

Nevertheless, sets A and B given above, used in conjunction with an appropriate list of candidate loci, should significantly reduce the necessity for wasted months spent 'fishing' for linkage. Those who reach

the end of these lists without a 'bite' can at least derive some comfort from the knowledge that, once they find their locus, it will probably be in a relatively gene-poor region, and thus the gene may prove easier to identify in the long run.

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1. Antonarakis, S.E. *Nature Genet.* **8**, 211–212 (1994).
2. Schuler, G.D. *et al.* *Science* **274**, 540–546 (1996).
3. Sullivan, L.S. & Daiger, S.P. *Molec. Med. Today* **2**, 380–386 (1996).

Table 1 • Génethon poly-CA markers which selectively screen EST rich regions of the human genome

Set A							
D1S513	D1S255	D1S447	D1S252	D1S305	D1S212	D1S510	D1S213
D2S291	D2S113	D2S152	D3S1581	D3S1290	D5S658	D6S273	D7S632
D7S479	D8S531	D8S270	D9S176	D9S282	D10S198	D11S1346	D11S1313
D11S987	D11S916	D11S1317	D11S908	D11S924	D12S361	D12S90	D12S84
D15S211	D16S496	D17S933	D17S806	D19S226	D19S223	D20S174	D22S282
Set B							
D1S464	D1S236	D1S248	D2S390	D3S1267	D4S1558	D4S395	D4S414
D5S668	D7S653	D7S480	D7S512	D9S165	D9S289	D10S537	D14S281
D14S271	D15S132	D15S155	D15S125	D17S938	D17S841	D17S948	D20S119
D22S275							

If each marker is assumed to screen the 10-cM interval surrounding it, 40 markers cover approximately one tenth of a 4-Morgan genome. Yet the markers listed in set A lie within 5 cM of at least 5,250 of the 20,104 ESTs mapped by Schuler and colleagues². Each marker in this set has within 5cM on either side of it an average of 125 ESTs, whereas one might expect a figure of around 50 ESTs per marker in a genome where EST distribution correlated with recombination frequency. Thus by searching one tenth of the genetic map, a researcher screens over a quarter of the localised ESTs. Use of set B covers a further 2,200 ESTs at 5-cM distance, with an average of 88 ESTs covered per marker. Use of both sets to screen approximately 17% of the genome would place markers

13 and 18 are relatively sparse in ESTs. Also chromosome 21, despite having a gene-rich long arm, appears unworthy of screening on the basis of the EST data. In contrast, chromosome 11 is EST rich with highly uneven distribution. Thus seven Génethon markers, covering less than 40% of this chromosome at the 5-cM level, account for just under 70% of the ESTs mapped to it. Chromosome 11 also boasts the most EST dense region, with 288 ESTs mapping in a 10-cM region in the vicinity of D11S1313. Predictably the HLA region on 6p comes a close second with 237 ESTs in the 10 cM around D6S273. In contrast, no ESTs have been assigned to the terminal 11 cM of 17q, and this on an otherwise relatively gene-rich chromosome. Chromosomes 1, 9, 15, 16, 17, 19, 20 and 22,