

P009 New methods for determining recombination hotspots experimentally

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The experimental detection and validation of recombination hotspots lags far behind the inference of hotspot locations from population genetic data. We have developed two suites of new methods for identifying and quantifying the recombinant haplotypes that result from homologous recombination. The first suite of techniques relies on the highly parallel haplotyping of single DNA molecules by clonal amplification in oil:water emulsions followed by the phased genotyping of individual variant nucleotides. The second suite of methods extends reverse-phase PCR to include real-time PCR. These methods can be applied to both allelic and non-allelic homologous recombination (NAHR). The simplest application of these methods is to genotype constitutive chromosomal rearrangements (caused by NAHR) that are otherwise difficult to assay. We have applied three of these methods to genotyping two chromosomal inversions. We are also applying these methods to identifying rare recombinant haplotypes against a background of non-recombinant haplotypes in a pool of sperm genomes. This latter application allows us to validate experimentally the existence of both NAHR and AHR hotspots in the male germline. We demonstrate the utility of these methods for assaying the known NAHR hotspot within the CMT1A-REPs.