

## Genetics and population analysis

# hotspot: software to support sperm-typing for investigating recombination hotspots

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Associate Editor: Oliver Stegle

Received on February 8, 2016; revised on March 24, 2016; accepted on April 7, 2016

### Abstract

**Motivation:** In many organisms, including humans, recombination clusters within recombination hotspots. The standard method for *de novo* detection of recombinants at hotspots is sperm typing. This relies on allele-specific PCR at single nucleotide polymorphisms. Designing allele-specific primers by hand is time-consuming. We have therefore written a package to support hotspot detection and analysis.

**Results:** hotspot consists of four programs: asp looks up SNPs and designs allele-specific primers; aso constructs allele-specific oligos for mapping recombinants; xov implements a maximum-likelihood method for estimating the crossover rate; six, finally, simulates typing data.

**Availability and Implementation:** hotspot is written in C. Sources are freely available under the GNU General Public License from <http://github.com/evolbioinf/hotspot/>

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**Supplementary information:** [Supplementary data](#) are available at *Bioinformatics* online.

## 1 Introduction

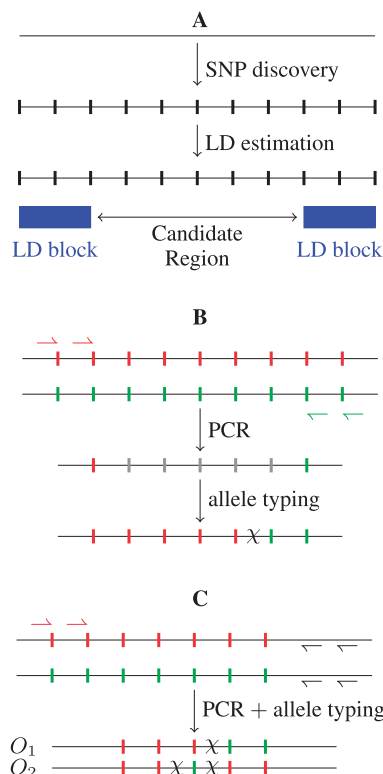
Understanding recombination is one of the classic problems in biology (Maynard Smith, 1978). In the past decade, the quantitative underpinnings of the debate about the function of recombination have dramatically improved as a result of population genomics based on genome sequencing. A major result of such studies is that in many organisms, including humans, recombination is localized in recombination hotspots (Hinch *et al.*, 2011; Myers *et al.*, 2005). Still, direct observation by typing of the products of recombination—usually sperm cells—is highly desirable to check and refine the results of population genetic inference (Jeffreys *et al.*, 2005; Odenthal-Hesse *et al.*, 2014).

Sperm typing is usually carried out after determining SNPs in a genomic region (Fig. 1A). Given a set of SNPs, linkage disequilibrium (LD) can be computed between pairs of them leading to the discovery of LD blocks punctuated by hotspot candidate regions a few kb wide. The best-known method for measuring the frequency of crossover at a candidate hotspot is the full-crossover assay (Fig. 1B), where allele-specific primers are designed against SNPs located upstream and downstream of the putative recombination hotspot (ASPs, Fig. 1B). Nested sets of phased ASPs are then used in

repulsion phase on pools of sperm DNA, such that only molecules affected by a crossover are amplified (Kauppi *et al.*, 2009). To map the crossover breakpoints, the SNPs of the amplification product are typed using allele-specific oligos (ASOs).

Recombination results in crossovers as well as non-crossovers, which are characterized by short, non-reciprocal gene conversion tracts. Both types of recombinants can be simultaneously detected by modifying the full-crossover assay such that one of the two sets of allele-specific primers is replaced by a set of universal primers (Fig. 1C). The resulting half-crossover assay allows the selective amplification of either haplotype targeted. Recombinant molecules are isolated by sequential ASO hybridization to each SNP within the hotspot interval using ASOs complementary to the haplotype not amplified. Under this protocol, hybridization products may still be due to crossovers (Fig. 1C, O<sub>1</sub>). However, any stretch of the haplotype not amplified that is detected without the exchange of flanking markers must be the result of a non-crossover event (Fig. 1C, O<sub>2</sub>).

SNP annotation and the design of ASPs, ASOs and universal primers are typically done by hand, which is slow and error-prone, especially when screening many hotspot candidates. We have written the software package hotspot to assist with this.



**Fig. 1** Crossover detection by sperm typing. **(A)** Pre-PCR steps consisting of sequencing, SNP discovery and linkage disequilibrium (LD) estimation for identifying LD blocks flanking hotspot candidates. **(B)** Full-crossover assay. Nested PCR is carried out across a hotspot candidate with allele-specific primers constructed using our program asp. This leads to positive PCR reactions indicating crossovers, and to negative PCR reactions indicating no crossover. The cartoon shows an example positive reaction, where the crossover region ( $\chi$ ) is identified using allele-specific oligos constructed with our program aso. **(C)** Half-crossover assay. Allele-specific primers (red) are paired with universal primers (black). PCR and SNP typing can then lead to the detection of crossover ( $O_1$ ) and non-crossover ( $O_2$ ) events. Adapted from Figs 20.1 and 20.5 by Kauppi *et al.* (2009)

## 2 Individual programs

hotspot consists of four programs: asp for designing allele-specific primers, aso for designing allele-specific oligos, xov for estimating the crossover rate and six for simulating crossover data. All four programs are command-line driven and run under UNIX operating systems such as Linux and OS-X.

asp takes three kinds of input: a list of candidate regions, the SNPs of the target genome and the genome sequence. Given these three inputs, asp returns candidate forward and reverse primers flanking both sides of the recombination hotspot. As explained above, these primers can be used for amplifying recombination products either in full-crossover assays (Fig. 1B), or half-crossover assays (Fig. 1C). The primers are constructed for every SNP in a 5 kb window adjacent to the candidate region. The 3'-end of each primer overlaps either of the two SNP alleles. asp optimizes primer length with respect to GC content. Apart from the primer sequences and the GC content, the program also prints out the melting temperature. Finally, asp can generate uniformly spaced 'universal' primers that do not intersect known polymorphisms for haplotype amplification in the half-crossover assay.

To look up SNPs, asp calls the program tabix (Li, 2010), which is part of the samtools package (Li *et al.*, 2009). This makes asp fast; for example, it takes only 10.8 s on a Xeon 2.60 GHz system to

construct forward primers for all 98 692 SNPs contained in the 5 kb upstream regions of 753 hotspot candidates identified on mouse chromosome 1 by Smagulova *et al.* (2011).

aso also takes as input a list of hotspot candidates, SNPs and genome sequences. Using tabix (Li, 2010), it then looks up each SNP in the candidate region and returns two short (e. g. 17 bp) oligos that straddle both alleles of the SNP. These are used to map crossover as well as non-crossover breakpoints ( $\chi$  in Fig. 1B and C). aso needs just 9.6 s to construct allele-specific oligos for the same 98 692 SNPs analyzed with asp in the previous paragraph.

Again, like asp, aso can generate evenly spaced oligos that do not overlap SNPs. These 'universals' tend to be longer than the allele-specific oligos, say 100 bp. They can be used as capture probes for in-solution enrichment. However, such oligos may come from low complexity regions in the genome, which would cause unspecific enrichment. To allow filtering for low complexity, aso also computes a measure based on the Lempel-Ziv complexity (Lempel and Ziv, 1976).

xov implements a maximum likelihood procedure for estimating the crossover probability from results of allele-specific PCR (Fig. 1B). In a typical screen,  $m$  experiments with different numbers of molecules,  $d_i$ , are carried out with  $n_i$  PCR reactions leading to the observation of  $k_i$  blanks. xov comes with example data to demonstrate how these quantities are entered.

six was written to check the accuracy of xov by simulating sperm typing results. It allows the user to modify all three relevant variables in the design of sperm typing studies: the number of experiments ( $m$ ), including the number of molecules per experiment ( $d_i$ ); the number of replicates per experiment ( $n_i$ ); and the crossover rate ( $\chi$ ). Apart from checking xov, six can be used to test the efficacy of experimental setups to detect a given recombination rate.

In conclusion, hotspot enables large-scale design of allele-specific oligos and accurate estimation of the rate of crossing over. In addition, it allows exploration of typing designs through simulation.

## Acknowledgment

We thank Angelika Börsch-Haubold for helpful comments.

*Conflict of Interest:* none declared.

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