

C. DESIGNING AND HANDLING OF PCR PRIMERS

Primers for genotyping assays are designed with MacVector 7.2.2 (Accelrys) and Oligo 4.0-s (MBI) computer software. MacVector software recognizes NCBI accession numbers and imports sequences directly from GenBank. The accession numbers and the information about sequences are typically obtained from original publications or ZFIN, NCBI, and Ensemble websites. In addition to primer sequences, MacVector also provides calculations and predictions about the T_m (melting temperature) of primers and the optimal annealing temperature for each primer set. Typically, we design primer sets with primer melting temperatures within 2°C of each other. The calculated annealing temperature is subsequently used in the PCR protocol. MacVector and Oligo 4.0-s programs are also used to review and, if necessary, optimize existing protocols that we have acquired from the provider of the line.

Primers are purchased in a lyophilized state. All primers are dissolved in water to a final concentration of 100 pmol/μl and stored at -20°C.

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For sample preparation and other PCR related protocols, visit <http://zebrafish.org/zirc/documents/protocols.php>

Zebrafish International Resource Center (ZIRC)

5274 University of Oregon
Eugene, OR 97403-5274, USA
Phone: 541-346-6028
Email: genotyping@zebrafish.org
Web: <http://zebrafish.org>