DegenPrimer: a software for *in silico* simulation of multiplex PCR with degenerate primers

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PCR with degenerate primers is a powerful tool that is often used in molecular studies of ecology and phylogeny of microorganism. The development of such primers, however, is a difficult process that is usually associated with time consuming trial-and-error routine. To address this issue we developed DegenPrimer – a software for *in silico* PCR simulation.

DegenPrimer performs sophisticated analysis of degenerate primers (Fig. 1), including: calculation of melting temperatures; prediction of stable secondary structures and primer dimers; cycle-by-cycle PCR simulation with any number or primers and matrices; primer specificity checks with automated BLAST queries and consequent PCR simulation using BLAST results as matrices; simulation of electrophoresis; and automated optimization of PCR conditions.

Aside from the sequences of the primers, matrices, and PCR conditions (such as Na^+ and Mg^{2+} concentrations) our PCR simulation takes into account concentrations of secondary structures, primer dimers, all annealing sites and alternative annealing conformations with mismatches, predicting not only the probable products but also their yields.

All predictions are based on the thermodynamics of the reaction system. Gibbs energies of annealing reactions are calculated using the nearest-neighbor model of the stability of oligonucleotide duplexes with mismatches (SantaLucia, 1998; SantaLucia & Hicks, 2004). Corrections for concentrations of divalent ions, dNTP and DMSO are performed as described by von Ahsen et al., 2001. All necessary thermodynamic parameters were obtained from different experimental studies (SantaLucia et al., 1996; Allawi & SantaLucia, 1997, 1998a, 1998b, 1998c; SantaLucia, 1998; Peyret et al., 1999; Bommarito et al., 2000; SantaLucia & Hicks, 2004).

Each analysis produces several reports that help to identify different problems that may be caused by some primers and primer combinations and select the best options available.

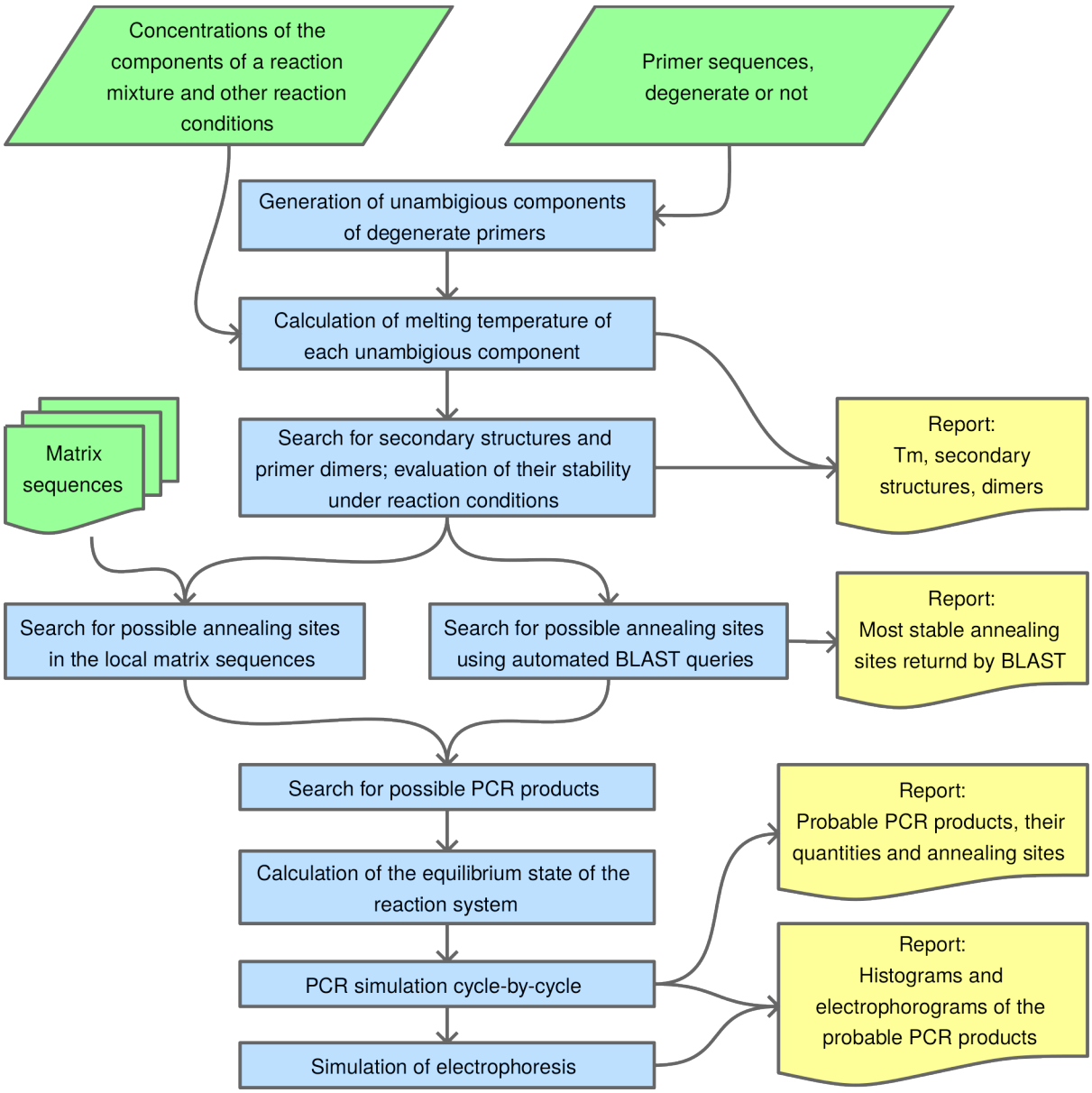

Fig. 1: DegenPrimer workflow

The accuracy of predictions made by DegenPrimer was tested in our laboratory during development of several systems of degenerate primers. In our experience, the predicted PCR results sometimes differed from reality (e.g. not all predicted side products were present), but the primers that we had chosen after initial analysis with DegenPrimer were always robust and with the desired specificity. Also, predicted melting temperatures were tested against experimental data available in literature. Linear regression analysis of the predicted vs observed Tm showed that 99% confidential interval of Tm prediction is less than 1.6°C.

DegenPrimer is written in Python for Linux and is licensed under GPLv3. Most calculations are highly parallelized, so DegenPrimer benefits from multi-core CPUs. The main program has command line interface and is useful for batch analysis and scripting. In addition we provide separate graphical interface that is more convenient for in-depth analysis of a particular primer system. The source code and all releases may be obtained at https://launchpad.net/degenprimer and https://launchpad.net/degenprimergui.

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