Supplementary methods

Detailed AFLP protocol

A protocol modified from that described by Wolf, PG (originally at http://bioweb.usu.edu/wolf/aflp_protocol.htm, now archived at http://archive.is/LCe6 [Feb 2014]) was used to generate amplified fragment length polymorphisms (AFLPs). Restriction enzymes, associated buffers and bovine serum albumin (BSA) were from New England Biosciences, Hitchin, UK; spermidine and fluorescent D2, D3, and D4 dye-labelled primers were from Sigma Aldrich, Gillingham, UK; adaptor and unlabeled primer sequences were obtained from Integrated DNA Technologies, Leuven, Belgium; the enzymes, T4 ligase and Taq polymerase (BIOTAQ), and associated buffers were from Bioline, London, UK. The initial restriction enzyme digestion step was modified as follows. For each sample, 10.7 ul milli-Q water, 2 ul EcoR1 buffer 10X, 0.2 ul BSA 10 ul ul^{-1}, 1.5 ul of 10 mM spermidine, 2.5 ul Dneasy extraction in AE, 0.1 ul Mse1 50 U ul^{-1}, 0.5 ul EcoR1 20 U ul^{-1}, were combined to a total volume of 20 ul followed by restriction digestion at 37 °C for 3 hrs, followed by a 65 °C for 20 mins enzyme deactivation step. The second adaptor ligation step was modified as follows. To each 20 ul digested sample, 2 ul of 50 uM Mse1 adaptor mix and 2 ul 5 uM EcoR1 adaptor mix was added. Then, for each sample, a separate mix of 3 ul T4 DNA ligase buffer 2X, 3 ul ATP 10 mM, and 0.5 ul T4 DNA ligase 10 U ul^{-1} was made up and combined with the digested sample and adaptor mix to a total volume of 30.5 ul followed by ligation at 20 °C (lab bench) for 2 hrs. Digested, ligated samples were diluted to 100 ul by adding 70 ul of $TE_{0.1}$ 1X buffer (TE 1X buffer with EDTA concentration reduced to 0.1 mM). Later pre-selective and selective PCR steps were similar to the Wolf lab protocol, except that pre-selective PCR product was diluted with 170 ul of $TE_{0.1}$ and that fluorescently labelled forward primers were multiplexed in the final selective PCR step by adding 0.05 ul of each of two labelled primers per sample and adjusting the total 20 ul PCR volume accordingly.