Sample preparation for fragment analysis experiments at TCAG

Ready-to-run service (electrophoresis of microsatellite, RFLP, t-RFLP, AFLP or other PCR fragments)

What you send to us:

Unpurified PCR products (10ul or more) should be sent to the facility in 96-well plates or 8-tube strips (if sample sizes are smaller). Please ensure that plates/tubes are well-sealed to avoid evaporation during transit. The facility will add the required sizing standards (ladder). The ABI 3730XL or 3100 instruments can detect fragments labelled with 6-FAM, HEX, VIC, PET or NED dyes. We require information for every sample (via spreadsheet or email) about expected sizes of fragments, type of repeats, dye labels used and marker names if applicable.

What you receive from us:

The user is provided with fragment sizes, allele names/numbers and peak heights if required (in an excel table), but we encourage every user to download a free copy of ABI's Peakscanner software (available from www.appliedbiosystems.com) so that FSA data files can be viewed. For microsatellite repeats that have not been previously validated (for both successful amplification and usefulness), we recommend sending a small number of samples for each marker of this category as a test before larger number of samples are submitted. PCR reactions can be multiplexed or pooled, but it is best to ensure that there are similar PCR efficiencies among fragments and that different coloured dyes are used. If two microsatellite repeats of the same colour are multiplexed/pooled, the fragments lengths should be at least 50bp apart so that separate analysis is possible. The facility does not provide advanced analysis of t-RFLP and AFLP fragments, but will provide an excel table of all observed fragments and their sizes along with the FSA files.

Full service microsatellite genotyping (using TCAG or custom primers)

What you send to us:

Genomic DNA should be provided at a concentration of 20-50 ng/ul. Volume will depend on number of markers to be genotyped. General guidelines: 10ul volume (1-4 markers) + 3-5 ul for each additional marker.

What you receive from us:

The user is provided with fragment sizes, allele names/numbers and peak heights if required (in an excel table). We also encourage every user to download a free copy of ABI's Peakscanner software (available from www.appliedbiosystems.com) so that FSA data files can be viewed.

SNaPshot SNP genotyping

What you send to us:

Already-completed SNaPshot reaction products should be cleaned with ExoSap and submitted at a volume of 5-10ul. If the facility is performing the SNaPshot reaction, please provide purified PCR products and extension primers in separate tubes.

What you receive from us:

The user is provided with genotype and peak heights (if required) for each sample in an excel table.

Taqman genotyping

What you send to us:

Genomic DNA should be provided at a concentration of 50 ng/ul. Volume will depend on number of SNPs to be genotyped. General guidelines: 10ul volume (1-4 markers) + 3-5 ul for each additional marker.

What you receive from us:

The user is provided with a genotype for each sample in an excel table.