

Prepping PCR Product for an ABI 3730xl Run

Initial considerations:

- a) Given the sensitivity of the 3730 to the spectral output of various dye sets/dyes, it is probably advisable to first run a dilution series on the machine with a number of samples per dilution. You can then use the results of the dilution series to determine the optimum volume of PCR product to load on the machine (the optimum volume should generally yield consistent peak-heights across all dyes/loci).
- b) The same can be said for the size standard. We have found that 0.25 to 0.20 ul/lane works well on the 3730xl. This may or may not be the case for you.
- c) Also, don't forget that if you are using a dye set other than G5 [e.g. an older dye set like DS-30 (FAM, NED, HEX, ROX)] you will likely need to run a spectral calibration for your particular machine. You should speak to the operator of the machine about this procedure.

Although hard to find, a rep. from ABI informed me that prepping the spectral matrix for the ABI 3130xl should go as follows:

- 1) In 1.5 ml tube, mix 993 ul Hi-Di formamide with 7ul DS-30 (or whichever) standard
- 2) Vortex, centrifuge (<1500g for a few seconds)
- 3) Heat tube at 95 C for 5 m
- 4) Place tube on ice for 2 m
- 5) Vortex, centrifuge (<1500g for a few seconds)
- 6) Transfer 10 ul from tube to each lane of a skirted plate for 3730
- 7) Proceed with spectral calibration

Stuff you may need:

DS-30 Matrix Standard	ABI part: 4345827
HiDi Formamide	ABI part: 4311320
96-well Skirted Plates (w/ barcode)	ABI part: 4306737
ROX500	ABI part: 401734

Prepping PCR Product:

- 1) You need to determine how many primer sets you will score, and how much of each PCR reaction you will be using (see stuff about dilution series above). If you are just getting to the dilution series, read on...
- 2) For simplicity's sake, let's assume you want to try a 1:1:1 dilution of 3 primers sets, each labeled with FAM; HEX; NED, respectively

- 3) A single-well mix will consist of 1 ul each PCR product with 0.25 ul ROX 500 (or whichever) standard and 6.75 ul HiDi formamide
 - 4) Assuming you are setting up a plate of reactions (100X), this will require you to make a “master mix” of 25 ul ROX and 675ul HiDi formamide. Mix these 2, vortex, spin down and add 7ul to each well of a 96-well (skirted) plate
 - 5) Add 1ul each PCR product to respective wells
 - 6) Vortex plate and spin down (<1500g for a few s)
 - 7) Heat at 95 C for 5 m
 - 8) Place on ice 2-5 m
 - 9) Vortex plate and spin down
 - 10) Load plate on 3730 and begin run
- Now, assuming you are setting up a dilution series, you will want to vary the volume of PCR product you are using. You may want to try a 0.5:1:1 (FAM:HEX:NED) or 0.5:0.5:1 ratio. If you are feeling brave, you can try a bunch of primers in a 0.5:0.5:1.0:1.0:1.0 dilution or something.
 - We typically setup the dilution series for ~16 samples to get an idea of the range in fluorescence we might see.
 - Remember: FAM is a beast and will fluoresce more than the other dyes (our experience is that HEX and NED are generally similar). Also keep in mind that guanine quenching of fluorescence can help you out here – we try to use FAM with primers that will (hopefully) quench the FAM a bit (e.g. those labelled primers with guanine bases at their 5' ends)
 - Remember if trying these ratios, you will need to adjust the volume of HiDi used accordingly (keep ROX volume the same). Therefore, a single well mix of ROX and HiDi for a 0.5:0.5:1 run would consist of 7.75ul HiDi, 0.25ul ROX, and your PCR product
 - I have it on good authority (other labs and ours) that HiDi formamide will yield the same results when diluted 1:1 with ddH₂O
 - It may be possible to get decent results using 0.15ul ROX per lane