

ABI 3130xl Genetic Analyzer Protocols for Genotyping DNA Samples

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The ABI 3130xl is a 16 capillary electrophoresis system that uses fluorescently labeled dyes for detection of DNA. There are four main steps required to genotype samples using the ABI 3130xl, 1) PCR, 2) ABI plate setup, 3) run on ABI machine, and 4) data analysis using genetic software. For further information see the ABI manuals. They are kept in a drawer next to the machine.

PCR Setup for ABI using **Direct Labeled Primers**

	<u>1 reaction</u>	<u>100 reactions</u>
10x buffer with MgCl ₂	1.2 ul	120 ul
dNTP (25mM)	0.96 ul	96
FAM Primer F&R 10uM/ul	0.3 ul	30
NED Primer F&R 10uM/ul	0.3 ul	30
PET Primer F&R 10uM/ul	0.3 ul	30
VIC Primer F&R 10uM/ul	0.3 ul	30
Taq polymerase (5units/ul)	0.18 ul	18
H ₂ O	5.96 ul	596
<u>DNA template</u>	<u>2.5 ul</u>	<u>----</u>
Total	12 ul	950 ul (dispense 9.5 ul per well)

PCR Setup for ABI using **M13 Labeled Primers**

	<u>1 reaction</u>	<u>100 reactions</u>
10x buffer with MgCl ₂	1.2 ul	120 ul
dNTP (25mM)	0.96 ul	96
Tailed Forward Primer 1uM/ul	0.96 ul	96
Reverse Primer 10uM/ul	0.72 ul	72
M13 Dye Labeled Primer 10uM/ul	0.72 ul	72
Taq polymerase (5units/ul)	0.18 ul	18
H ₂ O	5.26 ul	526
<u>DNA template</u>	<u>2.0 ul</u>	<u>----</u>
Total	12 ul	1000 ul (dispense 10 ul per well)

Using M13 labeled primers is cheaper alternative to order direct labeled primers from ABI. Order your forward and reverse primers from your favorite oligo provider and modify the forward primer sequence by adding an "M13" tail to the 5' end of the primer. For example:

5'-ACG ACG TTG TAA AAC GAC + primer sequence-3'

Do not modify the reverse sequence. Now you only need to order a set of four primers each labeled with a fluorescent dye from ABI that have the same M13 primer sequence you used to “tail” your forward primer. For our example:

5'-FAM- ACG ACG TTG TAA AAC GAC-3'
 5'-NED- ACG ACG TTG TAA AAC GAC-3'
 5'-PET- ACG ACG TTG TAA AAC GAC-3'
 5'-VIC- ACG ACG TTG TAA AAC GAC-3'

Resuspend primers to a stock concentration of 100 uM/ul and a working concentration of 10 uM/ul. The forward M13 tailed primer is used at a lower concentration of 1uM/ul. Touchdown thermocycler profiles work fine.

ABI plate setup

	1 reaction	100 reactions
PCR reaction*	2 ul *	----
Liz-250 Size Standard	0.25 ul	25 ul
Hi-Di Formamide	9.75	975
Total	12 ul	1000 ul (dispense 10 ul per well)

A size standard must be added to each PCR sample. The standard is labeled with an orange dye (LIZ) for dye set “G5”. The samples must also be suspended in formamide to denature the DNA. Prepare the formamide:size standard (FSS) mix on ice. For a 96 well plate, add 25 ul of size standard to 975 ul of Hi-Di Formamide and vortex. Pipette 10 ul of the FSS mix into each well of new, empty ABI plate. Vortex and centrifuge your PCR samples. Add 2 ul* of each PCR sample from your PCR plate to the FSS mix in the ABI plate. Place a rubber ABI septa on the plate. Vortex, centrifuge, and then denature for 95°C for 5 minutes. After denaturing place on ice for at least 5 minutes and then assemble plate into ABI plate sandwich. The sandwich is assembled in this order, the black baseplate, the clear reaction plate, the gray rubber septa, and then the white plate retainer. While the plate is cooling this is a good time to create or import your plate record on the ABI computer.

* This is assuming you ran a PCR with direct labeled primers. If you ran four separate M13 PCR's you can pool the four (FAM, NED, PET, VIC) PCR's into a pooled PCR plate and then add your 2 ul of pooled PCR reaction. Alternatively, good results have also been achieved by simply adding 1 ul from each of the four (FAM, NED, PET, VIC) M13 PCR reactions to 8 ul of the FSS mix giving a total volume in the ABI plate of 12 ul. Using this simpler method saves the use of an additional PCR plate but requires good pipetting skills and a good 1 ul pipette. The minimum amount of total volume in the ABI plate is 10 ul.

Plate Records

Prepare a new plate record for each plate to be run. The container name must be a new, unique name or the record will fail to import. The plate record stores the name of each sample, the instrument protocol, and the well position of each sample. Sample

names can not contain spaces, tabs, periods, slashes, or unusual characters. Use Excel to prepare the plate record and save the file as a tab-delimited text only file. Use your own name in the owner and results group fields. It is very helpful to name samples using the plant name (or individual number), the primer used, and the dye or color used for each primer.

ABI Runs

The array has 16 capillaries. For a 96-well plate, injections are made two columns at a time. A full 96-well plate requires 6 runs (loads) to inject all samples from the plate. The minimum number of samples is 16 and they must be loaded in columns 1 and 2 of the plate. If you have fewer than 16, put ddH₂O in the empty wells. Two plates can be loaded on the machine at one time. It takes approximately one hour to complete one run; therefore a full 96-well plate takes about 6 hours. Two 96-well plates take about 12 hours.

Place the ABI plate sandwich on the autosampler carefully. Do not push down too hard or you will damage the autosampler. The plate only goes on the autosampler only one way, the correct way, with the cutout of the plate facing the back of the machine. Please double check that all plates and reservoirs are fully seated and that all septa are in place. Also check that the anode and cathode buffer reservoirs are full of ABI buffer. After all checks have been made close the door and make sure to turn off the light.

Now you need to link your plates and run them. In the run scheduler panel of the ABI software, press the find all button to locate your plate record. Click on the plate record to select it then click on the correct plate to “link” your plate record to the plate on the autosampler. After your plate is linked you can run your plates by pressing the green arrow at the top of the computer screen.

Software analysis

This is by far the most time consuming step. Many different types of genetic analysis software exist. I am most familiar with GeneMarker. All of your data will be stored in a run folder according to the date of the run. You should copy the run data to a portable USB key drive. If you ran a full 96 well plate you will have 6 run folders. Make sure you get all of your data.

The raw files will not mean anything to you. You need to add them into a analysis software package and work with them there. I could write an entire book on using GeneMarker and won't here. I will offer a few tips. Make sure the sizing calibration looks good for all of the samples. Adjust any sizing peaks that you can. Make sure you can see amplification from all four dyes if you used all four dyes in your PCR. Set bins for alleles where appropriate and let the software do its job. Look at your parents for each primer to help you when setting bins. You can zoom in and out to look at poor amplification. The software will only call the alleles you tell it to. This becomes important if you have poor amplification with small intensity peaks or large amplification with out of range peaks.