

Prepping PCR Product for ABI 377 Run

- 1) Prepare loading mix for PCR product

	<u>1X Conc.</u>	<u>100X Conc.</u>
A) De-ionized formamide	3.0 μ l	300 μ l
B) 377 loading buffer	1.2 μ l	120 μ l
C) ROX 500	<u>0.3 μl</u>	<u>30 μl</u>
Total	4.5 μ l	450 μ l

- 2) Load mix (4.5 μ l per well) into 96-well plate (clean).

- 3) Prepare to load PCR product into wells. This gets kind of tricky:

For Primer 105 6-FAM 1.0 μ l per well @ 1:11 Dilution or
0.4 μ l undiluted (each can be tricky **see below**...might try 0.5 μ l PCR
product with 5.5 μ l ddH₂O)
For Primer 17 HEX 2.0 μ l per well
For Primer 69 NED 1.0 μ l per well

- 4) Make sure all 96 well plates are spun down prior to pipetting PCR product from plates. Otherwise, the pipettor will be VERY inconsistent.
- 5) When multiplexing (adding multiple dyes to one lane), add the same quantities of the above to each well of the 96-well plate containing the master mix described above. THOROUGHLY mix samples by pipetting up and down within the well when the last PCR product is added.
- 6) Cover plate with clear 96-well seal. Spin down now or prior to denaturing and loading sample.

* This is tricky for the following reasons: (1) when pipetting volumes lower than about 1 μ l, pipettor error can cause a serious problem for you when you pipet various volumes ranging from 0.4 to 0.6 μ l into the 377 load. This results in some bands that are ok, some that are not bright enough and some that are too bright. The 1:11 dilution is problematic because mixing the sample in the ddH₂O does not typically go very well. This is why a shift to a smaller, more manageable volume of water might be better. Conversely, the number of cycles for primer 105 could be reduced to 30 or 35 and the primer run at a volume of 1 μ l. It's up to you...