ABI PRISM® SNaPshot™ Multiplex Kit Quick Reference Card

This quick reference guide is intended for use only by appropriately trained laboratory personnel who have read the user's manual and are experienced with the protocol. For safety guidelines, please refer to the *ABI PRISM® SNaPshot™ Multiplex Kit Protocol* (P/N 4323357). For all chemcials in bold type below, please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Preparing the SNaPshot Control Reactions

Step	Action
1	Label two 0.2-mL MicroAmp® tubes: ♦ One for the positive control reaction ♦ One for the negative control reaction
2	Combine the following items in each tube.

Item	Positive Control (µL)	Negative Control (µL)
SNaPshot Multiplex Ready Reaction Mix	5	5
SNaPshot Multiplex Control Primer Mix	1	1
SNaPshot Multiplex Control Template	2	0
Deionized water	2	4
Total	10	10

Step	Action
3	Mix well, spin briefly, and immediately place on ice.

Preparing the SNaPshot Reactions

Step	Action
1	Combine the following ingredients on ice.

Item	One Sample (µL)
SNaPshot Multiplex Ready Reaction Mix	5
Pooled PCR products	3
Pooled SNaPshot primers	1
Deionized water	1
Total	10

Step	Action
2	Aliquot 10 µL into each MicroAmp tube/well and immediately place on ice.

Thermal Cycling the SNaPshot Reactions

Step	Action
1	Place the tubes in a GeneAmp® 9600 thermal cycler and set the volume to 10 µL.
2	Repeat the following for 25 cycles: ♦ 96 °C for 10 seconds ♦ 50 °C for 5 seconds ♦ 60 °C for 30 seconds **Note** The conditions can be modified to accommodate specific primers.
3	Rapid thermal ramp to 4 °C and hold until ready for post-extension treatment.
4	Continue with "Post-Extension Treatment," on the back of this card.

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Post-Extension Treatment

Step	Action
1	Add one of the following to the reaction mixture and incubate at 37 °C for 1 hour. ♦ 1.0 Unit of Shrimp AlkalinePhosphatase (SAP) or ♦ 1.0 Unit of Calf Intestinal Phosphatase (CIP)
2	Deactivate the enzyme by incubating at 75 °C for 15 minutes.
3	Place the sample at 4 °C.

Preparing the SNaPshot Products for the ABI PRISM 310 Genetic Analyzer, 3100 Genetic Analyzer, or 3700 DNA Analyzer

Step	Action
1	Add 9 µL of **HI-Di formamide** into each tube. **⚠ WARNING CHEMICAL HAZARD. Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Add 0.5 µL of SNaPshot products and 0.5 µL of GeneScan™120 LIZ™ size standard into each tube.
3	Vortex briefly and quick spin.
4	Denature the samples by placing them at 95 °C for 5 minutes.
5	Quick spin or tap the tubes or plates to bring liquid to the bottom of the tubes.
6	Place the samples at 4 °C until ready to load.
7	To run samples on the 310 Genetic Analyzer, verify that you have selected the following in the data collection software: a. GS STR POP-4 (1 mL) E5 module b. E5 matrix c. GeneScan-120 LIZ size standard (change the default color to orange) To run samples on the 3100 Genetic Analyzer, verify that you have selected the following in the data collection software: a. E5 dye set b. SNP36_POP4 module c. GeneScan-120 LIZ size standard Analysis (change the default color to orange) To run samples on the 3700 DNA Analyzer, verify that you have selected the following in the data collection software: a. E5 dye set b. Modified SNP1_1 POP5 module c. GeneScan-120 LIZ size standard Analysis (change the default color to orange)

Kit	Number of Reactions	Part Number
ABI PRISM® SNaPshot™ Multiplex Kit	100[a]	4323151
	1000[a]	4323154
	5000[a]	4323155

a. Contains Multiplex Control Template and Multiplex Control Primer Mix for 30 control reactions

Note The fluorescent dyes are assigned to the individual ddNTPs as follows:

ddNTP	Dye Label	Color of Analyzed Data
A	dR6G	Green
C	dTAMRA™	Black
G	dR110	Blue
T(U)	dROX™	Red