

Appendix S1. Comparison of incurred costs between the traditional method of individually labeling each primer and the dual labeling technique, using 10 primer pairs and 50 samples. Also shown below for comparison are total costs for 100 and 300 samples. In the table, steps that are relatively constant (i.e., not necessarily dependent on sample size or primer number) are shaded. All costs are in US\$.

Step	Individual primer method	Dual labeling method
Initial testing of unlabeled primers ^a	Forward primers: $\$5.50 \times 10 = \55 Reverse primers: $\$5.50 \times 10 = \underline{\$55}$ \$110	Forward primers (unlabeled): $\$5.50 \times 10 = \55 Reverse primers: $\$5.50 \times 10 = \55 Tagged forward primer: $\$5.50 \times 10 = \underline{\$55}$ \$165
Order forward fluorescent primers	VIC-labeled: $\$75 \times 2 = \150 NED-labeled: $\$75 \times 2 = \150 PET-labeled: $\$75 \times 3 = \225 6-FAM-labeled: $\$65^b \times 3 = \underline{\$195}$ \$720	VIC-labeled: $\$75 \times 1 = \75 NED-labeled: $\$75 \times 1 = \75 PET-labeled: $\$75 \times 1 = \75 6-FAM-labeled: $\$65^b \times 1 = \underline{\$65}$ \$290
PCR	<i>Per sample:</i> QIAGEN Mix ^c = \$0.254 Tips ^d = $\$0.0625 \times 2$ Plate ^e = $\underline{\$0.042}$ \$0.421 <i>For all samples:</i> $\$0.421 \times 10 \text{ primers} \times 50 \text{ samples} = \mathbf{\$210.50}$	<i>Per sample:</i> QIAGEN Mix ^c = \$0.254 Tips ^d = $\$0.0625 \times 2$ Plate ^e = $\underline{\$0.042}$ \$0.421 <i>For all samples:</i> $\$0.421 \times 2 \text{ primer mixes} \times 50 \text{ samples} = \mathbf{\$42.10}$

<p>Fragment analysis</p> <p><i>Per sample:</i></p> <p>LIZ size standard^f = \$0.539</p> <p>Formamide^g = \$0.0042</p> <p>Tips^d = \$0.0625 × 2</p> <p>Plate^e = \$0.042</p> <p>Fragment analysis^h = <u>\$0.708</u></p> <p style="text-align: right;">\$1.419</p> <p><i>For all samples:</i></p> <p>\$1.419 × 10 primers × 50 samples = \$709.50</p> <p><i>Number of 96-well plates required: 5.21</i></p> <p>Total cost \$1750</p> <p>(50 samples)</p> <p>Total cost \$2670</p> <p>(100 samples)</p> <p>Total cost \$6350</p> <p>(300 samples)</p>	<p><i>Per sample:</i></p> <p>LIZ size standard^f = \$0.539</p> <p>Formamide^g = \$0.0042</p> <p>Tips^d = \$0.0625 × 2</p> <p>Plate^e = \$0.042</p> <p>Fragment analysis^h = <u>\$0.708</u></p> <p style="text-align: right;">\$1.419</p> <p><i>For all samples:</i></p> <p>\$1.419 × 2 primer mixes × 50 samples = \$141.8</p> <p><i>Number of 96-well plates required: 1.04</i></p> <p>Total cost \$639</p> <p>Total cost \$823</p> <p>Total cost \$1559</p>
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^a Prices for forward and reverse primers are estimated at \$5.50 each, based on a typical 20–23 fragment size.

^b The price for a 6-FAM–labeled primer is typically less than other dyes because 6-FAM is not proprietary to Applied Biosystems and can be ordered elsewhere.

^c Prices for the QIAGEN kit (catalog no. 206143) are based on 5 µL needed for a 10-µL PCR reaction; \$254 for 5000 µL ($\$254/5000 \times 5 = \0.254).

^d Cost for tips per reaction are based on typical costs of \$60 for a pack of 960 tips ($\$60/960 = \0.0625 each).

^e Cost for space on a 96-well plate for PCR are based on \$40 for a pack of 10 plates ($\$40/10/96 = \0.042 per reaction well).

^f Cost for the LIZ-500 size standard is based on \$431 for 800 reactions ($\$431/800 = \0.539).

^g Cost for formamide is based on \$47.80 per 100 mL ($\$47.80/100 \text{ mL}/1000 \mu\text{L} \times 8.8 \mu\text{L} = \0.0042).

^h Fragment analysis on a 3730xl sequencer is based on costs at the Cornell University Biotechnology Resource Center of \$68 per plate ($\$68/96 = \0.708).