



# SitePreparationGuide

## OncoScan<sup>®</sup> CNV FFPE Assay Kit

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**Not for use in diagnostic procedures.**

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# Chapter 1

## OncoScan® CNV FFPE Assay Protocol Site Preparation Guide

This document provides an overview of how to prepare the laboratory for performing the OncoScan® CNV FFPE Assay Kit Protocol. It includes information on the recommended laboratory set up and includes the lists of reagents, consumables and equipment used in the OncoScan® CNV Assay.

### Laboratory Setup and Recommendations

We strongly recommend using TWO separate rooms when performing this protocol. The use of two separate rooms greatly reduces the risk of sample contamination due to the presence of previously amplified OncoScan products. These rooms are referred to as the:

- Pre-PCR Lab
- Post-PCR Lab

The high-level steps performed in each lab are presented in [Table 1.1](#).

**Table 1.1** OncoScan® CNV Assay Protocol Workflow

<b>Pre-PCR Lab Assay Steps</b>
Genomic DNA Preparation and Picogreen Quantitation of the Genomic DNA and Dilution
Anneal
First Stage PCR
<b>Post-PCR Lab Assay Steps</b>
Gel QC
Second Stage PCR
HaeIII Digest
GeneChip Array Hybridization
GeneChip Array Washing and Staining
GeneChip Array Scanning

## Pre-PCR Lab

The Pre-PCR Lab should be a low copy DNA template lab, and should be free of PCR product (amplicons). Hence no amplified product may be taken into the pre-PCR lab. Do not open the seal of the First stage PCR plate in the Pre-PCR Lab.



**NOTE:** The Post-PCR Lab has airborne contamination with the amplified probe-annealed template. It is strongly recommended not to enter the Post-PCR lab while doing the pre-PCR steps of the assay.

## To Help Prevent Sample Contamination

1. All of the reagents and master stocks required for the steps performed in the Pre-PCR Lab should be stored in this lab under the appropriate conditions.
2. Equipment dedicated to the OncoScan CNV FFPE Assay is required for each lab space. Do not move any equipment including cold blocks, ice buckets and pipettes between the Pre- and the Post-PCR Labs.
3. Always wear a fresh gown, booties, and gloves to prevent PCR carryover, and to minimize the risk of trace levels of contaminants being brought into the lab. Proper gowning procedure must be followed in the Pre-PCR Lab by using the steps given below:
  - Using the tacky mats to remove any dust and particulates from the bottom of the shoes.
  - Putting on disposable booties
  - Putting on bouffant head cover (cap)
  - Putting on the right size disposable laboratory gloves
  - Putting on disposable sterile lab coats
  - Wiping down the work bench area with 70% Ethanol before and after use
  - Frequently changing the gloves throughout the assay as needed

## Activities in the Pre-PCR Lab

1. Preparation of non-amplified genomic DNA
  - A. Extraction and Purification of genomic DNA
  - B. Picogreen® quantitation of the genomic DNA

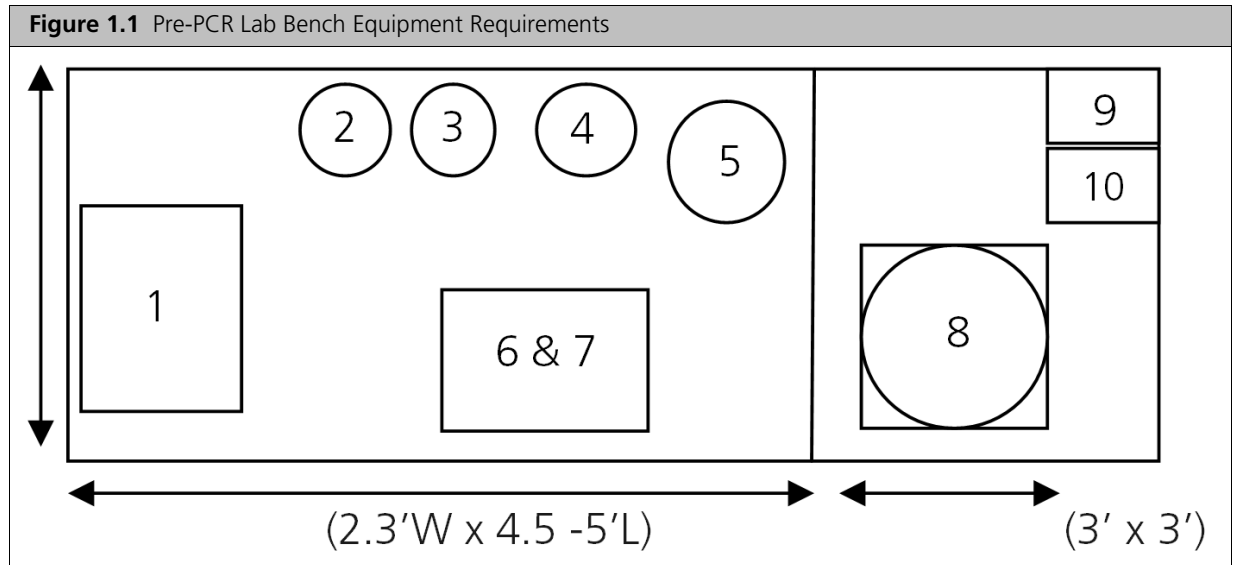


**NOTE:** If the Qubit® or Fluoroskan instrument is located in the Post-PCR lab, transfer only the volume needed for Picogreen quantitation and store the rest of the DNA in Pre-PCR Lab.

- C. Dilution or concentration of the genomic DNA as needed
2. Anneal reaction
3. Gap-Fill reaction
4. Exonuclease digestion reaction
5. Cleavage reaction
6. First Stage PCR reaction

## Equipment Required in Pre-PCR Lab

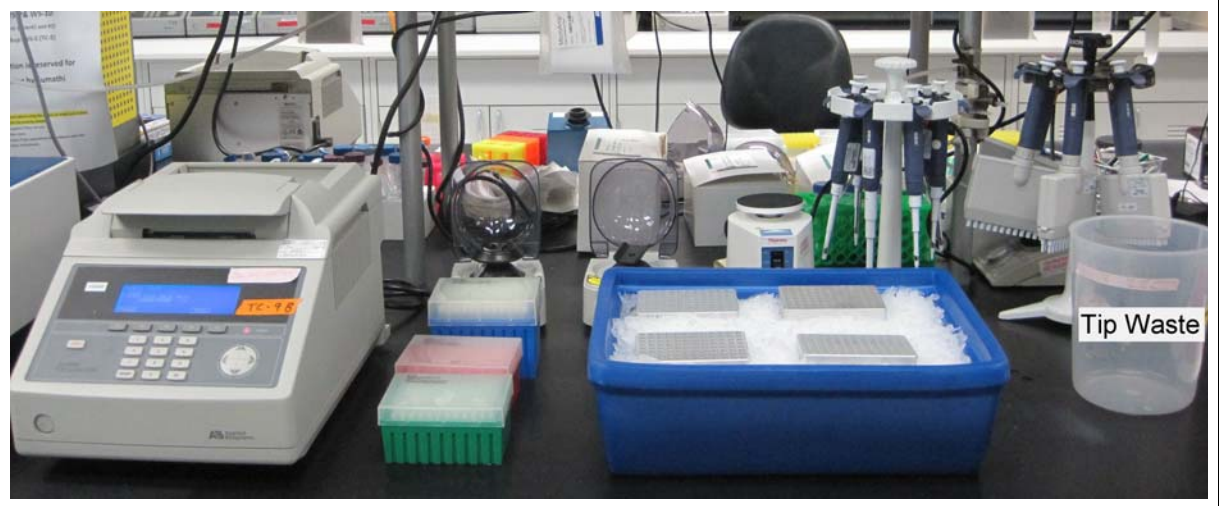
The major pieces of equipment required for this lab are given below. Refer to [Figure 1.1](#), [Figure 1.2](#) and [Figure 1.3](#).



### Pre-PCR Lab Equipment

1. GeneAmp® PCR System 9700 96-well Thermal Cycler or Veriti® Thermal Cycler, P/N 4375786<sup>1</sup>
2. Microfuge for 1.5 mL Eppendorf vials
3. Microfuge for strip tubes
4. Vortexer
5. Single/Multi- Channel Pipettes on stand [L-20, L-200, L-1000, L12-20, and L12-200]
6. Ice bucket
7. Cold blocks for 96-well PCR plates - 4 each
8. Plate centrifuge - refrigerated or non-refrigerated
9. Freezer, -20 °C
10. Refrigerator, 2-8 °C

<sup>1</sup> The Veriti 96-well Fast Thermal Cycler, P/N 4375305 is not compatible with this assay protocol. Use only one of the two recommended thermal cyclers listed above.

**Figure 1.2** Pre-PCR lab Bench Equipment - Recommended Setup**Figure 1.3** Pre-PCR Lab - Plate Centrifuge

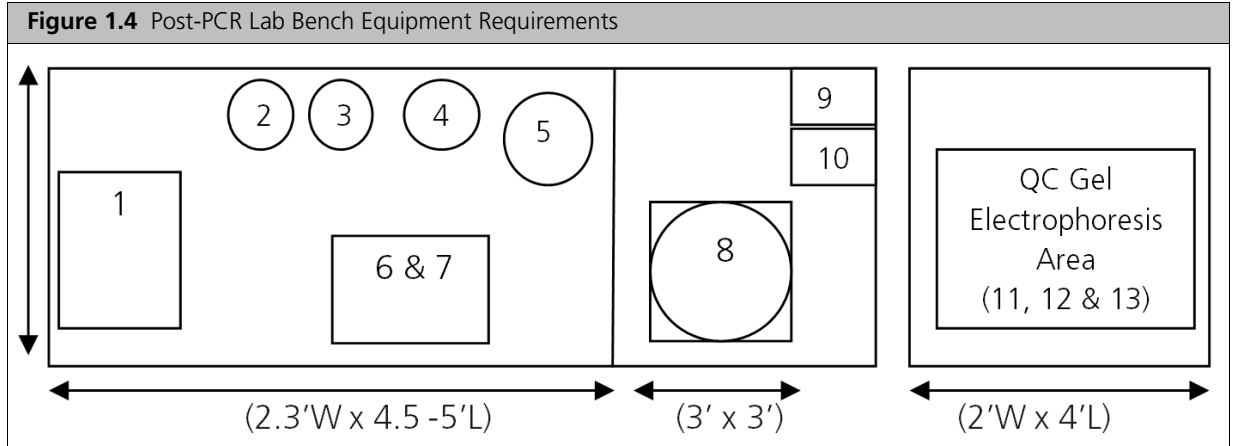
## Post-PCR Lab

### Activities in the Post-PCR Lab

1. Second Stage PCR amplification
2. QC gel of First stage PCR by agarose gel electrophoresis
3. HaeIII digest of Second Stage PCR
4. QC gel of HaeIII digest by agarose gel electrophoresis
5. Preparation of hybridization target
6. Target DNA hybridization onto GeneChip® Arrays
7. Washing and staining of arrays
8. Scanning of arrays

## Equipment Required in Post-PCR Lab

The major pieces of equipment required for this lab are given below. The bench top equipment requirements are the same as for the Pre-PCR Lab, however additional equipment are needed for processing QC gels. Refer to [Figure 1.4](#).



### Post-PCR Lab Equipment:

1. GeneAmp® PCR System 9700 96-well Thermal Cycler or Veriti® Thermal Cycler, P/N 4375786<sup>1</sup>
2. Microfuge for 1.5 mL Eppendorf vials
3. Microfuge for strip tubes
4. Vortexer
5. Single/Multi- Channel Pipettes on stand [L-20, L-200, L-1000, L12-20 (or L24-20), and L12-200 (or L24-200)]
6. Ice bucket
7. Cold blocks for 96-well PCR plates - 3 each
8. Plate centrifuge - refrigerated or non-refrigerated
9. Freezer, -20 °C
10. Refrigerator, 2-8 °C
11. Electrophoresis gel box or E-Gel instrument
12. Electrophoresis power supply
13. Gel Imager
14. GeneChip® Hybridization Oven 645
15. Fluidics Station 450 or Fluidics Station 450Dx connected to an Affymetrix® GeneChip® workstation
16. GeneChip® Scanner 3000 7G or GeneChip® Scanner 3000 Dx2 connected to an Affymetrix® GeneChip® workstation

<sup>1</sup> The Veriti 96-well Fast Thermal Cycler, P/N 4375305 is not compatible with this assay protocol. Use only one of the two recommended thermal cyclers.



## Reagents, Equipment, and Consumables Required

This section includes the vendor and part number information for the reagents, equipment and consumables that have been tested for use with the Affymetrix® OncoScan® CNV FFPE Assay Kit.

**!** **IMPORTANT:** This protocol has been optimized using the equipment, consumables and reagents listed in this site preparation guide. For the best results, we strongly recommend that you adhere to the protocol as described. Do not deviate from the protocol; do not substitute reagents or equipment.

## Reagents, Equipment and Consumables Available from Affymetrix®

### Affymetrix® Equipment and Software Required

**Table 1.2** Affymetrix Equipment and Software Required

ü	Item	Part Number
☐	GeneChip® Fluidics Station 450 — two or more units required or GeneChip® Fluidics Station 450 DX— two or more units required	00-0079  00-0335
☐	Tubing, Silicone peristaltic for GeneChip® Fluidics Station 450	Contact Affymetrix
☐	GeneChip® Hybridization Oven 645	00-0331
☐	GeneChip® Scanner 3000 7G System with Workstation and Autoloader or GeneChip® Scanner 3000 Dx2 System with Workstation and Autoloader	00-0215  00-0334
☐	GeneChip® Command Console® Software (AGCC) or Affymetrix® Molecular Diagnostic Software	Version 3.2 or higher  Version 1.1.1 or higher
☐	OncoScan® Console	Current version
☐	Chromosome Analysis Suite (ChAS)	Current version
☐	Nexus for Affymetrix® Software	Current version

## OncoScan® CNV Reagents and OncoScan® CNV Arrays Required

**Table 1.3** OncoScan® CNV Reagent Kit and OncoScan® CNV Arrays

ü	Item	Part Number
□	OncoScan® CNV FFPE Assay Kit Array and Reagent Kit Bundle, Sufficient for 24 samples and includes 48 OncoScan Arrays — Components in this kit are listed below.	902695
	<b>OncoScan® CNV Buffer C</b>	
	<ul style="list-style-type: none"> <li>■ Buffer C</li> </ul>	
	<b>OncoScan® CNV Copy Number Probe Mix 1.0 &amp; Controls</b>	
	<ul style="list-style-type: none"> <li>■ Positive Control (12ng/μL)</li> <li>■ Negative Control</li> <li>■ Copy Number Probe Mix 1.0</li> <li>■ Buffer A</li> </ul>	
	<b>OncoScan® CNV Gap Fill and 1st Stage PCR</b>	
	<ul style="list-style-type: none"> <li>■ Buffer A</li> <li>■ Gap Fill Enzyme Mix</li> <li>■ SAP, Recombinant (1 U/μL)</li> <li>■ dNTP Mix (A/T)</li> <li>■ dNTP Mix (G/C)</li> <li>■ Nuclease-Free Water</li> <li>■ Exo Mix</li> <li>■ Cleavage Buffer</li> <li>■ Cleavage Enzyme (2 U/μL)</li> <li>■ PCR Mix</li> <li>■ Taq Polymerase (5 U/μL)</li> </ul>	
	<b>OncoScan® CNV 2nd Stage PCR and Post PCR Processing</b>	
	<ul style="list-style-type: none"> <li>■ PCR Mix</li> <li>■ Taq Polymerase (5 U/μL)</li> <li>■ Buffer B</li> <li>■ HaeIII Enzyme (10 U/μL)</li> <li>■ Exo I Enzyme (20 U/μL)</li> <li>■ Nuclease Free Water</li> <li>■ Hybridization Mix</li> </ul>	
	<b>OncoScan® CNV Stain Reagents</b>	
	<ul style="list-style-type: none"> <li>■ Stain 1</li> <li>■ Stain 2</li> <li>■ Array Holding Buffer</li> </ul>	
	<b>Individual Bottles</b>	
	<ul style="list-style-type: none"> <li>■ Wash A</li> <li>■ Wash B</li> </ul>	
□	<b>Arrays</b>	
	<ul style="list-style-type: none"> <li>■ OncoScan® CNV Array, 4 x 12 pack</li> </ul>	

## Reagents, Equipment and Consumables Available from Other Suppliers

### Reagents Required for Gel QC Not Provided in OncoScan® CNV Reagent Kit

**Table 1.4** Reagents Required for Gel QC (Not included in the OncoScan® CNV FFPE Assay Kit)

Item	Part Number	Supplier
3% 96-well pre-cast agarose gel (as needed, based on the number of samples)	161-3062	Bio-Rad
50 bp DNA ladder	N3436S	New England Biolabs
Gel loading Dye, Blue (6X)	B7021S	New England Biolabs
100% Glycerol	16374	Affymetrix
EDTA, 0.5 M Solution	15694	Affymetrix
1X TE buffer Low EDTA, pH 8.0	75793	Affymetrix

**Table 1.5** Reagents and Equipment Required for Gel QC using E-Gels (Not included in the OncoScan CNV FFPE Assay Kit)

Item	Part Number	Supplier
Mother E-Base™ Device	EB-M03	Life Technologies
Daughter E-Base™ Device (optional for running multiple gels simultaneously)	EB-D03	Life Technologies
E-Gel® 48 4% Agarose Gels	G8008-04	Life Technologies
TrackIt™ Cyan/Orange Loading Buffer	10482-028	Life Technologies
50 bp DNA ladder	N3236S	New England Biolabs
Nuclease-free Water	71786	Affymetrix

## Miscellaneous Consumables Required

**Table 1.6** Miscellaneous Consumables Required

Item	Qty Needed per 24 Samples	Part Number	Supplier
0.2 mL PCR 12-tube strip with 12-cap strips, natural	10	1402-2400	USA Scientific
1/2" Laser Tough-Spots	as needed	RNBW-1100	Diversified Biotech
3/8" Laser Tough-Spots	as needed	RNBW-1000	Diversified Biotech
Micro-Amp Clear Adhesive Film	as needed	4306311	Applied Biosystems
Optical 96 Well Half-skirt plate	5	289196	E&K Scientific
MicroAmp Optical 96 well reaction plate (Optional for UK; compatible with ABI thermal cyclers)	5	N8010560	Invitrogen
VWR PCR Plate, 96-Well, Flat Plate	Optional (for gel QC)	82006-636	VWR
1.5 mL Safe-Lock Tubes, Natural	as needed	022363204	Eppendorf
1.5 mL Safe-Lock Tubes, Blue	as needed	022363247	Eppendorf
1.5 mL Safe-Lock Tubes, Amber	as needed	022363221	Eppendorf
Non-Stick RNase-free Microfuge Tubes (1.5 mL)	as needed	AM12450	Life Technologies
Non-Stick RNase-free Microfuge Tubes (2.0 mL)	as needed	AM12475	Life Technologies
25 mL Reagent Reservoir	as needed	89094-662	VWR
Pipette Tip Refills, 20 µL	as needed	GP-L10F	Rainin
Pipette Tip Refills, 200 µL	as needed	GP-L200F	Rainin
Pipette Tip Refills, 1000 µL	as needed	GP-L1000F	Rainin
15 mL Centrifuge tubes			VWR

## Equipment Required

When performing the pre-PCR stages of the OncoScan® CNV FFPE Assay Kit Protocol, great care should be taken to avoid sample contamination with PCR products.

**Table 1.7** Equipment Required

Item	Part Number	Supplier	Optional Part Number/ Manufacturer	Needed In	Needed In
Vortexer Maxi Mix II	M37615	Thermo Scientific	Vortex Genie G560 /VWR	Pre-PCR lab	Post-PCR Lab
Picofuge Galaxy Mini	C 1213	VWR	—	Pre-PCR lab	Post-PCR Lab
Strip tube Centrifuge Galaxy Mini	C 1213	VWR	—	Pre-PCR lab	Post-PCR Lab
Ice Pan, Maxi	89231-998	VWR	—	Pre-PCR lab	Post-PCR Lab
96-well Aluminum Cold Blocks	81001	BioSmith	—	Pre-PCR lab	Post-PCR Lab
GeneAmp® PCR system 9700 Thermal cycler (with Gold block)	N050200	Life Technologies	4375786* (Veriti 96-well) / Life Technologies	Pre-PCR lab	Post-PCR Lab
MicroAmp® Optical Film Compression Pad	4312639	Life Technologies		Pre-PCR lab	Post-PCR Lab
Single Channel Pipettes (entire LTS set)	L-2, L-20, L-200, L-1000	Rainin	—	Pre-PCR lab	Post-PCR Lab
12-Channel LTS Pipette (20 µL and 200 µL)	L12-20, L12-200	Rainin	—	Pre-PCR lab	Post-PCR Lab
24-Channel LTS Pipette (20 µL and 200 µL)	L24-20, L24-200	Rainin	—	Pre-PCR lab	Post-PCR Lab
MicroAmp® Clear Adhesive Film	4306311	Life Technologies	—	Pre-PCR lab	Post-PCR Lab
Plate Centrifuge 5810 or 5804	5810 / 5804	Eppendorf	—	Pre-PCR lab	Post-PCR Lab
Benchtop Cooler	89004-558	VWR	—	Pre-PCR lab	Post-PCR Lab
Gel Box (Wide-Mini sub cell GT basic system)	170-4489	Bio-Rad	—	—	Post-PCR Lab
Power Supply for Gel Electrophoresis	164-5070	Bio-Rad	—	—	Post-PCR Lab
AlphaImager HP System for gel imaging (with software)	Varies (Inquire)	Protein Simple	—	—	Post-PCR Lab
Hybridization Oven 645	00-0331	Affymetrix	—	—	Post-PCR Lab
Probe Array Cartridge Carriers	90-0356 to 90-0359	Affymetrix	—	—	Post-PCR Lab
GeneChip® Fluidics Station 450	Varies depending on location	Affymetrix	—	—	Post-PCR Lab
GeneChip® Scanner 3000 7G System w/Workstation and AutoLoader	Varies depending on location	Affymetrix	—	—	Post-PCR Lab
GeneChip® Command Console® Software (AGCC)	Inquire	Affymetrix	—	—	Post-PCR Lab

\* The Veriti 96-well Fast Thermal Cycler, PIN 4375305 is not compatible with this assay protocol. Use only one of the two recommended thermal cyclers listed above.

**Table 1.7** Equipment Required (Continued)

Item	Part Number	Supplier	Optional Part Number/ Manufacturer	Needed In	Needed In
<b>If preparing samples using QIAGEN FFPE Kit</b>					
Fume Hood			—		
Thermomixer® R with 1.5 mL adapter	5355-31595	Eppendorf	—		
Centrifuge 5415D	5425 50003	Eppendorf	5415C		
Vortexer Maxi Mix II	M37615	Thermo Scientific	Vortex Genie G560 VWR	Sample Preparation Lab (Clean or Pre-PCR)	
Picofuge Galaxy Mini	C 1213	VWR	—		
DNA120 Speedvac	DNA120-115	Thermo Scientific	DNA110		
Fluoroskan Ascent Microplate Fluorometer	5210470	Thermo Scientific	—		
Single Channel Pipettes (entire LTS set)	L-2, L-20, L-200, L-1000	Rainin	—		

## Supplier Contact List

**Table 1.8** Supplier Contact List

Supplier	Web Site Address (www not required for some addresses)
Affymetrix	<a href="http://www.affymetrix.com">www.affymetrix.com</a>
Applied Biosystems	<a href="http://www.appliedbiosystems.com">www.appliedbiosystems.com</a>
Bio-Rad	<a href="http://bio-rad.com">bio-rad.com</a>
Bio-Smith	<a href="http://biosmith.com">biosmith.com</a>
C.B.S. Scientific	<a href="http://www.cbsscientific.com">www.cbsscientific.com</a>
Clontech	<a href="http://www.clontech.com">www.clontech.com</a>
Diversified Biotech	<a href="http://divbio.com">divbio.com</a>
E&K Scientific	<a href="http://eandkscientific.com">eandkscientific.com</a>
Eppendorf	<a href="http://eppendorf.com">eppendorf.com</a>
ESCO	<a href="http://www.escoglobal.com">www.escoglobal.com</a>
Fisher Scientific	<a href="http://www.fishersci.com">www.fishersci.com</a>
Invitrogen	<a href="http://invitrogen.com">invitrogen.com</a>
Lonza	<a href="http://www.lonza.com">www.lonza.com</a>
Life Technologies	<a href="http://www.lifetechnologies.com">www.lifetechnologies.com</a>
New England Biolabs	<a href="http://www.neb.com">www.neb.com</a>
QIAGEN	<a href="http://www1.qiagen.com">www1.qiagen.com</a>
Rainin	<a href="http://www.rainin.com">www.rainin.com</a>

**Table 1.8** (Continued) Supplier Contact List

Supplier	Web Site Address (www not required for some addresses)
TekNova	<a href="http://teknova.com">teknova.com</a>
VWR	<a href="http://vwr.com">vwr.com</a>