NimbleGen Arrays User's Guide

AccuSNP Arrays

Version 1.0

For life science research only.
Not for use in diagnostic procedures.

Edition

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Preface

Intended Use

For life science research only. Not for use in diagnostic procedures.

AccuSNP arrays are used for single-nucleotide polymorphism (SNP) detection in sample populations.

Contact Information

Technical Support

If you have questions, contact your local Roche Microarray Technical Support. Go to *www.nimblegen.com/arraysupport* for contact information.

Manufacturer and Distribution

Manufacturer	Roche NimbleGen, Inc. Madison, WI USA
Distribution	Roche Diagnostics GmbH Mannheim, Germany
Distribution in USA	Roche Diagnostics Corporation Indianapolis, IN USA

Conventions Used in This Guide

Symbols

Symbol	Description
	Important Note. Information critical to the success of the procedure or use of the product. Failure to follow these instructions could result in compromised data.
	Information Note: Designates a note that provides additional information concerning the current topic or procedure.

Text

Conventions	Description
Numbered listing	Indicates steps in a procedure that must be performed in the order listed.
Italic type, blue	Identifies a resource in a different area of this manual or on a web site.
Italic type	Identifies the names of dialog boxes, windows, tabs, panels, views, or message boxes in the software.
Bold type	Identifies names of menus and controls (buttons, checkboxes, etc.) in the software.

Chapter 1. Before You Begin

The *AccuSNP User's Guide* describes the protocol for microarray-based single-nucleotide polymorphism (SNP) detection on an AccuSNP-12 array.

AccuSNP arrays are used to screen whether SNPs exist in a sample, are relevant to a condition and to help determine the characteristics of a group.

Array Workflow

Figure 1 lists the steps and the corresponding chapters in the workflow for AccuSNP arrays. It is important that you follow the steps in the exact order. Stopping points are indicated where you can stop in the protocol without affecting results. Refer to the specific chapter for more information.

The corresponding estimated time for each step is based on processing 12 samples and/or 1 slide. When applicable, incubation times are indicated between process times in Figure 1.

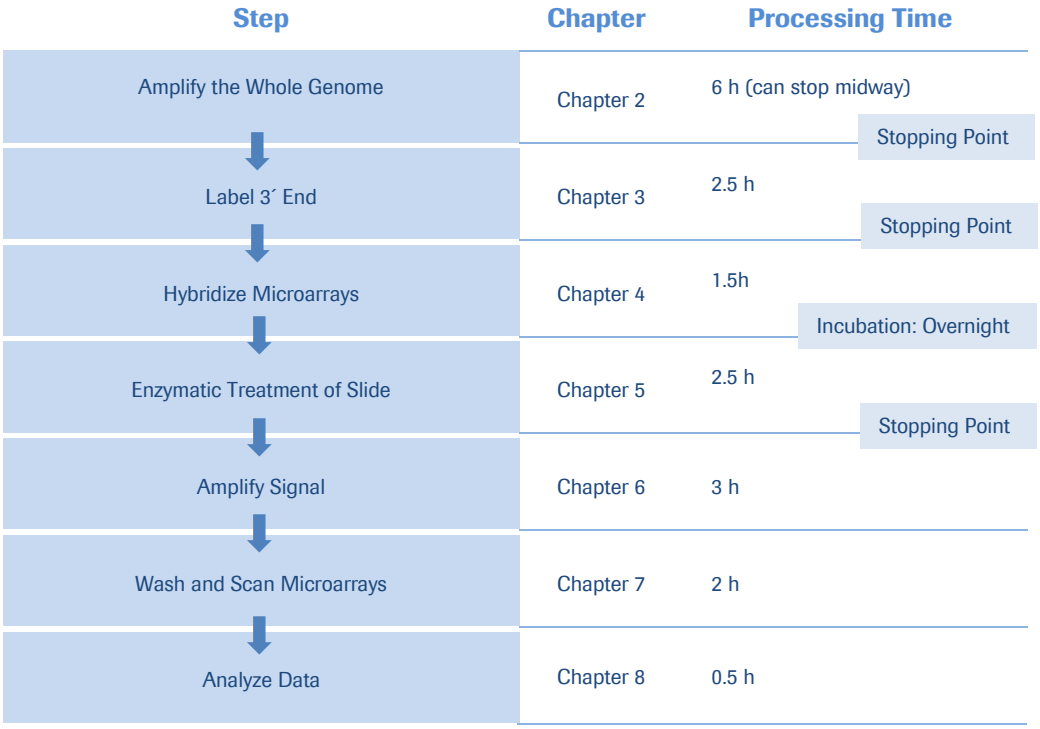


Figure 1: Workflow for NimbleGen AccuSNP Arrays

Checking for Updates

To verify that you are using the most up-to-date version of this guide to process your arrays, go to *www.nimblegen.com/lit/.*

Components Supplied

Component	Description
NimbleGen Arrays	As ordered
NimbleGen Mixers	n One HX12 mixer is provided per AccuSNP-12 slide.
Mixer Port Seals	For sealing fill and vent ports of NimbleGen mixers: n Mixer ports seals are provided with HX12 mixers.
CD/DVD	This *User's Guide* and NimbleGen design files are included in the Design Information CD/DVD.

Microarray Storage

NimbleGen AccuSNP slides are packaged with sorbent and must be stored at +2 to +8ºC for use by the expiration date. Once the seal is broken, use the NimbleGen AccuSNP slides immediately.

Reagent Kit Storage

Kit	Storage Temp
AccuSNP WGA Kit	-15 to -25ºC
AccuSNP Sample Preparation Kit	-15 to -25ºC
AccuSNP DNA Purification Kit	+15 to +25 ºC
AccuSNP Array Processing Kit	+2 to +8ºC
AccuSNP Enzyme Assay Kit	-15 to -25ºC
AccuSNP Detection Kit	+2 to +8ºC
NimbleGen Sample Tracking Control Kit	-15 to -25ºC

Environmental Requirements

Ozone

Cyanine dyes (Cy) are ozone sensitive. It is important that you regularly monitor ozone levels in the lab environment and take the necessary precautions to maintain atmospheric ozone levels below 5 ppb (parts per billion).

Ozone levels greater than 5 ppb can affect Cy dye signal degradation and result in higher PMT gain, higher noise, and lower signal-to-noise ratio in microarray data analysis.

To mitigate ozone problems in the lab environment, Roche NimbleGen recommends that you use an ozone scrubber and ozone protection box during the post-detection washing and drying steps of the workflow, as well as during microarray scanning.

Humidity

Cy dyes are sensitive to humidity. Take the necessary precautions to keep humidity levels below 40%.

Organic Solvents

Cy dyes are sensitive to organic solvents used in tissue fixation, such as formaldehyde and acetic acid. They also are sensitive to bleach and other cleaning chemicals. Maintain the necessary precautions to keep Cy dyes away from these types of chemicals. Perform the array workflow in an organic-solvents-free environment.

Light

Cy dyes are sensitive to light. Minimize light exposure of the dyes during use. Store in the dark when not in use.

Protocol Information and Safety

- Always be aware of possible hazards associated with reagents used or samples processed.
- Use appropriate protective clothing, safety goggles and gloves.
- All samples and reagents must be considered as potentially hazardous agents. Strictly apply appropriate safety precautions according to local, state and federal regulations.
- Thoroughly follow the instructions in this guide and review the material safety data sheets of all kits and reagents prior to their use.
- Dispose all reagent waste in accordance with all local, state and federal environmental, health, and safety laws and regulations.
- The AccuSNP backplates and slides are made of glass. Handle accessory plates and slides with care to prevent shattering and incision. The use of metal forceps may damage the glass slide and create a potentially hazardous condition (for example, broken glass) for the operator.

Required Equipment, Labware & Consumables

You assume full responsibility when using the equipment, labware, and consumables described below. These protocols are designed for the specified equipment, labware, and consumables.

AccuSNP Kits Required

Kit	Description
AccuSNP WGA Kit	Contains reagents for the amplification of genomic DNA targets in order to facilitate SNP detection.
AccuSNP Sample Preparation Kit	Contains components for the preparation of DNA targets and hybridization to AccuSNP arrays in order to facilitate SNP detection.
AccuSNP DNA Purification Kit	Designed to isolate amplified and fragmented genomic DNA from enzymatic steps during the preparation of DNA targets for hybridization on AccuSNP arrays.

Kit	Description
AccuSNP Array Processing Kit	Contains components for the preparation of DNA targets and processing of hybridized AccuSNP arrays in order to facilitate SNP detection.
AccuSNP Enzyme Assay Kit	Contains components to enzymatically treat hybridized AccuSNP slides in order to facilitate SNP detection.
AccuSNP Detection Kit	Contains components for the detection of biotin-labeled target hybridized to AccuSNP slides in order to facilitate SNP detection.
NimbleGen Sample Tracking Control Kit (optional)	Contains 12 Sample Tracking Controls (STCs). A unique STC is added to each sample before hybridization to a multiplex array, enabling you to confirm that the correct sample was hybridized to each array.

NimbleGen Equipment

Equipment	Supplier	Process Quantity	Catalog No.
NimbleGen Hybridization System 4*	Roche NimbleGen	4 slides	05 223 652 001 (110V) 05 223 687 001 (220V)
NimbleGen MS 200 Microarray Scanner	Roche NimbleGen	48 slides	05 394 341 001
NimbleGen Array Processing Accessories (Slide Rack, 4 Wash Tanks, 8 Slide Containers).	Roche NimbleGen	8 slides	05 223 539 001
AccuSNP Accessories Set ** n 8-manifold mounting plate n 8-manifold flow-through chamber which includes the 8 multifold lid, 8 multifold base, and joint rods (anchor rods) n rack TeFlow No Thermal Block GenePaint (chamber rack) n bowl reagent chamber rack (waste tray) n backplate (glass plates - 8) n spacers (500)	Roche NimbleGen	8 slides	06 343 341 001
Additional Replacements for the AccuSNP Accessories Set– as needed	Tecan		
n backplate (glass plates - 8)			30 059 045
n spacers (500)			10 760 815
n Spare Part Kit (contains springs for the 8-Multifold)			30 065 377

* NimbleGen Hybridization Systems include an accessory kit that contains a Precision Mixer Alignment Tool (PMAT), Mixer Disassembly Tool, Mixer Brayer, System Verification Assemblies, replacement O-rings, and forceps.

** Refer to *Appendix D* for photos of equipment and additional information.

Software

Program	Supplier	Catalog No.
DEVA Software v1.2 or later	Roche NimbleGen	Available for free download from: *http://www.nimblegen.com/products/software/deva/index.html*

Standard Laboratory Equipment

Equipment	Supplier	Catalog No.
Compressed Gas Nozzle	TeqCom	TA-N2-2000
Desiccator	Multiple Vendors	
Electrophoresis System	Multiple Vendors	
Heat Block (capable of temperatures to +98°C)	Multiple Vendors	
Inert gas (nitrogen or argon)	Multiple Vendors.	
Microcentrifuge (capable of at least 12,000 x g)	Multiple Vendors.	
Microman M10 Pipette	Gilson	F148501
Rectangle 9" x 13" glass dish	Multiple Vendors	
Spectrophotometer	NanoDrop	ND-1000 or newer
Vacuum Concentrator (speed vacuum)	Multiple Vendors.	
Microarray dryer that meets these specifications: n Minimum of 4,000 revolutions per minute (rpm). n Capable of drying arrays in 30 seconds or less. n Supplied with a slide holder or cassette for 1x3 inch slide from the manufacturer.	ArrayIt	MHC 110V MHC 220V
Vortex Mixer	Multiple Vendors	
Water baths	Multiple Vendors	

Roche NimbleGen Consumables & Accessories

Component	Package Size / Process Quantity	Catalog No
AccuSNP WGA Kit Contents: n Klenow n dNTP Mix n Random Nonamer n Reaction Buffer, 10x	24 samples	06 300 120 001
AccuSNP Sample Preparation Kit [1] Contents: n TdT Reaction Buffer, 5x n $CoCl_2$, 25 mM n Biotin-16-dUTP n Terminal Transferase, rec n Dephosphorylation Buffer, 10x n Alignment Oligo n 2x Hybridization Buffer n Hybridization Component A	96 samples	06 300 154 001
AccuSNP DNA Purification Kit	24 samples	06 300 189 001

Component	Package Size / Process Quantity	Catalog No
Contents: n Binding Buffer n Binding Enhancer n Wash Buffer n Elution Buffer n High Pure Spin Filter tubes n High Pure Collection tubes		
AccuSNP Array Processing Kit Contents: n 10x Wash Buffer I n 10x Wash Buffer II n 10x Wash Buffer III n DTT n Phosphatase, alkaline	96 samples	06 300 197 001
AccuSNP Enzyme Assay Kit [1] Contents: n 20x AccuSNP Array Buffer n AccuSNP Ligase n AccuSNP Assay Enzyme n Biotin Alignment Oligo	24 samples	06 297 579 001
AccuSNP Detection Kit Contents: n Cy3 Streptavidin n Biotinylated Anti-Streptavidin	96 samples	06 303 536 001
NimbleGen Sample Tracking Control Kit [2] Contents: n Control 1, 3, 5, 7, 9,11, 13, 15, 16, 17, 18, 20	Perform up to 384 hybridizations (or up to 32 AccuSNP slides) with the components of this kit.	05 223 512 001
BSA, molecular biology grade	20 mg/ml	Available from Roche 10 711 454 001
NAD Grade 1, free acid	1 g	Available from Roche 10 127 965 001
NimbleGen HX12 Mixer	10 mixers	05 223 768 001
10x PBS	4 l	Available from Roche 11 666 789 001
Western Blocking Reagent	100 ml	Available from Roche 11 921 673 001

[1] The Alignment Oligo in the AccuSNP Sample Preparation Kit is a mixture of Cy3 and Cy5 labeled 48 mer oligonucleotides that hybridize to alignment features on NimbleGen arrays. It is required for proper extraction of array data from the post-hybridization scanned image. The Biotin Alignment Oligo, in the AccuSNP Enzyme Assay Kit, is a biotin labeled 48 mer oligonucleotide that is ligated to alignment features on NimbleGen AccuSNP arrays and detected during the AccuSNP workflow. It is required for proper extraction of array data from the final scanned image.

[2] Twelve Sample Tracking Controls (STCs) are provided. Each STC is a Cy3-labeled 48 mer oligonucleotide. When a unique STC is added to each sample before hybridization to a multiplex array, the STC can be used to confirm that the correct sample was hybridized to each array.

Other Vendors' Reagents/Consumables

The following components are available from other vendors:

Component	Supplier	Catalog No.
0.5 M EDTA, molecular biology grade	Multiple Vendors	
0.2 ml thin-walled microcentrifuge tubes	Multiple Vendors	
1.5 ml microcentrifuge tubes	Multiple Vendors	
15 ml conical tubes	Multiple Vendors	
30 ml Slide Staining Container	Evergreen Scientific	240-5400—B8K
5 N Sodium hydroxide, molecular biology grade	Multiple Vendors	
50x Denhardt's Solution	Sigma	D2532
Alconox Powder Detergent	Multiple Vendors	
Compressed Nitrogen or Argon Gas (for cleaning array surface)[1]	Multiple Vendors	
Cotton Swabs	Multiple Vendors	
CP10 Pipette Tips	Gilson	192 tips (F148412) 960 tips (F148312)
Ethanol, absolute (100%)	Multiple Vendors	
Glacial acetic acid	Multiple Vendors	
PCR grade water	Multiple Vendors	
Water: reagent grade, ACS, nonsterile, type 1	VWR	RC915025

[1] Roche NimbleGen recommends using a compressed gas nozzle to gently blow compressed nitrogen or argon gas across arrays to remove any dust or debris. The use of canned compressed air for this purpose is not recommended and could compromise array and data quality.

Notes

Chapter 2. Performing Whole Genome Amplification

Chapter 2 describes how to amplify your DNA samples using an AccuSNP WGA kit. The samples will then be fragmented, dephosphorylated and purified to prepare the amplified DNA for 3´ end labeling.

Preparing Samples

The samples requirements are listed below:

- Use purified, intact genomic DNA (gDNA) for optimal sample processing and hybridization.
- Roche NimbleGen recommends starting with 1.0 µg of gDNA for each sample.
- Prepare samples at a concentration of ≥ 160 ng/µl in PCR grade water or 1x TE buffer (10 mM *Tris*-HCl and 0.1 mM *EDTA*, pH 7.5 - 8.0).
- Verify that samples have an $A_{260}/A_{280} \geq 1.8$ and $A_{260}/A_{230} \geq 1.9$ for optimal labeling yields.

Roche NimbleGen recommends analyzing 1.5 µl of your gDNA on a NanoDrop spectrophotometer to measure the concentration and A260/A280 and A260/A230 ratios.

Performing Sample QC

To determine the quality of your samples, analyze 250 ng of gDNA on a 1% agarose gel to ensure they do not show any signs of RNA contamination or degradation.

Genomic DNA should appear as a single prominent band greater than 12 Kb. If the sample appears as more than one band or as a smear, the DNA may be degraded or have a contaminant that could affect the amplification procedure. RNA contamination will result in a smear less than 200 bp.

AccuSNP Kits & Accessories Required

The following AccuSNP kit components are required for this portion of the protocol:

Kit	Vial No.	Component
AccuSNP WGA Kit	vial 1	Klenow fragment (3´ → 5´ exo-)
	vial 2	dNTP Mix
	vial 3	Random Nonamers
	vial 4	Reaction Buffer, 10x
AccuSNP Sample Preparation Kit	vial 5	Dephosphorylation Buffer, 10x
AccuSNP Array Processing Kit	vial 5	Phosphatase, alkaline (AP)

Kit	Vial No.	Component
AccuSNP DNA Purification Kit	bottle 1	Binding Buffer
	bottle 2	Binding Enhancer
	bottle 3	Wash Buffer
	bottle 4	Elution Buffer
		High Pure Filter Tubes
		High Pure Collection Tubes

Materials and Equipment Required

The materials and equipment that are required for this portion of the protocol are as follows:

Materials
0.2 ml thin-walled microcentrifuge tubes
1.5 microcentrifuge tubes
5 N Sodium hydroxide, molecular biology grade
Ethanol, absolute
Glacial acetic acid
PCR grade water
Water: reagent grade, ACS, nonsterile, type 1

Equipment
Microcentrifuge (capable of at least 12,000 x g)
Thermocycler (capacity for at least eight 0.2 ml tubes)
Spectrophotometer

Before you Begin

The following three preparations should be completed before you begin the protocol.

Preparation 1

Solution	Quantity	Instructions
AccuSNP DNA Purification Kit Wash Buffer (bottle 3 – cap color blue)	50 ml	**1.** Add the volume of 100% ethanol to the bottle of concentrated High Pure Wash Buffer as indicated on the label. n Each bottle of Wash Buffer from the 24-sample AccuSNP DNA Purification Kit requires the addition of 40 ml absolute ethanol.

Preparation 2

Solution	Quantity	Instructions
2N Acetic Acid	1.0 ml	1. Add 114.3 µl glacial acetic acid to 885.7 µl PCR grade water. Mix well.

Preparation 3

Solution	Quantity	Instructions
Random nonamers	1,344 µl	1. Briefly centrifuge a vial containing the random nonamers (vial 3). Add 1344 µl of PCR grade water to the primers. Let stand 2 minutes at room temperature (+15 to +25°C). Vortex well. Quick-spin to collect contents in bottom of the tube. Aliquot 50 µl individual reaction volumes in 0.2 ml thin-walled microcentrifuge tubes and store at -15 to -25°C. Diluted primers are stable for up to 12 months at -15 to -25°C.

The following protocol is intended to process 12 samples at a time. The steps need to be performed in order.

Step 1. Assembling the Amplification Reaction for Each Sample

1. Thaw all the components of the AccuSNP WGA kit on ice and store on ice until ready to use.
2. For each sample, transfer 1 µg of template DNA to a 0.2 ml tube containing diluted random nonamers.

Components	Amount
Template DNA	1 µg
Random primers	50 µl

3. Add 6.25 µl 10x Reaction Buffer to each tube containing the template DNA and primers. Bring the final volume to 62.5 µl with PCR grade water.

Components	Amount
Template DNA & Random primers	step 1.2
Reaction Buffer, 10x (vial 4)	6.25 µl
PCR grade water	to volume (62.5 µl)
Total Volume	**62.5 µl**

4. Mix by pipetting up and down 10 times.
5. Quick-spin to collect contents in the bottom of the tube.

Step 2. Denaturing Each Template DNA Sample

1. Heat-denature samples in a thermocycler at +98°C for 10 minutes. Quick chill in an ice-water bath for 2 minutes.

Quick-chilling after denaturation is critical for high-efficiency DNA amplification.

Step 3. Preparing Amplification Master Mix

1. Prepare the following amplification master mix for the twelve samples prepared in Step 1.

Keep all reagents and dNTP/Klenow master mix on ice. Do not vortex after addition of Klenow.

Components	Per Single Reaction	Master Mix Volume 12 reactions (x12.5)
dNTP Mix (vial 2)	12.5 µl	156.3 µl
PCR grade water	41.25 µl	515.6 µl
Reaction Buffer, 10x (vial 4)	6.25 µl	78.1 µl
Klenow (vial 1)	2.5 µl	31.3 µl
Total Volume	**62.5 µl**	**781.3 µl**

2. Mix well by pipetting up and down 10 times.
3. Add 62.5 µl of the master mix to each of the denatured samples prepared in *Step 2* above

Components	Volume
Denatured Template DNA (*Step 2.1*)	62.5 µl
Amplification Master Mix (*Step 3.1*)	62.5 µl
Total Volume	**125 µl**

4. Mix well by pipetting up and down 10 times.

Do not vortex after addition of Klenow.

5. Quick-spin to collect contents in bottom of the tube.
6. Incubate for 2 hours at +37°C in a thermocycler with a heated lid.
7. Stop the reaction by incubating the samples for 20 minutes at +75°C in a thermocycler.

STOP POINT: Proceed to *Step 4* or store the samples at -15 to -25°C.

Step 4. Performing DNA Fragmentation

1. Add 3.1 µl 2 N Acetic Acid to the amplified samples prepared in *Step 3* above.

Components	Volume
Amplified DNA *(Step 3.7)*	125 µl
2 N Acetic Acid	3.1 µl
Total Volume	**128.1 µl**

2. Vortex briefly.
3. Quick–spin to collect contents in bottom of the tube.
4. Incubate for 22 minutes in a thermocycler with the block set at +95°C and a heated lid.

Incubation time and temperature are critical. An incubation time longer or shorter than 22 minutes will affect the size of the DNA fragments generated and affect data quality. The thermocycler should be used as a heat block for this step. Over fragmentation has been observed if the thermocycler is programmed to incubate the samples at +95°C.

5. Quick–spin to collect contents in bottom of the tube.
6. Chill samples by placing on ice for 2 minutes.

Incubation time and temperature are critical. Samples should be chilled on ice; do not follow the 22 minute incubation mentioned above with a +4°C incubation in the thermocycler.

7. Immediately add 3.1 µl of 5 N NaOH to each tube.

Reagents	Volume
Amplified DNA *(Step 4.6)*	128.1 µl
5 N NaOH	3.1 µl
Total Volume	**131.2 µl**

8. Vortex briefly.
9. Quick–spin to collect contents in bottom of the tube.
10. Incubate for 40 minutes at +95°C in a thermocycler with a heated lid.
11. During the incubation step, thaw the 10x Dephosphorylation Buffer that is required for the next part of the protocol.
12. Quick–spin to collect contents in bottom of the tube.
13. Chill samples by placing on ice for 2 minutes.

Incubation time and temperature are critical. Do not extend the 2 minute incubation time as over-fragmentation of the DNA may result.

14. Proceed directly to *Step 5* below. Do not stop or store the samples at this step.

Step 5. Performing DNA Dephosphorylation

1. To each fragmented DNA sample from *Step 4* above, add 15 µl Dephosphorylation Buffer and 2 µl Alkaline Phosphatase.

Reagents	Volume
DNA Fragmentation Reaction (*step 4.14*)	131.2 µl
Dephosphorylation Buffer, 10x (vial 5)	15 µl
Alkaline Phosphatase (vial 5)	2 µl
Total	**148.2 µl**

2. Mix by pipetting up and down 10 times.
3. Quick–spin to collect contents in bottom of the tube.
4. Incubate for 1 hour at +50°C using thermocycler with heated lid.
5. Heat inactivate the enzyme. Incubate for 2 minutes at +95°C using a thermocycler with a heated lid.
6. Proceed directly to *Step 6.* Do not stop or store the samples at this step.

Step 6. Performing DNA Purification

1. Spin sample and transfer the entire contents to a labeled 1.5 ml tube. Add 296.4 µl Binding Buffer to each sample.

Component	Volume
DNA Dephosphorylation Reaction (*step 5.5*)	148.2 µl
Binding Buffer (bottle 1)	296.4 µl
Total	**444.6 µl**

The order of addition of reagents is important. The binding reagents (Binding Buffer and Binding Enhancer) must be added to the sample sequentially. DNA purification performance will significantly decrease if the order of addition is not followed according to the tables.

2. Vortex well.
3. Quick–spin to collect contents in bottom of the tube.
4. Add 444.6 µl Binding Enhancer to each sample.

Component	Volume
Reaction Volume from *step 6.3*	444.6 µl
Binding Enhancer (bottle 2)	444.6 µl
Total	**889.2 µl**

5. Vortex well.
6. Quick–spin to collect contents in bottom of the tube.
7. Transfer the sample to the upper reservoir of an assembled High Pure Filter Tube (filter tube placed in a collection tube).
8. Centrifuge at 12,000 x g for 60 seconds.
9. Discard the flow through.
10. Place the High Pure Filter Tube into the same High Pure Collection Tube.
11. Centrifuge at 12,000 x g for 30 seconds.
12. Discard the flow through.

13. Add 500 µl of prepared Wash Buffer (bottle 3) to the High Pure Filter Tube.
14. Centrifuge at 12,000 x g for 1 minute.
15. Discard the flow through.
16. Return the High Pure Filter Tube into the same sample Collection Tube.
17. Add 200 µl of prepared Wash Buffer (bottle 3) to the High Pure Filter Tube.
18. Centrifuge at 12,000 x g for 1 minute.
19. Discard the flow through.
20. Place High Pure Filter Tube back into the same sample Collection Tube.
21. Centrifuge at 12,000 x g for 1 minute to dry the column.
22. Place the High Pure Filter Tube into a 1.5 ml microcentrifuge tube.
23. Add 50 µl Elution Buffer (bottle 4) directly to the center of the High Pure Filter.
24. Centrifuge at 12,000 x g for 1 minute.
25. Remove eluant from 1.5 ml microcentrifuge tube and re-apply directly to the center of the High Pure Filter.
26. Replace the High Pure Filter Tube into the same 1.5 ml microcentrifuge tube.
27. Centrifuge at 12,000 x g for 1 minute.
28. Discard the High Pure Filter Tube.
29. Centrifuge the eluant at 12,000 x g for 3 minutes.
30. Avoid disturbing the pellet, transfer the supernatant (50 µl) to a clean 1.5 ml microcentrifuge tube. Discard pellet.

If pellet is disturbed repeat *steps 6.29* and *6.30* above.

31. Quantify the DNA sample on a NanoDrop spectrophotometer:
    - Expected Yield > 75 µg
    - A_{260}/A_{280} ratio ≥ 1.8
    - A_{260}/A_{230} ratio ≥ 1.9

If sample(s) do not meet A260/A280 or A260/A230 recommendations, then sample purification should be repeated. If sample(s) do not meet expected yield, repeat *Step 1.1* through *Step 6.31*.

32. FOR INEXPERIENCED USERS: Visualize 0.5-1 µg of each sample on a 4% agarose gel using an appropriate DNA marker (Figure 2).
    - Size range of the samples should be between 25 – 125 bp.
    - Majority of the fragments between 40-80 bp.

If this DNA fragment size range is not visualized as expected on the 4% agarose gel, repeat *Step 1.1* through *Step 6.31*.

33. STOP POINT: Proceed to *Chapter 3* or store the sample at -15 to -25°C (up to 1 month).

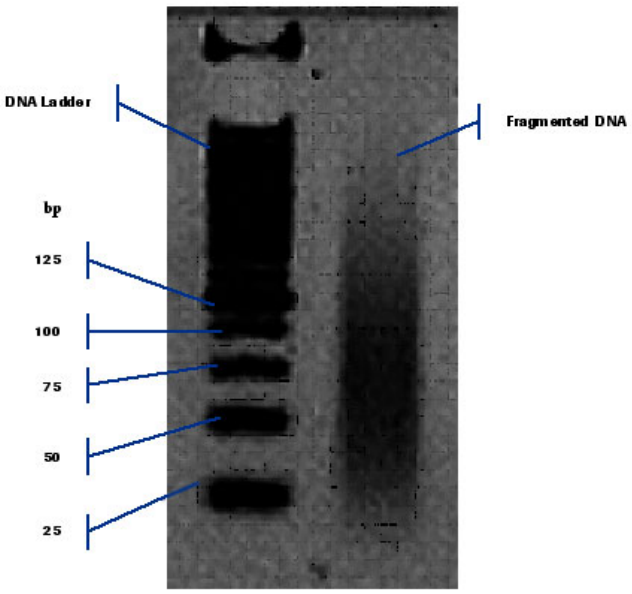


Figure 2: Evaluating your Fragmented DNA Sample. This figure illustrates a successfully fragmented sample. The first lane is a standard ladder of different sized DNA fragments. The goal is to have the size of the fragments in your DNA sample in the 25 to 125 bp range, with the majority of the DNA fragments in the intermediate range. If the majority of the sample is in the low range, the DNA fragments are over fragmented. If the majority of the sample is above the recommended range, the DNA fragments have not been fragmented enough.

Chapter 3. Performing End Labeling DNA

Chapter 3 describes how to label and clean up the DNA samples you amplified in *Chapter 2*. By using the NimbleGen AccuSNP Sample Preparation kit and AccuSNP DNA Purification kit, you are adding biotin to the 3´ end of the DNA fragments to allow visualization on the array after signal amplification.

AccuSNP Kits & Accessories Required

The AccuSNP kit components that are required for this portion of the protocol are as follows:

Kit	Components	Vial/Bottle No.
AccuSNP Sample Preparation Kit	TdT Reaction Buffer, 5x	vial 1
	CoCl2, 25 mM	vial 2
	Biotin-16-dUTP	vial 3
	Terminal Transferase, rec.	vial 4
DNA Purification Kit	Binding Buffer	bottle 1
	Binding Enhancer	bottle 2
	Wash Buffer	bottle 3
	Elution Buffer	bottle 4
	High Pure Filter Tubes	
	High Pure Collection Tubes	

Materials and Equipment Required

The following materials and equipment are required for this portion of the protocol:

Materials
0.2 ml thin-walled microcentrifuge tubes
1.5 ml microcentrifuge tubes
0.5 M EDTA , molecular biology-grade (pH 7.5 to 8.5)

Equipment
Thermocycler
Vacuum Concentrator (Speed Vacuum)
Microcentrifuge
Spectrophotometer

Step 1. Performing 3′ End-Labeling

1. Thaw all the required components of the AccuSNP Sample Preparation Kit on ice and store on ice until ready to use.
2. Prepare the following 3′ end-labeling master mix to process the 12 samples. Add the following reagents to a 1.5 ml microcentrifuge tube on ice.


Keep all reagents and 3′ end-labeling master mix on ice.

Component	Vial No.	Per single reaction	Master Mix Volume 12 reactions (x12.5)
TdT Buffer, 5x	vial 1	20 µl	250 µl
25 mM $CoCl_2$	vial 2	20 µl	250 µl
10 mM Biotin-dUTP	vial 3	1.6 µl	20 µl
Terminal Transferase	vial 4	2 µl	25 µl
Total Volume		**43.6 µl**	**545 µl**

3. Pipette up and down gently to mix.
4. Add 43.6 µl of the 3′ end-labeling master mix to 75 µg of each fragmented and dephosphorylated DNA sample in a 0.2 ml thin-walled tube.

Reactions	Volume
Fragmented and dephosphorylated DNA *(chapter 2. step 6.31)*	75 µg
3′ end-labeling reaction master mix	43.6 µl
PCR grade water	To volume
Total	**100 µl**

5. Mix by pipetting up and down 10 times.
6. Quick–spin to collect contents in bottom of the tube.
7. Incubate for 1 hour at +37°C in a thermocycler with a heated lid.
8. Incubate for 2 minutes at +95°C in a thermocycler with a heated lid.
9. Add 1 µl of 0.5M EDTA.

Step 2. Performing DNA Purification

1. Quick–spin to collect contents in bottom of the tube.
2. Transfer sample to a 1.5 ml microcentrifuge tube.
3. Add 200 µl of Binding Buffer to each sample.

Components	Volume
Reaction volume from *(step 1. 7)*	100 µl
Binding Buffer (bottle 1)	200 µl
Total	**300 µl**

The order of addition of reagents is important. The binding reagents (Binding Buffer and Binding Enhancer) must be added to the sample sequentially. DNA purification performance will significantly decrease if the order of addition is not followed according to the tables.

4. Vortex well. Quick–spin to collect contents in bottom of the tube.
5. Add 300 µl of Binding Enhancer to each sample.

Components	Volume
Reaction volume from (*step 2.3*)	300 µl
Binding Enhancer (bottle 2)	300 µl
Total	**600 µl**

6. Vortex well. Quick–spin to collect contents in bottom of the tube.
7. Transfer the sample (*step 2.6*) to the upper reservoir of an assembled High Pure Filter Tube.
8. Centrifuge at 12,000 x g for 60 seconds.
9. Discard the flow through.
10. Place the High Pure Filter Tube into the same sample collection tube.
11. Add 500 µl of prepared Wash Buffer (bottle 3) to the High Pure Filter Tube.
12. Centrifuge at 12,000 x g for 1 minute.
13. Discard the flow through.
14. Add 200 µl of prepared Wash Buffer (bottle 3) to the High Pure Filter Tube.
15. Centrifuge at 12,000 x g for 1 minute.
16. Discard the flow through.
17. Place High Pure Filter tube back in Sample Collection tube.
18. Centrifuge at 12,000 x g for 1 minute to dry the column.
19. Place the High Pure Filter Tube into a new 1.5 ml microcentrifuge tube.
20. Add 50 µl Elution Buffer (bottle 4) directly to the center of the High Pure Filter Tube.
21. Centrifuge at 12,000 x g for 1 minute.
22. Remove the eluant from 1.5 ml microcentrifuge tube and re-apply directly to the center of the High Pure Filter Tube.
23. Replace the High Pure Filter Tube into the same 1.5 ml microcentrifuge tube.
24. Centrifuge at 12,000 x g for 1 minute.
25. Discard the High Pure Filter Tube.
26. Centrifuge the eluant at 12,000 x g for 3 minutes.
27. Avoid disturbing the white pellet, transfer the supernatant (50 µl) to a new 1.5 ml microcentrifuge tube. Discard pellet.

28. Quantify the DNA sample on a NanoDrop spectrophotometer:

- Expected Yield > 50 µg
- A260/A280 ratio ≥ 1.8
- A260/A230 ratio ≥ 1.9

If sample(s) do not meet A260/A280 or A260/A230 recommendations, then sample purification should be repeated. If sample(s) do not meet expected yield, repeat *Chapter 2* and *Chapter 3*.

29. FOR INEXPERIENCED USERS: Remove 2 µg of labeled and purified DNA sample and perform a gel-shift assay (*Appendix A*) to measure the effectiveness of the biotin labeling step.

Step 3. Preparing Each Sample for SNP Microarray Hybridization

1. Transfer 50 µg of each labeled DNA *(step 2.26)* into a 1.5 ml microcentrifuge tube.

2. Dry samples in a vacuum concentrator on low heat.

Dried sample pellet should be clear and colorless. If pellet is white, this is evidence of carryover of fleece from the purification column. Samples with white pellets should be re-suspended in 50 µl of PCR grade water and then centrifuged at 12,000 x g for 10 minutes. Avoid disturbing the white pellet, transfer the supernatant (50 µl) to a new 1.5 ml microcentrifuge tube.

STOP POINT: Proceed to *Chapter 4* or store the DNA sample pellets at -15 to -25°C for up to 1 month.

Chapter 4. Hybridizing & Washing

Chapter 4 describes the NimbleGen protocol for sample hybridization and array washing using NimbleGen AccuSNP arrays in preparation for the enzymatic treatment of the arrays for SNP detection.

Requirements

- For the hybridization protocol, you must use a NimbleGen Hybridization System. Refer to the *NimbleGen Hybridization System Operator's Manual* for specific instructions on its use.
- This protocol requires the use of the NimbleGen MS200 Scanner. Refer to the *NimbleGen MS 200 Microarray Scanner Operator's Manual* for specific instructions on its use.
- The Alignment Oligo and Sample Tracking Controls (STCs) provided in the AccuSNP Sample Preparation and Sample Tracking Control Kits, respectively, are labeled with Cy dyes, which are sensitive to photobleaching and freeze-thawing. After thawing stock tubes for the first time, aliquot the Alignment Oligo and STCs into single-use volumes and freeze at -15 to -25°C. Protect tubes from light.

AccuSNP Kits & Accessories Required

The AccuSNP kit components that are required for this portion of the protocol are as follows:

Kit	Vial or Bottle No	Components
AccuSNP Sample Preparation Kit	vial 6	Alignment Oligo
	vial 7	Hybridization Buffer, 2x
	vial 8	Hybridization Component A
AccuSNP Enzyme Assay Kit	vial 4	Biotin Alignment Oligo
AccuSNP Array Processing Kit	bottle 1	10x Wash Buffer I
	bottle 2	10x Wash Buffer II
	bottle 3	10x Wash Buffer III
	vial 4	DTT
NimbleGen Sample Tracking Control Kit		Sample Tracking Control 1, 3, 5, 7, 9, 11, 13, 15, 16, 17, 18, 20

Materials and Equipment Required

The materials and equipment required for this portion of the protocol are as follows:

Materials
Inert gas
Water, reagent grade
50x Denhardt's Solution, molecular biology-grade
HX12 Mixer
Port Seals (12 for each slide)
SNP Array 12 arrays/slide
Microman M10 tips
Cotton swabs
Equipment
Hybridization System
Microarray Dryer
MS200 Scanner
Hybridization System Accessory Kit (Hybridization System Disassembly Tool, metal forceps, Gasket Brayer)
Precision Mixer Alignment Tool (PMAT)
Compressed Gas Nozzle
Microcentrifuge
+42°C and +95°C Heat Blocks
Microman M10 Pipette

Before you Begin

1. Set the Hybridization System to +42°C.
2. With the cover closed, allow at least 3 hours for the temperature to stabilize.

Be aware that the temperature of the Hybridization System could fluctuate during stabilization.

Step 1. Preparing the Hybridization Reaction

1. Ensure that you have heat blocks set at +42°C and +95°C.
2. Resuspend each dried sample pellet in 2.7 µl of a Sample Tracking Control. Use PCR grade water to resuspend the sample pellet, if the STCs are not used.
3. Vortex well and spin to collect contents in bottom of tube.
4. Record the Sample Tracking Control used with each sample.
5. Using components from the AccuSNP Kits, prepare the hybridization solution master mix by adding the following reagents in a 1.5 ml tube.

The table below will generate enough hybridization solution master mix to process 12 samples or 1 AccuSNP slide.

Component	Volume
2x Hybridization Buffer (vial 7)	88.5 µl
Hybridization Component A (vial 8)	35.4 µl
Alignment Oligo (vial 6)	1.8 µl
Biotin Alignment Oligo (vial 4)	1.8 µl
50x Denhardt's Solution	3.6 µl
Total	**131.1 µl**

6. Add 7.3 µl of the hybridization solution master mix to each tube containing 2.7 µl of resuspended DNA sample.

Component	Volume
Resuspended sample (*from step 1.2*)	2.7 µl
Hybridization solution (*from step 1.5*)	7.3 µl
Total	**10 µl**

7. Vortex well (approximately 15 seconds) and spin to collect contents in bottom of the tube. Incubate at +95°C for 5 minutes, protected from light.
8. Place the tubes at +42°C (in the Hybridization System sample block or heat block) for at least 5 minutes or until ready for sample loading onto AccuSNP array.
9. Vortex and spin prior to loading.

Step 2. Preparing Mixers

1. Locate the mixer. Remove from its package.

For best results, use a compressed gas nozzle to gently blow compressed nitrogen or argon gas across the mixer and slide to remove any dust or debris. The use of canned aerosol compressed air for this purpose is not recommended and could compromise array and data quality.

Load samples within 30 minutes of opening the vacuum-packaged mixer to prevent the formation of bubbles during loading and/or hybridization.

2. Position the Precision Mixer Alignment Tool (PMAT) with its hinge on the left. Open the PMAT (Figure 3).
3. Snap the mixer onto the two alignment pins on the lid of the PMAT with the tab end of the mixer toward the inside hinge and the mixer's adhesive gasket facing outward.
4. While pushing back the plastic spring with a thumb, place the slide in the base of the PMAT so that the barcode is on the right and the corner of the slide sits against the plastic spring. The NimbleGen logo and barcode number should be readable. Remove your thumb and make sure the spring is engaging the corner of the slide and the entire slide is registered to the edge of the PMAT to the rightmost and closest to you. In addition, be sure that the slide is lying flat against the PMAT. Gently blow compressed nitrogen or argon gas across the mixer and slide to remove dust.

While holding the slide by the edges, place the slide into the PMAT base using gloved fingers. The use of metal forceps may damage the glass slide and create a potentially hazardous condition (for example, broken glass) for the operator.

Take care to align the slide correctly in the PMAT. Incorrectly aligned slides may result in inaccurate attachment of the mixer and may affect the array features, or may not fit well into the Mixer Disassembly Tool used to remove the mixers after hybridization.

5. Using forceps, remove the backing from the adhesive gasket of the mixer and close the lid of the PMAT so that the gasket makes contact with the slide.
6. Lift the lid by grasping the long edges of the PMAT while simultaneously applying pressure with a finger through the window in the lid of the PMAT to free the mixer-slide assembly from the alignment pins.

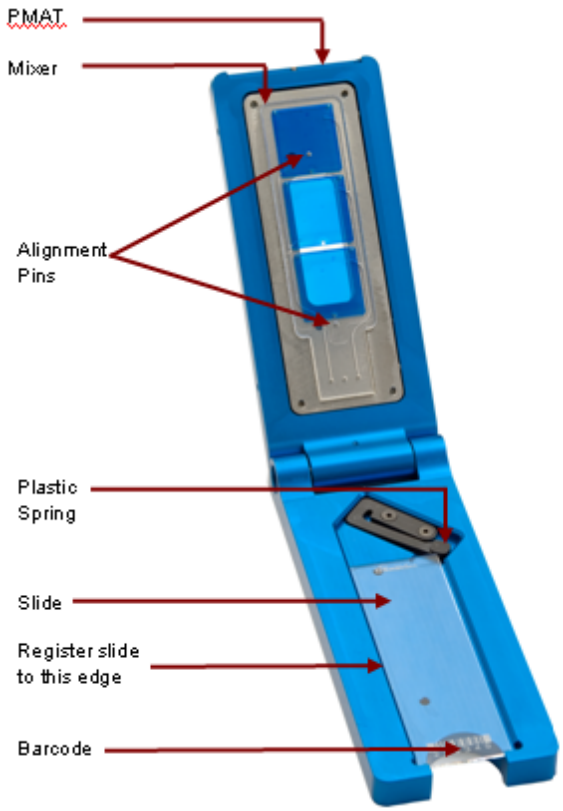


Figure 3: PMAT with Mixer and Slide. For photographic purposes only, blue coloring was used to show the location of the mixer's hybridization chambers. The hybridization chambers of the mixer you receive will not be blue.

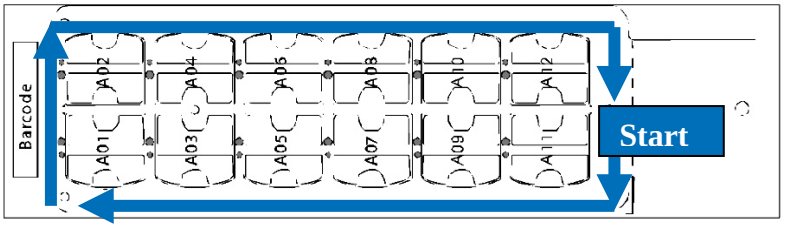
7. Remove the mixer-slide assembly from the PMAT.
8. Place the mixer-slide assembly on the back of a +42°C heating block for 5 minutes to facilitate adhesion of the mixer to the slide.
9. Rub the Mixer Brayer over the mixer with moderate pressure to adhere the adhesive gasket and remove any bubbles. Use the pattern depicted below to rub the borders between the arrays and around the outside of the arrays. The adhesive gasket will become clear when fully adhered to both surfaces.

10. Place the mixer-slide assembly in the slide bay of the Hybridization System.

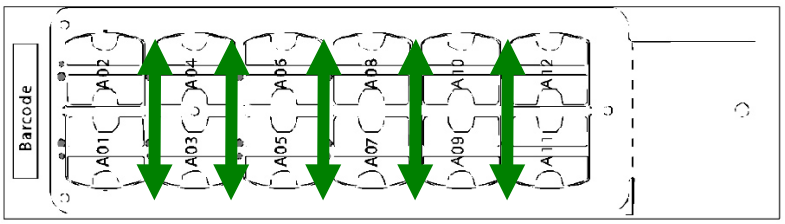
Performing Braying

The following steps are to illustrate braying or sealing the edges of the slides:

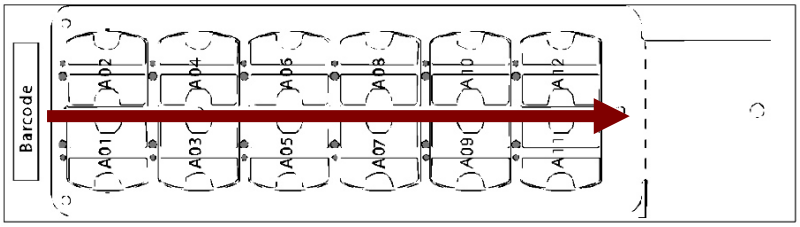
1. Complete the first step outlined below in blue.



2. Complete the second step shown below in green.



3. Complete the third step shown below in red.



4. Then repeat Steps 1 – 3.

Step 3. Loading and Hybridizing Samples

1. Keep the following in mind before loading sample:
   - When pipetting the sample before loading, leave residual volume in the sample tube to avoid introducing bubbles.
   - After aspirating the designated sample volume, inspect the pipette tip for air bubbles. Dispense and reload the pipette if bubbles exist.

   Keep the following in mind when loading sample:
   - Keep the pipette tip perpendicular to the slide to avoid possible leakage at the fill port.
   - Apply gentle pressure of the tip into the port to ensure a tight seal while loading the sample.
2. Slowly aspirate 8 µl of the hybridization reaction into a pipette tip using a positive displacement Gilson Microman M10 pipette.

3. Load sample into a fill port (Figure 4)
   - Due to the close proximity of the fill and vent ports, do not overfill the arrays.
   - Load sample until it enters the vent port channel.
   - Do not allow sample to overflow the surface of the mixer.
   - Dry any overflow from the fill and vent ports with a cotton swab after loading the array. Use a fresh cotton swab for each loaded sample.
   - It is not unusual for small bubbles to form in the corners of the mixer-slide assembly during loading. These bubbles will dissipate upon mixing and will not compromise the data.
4. Use one mixer port seal to cover both the fill and vent ports on the mixer, filling and sealing one chamber at a time. Press the mixer port seal, using uniform pressure across the seal to adhere. Use forceps to press the mixer port seal around the fill and vent ports to ensure it is adhered in those areas.

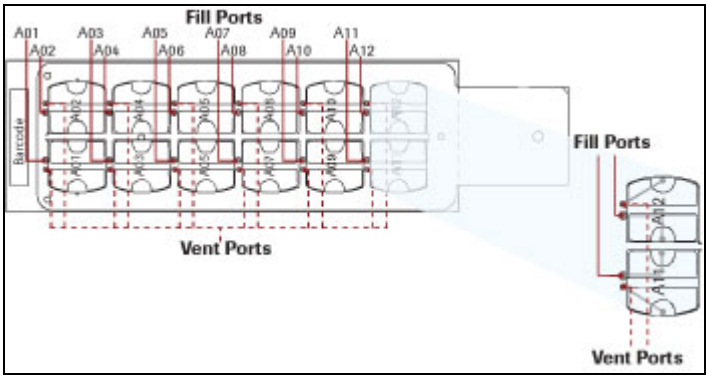


Figure 4. AccuSNP Slide with Mixer

5. Close the bay clamp.
6. Turn on the Mixing Panel on the Hybridization System. Set the mix mode to B and press the mix button to start mixing. Confirm that the Hybridization System recognizes the slide in each occupied bay (its indicator light becomes green).
7. Approximately 10 minutes after starting the Hybridization System:
   - Ensure the mix mode is set to B.
   - Ensure a green light is displayed for all occupied stations.
8. Allow samples to hybridize in the Hybridization System at +42°C for 16 – 20 hours.

Step 4. Washing Post-Hybridization SNP Arrays

To ensure high quality data, it is important to proceed through all the washing and drying steps without interruption..

1. Prepare Solutions.

Quantity	Solution	Instructions
1.2 ml	1 M DTT	Reconstitute the DTT (vial 4) from the AccuSNP Array Processing kit. In a fume hood, prepare 1 M DTT solutions by adding 1.2 ml of PCR grade water to each tube of dry DTT. After reconstitution, store the 1 M DTT solutions at -15 to -25°C.

2. Prepare 1x Wash Buffers I, II, and III.

1x Wash Buffer I	Volume
Reagent grade water	486 ml
10x Wash Buffer I	54 ml
1 M DTT	54 µl
Total Volume	**540 ml**

1x Wash Buffer II	Volume
Reagent grade water	243 ml
10x Wash Buffer II	27 ml
1 M DTT	27 µl
Total Volume	**270 ml**

1x Wash Buffer III	Volume
Reagent grade water	243 ml
10x Wash Buffer III	27 ml
1 M DTT	27 µl
Total Volume	**270 ml**

3. Dispense 270 ml of 1x Wash Buffer I, 1x Wash Buffer II, and 1x Wash Buffer III each into separate, labeled NimbleGen Array Accessory Wash Tanks and hold at room temperature.
4. Prepare a workspace with the buffers and paper towel for blotting.
5. Place a slide holder into the wash tank containing the room temperature 1x Wash Buffer I.
6. To facilitate the removal of the mixer, heat a shallow dish containing Wash Buffer I to +42°C. Roche NimbleGen recommends measuring the temperature of Wash I at every use.
7. Insert the Mixer Disassembly Tool into the shallow dish containing warm Wash Buffer I. Remove the mixer-slide assembly from the Hybridization System and load into the Mixer Disassembly Tool submerged in the pre-heated 1x Wash Buffer I.

Do not allow the mixer-slide assembly to cool before removing the mixer. If processing more than one AccuSNP slide, keep power on to the Hybridization System's heat block and mixer system during mixer-slide disassembly, and transfer each mixer-slide assembly one at a time to Wash I for immediate removal of the mixer.

8. With the mixer-slide assembly submerged, carefully peel the mixer off the slide (Figure 5). It is important to hold the Mixer Disassembly Tool flat while removing the mixer and to avoid any horizontal movement or scraping with the mixer across the slide. Do not touch the array surface of the slide.

The mixer is extremely flexible. Peel the mixer off slowly to avoid breaking the slide.

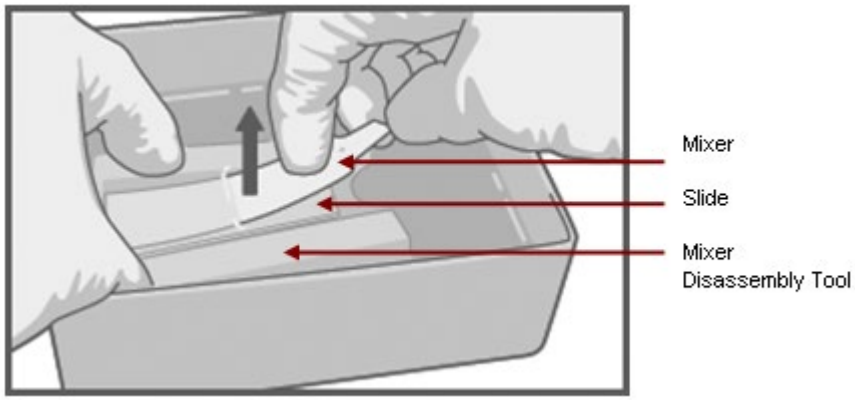
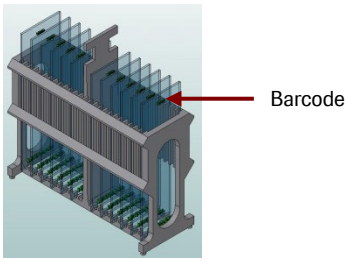


Figure 5. Using the Mixer Disassembly Tool to Remove a Slide from a Mixer

9. Working quickly, discard the mixer and remove the slide from the Mixer Disassembly Tool. Gently agitate the slide for 10 - 15 seconds in the shallow dish containing warm Wash I to quickly remove the hybridization buffer.

10. Transfer the slide to the slide rack submerged in Wash Buffer I so that the slide barcode is at the top of the slide rack.



11. Wash for an additional 2 minutes in Wash Buffer I.. Shake the slide container at least 20 times every 10 seconds.

At several times during the wash, rock the wash tank so the wash solution covers and cleans the top of the slide(s).

12. Remove the slide rack from the dish and quickly blot the edges of the slide several times using paper towels to minimize buffer carryover. Transfer the slide to Wash Buffer II and wash for 1 minute with vigorous, constant agitation.

Do not allow the slide to dry between wash steps.

13. Transfer the slide to Wash Buffer III and wash for 15 seconds with vigorous, constant agitation.

14. Remove the slide from Wash Buffer III. Immediately spin dry in an ArrayIT microarray dryer per the manufacturer's recommendation for 30 seconds.

15. Remove the slide from the microarray dryer. Blot the edges of the slide with lint-free paper to remove any residual moisture.

16. Scan the slides at 5 µm resolution at a gain setting (such as 50%) to achieve the desired alignment oligo intensity that is less than saturation (ideally between 25,000-50,000). Adjust the scanner gain setting as needed to achieve the optimal alignment oligo signal intensity. Refer to the Roche NimbleGen *MS200 Scanner User's Guide* for more information.

This post-hybridization scan allows for the monitoring of the Cy-labeled alignment oligos and STCs. These alignment oligos, in addition to providing array gridding information, can be used as an indirect check that the sample hybridized to the microarray. Analysis of this image is presented in *Chapter 8. Step 3* to evaluate the sample tracking controls.

The biotin-labeled alignment oligo, which was also added to the hybridization solution master mix, will be detected by the final scan of the microarray (*Chapter 7*). To be detected, these alignment oligos require the on-slide enzymatic treatment and signal amplification steps. These oligos provide gridding information for the array image containing the SNP data, as well as serve as a measure of the performance of the enzymatic and signal amplification steps.

17. Proceed directly to *Chapter 5. Performing Array Enzymatic Treatment.*

Notes

Chapter 5. Performing Array Enzymatic Treatment

Chapter 5 describes the steps for the enzymatic treatment of NimbleGen AccuSNP Arrays. This enzymatic treatment facilitates the detection of the SNPs present in the samples.

The protocol is written for the processing of one slide. However, at this point in the SNP Analysis workflow, up to four AccuSNP slides can be processed together through the remaining SNP analysis steps: *Chapter 5: Performing Array Enzymatic Treatment* and *Chapter 6: Performing Signal Amplification*

AccuSNP Kits & Accessories Required

The AccuSNP kit reagents that are required for this portion of the protocol are as follows:

Kit	Vial No.	Product Components
AccuSNP Enzyme Assay Kit	vial 1	20x AccuSNP Array Buffer
	vial 2	AccuSNP Ligase
	vial 3	AccuSNP Assay Enzyme
AccuSNP Array Processing Kit	bottle 1	10x Wash Buffer I
	bottle 2	10x Wash Buffer II
	bottle 3	10x Wash Buffer III
	vial 4	DTT

Materials and Equipment Required

The materials and equipment required for this portion of the protocol are as follows:

Materials
10x PBS
20 mg/ml BSA
NAD Grade 1, free acid
Western Blocking Reagent
PCR grade water
Reagent grade water
30 ml Slide Staining Container.
Equipment
+50°C and +98°C Water Baths

Before You Begin

1. Ensure that you have water baths set at +50°C and +98°C.

Prepare the following solution:

Quantity	Solution	Instructions
5 ml	200 mg/ml (300 mM) NAD	Resuspend 1 g of NAD in 5 ml of PCR grade water. Prepare single use vials by aliquoting 60 µl to several individual tubes. Store the aliquots at -15 to -25ºC.

Step 1. Preparing Enzymatic Buffers

This protocol makes enough reagent to process up to 4 slides.

1. Label 2 slide containers – A and B.
2. Prepare 1x AccuSNP Array Buffer according to the following table in the 30 ml slide container labeled A.

Components	Volume
20x AccuSNP Array Buffer (vial 1)	1.5 ml
20 mg/ml BSA	150 µl
300 mM NAD	50 µl
PCR grade water	28.3 ml
Total Volume	**30 ml**

3. Pre-heat the prepared 1x AccuSNP Array Buffer for 20 minutes in a +50ºC water bath.
4. Pre-heat 270 ml of reagent-grade water in a 270 ml wash tank with lid in a +98ºC water bath.
5. Prepare the Blocking / Wash Buffer according to the following table in the 30 ml slide container labeled B.

Components	Volume
1x PBS	27 ml
Western Blocking Reagent	3 ml
Total Volume	**30 ml**

Step 2. Performing AccuSNP Array Enzymatic Treatment

1. Place the AccuSNP slide from *Chapter 4. Step 4.15* into the slide container B containing the prepared Blocking Buffer.
2. Incubate for 10 minutes at room temperature (+15 to +25ºC).
3. Add the AccuSNP enzyme assay components to the pre-heated 1x AccuSNP Array Buffer in container A.

Components	Volume
Pre-heated 1x AccuSNP Array Buffer (from *Step 1.3*)	30 ml
AccuSNP Ligase (vial 2)	2.7 µl
AccuSNP Assay Enzyme (vial 3)	9.43 µl
Total Volume	**~30 ml**

4. Mix by inverting the slide container 3-4 times.

5. Transfer the slide from the blocking buffer (*Step 2.2*) in slide container B to the slide container A containing the Enzyme Assay Mix (prepared in *Step 2.3*).

If processing more than one slide, ensure there are no bubbles between slides. If bubbles occur, tap container lightly on benchtop to dislodge them before proceeding.

6. Incubate the slide in the AccuSNP Enzyme Assay Buffer for 1 hour at +50°C.
7. During the 1 h incubation, prepare fresh wash buffers as indicated in *Step 3*.

Step 3. Preparing Post-Enzymatic Treatment Washes

1. Prepare 1x Wash Buffers I, II, and III by combining the following:

1x Wash Buffer I	Volume
Reagent grade water	243 ml
10x Wash Buffer I	27 ml
1 M DTT	27 µl
Total Volume	**270 ml**

1x Wash Buffer II	Volume
Reagent grade water	243 ml
10x Wash Buffer II	27 ml
1 M DTT	27 µl
Total Volume	**270 ml**

1x Wash Buffer III	Volume
Reagent grade water	243 ml
10x Wash Buffer III	27 ml
1 M DTT	27 µl
Total Volume	**270 ml**

2. Dispense 270 ml of 1x Wash Buffer I, 1x Wash Buffer II, and 1x Wash Buffer III each into separate, labeled NimbleGen Array Accessory Wash Tanks and hold at room temperature.
3. Dispense 270 ml room temperature reagent grade water into a wash tank.
4. Prepare a workspace with a setup of buffers, +98°C water, room temperature water, and paper towels for blotting.

Step 4. Performing Post-Enzymatic Treatment Washes

To ensure high quality data, it is important to proceed through all the washing and drying steps without interruption.

1. Transfer the slide from the AccuSNP Enzyme Assay Buffer into a slide rack submerged in 1x Wash Buffer I. Do not let the slide dry.
2. Agitate the slide rack vigorously for 2 minutes. Bubbles/foam should form in the buffer to indicate agitation is vigorous enough.
3. Quickly blot the slide rack on a stack of paper towels, and transfer the slide rack into the wash tank containing 1x Wash Buffer II.

Do not allow the slide to dry between wash steps.

4. Agitate the slide rack vigorously in 1x Wash Buffer II for 1 minute. Bubbles/foam should form in the buffer to indicate agitation is vigorous enough.
5. Remove the slide rack from the dish and quickly blot the slide rack on a stack of paper towels, and transfer the slide rack into the wash tank containing 1x Wash Buffer III.
6. Agitate the slide rack vigorously in 1x Wash Buffer III for 15 seconds.
7. Transfer the slide rack to the pre-warmed H_2O wash tank in the +98°C water bath.
8. Place handle extender onto slide rack.
9. Keeping the tank in the water bath and holding on to its rim with one hand, agitate the slide rack for 2 minutes.
10. Transfer the slide rack into the wash tank containing room temperature water and agitate gently and briefly, just enough to cool the slide. Immediately spin dry in an ArrayIT microarray dryer per the manufacturer's recommendation for 30 seconds.
11. Remove the slide from the microarray dryer. Blot the edges of the slide with lint-free paper to remove any residual moisture. Proceed to *Chapter 6. Performing Signal Amplification* or store dried slide at room temperature in its original case in a dark desiccator. Slide may be stored up to overnight.

Chapter 6. Performing Signal Amplification

Chapter 6 describes the steps necessary to detect the biotin-labeled sample on the AccuSNP Array.

Contact Tecan for replacement parts for the spacers and backplates. Refer to *Appendix D* for more information.

To avoid damage to the springs of the assembly, be aware of the following:

- The springs of the 8-Multifold are very sensitive parts. They provide optimal tightness of the chamber by holding the flow-through chamber together.
- Inappropriate handling during assembly, dismantling and cleaning can result in deformed springs. In this state, a tight chamber during the entire flow through process can't be guaranteed.
- During assembly and dismantling do not touch, bend or lift the springs of the 8-Multifold lid.
- Cleaning should only be done by rinsing with distilled water and ethanol. Please do not clean the springs mechanically.
- Deformed springs can be exchanged with new springs from the spare part kit.

Be aware of the following before you begin:

- Cy3 is used in the signal amplification step.
- Cy dyes are light sensitive. Be sure to minimize light exposure of the dyes during use and store in the dark when not in use.
- Cy dyes are ozone sensitive. Take the necessary precautions to keep atmospheric ozone levels below 5 ppb (parts per billion).
- Cy dyes are humidity sensitive. Take the necessary precautions to keep humidity levels below 40%.

AccuSNP Kits & Accessories Required

The AccuSNP reagents that are required for this portion of the protocol are as follows:

Kit	Vial No	Components
AccuSNP Detection Kit	vial 1	Cy3-Streptavidin
	vial 2	Biotinylated Anti-Streptavidin

Materials and Equipment Required

The materials and equipment required for this portion of the protocol are as follows:

Materials
10x PBS
Western Blocking Reagent
Alconox Powder Detergent
PCR grade water
Reagent grade water
0.2 ml microcentrifuge tubes
1.5 ml microcentrifuge tubes
Rectangle 9x13 inch Glass Dish
15 ml conical tubes
Equipment
MS 200 Microarray Scanner
8-manifold mounting plate
8-manifold flow-through chamber which includes the 8 multifold lid, 8 multifold base, and joint rods (anchor rods)
Rack TeFlow No Thermal Block GenePaint (chamber rack)
backplate *
spacers
Chamber Rack Waste Tray

* Refer to *Appendix C. Cleaning Protocol for Glass Backplate* for more information.

Step 1. Before you Begin

Ensure that the glass plates have been cleaned as described in Appendix C.

The following solutions need to be prepared before starting the protocol.

Solution	Quantity	Instructions	Components	Volume
1x PBS	500 ml	Combine the following:	n 10x PBS	50 ml
			n Reagent grade water	450 ml

Solution	Quantity	Instructions
Streptavidin-Cy3	75 µl	n Add 75 µl PCR grade water to the lyophilate (vial 2). n Prepare single use aliquots by pipetting 9 µl into 8 new 0.2 ml tubes. n Store aliquots at -15 to -25°C, protected from light.

Step 2. Preparing Signal Amplification Buffers

1. Prepare the Signal Amplification Blocking/Wash Buffer in a 30 ml slide container. Mix by swirling. Hold at room temperature.

This buffer is for single use only. Discard after use.

Components		Volume
Blocking/Wash Buffer	1x PBS	13.5 ml
	Western Blocking Reagent	1.5 ml
	Total Volume	**15 ml**

2. Prepare the Streptavidin-Cy3 reagent in a 15 ml conical tube. Mix by swirling. Hold at room temperature.

This buffer is for single use only. Discard after use.

Components		Volume
Streptavidin-Cy3 Reagent	10x PBS	800 µl
	Western Blocking Reagent	800 µl
	Streptavidin-Cy3	8 µl
	PCR grade water	6.4 ml
	Total Volume	**8 ml**

3. Prepare fresh Biotinylated Anti-Streptavidin Reagent in a 15 ml conical tube. Mix by swirling. Hold at room temperature.

This reagent is for single use only. Discard after use.

Components		Volume
Anti-Streptavidin Reagent	10x PBS	200 µl
	Western Blocking Reagent	200 µl
	Biotinylated anti-Streptavidin (vial 2)	40 µl
	PCR grade water	1.56 ml
	Total Volume	**2 ml**

Step 3. Assembling the Flow Through Chamber

For more information about how to assemble the flow-through chamber, refer to *Appendix D.*

1. Assemble the 8–Multifold Base onto the Mounting Plate. Align the "1" and "8" labels etched on the 8–Multifold Base with the "1" and "8" labels etched on the Mounting Plate. Place the 8–Multifold Base onto the Mounting Plate. The 8–Multifold Base should be secure and should not shift on the base.
2. Place the Mounting Plate assembly into a 9x13 inch rectangular glass dish so that the numbers are closest to you.
3. Dispense reagent grade water so the liquid level reaches half-way up the combs (Figure 6) located at the back of the assembly.

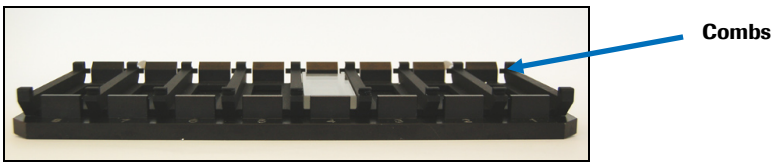


Figure 6. Illustration of Combs

4. With the reservoir opening facing up, set the glass backplate at an angle with the flat bottom against the numbered side of the 8–Multifold base. Slowly lower the glass backplate down onto the 8–Multifold Base so that it is partially submerged in the nuclease-free water, such that the reservoir end of the backplate is at the top of the assembly. The backplate will fit securely.
5. Repeat this step for each slide that will be processed.
6. Remove and discard the white backing from a clear spacer and place the clear spacer onto the top of the glass backplate.

Do not use the white spacer backing.

7. Hook the ends of the clear spacer around the combs of the Mounting Plate (Figure 7). The combs are at the lips at the top and bottom plate.

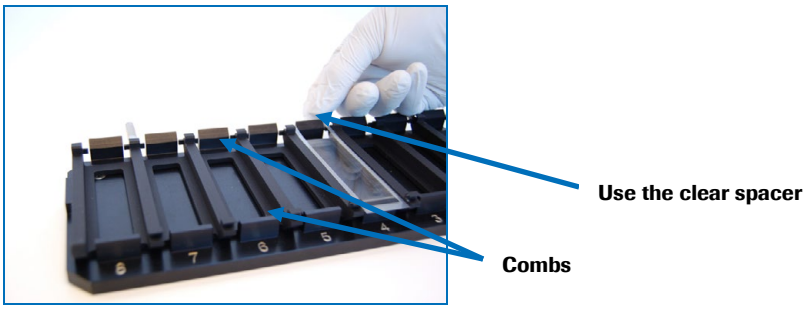


Figure 7. Hook the Spacer

8. Ensure that the clear spacer is wet and submerged in the water before proceeding. If not, use a finger to wet the spacer and eliminate any bubbles.
9. Grip the microarray slide by the sides and place it array side down with the "NimbleGen" label next to the numbers etched in the Multifold Base, on top of the clear spacer (Figure 8). The slide should rest on the notch located near the numbers etched in the mounting plate. The barcode will be at the top of the slide.

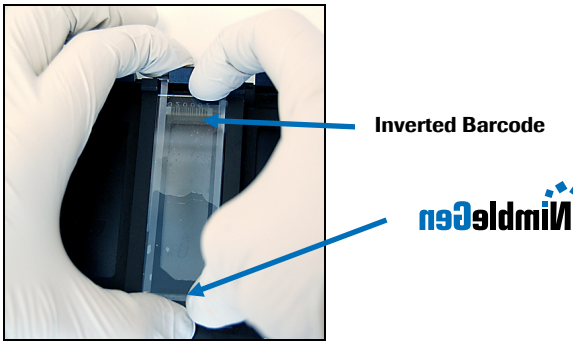


Figure 8. Placing the Microarray Slide, indicating appearance of etching when the slide is placed in the correct orientation.

10. Assemble the 8- Multifold Lid on top of the microarray slide. Align the "1" and "8" labels etched in the Mounting Frame with the "1" and "8" labels etched in the Mounting Base.
11. Lift up the assembly with one hand, while holding the lid firmly to the base. With the other hand, slide an anchoring rod through the top combs of the assembly until the flat head of the anchoring rod is flush with the side of the flow chamber.
12. Slide the second anchoring rod through the bottom combs of the assembly until the flat head of the anchoring rod is flush with the side of the flow chamber.
13. Using the transport screws, lift the flow-through assembly. Rotate the assembly so that the number 1 is on the left and the top is facing away from you.
14. Place the assembly into the chamber rack. Hook the lip of the chamber over the lip of the chamber rack.

The mounting base will remain in the glass container.

Step 4: Performing Signal Amplification

Air bubbles should be avoided through the signal amplification step. If bubbles are present, use a pipette tip to remove the bubbles. See *Appendix B* for a laboratory worksheet to track the completion of the signal detection steps.

1. Dispense 500 µl Blocking/Wash Buffer (from *Step 2.1*) into the reservoir of the flow-through chamber. Allow the Blocking/Wash Buffer to flow down over the slide by gravity.
2. Repeat step 4.1 five times, for a total of 3 ml Blocking/Wash Buffer.
3. Incubate the AccuSNP slide for 10 minutes.
4. Dispense 500 µl Streptavidin-Cy3 reagent (from *step 2.2*) into the reservoir of the flow-through chamber. Allow the Streptavidin-Cy3 to flow down over the slide by gravity.
5. Repeat step 4 three times, for a total of 2 ml Streptavidin-Cy3 reagent.
6. Incubate the AccuSNP slide for 10 minutes.
7. Dispense 500 µl Blocking/Wash Buffer into the reservoir of the flow-through chamber. Allow the Blocking/Wash Buffer to flow down over the slide by gravity.
8. Repeat step 7 five times, for a total of 3 ml Blocking/Wash Buffer.

9. Incubate AccuSNP slide for 10 minutes.
10. Dispense 500 µl Anti-Streptavidin reagent (from *step 2.3*) into the reservoir of the flow-through chamber. Allow the Anti-Streptavidin reagent flow down over the slide by gravity.
11. Repeat step 10 once more for a total of 1 ml of Anti-Streptavidin reagent
12. Incubate the AccuSNP slide for 10 minutes.
13. Dispense 500 µl Blocking/Wash Buffer into the reservoir of the flow-through chamber. Allow the Blocking/Wash Buffer to flow down over the slide by gravity.
14. Repeat step 13 five times for a total of 3 ml of Blocking/Wash Buffer
15. Incubate the AccuSNP slide for 10 minutes.
16. Repeat steps 4.4-4.15.
17. Dispense 500 µl Streptavidin-Cy3 reagent into the reservoir of the flow-through chamber. Allow the Streptavidin-Cy3 to flow down over the slide by gravity.
18. Repeat step 17 three times for a total of 2 ml of Streptavidin-Cy3 reagent
19. Incubate the Streptavidin-Cy3 on the microarray slide for 10 minutes.

Step 5. Disassembling the Flow-Through Chamber

1. Prepare a workspace on a lab bench with the following reagents dispensed into 270 ml separate wash tanks: 270 ml 1x PBS and 270 ml reagent grade water.
2. Remove the flow-through assembly from the chamber rack and place back onto the mounting plate on a laboratory bench with the etched numbers facing up. Ensure that the clear spacer is located around both combs.
3. Pull to remove the anchor rods.
4. Grip, lift, and remove the top assembly frame.
5. While holding the ends of the spacer with one hand, grip the microarray slide on the side and lift from the flow-through assembly.
6. Place the microarray slide into a slide rack in the wash tank containing 270 ml 1x PBS.

Step 6. Performing Post Signal Amplification Washes

1. Vigorously agitate the slide rack to wash the slide (from *step 5.6*) in 1x PBS for 2 minutes.
2. Transfer the microarray slide into the wash tank containing water. Agitate for 5 seconds. Immediately spin dry in an ArrayIT microarray dryer per the manufacturer's recommendation for 30 seconds.
3. Remove the slide from the microarray dryer. Blot dry the edges of the slide with lint-free paper to remove any residual moisture.
4. Proceed directly to *Chapter 7. Scanning One-Color Arrays*.

Chapter 7. Scanning One-Color Arrays

Chapter 7 describes the protocol for scanning one-color NimbleGen arrays with the MS 200 Microarray Scanner and the MS 200 Data Collection Software.

Before You Begin

Before starting these procedures, review the information in the NimbleGen MS 200 Microarray Scanner Operator's Manual (available at *www.nimblegen.com/products/instruments/* under Literature) or the online help available via the MS 200 Data Collection Software. These materials provide more detailed instructions on using the scanner, control unit (computer), and software than provided in this chapter.

Keep arrays in a dark desiccator until you are ready to scan them.

When handling slides, wear powder-free gloves and use care to touch only the slide's edges.

To mitigate ozone problems in the lab environment, Roche NimbleGen recommends that you place an ozone scrubber and ozone protection box over the scanner during scanning. At ozone levels of less than 5 ppb, it is possible to rescan the arrays without any photobleaching effects. However, in the presence of ozone levels that are greater than 5 ppb, Cy dye signal degradation can occur over time or during rescanning of the same slides.

Step 1. Starting the Scanner

1. Start the control unit and log into your user account as msOperator or other account as set up by your system administrator.

   Account: **msOperator**
   Password: **1-msOperator**

2. Turn on the scanner using the power switch on the left side. Ensure that the main power switch on the left rear of the scanner is also in the on position.
3. Using a compressed gas nozzle, gently blow compressed nitrogen or argon gas across the slide to remove any dust or debris from the arrays. Do not use canned aerosol compressed air for this purpose as it could compromise data quality.
4. Insert slides into the Slide Magazine.
5. Insert the slide magazine with loaded slides into the scanner.

Step 2. Starting the Software and Turning on the Lasers

1. Launch the MS 200 Data Collection Software. Ensure that the software has completely loaded before continuing.

2. Turn on the lasers by clicking the **Green Laser** button in the Laser Control (Figure 9) pane.

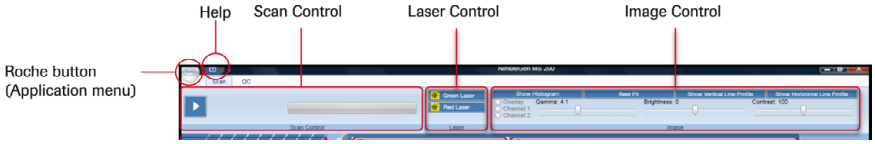


Figure 9: Top of Data Collection Workspace, showing Scan Control, Laser Control, and Image Control

3. Confirm that the Magazine Control pane (Figure 10) displays a green box appears in the *Slide Present* field for each slide loaded into the slide magazine.

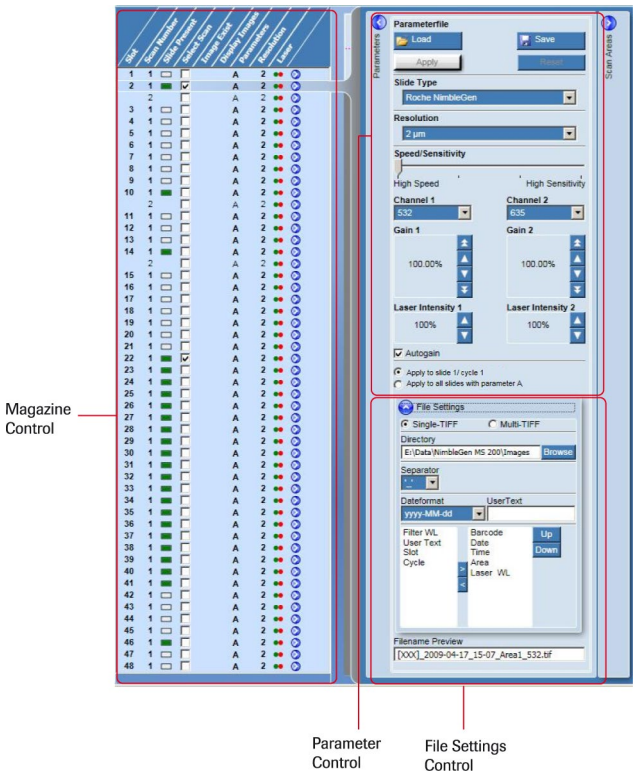


Figure 10: Magazine Control, Parameter Control, and File Setting Control

Step 3. Setting Scan Parameters

1. If not done already, download the AccuSNP session file from *www.nimblegen.com/accusnp.sessx* and save this file in the folder Computer>Data(E:)>Data>NimbleGen MS 200>Sessions.
2. Click the Roche button in the upper left-hand corner of the MS200 Data Collection Software window to display the Application menu (Figure 9).
3. Select **Open**.
4. In the Sessions folder, select the AccuSNP.sessx file and open. This will add twelve (12) unique scanning cycles to each slide in the Magazine Control Window.
5. In the Magazine Control pane (Figure 10) select the slides and arrays to be scanned.
6. The cycle number of each array corresponds to the subarray number (subarray A01 is scanned in cycle 1, subarray A02 is scanned in cycle 2, etc.)

7. If necessary, use the File Settings Control pane (Figure 10) to change the path to which the image files will be saved. The default path is *Computer>Data(E:)>Data>NimbleGen MS 200>Sessions*. To change the path, click **Browse** to open a dialog box to specify a location in the directory and click **OK** to confirm.

8. Do not change any other parameter settings.

Step 4. Scanning the Slides

1. Click the **Start/Stop Scan** button ( ) in the Scan Control pane (Figure 9).

2. When prompted, specify the folder and file naming to save the session file. Do not save over the AccuSNP.sessx.

3. Once scanning begins, the Parameter Control and Area Definition Control pane closes. The Image View, Slide View, and Spot View opens (Figure 11: Example of a Run Time Window

4. The current task is identified in the progress bar.

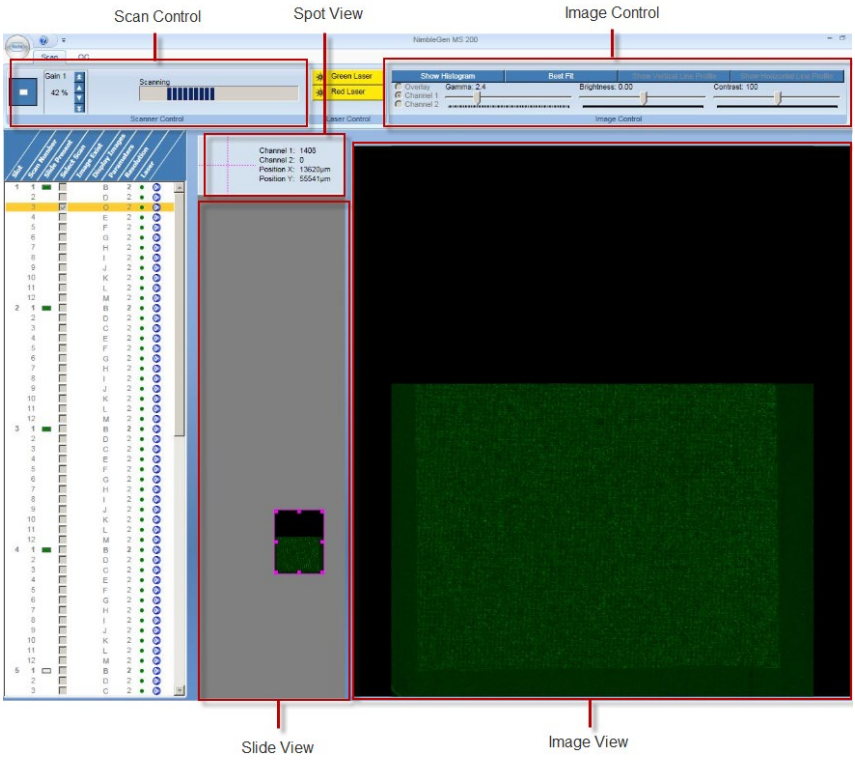


Figure 11: Example of a Run Time Window

5. Once scanning is completed, the light in the upper-left corner of the insert/eject magazine button on the scanner becomes green. The scanner unlocks its stacker cover, and the slide magazine can be removed.

Notes

Chapter 8. Extracting Data with DEVA Software

Chapter 8 describes the general steps involved in importing a scanned image and extracting the data using DEVA software. Refer to the *DEVA Software User's Guide* for computer system requirements and detailed information on using the software.

Setting up a Project

To begin automatically extracting data from your images using DEVA, you must set up a new project according to instructions in Chapter 3 of the *DEVA Software User's Guide*.

Selecting Analysis Workflows

DEVA software will run analyses on the data from your bursted and aligned images using the specified application type: AccuSNP.

Chapter 4 of the *DEVA Software User's Guide* provides information on how to:

- Select a workflow
- Use the *Analysis Workflow* window
- View examples of workflows
- Create customized workflows for your applications

Loading Designs

- DEVA software analyzes your scanned array images using the correct design files by matching the barcode in the design file(s) to the barcodes on the scanned image(s). After initially uploading the design (.zip) file, the design will continue to be used for new images, as long as the barcodes match and a default workflow analysis has been selected.
- Chapter 5 of the *DEVA User's Guide* provides information on how to load design files and add a design file to a slide barcode.

Importing Annotation

Many annotation files will load automatically as part of the NimbleGen Array design (.zip) file. However, if other annotation files are needed, you can load them manually.

Chapter 6 of the *DEVA User's Guide* provides information on how to select, load, and view annotations.

Monitoring Image Processing

DEVA software will automatically upload and process images that are placed in the Image Directory. The *DEVA Software User's Guide* provides information on how to monitor image processing.

Reviewing Processed Slides

The *DEVA Software User's Guide* provides information on how to view all of the slides that have been uploaded by DEVA software, and were successfully burst (if multiplex) and aligned.

Even though DEVA software confirms alignment, Roche NimbleGen recommends that you manually review the thumbnails and the entire image to confirm correct alignment.

Adding Information to the Hybridization Plan

The Hybridization Plan is useful for recording details about your array experiment. You can create a plan in DEVA software after you have imported a design file.

Chapter 7 of the *DEVA Software User's Guide* provides information on creating a hybridization plan

Reviewing Experimental Results

DEVA software will automatically run the default analyses for each application. When the jobs have completed, you can view the results. Note that the workflows in DEVA software do not include the generation of the promoter reports that map peak data to annotation files.

Chapter 9 of the *DEVA User's Guide* provides information on how to access and view the analysis results.

Confirming Experimental Integrity

As noted in this guide, you should add a unique STC to each sample prior to loading onto an AccuSNP-12 array. This control hybridizes to probes on the microarray, and enables you to confirm the sample identity on each array and ensure integrity of the experiment. STC probes are placed as repeating sets of 20 along the perimeter of each array and as two 4 x 5 blocks in the upper left corner and in the center of the array (Figure 12).

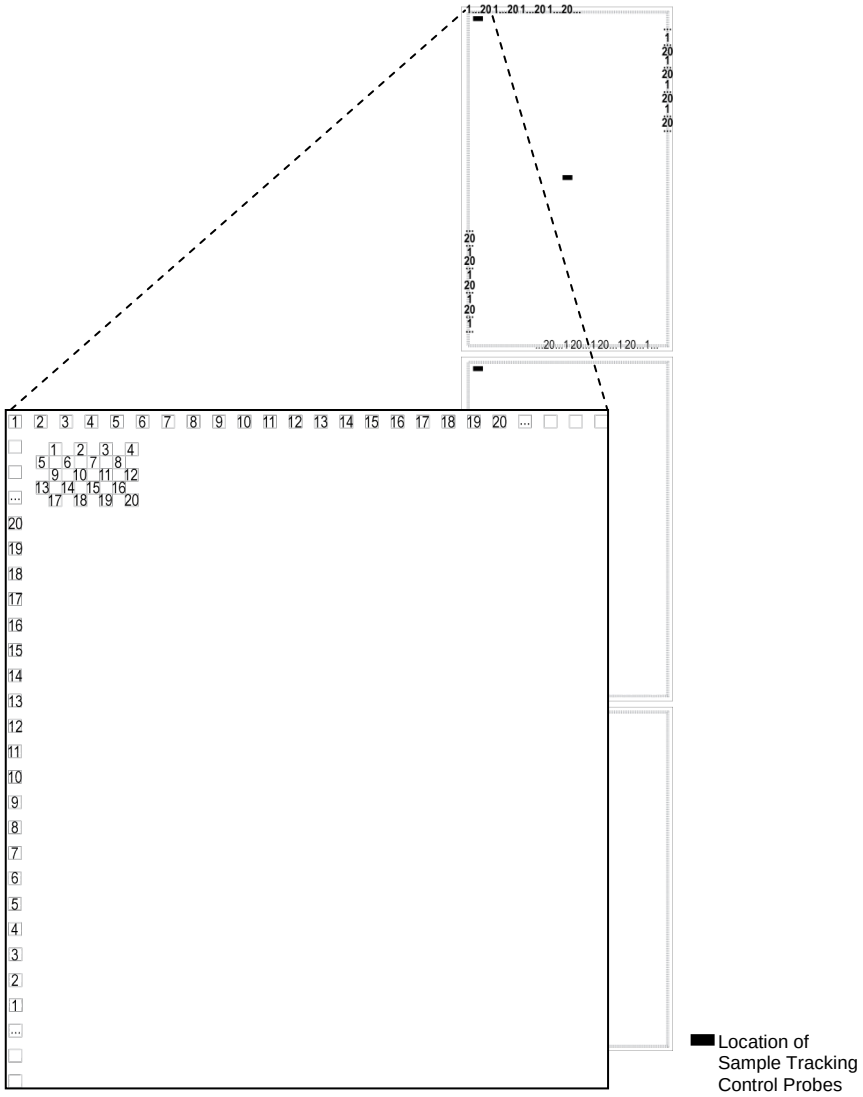


Figure 12. Location and Numbering of Sample Tracking Control Probes on an AccuSNP-12 Array

Roche NimbleGen recommends that you perform a sample tracking analysis and then visually check the STC features along the perimeter to confirm that the correct sample has been added to each array. You can perform the sample tracking analysis by generating a Sample Tracking Control Report. Visually check the STC features along the perimeter by using the following steps:

1. From the *Processed Data Slides* tab in the DEVA software, select the slide barcode of interest.
2. Click **Alignment Details**.
3. Select the desired image.
4. Click **Adjust Alignment**.

5. If necessary, zoom into the upper left corner of the array with the zoom tool.
6. Locate the repeating set of 20 features along the perimeter of the array. Figure 13 shows an example of an array hybridized with a sample containing STC 1.

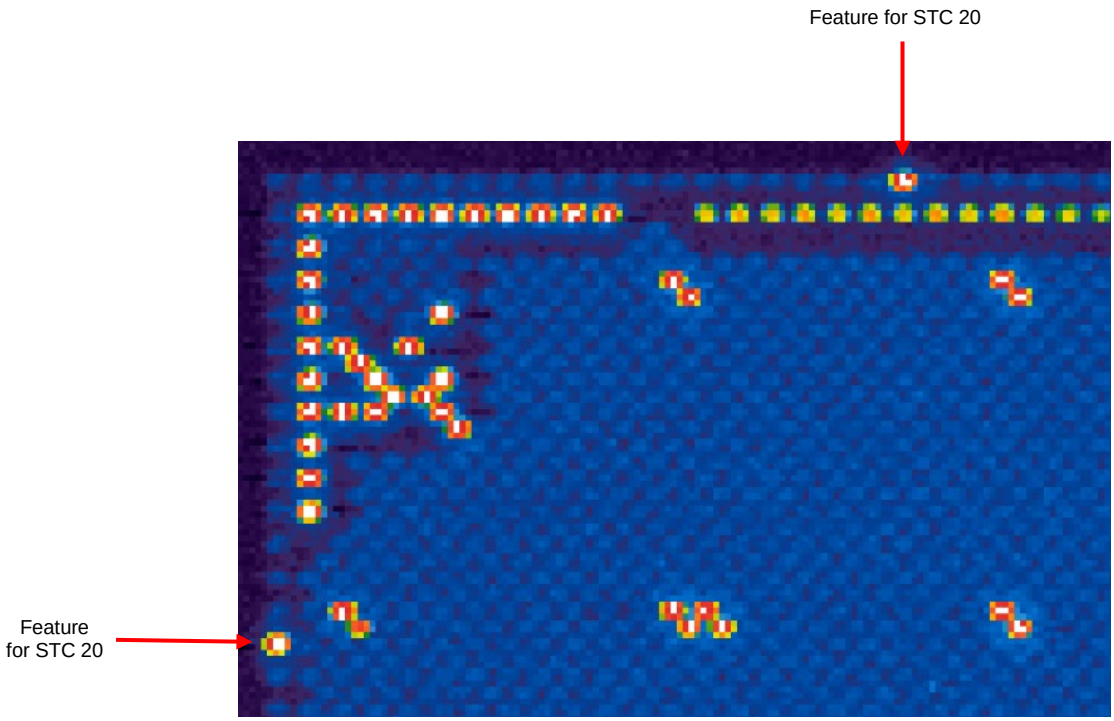


Figure 13: Example of an Array Hybridized with Sample Containing STC 20

7. Survey the entire perimeter of the array. If more than one STC is visible, the integrity of your data could be compromised due to cross-contamination that occurred during sample preparation, loading, or hybridization. Roche NimbleGen recommends that you repeat experiments that indicate cross-contamination.

Appendix A. Gel Shift Assay for Assessment of 3´ Biotin End Labeling Efficiency

Recommended Reagents

- Streptavidin (Vector Labs, catalog # SA-5000, 1 mg lyophilate)
  a. Resuspend lyophilate in 100 µl 1x PBS (final concentration - 10 mg/ml)
  b. Prepare single use aliquots and store at -15 to -25ºC.
  c. For the assay, thaw aliquot(s) on ice. Discard any remaining reagent. Do not re-freeze.
- 4% agarose gel (*e.g.* Invitrogen E-Gel 4% HR Agarose, cat # G501804)
- DNA marker (*e.g.* Invitrogen E-Gel 25 bp Ladder, cat # 10488-095)

Procedure

1. For each biotin labeled sample, aliquot 2 µg of purified DNA to a new tube (see *Chapter 3 step 2.27*).
2. Bring volume to 36 µl with 1x PBS.
3. Transfer 17 µl of the diluted DNA to 2 new tubes.
4. To one tube, add 1.0 µl of 10 mg/ml streptavidin.
5. To the other tube, add 1.0 µl of 1x PBS.
6. Pulse vortex each tube. Quick spin.
7. Incubate at room temperature for 30 minutes.
8. Prepare E-Gel according to the manufacturer's instructions.
9. Load the entire volume of each sample (18 µl, ~ 1 µg) into a well of the 4% agarose gel. Include a well with DNA marker, if desired.
10. Run the gel for 15 minutes using the manufacturer's instructions.
11. After electrophoresis, analyze your results. Use a transilluminator and /or gel documentation system to capture an image of the ethidium-bromide stained DNA. For each sample, compare the lanes loaded with DNA with and without the addition of the streptavidin. A successful labeling reaction is indicated by a majority (≥ 70%) of the DNA being shifted by the streptavidin (reduced electrophoretic migration). If desired, densitometry can be used to estimate the percentage of DNA shifted by the streptavidin (or labeling efficiency).

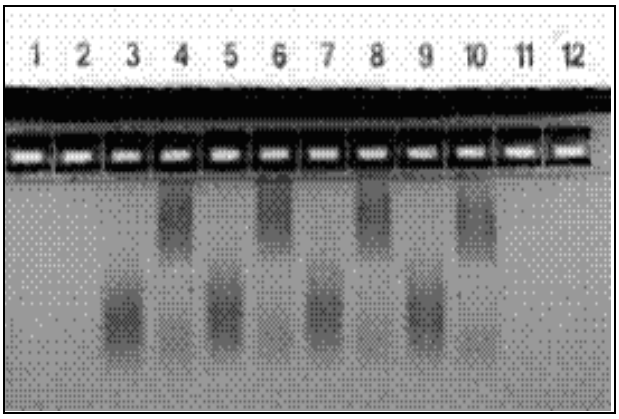


Figure A-1 – Assessment of Biotin Labeling Efficiency using a Streptavidin Gel Shift Assay. This figure illustrates assay results for efficiently labeled DNA samples. Four different DNA samples were assayed here. For each sample, the DNA aliquot in PBS (without the addition of streptavidin; lanes 3, 5, 7 and 9) is resolved alongside its counterpart aliquot containing streptavidin (lanes 4, 6, 8 and 10). The majority of the DNA sample is bound by the streptavidin and displays reduced electrophoretic migration (or a gel shift), compared to its control (no streptavidin) lane.

Appendix B. Signal Amplification Worksheet

The Signal Amplification Worksheet is for the AccuSNP protocol (*Chapter 6. Step 4*). Check the boxes as you complete the dispensing and incubation steps.

Signal Amplification Worksheet										
Buffer	**Protocol Steps**	**Loading Amount**	**Number of Dispensing Steps**						**Total Volume**	**10 Min. Incubation**
			1	**2**	**3**	**4**	**5**	**6**		
Blocking / Washing	1-3	500 µl							3 ml	
Cy3 Streptavidin	4-6	500 µl							2 ml	
Blocking / Washing	7-9	500 µl							3 ml	
Anti-Streptavidin	10-12	500 µl							1 ml	
Blocking / Washing	13-15	500 µl							3 ml	
Cy3 Streptavidin	Repeat 4-6	500 µl							2 ml	
Blocking / Washing	Repeat 7-9	500 µl							3 ml	
Anti-Streptavidin	Repeat 10-12	500 µl							1 ml	
Blocking / Washing	Repeat 13-15	500 µl							3 ml	
Cy3 Streptavidin	17-19	500 µl							2 ml	

Figure B-1. Signal Amplification Worksheet

Notes

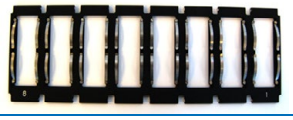
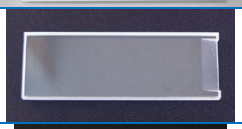
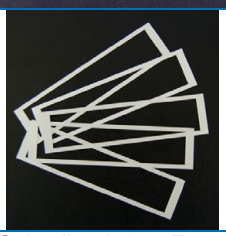
Appendix C. Cleaning Protocol for Glass Backplate

1. Prepare a 1% dilution (2.5 g per 250 ml) of Alconox Powder Detergent in water.
2. Dip each glass plate into the solution.
3. Remove the glass plate from the detergent solution and wipe with a Kimwipe.
4. Rinse completely using deionized water.
5. Allow to air dry.
6. Rinse glass plate with 70% Ethanol.
7. Dry with laboratory air gun.

Notes

Appendix D. Flow-Through Chamber

The following required items are available from Roche. Select replacement items are available directly from Tecan.

AccuSNP Accessories Set n The accessory set includes the items below.	06 343 341 001
8-Manifold Mounting Plate	
8-Multifold Lid	
8-Multifold Base	
Joint Rods (anchor rods)	
TeFlow Module Chamber Rack	
Bowl Reagent Chamber Rack (waste tray)	
Backplate (glass plates - 8)	
Spacers (500)	
Additional Replacements – as needed n Backplate (glass plates - 8) n Spacers (500) n Spare Part Kit (contains springs for the 8-Multifold)	Order directly from Tecan 30059045 10760815 30065377

To avoid damage to the springs of the assembly, be aware of the following:

- The springs of the 8-Multifold are very sensitive parts. They provide optimal tightness of the chamber by holding the flow through chamber together.
- Inappropriate handling during assembly, dismantling and cleaning can result in deformed springs. In this state, a tight chamber during the entire flow through process can't be guaranteed.
- During assembly and dismantling do not touch, bend or lift the springs of the 8-Multifold lid.
- Cleaning should only be done by rinsing with distilled water and ethanol. Please do not clean the springs mechanically.
- Deformed springs can be exchanged with new springs from the spare part kit.

Assembling the Mounting Plate

1. Assemble the 8–Multifold Base onto the Mounting Plate. Align the "1" and "8" labels etched on the 8–Multifold Base with the "1" and "8" labels etched on the Mounting Plate.

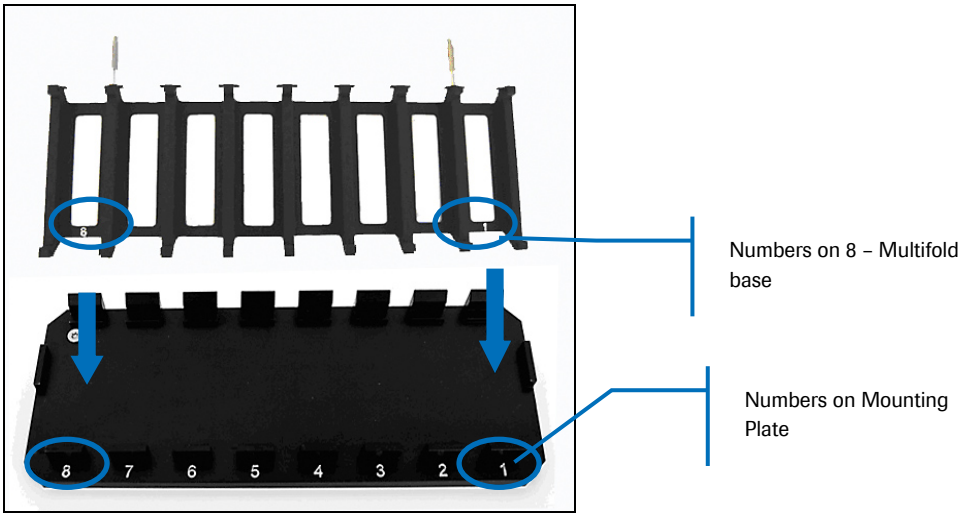


Figure D-1. Assembling the Mounting Plate

2. Place the 8-Multifold Base onto the Mounting Plate. The 8–Multifold Base should be secure and should not shift on the base.

Figure D-2. Placing the 8 Multifold Base onto the Mounting Plate

3. Place the mounting plate assembly into a 9x13 inch rectangular glass dish so that the numbers are closest to you. Slowly lower the glass backplate down onto the 8-Multifold base so that it is partially submerged in the reagent grade water.

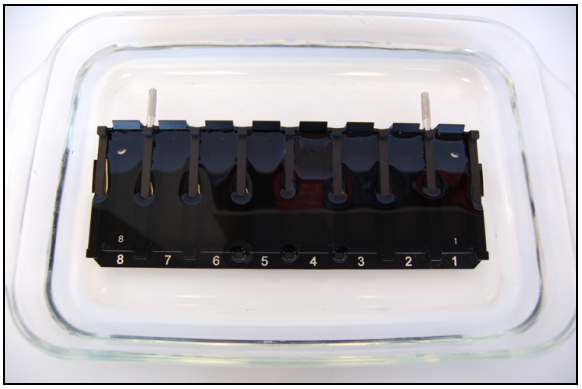


Figure D-3. Placing the Assembly into a 9x13" Glass Dish

4. Dispense the water so the liquid level reaches half-way up the combs located at the back of the assembly.

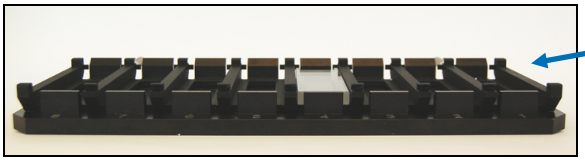


Figure D-4. Filling Water Half-way up the Combs

Putting on the Glass Plate

1. With the reservoir opening facing up, set the glass backplate at an angle with the flat bottom against the numbered side of the 8-Multifold base. Slowly lower the glass backplate down onto the 8-Multifold base so that it is partially submerged in the reagent grade water, such that the reservoir end of the backplate is at the top of the assembly. The backplate will fit securely.

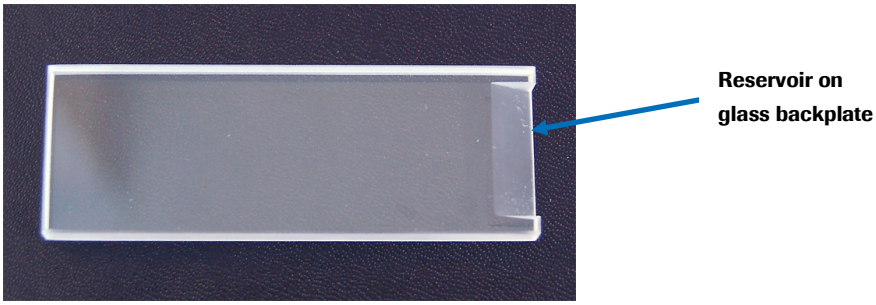


Figure D-5. Glass Backplate

2. Repeat this step for each slide to be processed.

Putting on the White Spacer

1. Remove and discard the white backing from a clear spacer and place the clear spacer onto the top of the glass backplate.

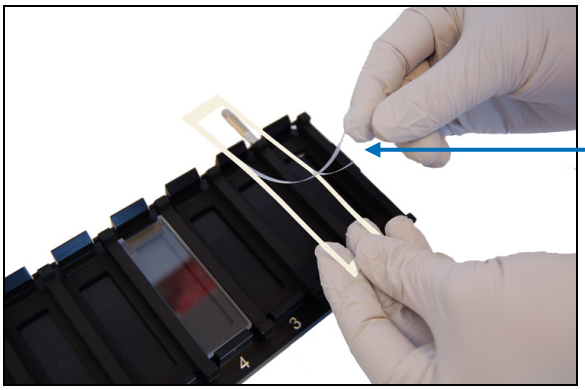


Figure D-6. Putting on the Clear Spacer

Do not use the white spacer backing.

2. Hook the ends of the clear spacer around the combs of the 8-Multifold Base. The combs are the lips at the top and bottom of the plate.

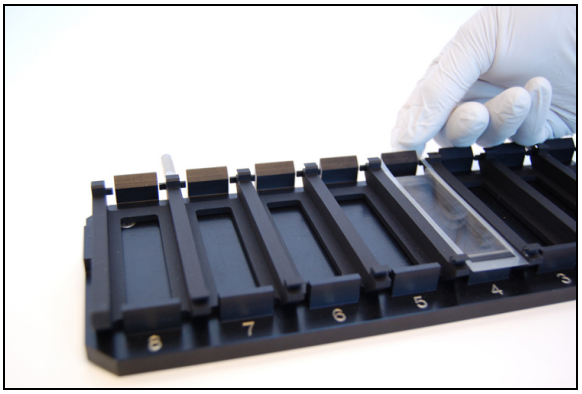


Figure D-7. Hooking the Spacer Around the Combs

3. Ensure that the clear spacer is wet and submerged in the reagent grade water before proceeding. If not, use a finger to wet the spacer and eliminate any bubbles.
4. Grip the microarray slide by the sides and place it array side down with the "NimbleGen" label next to the numbers etched in the Multifold Base, on top of the clear spacer. The slide should rest on the notch located near the numbers etched in the Mounting Plate. The barcode will be at the top of the slide.

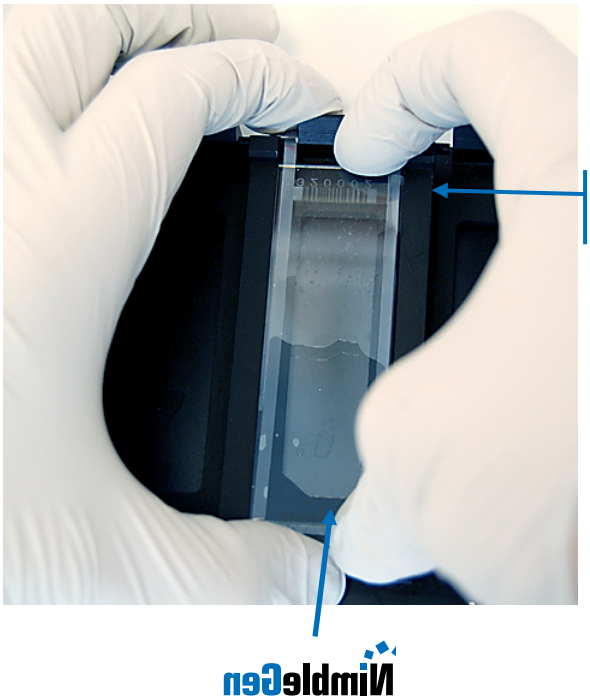


Figure D-8. Placing the Microarray Slide

Putting on the 8- Multifold Lid

1. Assemble the 8-Multifold Lid on top of the microarray slide(s) in the Multifold Base. Align the "1" and "8" labels etched in the Mounting Frame with the "1" and "8" labels etched in the Mounting Plate.

Figure D-9. Putting on the 8-Multifold Lid

2. Lift up the assembly with one hand, while holding the lid firmly to the base. With the other hand, slide an anchoring rod through the top combs of the assembly until the flat head of the anchoring rod is flush with the side of the flow chamber.
3. Slide the second anchoring rod through the bottom combs of the assembly until the flat head of the anchoring rod is flush with the side of the flow chamber.
4. Using the transport screws, lift the flow-through assembly.

Putting the Flow-through Assembly into the TeFlow Module Chamber Rack

1. Rotate the assembly so that the number 1 is on the left and the top is facing away from you.

Figure D-10. Flow-through Assembly and Chamber Rack

2. Place the assembly into the chamber rack. Hook the lip of the chamber over the lip of the chamber rack.

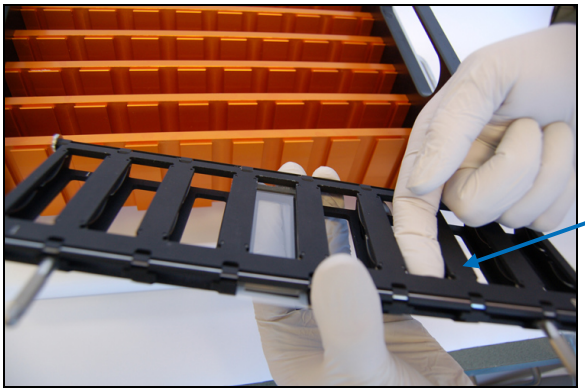


Figure D-11. Putting the Assembly into the Chamber Rack

The mounting base will remain in the glass container.

Continue the protocol following *Chapter 6. Step 4. Signal Amplification.*

Appendix E. Limited Warranty

ROCHE NIMBLEGEN, INC.
NIMBLEGEN ARRAYS

1. Limited Warranty

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