USER GUIDE

# Ovation® WGA System

PART NO. 6100-12


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# I. Introduction

## A. Background

The Ovation WGA System provides a robust and simple method for preparing amplified DNA (SPIA Product) from small amounts of genomic DNA, suitable for a variety of downstream applications, including copy number change analysis using real-time quantitative PCR (q-PCR) or microarrays. Because of the simplicity and robustness of the procedure, accurate and quantitative results are obtained with minimal sample or allele dropouts and without the need for customization or optimization.

The Ovation WGA System is powered by SPIA (Single Primer Isothermal Amplification), a robust isothermal strand displacement amplification process developed by NuGEN. Starting with 10 to 50 ng genomic DNA, microgram quantities of amplified SPIA Product can be prepared in approximately 4 hrs.

The Ovation WGA System (Part No. 6100-12) provides optimized reagent mixes to process 12 DNA samples. It is recommended to include control DNA samples when first using this product (not provided).

## B. How the System Works

The Ovation WGA System employs a three-step process that generates amplified DNA from as little as 10 ng of genomic DNA (see Figure 1).

1. **Denaturation and SPIA Template Synthesis**
   Genomic DNA (gDNA) is denatured, and a chimeric DNA/RNA primer mix (WG Primer) hybridizes uniformly across the input gDNA. The RNA portion of the WG Primer includes a unique sequence that serves as the tag for the subsequent SPIA process in Step 3. DNA polymerase creates the first SPIA template strand by extending from the chimeric WG Primer. The second strand is then synthesized in the same tube without additional reagent addition to form the resulting double-stranded DNA (SPIA Template) with a DNA/RNA heteroduplex at one end that contains the unique tag sequence.

2. **Purification of SPIA Template**
   The SPIA Template is purified away from the excess primers with magnetic beads prior to SPIA.

3. **SPIA**
   SPIA is a robust isothermal strand displacement amplification process that uses a second DNA/RNA chimeric primer (SPIA Primer) containing the tag sequence, DNA polymerase and RNase H in a homogeneous isothermal assay providing highly efficient amplification of DNA sequences.

   RNase H is used to degrade the RNA portion of the DNA/RNA heteroduplex introduced during the SPIA Template Synthesis reaction in Step 1. This results in the exposure of a DNA tag sequence that is now available for binding to a second SPIA Primer. DNA polymerase then initiates replication at the 3′ end of the primer, displacing the existing forward strand. The RNA portion at the 5′ end of the newly

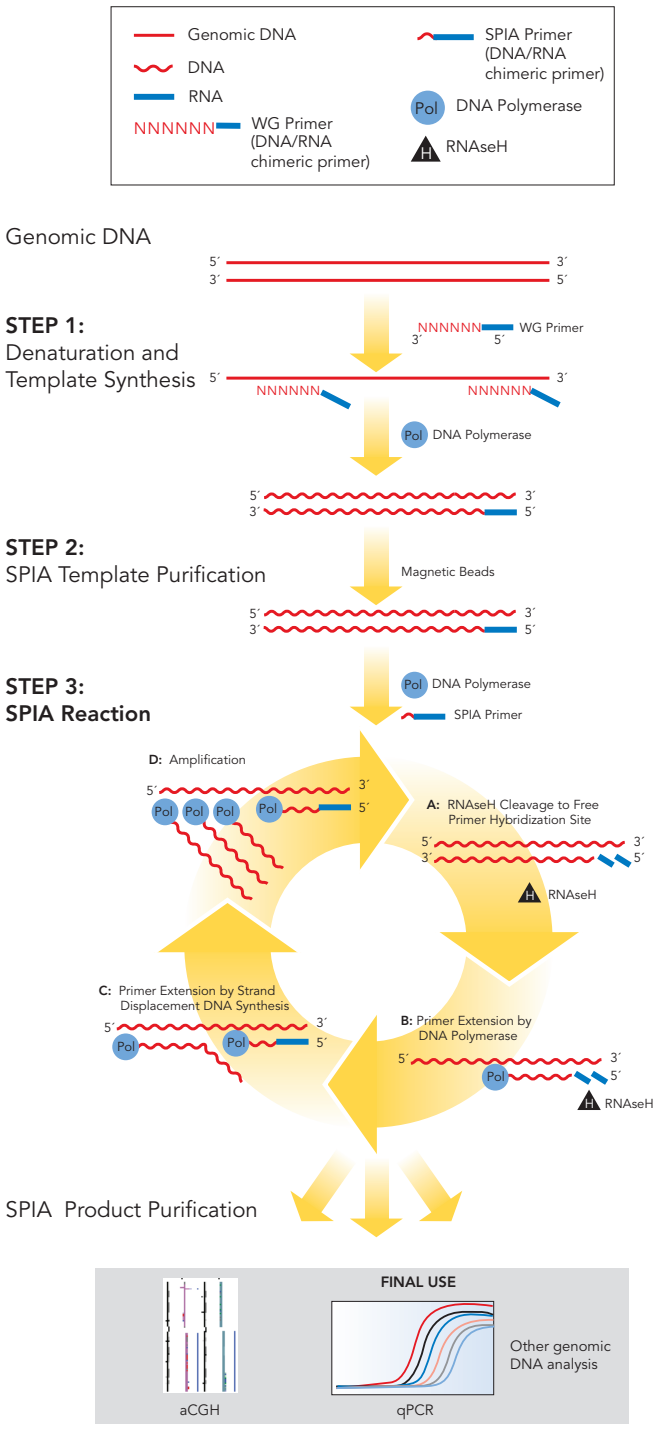
# I. Introduction

synthesized strand is again removed by RNase H, exposing part of the unique priming site for initiation of the next round of DNA synthesis.

The process of SPIA DNA/RNA primer binding, DNA replication, strand displacement and RNA cleavage is repeated, resulting in rapid accumulation of DNA with sequence complementary to the original gDNA. An average gDNA amplification of 500-fold is observed with 10 ng of starting gDNA within four hours.

# I. Introduction

**Figure 1. Schematic of the Ovation WGA System.**



# I. Introduction

## C. Performance Specifications

The Ovation WGA System synthesizes microgram quantities of amplified DNA starting with total gDNA input amounts of 10 to 50 ng. In approximately four hrs, the Ovation WGA System can produce 3 to 6 µg of amplified DNA (SPIA Product) ready for various downstream genomic analyses. When used with intact gDNA samples, the size of the majority of the DNA products produced by the amplification process is between 50 bases and 1.5 Kb. This whole genome approach results in densely overlapping amplified DNA fragments representing the entire genome.

## D. Quality Control

Each Ovation WGA System lot is tested to meet specifications of amplification performance.

## E. Storage and Stability

The Ovation WGA System is shipped on dry ice and should be unpacked immediately upon receipt.

**Note:** This product contains components with multiple storage temperature requirements.

- Vials labeled Agencourt® RNAClean® XP Beads (clear cap) should be removed from the top of the shipping carton upon delivery and stored at 4°C.
- All other components should be stored at –20°C on internal shelves of a freezer without a defrost cycle.

Kits handled and stored according to the above guidelines will perform to specifications for at least six months.

## F. Material Safety Data Sheet (MSDS)

An MSDS for this product is available on the NuGEN website atwww.nugeninc.com/nugen/index.cfm/support/user-guides/.

# II. Components

## A. Reagents Provided

**Table 1. Denaturation and Template Synthesis Reagents (Part No. 6100-12)**

| COMPONENT | 6100 PART NUMBER | VIAL CAP | VIAL NUMBER |
|---|---|---|---|
| Denaturing Buffer | S01271 | Blue | DA1 |
| WG Primer | S01272 | Blue | DA2 |
| Template Synthesis Buffer | S01273 | Blue | DA3 |
| Template Synthesis Enzyme | S01274 | Blue | DA4 |

**Table 2. SPIA Reagents (Part No. 6100-12)**

| COMPONENT | 6100 PART NUMBER | VIAL CAP | VIAL NUMBER |
|---|---|---|---|
| SPIA Primer | S01275 | Red | DB1 |
| SPIA Buffer | S01276 | Red | DB2 |
| SPIA Enzyme | S01277 | Red | DB3 |

**Table 3. Additional Reagents (Part No. 6100-12)**

| COMPONENT | 6100 PART NUMBER | VIAL CAP | VIAL NUMBER |
|---|---|---|---|
| Nuclease-free Water | S01001 | Green | D1 |
| Agencourt RNAClean XP Beads | S01307 | Clear, Colorless | — |

**Note:** The reagents in the Ovation WGA System may have similar labels and names to reagents in other kits manufactured by NuGEN. However, unless the vial part numbers are identical, these reagents do not have exactly the same composition and therefore are not interchangeable.

# II. Components

## B. Additional Equipment, Reagents and Labware

### Required Materials

- **Equipment**
  - Microcentrifuge for individual 1.5 mL and 0.5 mL tubes
  - 0.5–10 µL pipette, 2–20 µL pipette, 20–200 µL pipette, 200–1000 µL pipette
  - Vortexer
  - Thermal cycler with 0.2 mL tube heat block, heated lid, and 100 µL reaction capacity
  - Appropriate spectrophotometer and cuvettes, or Nanodrop® UV-Vis Spectrophotometer
- **Reagents**
  - Ethanol (Sigma-Aldrich, Cat. #E7023), for purification steps
- **Supplies and Labware**
  - Nuclease-free pipette tips
  - 1.5 mL and 0.5 mL RNase-free microcentrifuge tubes
  - 8 X 0.2 mL strip PCR tubes or 0.2 mL thin-wall PCR plates
  - Agencourt® SPRIPlate® 96R, Ring Magnet Plate (Beckman Coulter, Cat. #A29164) or Agencourt SPRIPlate Ring Super Magnet Plate (Beckman Coulter, Cat. #A32782)
  - Purification options for final SPIA product purification (select one option):
    - Agencourt® RNAClean® XP Beads (Beckman Coulter, Cat. #63987)
    - MinElute® Reaction Cleanup Kit (QIAGEN, Cat. #28204)
    - QIAquick® PCR Purification Kit (QIAGEN, Cat. #28104)
    - DNA Clean & Concentrator™-25 (Zymo Research, Cat. #D4005)
  - Disposable gloves
  - Kimwipes
  - Ice bucket
  - Cleaning solutions such as DNA-OFF™ (MP Biomedicals, Cat. #QD0500) and RNaseZap® (Ambion, Cat. #AM9780)
- **Optional Materials**
  - Agilent 2100 Bioanalyzer or materials and equipment for electrophoretic analysis of DNA
  - Real-time PCR system

### To Order

- Ambion, Inc., www.ambion.com
- Beckman Coulter, www.beckmancoulter.com
- MP Biomedicals, www.mpbio.com
- QIAGEN Inc., www.qiagen.com
- Sigma-Aldrich, Inc., www.sigmaaldrich.com
- Zymo Research, www.zymoresearch.com

# III. Planning the Experiment

## A. Input DNA Requirements

1. **gDNA Quantity**
   gDNA input must be at least 10 ng. Inputs greater than 50 ng per reaction may inhibit amplification, while lower amounts of input will potentially result in insufficient yields, and reduced copy number difference detection depending on required analytical platforms.

2. **gDNA Purity**
   gDNA samples must be free of contaminating proteins and other cellular material, organic solvents (including phenol and ethanol) and salts used in many DNA isolation methods. Use of a commercially available system for preparing small amounts of DNA that does not require organic solvents is recommended.

   If a method including organic solvents is used, we recommend column purification after isolation. Methods that use RNases to degrade RNA co-isolated with the desired DNA can be problematic as the presence of RNase in the gDNA sample can inhibit amplification, resulting in low yields and/or decreased detection of copy number changes.

   One way to measure DNA purity is to assess the ratio of absorbance readings at 260 nm and 280 nm. The A260:A280 ratio for DNA samples of acceptable purity should be in excess of 1.8. DNA samples with lower ratios may result in low amplification yield.

3. **gDNA Integrity**
   DNA samples of high molecular weight with little or no evidence of degradation will amplify very well with this product. Use of degraded samples may result in lowered yield.

## B. Using Nuclease-free Techniques

Nuclease contamination from equipment and the work environment will lead to experimental failure. Follow these guidelines to minimize contamination:

- Wear disposable gloves and change them frequently.
- Avoid touching surfaces or materials that could introduce nucleases.
- Use only the reagents provided and recommended.
- Clean and decontaminate work areas and instruments, including pipettes, with commercially available decontamination reagents.
- Use only new nuclease-free pipette tips and microcentrifuge tubes.

## C. SPIA Product Storage

The unlabeled SPIA Product may be stored at –20°C for at least six months.

# IV. Protocol

## A. Overview

**The Ovation WGA process is performed in three stages:**

| | |
|---|---|
| 1. Sample denaturation and template synthesis | 1.5 hours |
| 2. Template purification | 0.75 hours |
| 3. SPIA amplification, purification and quantitation | 1.75 hours |
| **Total time to purified SPIA DNA** | **~4 hours** |

## B. Protocol Notes

- We recommend the routine use of a positive control DNA. Especially during the first time you set up an amplification reaction, the use of a positive control DNA will allow the establishment of a baseline of performance and provide the opportunity to become familiar with the bead purification step. This step may be unfamiliar to many users, and can be especially prone to handling variability in using the magnet plate, so a practice run with the plate is highly recommended.
- Due to the high sensitivity inherent in this amplification system, we strongly recommend taking measures to minimize the potential for contamination of amplification reactions by carryover of nucleic acids or other laboratory contaminants. The two steps to accomplish this are: 1. Designating separate workspaces for "pre-amplified" and "post-amplified" steps and materials and 2. Implementing routine cleanup protocols for workspaces as standard operating procedure. A detailed set of these recommendations is listed in the Appendix.
- Use the water provided with the kit (green: D1) or an alternate source of nuclease-free water. We do not recommend the use of DEPC treated water with this protocol.
- Always keep thawed reagents and reaction tubes on ice unless otherwise instructed.
- After thawing and mixing buffer mixes, if any precipitate is observed, re-dissolve it completely prior to use. You may gently warm the buffer mix for two min at room temperature followed by brief vortexing. Do not warm any enzyme mixes.
- The reagent volumes recovered greatly depend on the number of batches processed with each kit. Set up no fewer than three reactions at a time.
- When placing small amounts of reagent into the master mix or reaction tube, gently pipette up and down several times to ensure complete transfer.
- When instructed to pipette mix, gently aspirate and dispense a volume of at least half of the total reaction mix volume. Repeat a minimum of five times to ensure complete mixing.
- Allow thermal cycler to reach the initial incubation temperature before placing samples in the block.
- When working with more than one sample, excess master mix may be needed.
- Components of this NuGEN product should not be used or combined with any other types of Ovation System products and vice versa.

- Use only fresh ethanol stocks to make 70% ethanol used in the template bead purification (Section G), and 80% ethanol for washes in the amplified SPIA Product purification protocols (Section I, Appendix A). Make the ethanol mixes fresh, as well, carefully measuring both the ethanol and water with pipettes. Lower concentrations of ethanol in wash solutions will result in loss of yield as the higher aqueous content will dissolve the DNA and wash it off the beads or column.

## C. Agencourt® RNAClean® XP Purification Beads

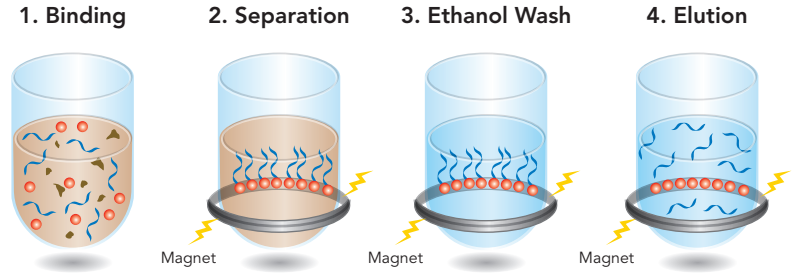
### Tips and Notes Relevant to the Template DNA Cleanup, Section F:

There are significant modifications to the Agencourt RNAClean XP kit standard procedure; therefore you must follow the procedures outlined in this user guide for the use of these beads with the Ovation WGA System. However you may review the Beckman Coulter user guide to become familiar with the manufacturer's recommendations.

The bead purification steps used in this kit are:

1. Binding of DNA to Agencourt RNAClean XP beads
2. Magnetic separation of beads from supernatant
3. Ethanol wash of bound beads to remove contaminants
4. Elution of bound DNA from beads

**Figure 2. Agencourt RNAClean XP Bead purification process overview.**




Reproduced from original picture from Agencourt/Beckman Coulter Genomics

### Additional Tips and Notes

- Remove beads from 4°C and leave at room temperature for at least 15 min before use, ensure that they have completely reached room temperature. Cold beads will result in reduced recovery.
- Fully resuspend beads by inverting and tapping before adding to sample.
- Note that we recommend using **1.6 volumes** (32 µL) of RNAClean XP beads. This is different from the standard Beckman Coulter protocol.

- It is critical to let the beads separate on the magnet for a full five min. Removing binding buffer before the beads have completely separated will impact yields.
- After the binding step has been completed, it is important to minimize bead loss when removing the binding buffer. With the samples placed on the magnet, remove only 40 µL of the binding buffer from each sample. Some liquid will remain at the bottom of the tube but this will minimize bead loss.
- Any significant loss of beads bound to the magnet during the ethanol washes will impact yields, so make sure the beads are not lost with the wash.
- Ensure that the ethanol wash is freshly prepared from fresh ethanol stocks at the indicated concentration. Lower percent ethanol mixes will reduce recovery.
- During the ethanol washes, keep the samples on the magnet. The beads should not be allowed to disperse; the magnet will keep the beads on the walls of the sample wells or tubes in a small ring.
- It is critical that all residual ethanol be removed prior to continuing with the SPIA amplification. Therefore, when removing the final ethanol wash, first remove most of the ethanol, then allow the excess to collect at the bottom of the tube before removing the remaining ethanol. This reduces the required bead air drying time.
- After drying the beads for 15 min, inspect each tube carefully and make certain that all the ethanol has evaporated before proceeding with the amplification step.
- It is strongly recommended that strip tubes or partial plates are firmly placed when used with the magnet plate. We don't advise the use of individual tubes as they are not very stably supported on the magnet plates.

### D. Programming the Thermal Cycler

Use a thermal cycler with a heat block designed for 0.2 mL tubes, equipped with a heated lid, and with a capacity of 100 µL reaction volume. Prepare the programs shown in Table 4, following the operating instructions provided by the manufacturer. For thermal cyclers with an adjustable heated lid, set the lid temperature to 100°C only when sample temperature reaches above 30°C. For thermal cyclers with a fixed temperature heated lid (e.g., ABI GeneAmp® PCR 9600 and 9700 models), use the default settings (typically 100 to 105°C).

**Table 4. Thermal Cycler Programming**

| | |
|---|---|
| **DENATURATION** | |
| **Program 1** Denaturation | 95°C – 3 min, hold at 4°C |
| **TEMPLATE SYNTHESIS** | |
| **Program 2** Template Synthesis | 4°C – 1 min, 2 cycles* (23°C – 10 min, 35°C – 10 min, 57°C – 10 min), 70°C – 5 min, hold at 4°C |
| **SPIA AMPLIFICATION** | |
| **Program 3** SPIA Amplification | 4°C – 1 min, 47°C – 90 min, 95°C – 5 min hold at 4°C |

**Important Note:** Carry out steps E (Denaturation and Template Synthesis) through G, step #7 (SPIA) in a pre-amplification workspace using dedicated pre-amplification consumables and equipment. Wipe all surfaces, equipment and instrumentation with a DNA decontaminant solution such as DNA-OFF to avoid the potential introduction of previously amplified DNA into new amplifications. For more information on our recommendations for workflow compartmentalization and routine lab cleanup please refer to Appendix D of this user guide. If you have any questions on this important topic, please contact NuGEN Technical Services (techserv@nugeninc.com, or 888-654-6544).

## E. Sample Denaturation and Template Synthesis

1. Obtain Denaturing Buffer (blue: DA1), WG Primer (blue: DA2), Template Synthesis Buffer (blue: DA3), Template Synthesis Enzyme (blue: DA4) and Nuclease-free Water (green: D1) from –20°C storage.
2. Flick DA4 to mix, then spin down contents for two seconds in a microcentrifuge. Place on ice.
3. Vortex DA1, DA2, and DA3 for two seconds, then spin in a microcentrifuge for two seconds. Place on ice.
4. Add 2 µL of DA1 to a 0.2 mL PCR tube.
5. Add 8 µL of gDNA sample (at least 10 ng) to DA1.
6. Cap and spin tubes for two seconds then return to ice.
7. Place tubes in a pre-warmed thermal cycler programmed to run Program 1 (Denaturation; see Table 4):

95°C – 3 min, hold at 4°C

8. Immediately after 95°C step remove tubes from the thermal cycler and place on ice for 5 minutes to snap cool.
9. Once Denaturation (Step 7) is complete, prepare the Template Synthesis Master Mix in a 0.5 mL capped tube, according to the volumes shown in Table 5.

**Table 5. Template Synthesis Mix (volumes listed are for a single reaction)**

| WG PRIMER (BLUE: DA2) | TEMPLATE SYNTHESIS BUFFER(BLUE: DA3) | TEMPLATE SYNTHESIS ENZYME(BLUE: DA4) |
|---|---|---|
| 2.0 µL | 7.0 µL | 1.0 µL |

*Mix by pipetting and spin down the master mix briefly. Place on ice. Use immediately.*

10. Add 10 µL of the Template Synthesis Master Mix to each sample tube.
11. Mix by pipetting five times, then spin for two seconds.
12. Place tubes in a pre-cooled thermal cycler programmed to run Program 2(Template Synthesis; see Table 4):

    4°C – 1 min, 2 cycles* (23°C – 10 min, 35°C – 10 min, 57°C – 10 min),
    70°C – 5 min, hold at 4°C
13. Remove tubes from the thermal cycler and spin for two seconds to collect condensation. Place on ice.
14. Proceed immediately to Template Purification.
15. ation and place on ice.
16. Continue immediately with the Ligation protocol.

## F. Template Purification

*The purification beads should be removed from 4°C and left at bench top to reach room temperature well before the start of purification.*

1. Ensure the Agencourt RNAClean XP beads have completely reached room temperature before proceeding.
2. Resuspend beads by inverting and tapping the tube. Ensure beads are fully resuspended before adding to sample.
3. After resuspending do not spin the beads. A large excess of beads is provided, therefore it is not necessary to recover any beads trapped in the cap.

*Best results can be obtained by using fresh 70% ethanol in wash steps.*

4. Prepare a 70% ethanol wash solution. It is critical that this solution be prepared fresh on the same day of the experiment from a recently opened stock container. Measure both the ethanol and the water components carefully prior to mixing. Failure to do so can result in a higher than anticipated aqueous content which may reduce amplification yield.

5. At room temperature, add 32 µL (1.6 volumes) bead suspension to each reaction and mix by pipetting up and down 10 times.
6. Incubate at room temperature for 10 min.
7. Transfer tubes or plate to magnet plate and let stand 5 min or until solution is completely cleared of beads.

! *Minimize bead loss by leaving a residual volume of binding buffer after completion of the binding step.*

8. Keeping the tubes or plate on the magnet, carefully remove only 40 µL of the binding buffer and discard it. Leaving some of the volume behind minimizes bead loss at this step.

   **Note:** The beads should not disperse; instead they will stay on the walls of the wells as a small ring. Significant loss of beads at this stage will impact SPIA Product yields, so ensure beads are not removed with the binding buffer or the wash.

9. With the plate still on the magnet, add 200 µL of freshly prepared (prepared on the same day), carefully measured 70% ethanol and let stand for 30 seconds.
10. Remove the 70% ethanol wash using a pipette.
11. Repeat the 70% ethanol wash two more times.

! *Ensure that all residual ethanol is removed prior to continuing with the SPIA amplification.*

   **Note:** With the final wash, it is critical to remove as much of the ethanol as possible. Use at least 2 pipetting steps to allow excess ethanol to collect at the bottom of the tubes after removing most of the ethanol in the first pipetting step.

12. Air dry the beads on the magnet for 15 min. Inspect each tube carefully to ensure that all the ethanol has evaporated. It is critical that all residual ethanol be removed prior to continuing with the SPIA step.
13. Proceed immediately with SPIA with the template DNA still bound to the dry beads.

## G. SPIA

! *Use SPIA Master Mix immediately after preparation.*

1. Obtain the SPIA Primer (red: DB1), SPIA Buffer (red: DB2) and SPIA Enzyme (red: DB3) from –20°C storage.
2. Thaw DB1 and DB2 at room temperature, mix by vortexing for two seconds and spin in a microcentrifuge for two seconds. Place on ice.
3. Thaw DB3 on ice and mix the contents by inverting gently five times. Ensure the enzyme is well mixed without introducing bubbles. Spin in a microcentrifuge for two seconds and place on ice.
4. Prepare the SPIA Master Mix by sequentially combining DB2, DB1 and DB3 in an appropriately sized capped tube according to the volumes shown in Table 6. Make sure the addition of DB3 is done at the last moment.

! *Mix by pipetting and spin down the master mix briefly. Place on ice. Use immediately.*

**Table 6. SPIA Mix (volumes listed are for a single reaction)**

| SPIA BUFFER (RED: DB2) | SPIA PRIMER (RED: DB1) | SPIA ENZYME(RED: DB3) |
|---|---|---|
| 20 µL | 10 µL | 10 µL |

5. Add 40 µL of the SPIA Master Mix to each tube containing the template DNA bound to the dried beads. Use a pipette set to 20 µL and mix well by pipetting up and down at least 8-10 times. Attempt to get the majority of the beads in suspension and remove most of the beads from the tube walls.

   **Note:** Beads may not form a perfectly uniform suspension, but this will not affect the reaction. The addition of SPIA Master Mix will elute the DNA template off the beads.

6. Place the tubes in a pre-warmed thermal cycler programmed to run Program 3 (SPIA Amplification; see Table 4):

   24°C – 1 min, 47°C – 90 min, 95°C – 5 min hold at 4°C

7. Remove tubes from the thermal cycler, spin for two seconds to collect condensation, then place on ice. Do not re-open tubes or plate in the pre-amplification workspace.

**Important Note:** At this point, the tubes or plate should be removed from the pre-amplification workspace. Carry out all remaining steps in a post-amplification workspace using dedicated post-amplification consumables and equipment. Take care to avoid contamination of your pre-amplification workspace with amplified SPIA Product present in the post-amplification workspace. For more information on our recommendations for workflow compartmentalization and routine lab cleanup, please refer to Appendix D of this user guide. If you have any questions on this important topic, please contact NuGEN Technical Services (techserv@nugeninc.com, 888.654.6544).

8. Transfer tubes or plate to magnet plate and let stand for five min to completely clear the solution of beads.

   **Note:** If using the Agencourt RNAClean XP Bead method for final SPIA Product cleanup, it is not necessary to remove the beads.

9. Carefully remove all of the cleared supernatant containing the eluted DNA and transfer to a fresh tube. The beads may now be discarded.

10. At this stage, the SPIA Product may be purified or stored at –20°C.

## H. Purification of SPIA Product

Amplified SPIA Product can be purified using various methods listed in Appendix A. Purification is required if the amplified DNA is intended for use in fragmentation and labeling reactions.

Selection of the optimum purification option can depend on many factors. Please contact the NuGEN Technical Support team for assistance in selecting the appropriate purification option for your application.

We recommend that the amplified SPIA Product be purified prior to q-PCR analysis.

## I. Measuring SPIA Product Yield and Purity

1. Mix the sample by brief vortexing and spinning prior to checking the concentration.
2. Measure the absorbance at 260, 280 and 320 nm. You may need to make a 1:20 dilution of the cDNA in water prior to measuring the absorbance.
3. **Purity:** Subtract the A320 value from both A260 and A280 values. The adjusted (A260–A320) / (A280–A320) ratio should be >1.8.
4. **Yield:** Assume 1 A260 unit = 33 µg/mL.

   To calculate: (A260–A320 of diluted sample) X (dilution factor) X 33 (concentration in µg/mL of a 1 A260 unit solution) X 0.03 (final volume in mL) = total yield in micrograms

   **Note:** Alternatively, you may measure the concentration and purity of SPIA DNA with a Nanodrop by setting 1 A260 unit of ssDNA = 33 µg/mL as the constant.
5. The purifed cDNA may be stored at –20°C, used directly in q-PCR analysis or labeled for subsequent aCGH analysis.

# V. Technical Support

For help with any of our products, please contact NuGEN Technical Support at 650.590.3674 (direct) or 888.654.6544, option 2 (toll-free, U.S. only). You may also send faxes to 888.296.6544 (toll-free) or email techserv@nugeninc.com.

In Europe contact NuGEN at +31(0)135780215 (Phone) or +31(0)135780216 (Fax) or email europe@nugeninc.com.

In all other locations, contact your NuGEN distributor for technical support.

# VI. Appendix

## A. Purification Protocols for SPIA DNA Product

There are four currently supported alternatives for carrying out the final purification of SPIA Product. Listed alphabetically, they are 1) Beckman Coulter Agencourt RNAClean XP Purification Beads, 2) QIAGEN MinElute Reaction Cleanup Kit, 3) QIAGEN QIAquick PCR Purification Kit, and 4) Zymo Research DNA Clean & Concentrator-25.

The procedures given below are specifically adapted for use with NuGEN products, and may differ significantly from the protocols published by the manufacturers. Failure to follow the purification procedures as given below may negatively impact your results.

**Agencourt RNAClean XP Purification Kit** (instructions for a single reaction)

Stop after SPIA (step G.8). It is not necessary to remove the beads from the SPIA reactions. Begin purification as follows:

! *Best results are obtained by using fresh 80% ethanol in wash steps. Lower percent ethanol mixes will reduce recovery.*

1. Obtain the RNAClean XP bottle from 4°C and allow to reach room temperature. Shake the RNAClean XP bottle well to resuspend the magnetic beads.
2. Prepare an 80% ethanol wash solution. It is critical that this solution be prepared fresh on the same day of the experiment from a recently opened stock container. Measure both the ethanol and the water components carefully prior to mixing. Failure to do so can result in a higher than anticipated aqueous content, which may reduce recovery yield.
3. Add 72 µL of resuspended RNAClean XP beads (1.8 times the sample volume) to the 40 µL SPIA reaction.
4. Mix the sample and beads thoroughly by pipetting up and down 10 times.
5. Incubate sample/bead mixture at room temperature for five min.
6. Place the samples (containing beads) on the SPRIPlate 96R magnet plate for 10 min or until the solution appears clear.

   **Note:** If the surface tension traps a small halo of beads suspended at the liquid surface, use a multi-channel pipette and gently pipette 10–15 µL up and down at the liquid surface to break the tension and allow the beads to sink to the magnet ring.
7. Using a multi-channel pipette, remove and discard the supernatant. Do not disturb the ring of magnetic beads.
8. With the samples still on the magnet plate, add 200 µL of freshly prepared 80% ethanol to each well of the reaction plate and incubate for 30 seconds or until the solution clears. Add slowly as to not disturb the separated magnetic beads.
9. Using a multi-channel pipette, remove and discard the ethanol.
10. Repeat the 80% ethanol wash. Ensure as much ethanol as possible is removed from the plate.

# VI. Appendix

11. Remove the reaction tubes or plate from the magnet and air dry the reaction plate on bench top for no more than two min. If the beads dry too long, they are difficult to resuspend.

*Use nuclease-free water at room temperature to elute sample.*

12. With the plate on the bench top, add 40 µL of room temperature Nuclease-free Water (green: D1) to each well. Holding the plate firmly, very carefully vortex for 30 seconds or use a plate shaker set to medium speed. Ensure the beads are fully resuspended; vortex longer if necessary. Alternatively, the beads may be resuspended by repeated pipetting.
13. Replace reaction tubes or plate on the plate magnet; allow the beads to separate for five min or until the solution clears.
14. Using a multi-channel pipette, remove the eluted sample and place into a fresh reaction tube or plate. There should be approximately 40 µL of purified SPIA Product.

    **Note:** Small amounts of magnetic bead carryover may interfere with sample quantitation. Take care to minimize bead carryover.
15. Continue with the Measuring SPIA Product Yield and Purity protocol or store the purified SPIA Product at –20°C.

**QIAGEN MinElute Reaction Cleanup Kit** (instructions for a single reaction)

**Important Points Before Starting:**

- The ERC buffer is considered hazardous, according to QIAGEN, and an MSDS may be consulted.
- Add the appropriate amount of 100% ethanol to Buffer PE before use (see bottle label for volume).
- All centrifuge steps are carried out at maximum speed in a conventional tabletop microcentrifuge at room temperature.

*100% ethanol must be added to the QIAGEN Buffer PE upon first use. Failure to do so will result in low amplification yields.*

1. Into a clean 1.5 mL tube, add 300 µL of ERC buffer from the QIAGEN kit.
2. Add the entire amount of amplified SPIA Product to the tube. Vortex gently for five seconds, then spin down for two seconds.
3. Obtain one MinElute spin column and insert into a collection tube.
4. Load the entire solution of sample and buffer onto column.
5. Centrifuge for one min at maximum speed. Discard flow-through.
6. Place column back in the same collection tube. Add 750 µL of Buffer PE with ethanol.
7. Centrifuge for one min at maximum speed. Discard flow-through.
8. Centrifuge again for two min at maximum speed to remove all residual Buffer PE with ethanol.

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9. Remove the MinElute spin column from the centrifuge. Discard flow-through, along with the collection tube.
10. Blot the column tip onto a filter paper to remove any residual wash buffer from the tip of the column.

    **Note:** Blotting of the column tip MUST be done prior to transferring the column to a clean 1.5 mL microcentrifuge tube. Failure to do so may result in a small quantity of wash buffer in your final eluted sample.
11. Place the MinElute spin column in clean 2.0 mL collection tube.
12. Add 15 µL of Nuclease-free Water (green: D1) to the center of each MinElute spin column Do not use cold water!
13. Let columns stand for one min at room temperature.
14. Centrifuge at maximum speed for one min.
15. Collect sample. There should be approximately 15 µL of purified DNA.
16. Mix sample by vortexing, then spin briefly.
17. Continue with the Measuring SPIA Product Yield and Purity protocol or store the purified SPIA Product at –20°C.

**QIAGEN QIAquick PCR Purification Kit** (instructions for a single reaction)

1. In a clean 1.5 mL tube, add 200 µL of PB buffer from the QIAGEN system.
2. Add the 40 µL of amplified SPIA DNA product to the tube.
3. Vortex for five seconds and spin down for two seconds.
4. Obtain one QIAquick spin column and insert into a collection tube.
5. Load the entire 240 µL of sample onto the column.
6. Centrifuge column in a collection tube for one min at 13,000 rpm (~17,900 x g).
7. Discard flow-through. Place the column back in the same collection tube. Add 700 µL of 80% Ethanol.

   **Note:** Use fresh 80% ethanol. Lower percent ethanol mixes will reduce recovery.
8. Centrifuge the column for one min at 13,000 rpm. Discard flow-through.
9. Repeat steps 7 and 8 once.
10. To remove remaining liquid, centrifuge column for one additional minute at 13,000 rpm.
11. Remove the column from the centrifuge. Discard flow-through with the collection tube.

! *Best results are obtained by using fresh 80% ethanol in wash steps. Lower percent ethanol mixes will reduce recovery.*

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12. Blot the tip of the column onto a filter paper in order to remove any residual wash buffer from the tip of the column.

    **Note:** Blot column tip prior to transferring it to a new tube to prevent any wash buffer from transferring to the eluted sample.

13. Place the column in a clean 2.0 mL collection tube, appropriately labeled.

*Use nuclease-free water at room temperature to elute sample.*

14. Add 40 µL of Nuclease-free Water (green: D1) to the center of each column. Do not use cold water!
15. Let columns stand for five min at room temperature to elute purified DNA.
16. Centrifuge at 13,000 rpm for one min to collect sample. There should be approximately 40 µL of purified SPIA Product.
17. Mix sample by vortexing, then spin briefly.
18. Continue with the Measuring SPIA Product Yield and Purity protocol or store the purified SPIA Product at –20°C.

**Zymo Research DNA Clean & Concentrator™-25** (instructions for a single reaction)

1. In a clean 1.5 mL tube, add 320 µL of DNA Binding Buffer.
2. Add 40 µL of amplified SPIA Product.
3. Vortex and spin down briefly.
4. Obtain one Zymo-Spin II Column and place it into a collection tube.
5. Load the entire volume of the sample (360 µL) onto the Zymo-Spin II Column.
6. Centrifuge column in the collection tube for 10 seconds at >10,000 x g in a microcentrifuge.

   **Note:** Be sure to wait until rotor achieves desired speed before starting timer for spins less than one min in this procedure.

7. Discard flow-through. Place the Zymo-Spin II Column back in the same collection tube.
8. Wash sample by adding 200 µL of room temperature 80% ethanol. Do not use the wash buffer provided with the Zymo columns. Note: Use fresh 80% ethanol. Lower percent ethanol mixes will reduce recovery.
9. Centrifuge column in the collection tube for 10 seconds at >10,000 x g in a microcentrifuge. Discard flow-through.

*Best results are obtained by using fresh 80% ethanol in wash steps. Lower percent ethanol mixes will reduce recovery.*

10. Add 200 µL of room temperature 80% ethanol.
11. Centrifuge column in the collection tube for 90 seconds at >10,000 x g in a microcentrifuge. Discard flow-through.

12. Blot the column tip onto a filter paper to remove any residual wash buffer from the tip of the column.

    **Note:** Blot column tip prior to transferring it to a new tube to prevent any wash buffer from transferring to the eluted sample.

13. Place the Zymo-Spin II Column in a clean 1.5 mL microcentrifuge tube.
14. Add 40 µL of room temperature Nuclease-free Water (green: D1) to the center of each Zymo-Spin II Column. Do not use cold water!
15. Let columns stand for one min at room temperature.
16. Centrifuge column and microcentrifuge tube for 30 seconds at >10,000 x g in a microcentrifuge.
17. Collect sample. There should be approximately 40 µL of purified SPIA Product.
18. Mix sample by vortexing, then spin briefly.
19. Continue with the Measuring SPIA Product Yield and Purity protocol or store the purified SPIA Product at –20°C.

## B. Labeling and Hybridization to Agilent CGH Arrays

NuGEN recommends labeling 2 µg of SPIA product with the Invitrogen BioPrime® total for Agilent® aCGH (Invitrogen Cat# A10963-010 or A10963-011) system, according to manufacturer's instructions. SPIA product does not need to be restriction digested prior to labeling. Users of Agilent 4x44k and 8x15k CGH Arrays may elute the labeled SPIA product in a smaller volume of 30 µL.

Follow instructions in Agilent CGH manual G4410-90010 for array set up, hybridization and wash.

## C. Proteinase K Treatment of Genomic DNA

1. On ice, mix together 1 µL 50X TE, 23 µL Nuclease-free Water (green:D1) and 1 µL Proteinase K (NEB Cat #P8102S 20 mg/mL).
2. Add 25 µL DNA sample (minimum 25 ng total) and mix by pipetting.
3. Incubate at 37°C for 30 minutes and return to ice.
4. Purify with QIAGEN MinElute Spin Column according to manufacturer's instructions.

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## D. Prevention of Cross-Contamination

Due to the high sensitivity inherent in our amplification systems, we have a set of recommendations designed to minimize the potential for contamination of amplification reactions by carryover of nucleic acids or other laboratory contaminants. We strongly recommend implementing these procedures, especially for high-throughput environments typical in today's gene expression laboratories. Our two general recommendations are first to designate separate workspaces for pre-amplified and post-amplified steps and materials. This provides the best work environment for processing DNA using NuGEN's highly sensitive amplification protocols. Our second recommendation is to implement routine cleanup protocols for workspaces as standard operating procedure. This will prevent contamination by amplification products, intermediaries and exogenous nucleic acids from spreading through laboratory workspaces. Details regarding establishing and maintaining a "clean" work environment are listed below:

1. Designate a pre-amplification workspace separate from the post-amplification workspace or general lab areas:
    a. Post-amplification includes all steps and materials related to the handling of the final amplified SPIA Product, purification, array hybridization and any other analytical work. This also includes any work and materials related to other non-NuGEN protocols.
    b. Pre-amplification includes all steps and materials related to DNA sample handling and dilution, denaturation and hybridization, template synthesis, purification and SPIA setup.
    c. Ideally, the pre-amplification workspace would be in a separate work room. If this is not possible, ensure that the pre-amplification area is sufficiently distant and not in the path of post-amplification work.
    d. We recommend the use of a "PCR Workstation" enclosure with UV illumination dedicated for NuGEN pre-amplification protocol.
    e. Materials and consumables for pre-amplification work should be regularly exposed to UV illumination to control nucleic acid surface contamination.
2. Establish and maintain a clean work environment:
    a. Initially clean the entire lab thoroughly with DNA OFF and RNaseZap.
        i. In the pre-amplification area, remove all small equipment, and then clean every surface that may have been handled without gloves (drawer handles, key pads, etc.). Before reintroducing any equipment, clean every piece of equipment thoroughly. Especially clean wells of thermal cycler(s) and magnetic plate(s) with a Q-tip or by filling with decontamination solution.
    b. Always wear gloves and don fresh gloves upon entry into this controlled area. Frequently change gloves while working in the pre-amplification area, especially prior to handling stock reagents.

c. Stock this area with clean (preferably new) equipment (pipettes, racks, consumables) that has not been exposed to a post-amplification workspace.

d. Make it a policy to carry out continual, regular decontamination of all workspaces.

e. Capture waste generated in both pre- and post-amplification areas (tips, columns, wash solutions from beads and columns, tubes, everything) in sealable plastic bags and dispose of promptly after each experiment to avoid waste spillage.

f. Do not open amplified product reaction vessels in the pre-amplification workspace.

3. Utilize negative controls in order to detect and troubleshoot contamination issues. The clearest indication that an amplification reaction is contaminated is the appearance of significant amounts of amplified product in a "negative" control or no template control (NTC). We recommend using negative controls to confirm suspected contamination issues; however, it is not necessary to run them as standard procedure as the NTC product can contribute to cross-contamination if used regularly.

   a. In the absence of contamination:

      i. NTC yields for Ovation WGA System amplifications are typically at or below 0.5 µg.

      ii. Products generated from uncontaminated NTC reactions do not yield significant array hybridization even when applied to arrays at standard input amounts.

      iii. Bioanalyzer trace of this normal NTC product is very characteristic.

   b. In the presence of contamination:

      i. NTC yields are generally significantly higher than three µg, making NTC results the most reliable indicator of contamination.

      ii. Contaminated NTC yields can be as high as or even higher than template containing reactions (i.e., your experimental samples or positive controls).

      iii. The Bioanalyzer traces of contaminated NTC reactions can look significantly different than the typical non-contaminated NTC reaction traces.

4. When contamination is detected in reactions containing templates:

   a. The amount of product generated from a template containing amplification reaction may or may not be affected, depending on the source of the contamination.

   b. The Bioanalyzer trace of the amplified product may or may not look altered.

   c. The copy number changes detectable on arrays run with amplified product generated from a contaminated sample may be lower than expected.

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## E. Frequently Asked Questions (FAQs)

**Q1. What materials are provided with the Ovation WGA System?**
The Ovation WGA System provides all necessary primers, buffers and enzymes for denaturation and template synthesis, template purification and SPIA amplification.

**Q2. What equipment is required or will be useful?**
The Beckman Coulter SPRIPlate 96R Ring Magnet Plate, or SPRIPlate Ring Super Magnet Plate, is required for the template purification step. Other required equipment includes a microcentrifuge, pipettes, vortexer, thermal cycler and a UV/Vis spectrophotometer. An Agilent Bioanalyzer or a similar instrument may be used for quality control.

**Q3. What additional consumables do the user need?**
For the SPIA Product purification step, purification columns or magnetic beads are required.

**Q4. What is the polymerase and fidelity of the assay?**
The DNA polymerase used in the SPIA amplification reaction is a standard strand-displacement polymerase. Due to the nature of the amplification technology, SPIA has inherently higher fidelity compared to other procedures. This is because each amplified product is generated from an original template, thereby, amplification products themselves are not amplifiable. As a result, the process prevents reproduction of the promulgation of an error introduced in earlier cycles, a source of concern in exponential amplification systems.

**Q5. Have you tested your kits on degraded genomic DNA samples?**
No. At this time, we only support the use of the Ovation WGA System with good quality, high purity gDNA. Use of degraded input DNA may result in lower yields and the inability to detect subtle copy number changes.

**Q6. What does a no template control sample look like after amplification?**
No template control samples should have a yield of less than 0.5 µg consisting of low molecular weight product <100 nt.

**Q7. Does the Ovation WGA System work for FFPE samples?**
We have not tested FFPE derived DNA and do not currently support use of FFPE derived gDNA as input for this system.

**Q8. What is the length of the amplified SPIA Product?**
The amplified SPIA Product from good quality DNA ranges from 50 nt to 1500 nt, with an average size of approximately 350 nt.

**Q9. How long does the assay take to complete?**
Approximately four hrs.

**Q10. Does the Ovation WGA System amplify GC-rich genomic regions?**
We have not identified any regions of genomic DNA that the kit systematically does not amplify.

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**Q11. How can I label SPIA products for CGH analysis?**
NuGEN has obtained high-quality data using the Invitrogen BioPrime DNA labeling system for use on Agilent's CGH arrays.

**Q12. How much SPIA Product do I need for q-PCR analysis?**
This will depend on how many loci you wish to interrogate. NuGEN typically uses 10 ng of SPIA Product as template for TaqMan analysis.

**Q13. Can the Ovation WGA System be used for SNP genotyping or sequencing analysis?**
This system is intended as a generic WGA, suitable for a variety of downstream genomic analyses. Much of the NuGEN validation work has been performed using copy number change analysis on q-PCR and Agilent CGH arrays.

**Q14. What genomic DNA isolation methods are compatible with the Ovation WGA System?**
Various genomic DNA isolation techniques that produce high-quality and high-purity gDNA should be compatible with NuGEN Ovation WGA System. QIAGEN DNeasy columns were used extensively in the development of the Ovation WGA System. Follow manufacturer's recommendations carefully to obtain optimum results.

**Q15. What are the recommended storage conditions for the SPIA Product?**
SPIA Product may be stored at –20°C. Ensure the vials are well sealed and avoid multiple freeze-thaw cycles.

**Q16. Are labeling reagents included in the Ovation WGA System?**
No. This kit only includes the reagents necessary for generating SPIA Product (amplified DNA). Labeling and hybridization reagents must be purchased separately.

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## F. Update History

This document, the the Ovation WGA System User Guide (M01101 v1.1), is an update to address the following topics:

| Description | Section | Page(s) |
|---|---|---|
| Updated SPIA technology description. | I.A., I.B. | 1, 3 |
| Updated contact information for NuGEN Technical Support | V. | 18 |
| Changed part number for Agencourt RNAClean XP Purification Beads from 1200-01 to S01307 | VI.A. | 17 |


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