ABI 433A Peptide Synthesis

3 mL Reaction Vessel

User's Manual

Applied Biosystems

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Contents

1 About This Manual

This manual describes how to install and use the 3 mL Reaction Vessel System on the ABI 433A Peptide Synthesizer. This manual contains information that you might need to refer to from time to time, so it is recommended that you insert this manual at the back of the *ABI 433A Peptide Synthesizer User's Manual* for future reference.

Contents of the Manual

Section 1 About this Manual Briefly describes each section of this manual, explains the User Attention Words, and tells how to get help.

Section 2 Introduction Describes the purpose of the 3 mL Reaction Vessel System and provides brief chemistry information.

Section 3 Measuring Loop Installation Gives the procedure for changing and calibrating the new Variable Measuring Loop required for use with the 3 mL Reaction Vessel.

Section 4 Chemistry Provides information about reagents, bottle positions, preparing solutions, synthesis setup and example.

Section 5 Cycles and Modules Describes the new cycles and modules designed to be used specifically with the 3mL Reaction Vessel.

Appendix Contains plumbing schematics for the new 0.125 mL and 0.500 mL measuring loop configurations.

User Attention Words

Throughout the 3 mL Reaction Vessel User's Manual, four kinds of information are set off from the regular text. Each "User Attention Word" requires a particular level of observation or action that is significant to the user's safety or to proper instrument operation.

Note	*Used to call attention to information.*

IMPORTANT	*Indicates information that is necessary for proper instrument operation.*

Caution	***Damage to the instrument could result if you do not comply with this information.***

WARNING	***Physical injury to the user or other persons could result if these precautions are not implemented.***

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2 Introduction

About the 3 mL Reaction Vessel System

The 3 mL Reaction Vessel System makes it possible for the ABI 433A to synthesize peptides and peptide analogues on the 5-, 10-, and 20-µmol scale. These scales of synthesis are very useful when using expensive monomers such as glycosylated amino acids, isotopically labeled amino acids, and peptide nucleic acid (PNA) monomers.

The 3 mL Reaction Vessel System is established by installing the 3 mL Reaction Vessel kit (P/N 402067), which contains the following components:

- 3 mL Reaction Vessel (3 mL RV)
- tubing kit containing tubing required to make a 0.125-mL and 0.50-mL Variable Measuring Loop
- SynthAssist™ disk containing the Variable Measuring Loop Cycles
- boxes of empty amino acid cartridges
- this manual

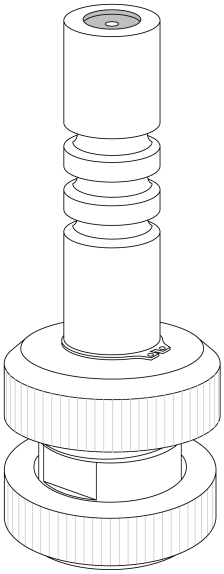
The cycles use HBTU activation strategy for both Fmoc and Boc chemistries. This approach is equivalent to Applied Biosystems *FastMoc* cycles when using Fmoc amino acids. However, we refer to these cycles as either Fmoc or Boc cycles because modules from both chemistries are present within a single chemistry file. This is useful when both Fmoc and Boc deprotections are required for one synthesis.

The 3 mL Reaction Vessel System is designed primarily for installation in the ABI 433A Peptide Synthesizer. The 3-mL Reaction Vessel System will also function on the ABI 431A Peptide Synthesizer if equipped as follows:

- The ABI 431A has the Feedback Monitoring kit
- The ABI 431A has the vortexer bracket that accepts RTF-style (Recessed Tab Filter) reaction vessels. (For information about whether your instrument accepts RTF-style reaction vessels, see *3 mL RV Installation Overview* on page 2-3.)

Note	*For information about upgrading or retrofitting an ABI 431A, contact Applied Biosystems Technical Support. (Refer to Technical Support on page 1-3.)*

3 mL Reaction Vessel



3 mL Reaction Vessel Part Number: 402776

Filter (Box of 30) Part Number: 401524

Use with 5-µmol, 10-µmol, and 20-µmol scale syntheses

Resin-sampling version is not available

Note	*The bottom cap of the 3 mL Reaction Vessel has three dots for identification.*

3 mL RV Installation Overview

This section describes the process of installing the hardware and software components of the 3 mL RV kit. Each step in the installation process must be performed in the order listed in this section to ensure that the 3-mL RV System functions properly. Carry out the following steps in the installation process:

1. Check ABI 431A for proper vortexer bracket (ignore for ABI 433A)

Note	*If you plan to use the 3 mL RV on an ABI 431A that has had the Monitoring Upgrade installed, verify that the synthesizer is equipped with the vortexer bracket that accepts RTF-style Reaction Vessels (RTF=Recessed Tab Filter). If you have an ABI 433A, ignore this instruction because all ABI 433A instruments have the proper vortexer bracket.*

If the vortexer bracket does not accept RTF-style Reaction Vessels, do not proceed any further. You need to upgrade the synthesizer's vortexer bracket before you can use the 3 mL RV. For more information, contact Technical Support (Refer to *Technical Support* on page 1-3).

2. Verify the contents of the 3 mL RV kit

The 3 mL Reaction Vessel System kit contains a number of different parts. Before you begin to install any components, take a minute to inventory the kit to verify that it is complete. Check the packing list(s) in the 3 mL Reaction Vessel System kit to verify that all the pieces are included before you begin any installation procedure. If your kit is missing any parts, contact Applied Biosystems Technical Support (Refer to *Technical Support* on page 1-3).

3. Inspect the RV

Caution	***The pieces of the 3 mL and 8 mL Reaction Vessels are not interchangeable. Do not mix parts from the two Reaction Vessels.***

Because the 3 mL Reaction Vessel (RV) is similar in shape to the 8 mL RV, the top and bottom caps of the 3 mL RV both have distinctive markings for easy recognition: the top cap of the 3 mL RV has 3 rings and the bottom cap has 3 dots.

4. Copy the new software modules to your hard disk

To copy the new software modules, drag the Variable Loop Folder from the disk in the kit onto your hard drive. Put the new cycles in the SynthAssist chemistry folder.

IMPORTANT *Once you install the Variable Measuring Loop, you must always use the new cycles included on the disk in the kit. The old cycles will not function properly with the Variable Measuring Loop hardware.*

5. Remove the old cycles from the Macintosh hard drive
The old cycles will not function properly with the new Measuring Loop hardware. Always use the new cycles from this point on.

6. Install the Variable Measuring Loop
For the procedure describing how to install the Variable Measuring Loop, Refer to *Installation Procedure* on page 3-3.

7. Remove the Conductivity Cell
If you are going to run cycles ***without*** monitoring, remove the Conductivity Cell. For instructions on removing the Conductivity Cell, refer to the *ABI 433A User's Manual*, Section 8: System Description.

8. Assemble the 3 mL RV
Install the filter, add the resin, and tighten the caps on the RV (see *To assemble the 3 mL RV:* on page 2-5).

9. Run a test peptide
Before you use expensive monomers, make a simple peptide to test the new synthesizer setup. For information on running a test synthesis, refer to *Synthesis Setup for the 3 mL Reaction Vessel* on page 4-15 and *Test Synthesis Example* on page 4-18. Use the normal synthesis procedure, with the following two exceptions:

- Prepare Bottle 7 and Bottle 8 reagent solutions according to your intended scale of synthesis (refer to the tables of concentrations on page 4-5).
- Prepare the amino acid solutions (Refer to *Bulk Amino Acid Solutions* on page 4-8).

10. Ready to go
If the test synthesis is satisfactory, you may proceed to your small-scale syntheses using the 3 mL RV.

How to Assemble the 3 mL RV

WARNING	***CHEMICAL HAZARD. To prevent serious chemical burns and eye damage, make sure a plug is inserted into the bulkhead fitting (ABI 431A) or the sliding cover flap covers the bulkhead fitting (ABI 433A). Even though the 3 mL RV cycles are written without resin sampling functions, hazardous solvents such as DCM, NMP, or DMF may squirt out of the resin sampler bulkhead fitting AT EYE LEVEL. Always wear protective lab coat, chemical-resistant gloves, and safety goggles.***

To assemble the 3 mL RV:

1. Hold the RV in a vertical position and place an RV filter on the protruding "knife edge" found just inside the openings at either end of the RV (Figure 2-1). The filter forms a seal with the knife edge when the RV cap is screwed in place.

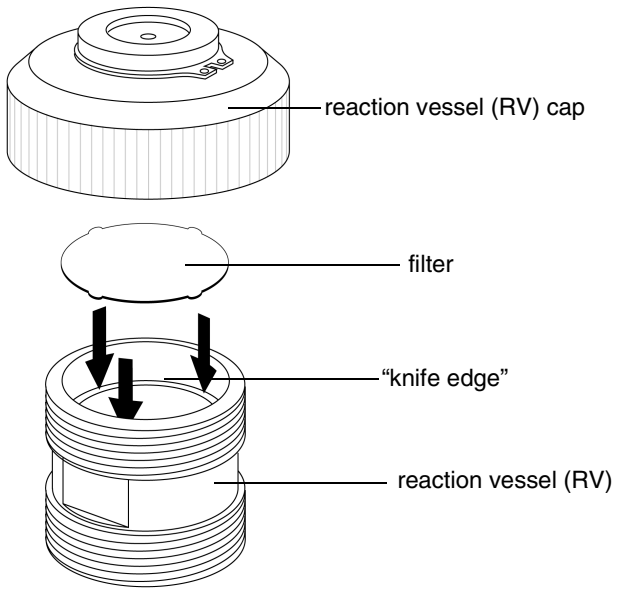


Figure 2-1. Placing RV filter on inner knife edge of reaction vessel

2. Screw on the RV cap, making sure to hold the RV in a vertical position at all times.

Caution	***Hold the RV in a vertical position when screwing on the RV cap. If you turn the RV on its side while tightening its cap, the filter may become crooked and form an imperfect seal. As a result, resin may escape and clog the in-line filter.***

Tighten the cap until you feel a firm resistance. This resistance indicates that the primary seal is forming between the filter and the recessed knife edge.

Visually check the filter placement by looking through the open end of the RV. The surface of the filter should be flat and smooth, with no protrusions beyond the knife edge.

Use the black, open-ended wrench to tighten and loosen both caps on 3 mL RV (see Figure 2-2).

Caution	***Reaction vessels are designed to be tightened by hand. Use only your hands or the ABI-supplied black wrench to tighten the 3 mL RV caps.***

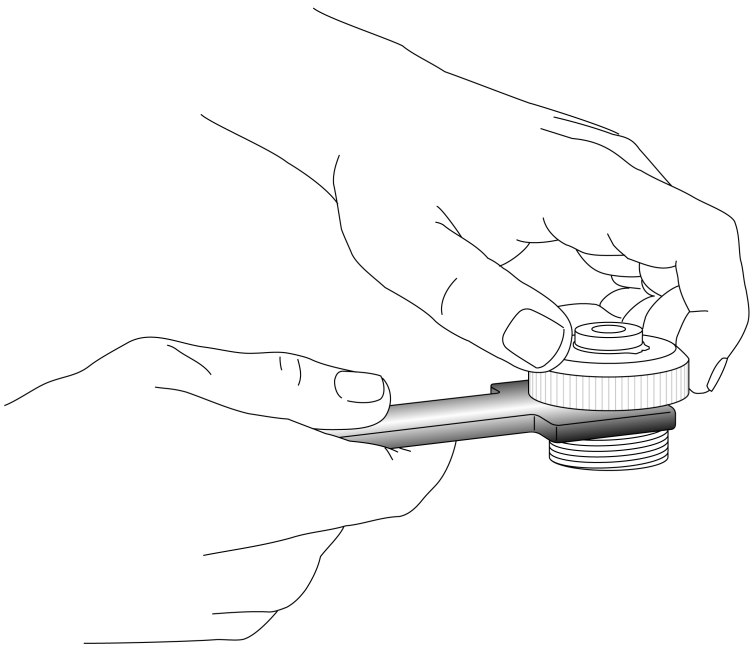


Figure 2-2. Use the open-ended wrench to tighten the 3 mL RV caps

3. Add the appropriate amount of resin to the 3 mL RV (Figure 2-3) Refer to Section 4 : *Chemistry* for resin quantity specifications.

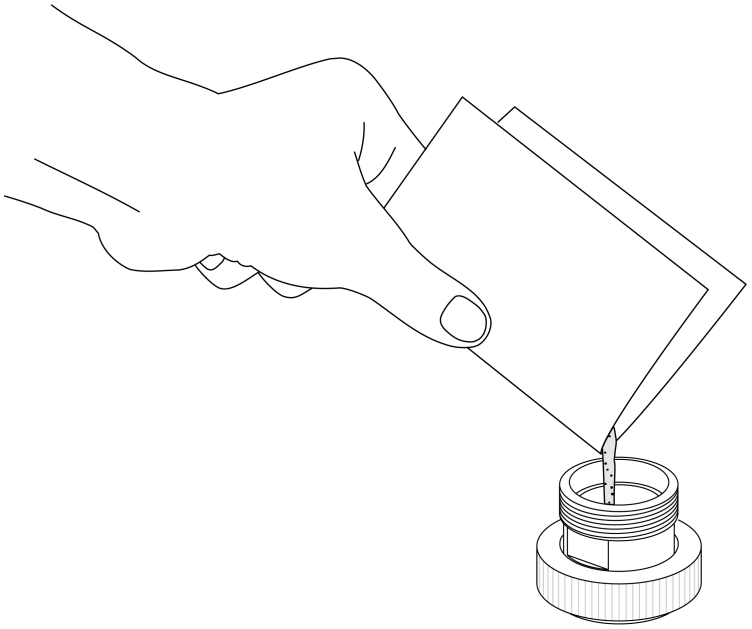


Figure 2-3. Filling the 3 mL RV with resin

4. Place a filter on the knife edge of the open end of the 3 mL RV. Tightly screw on the cap, using the procedure described in step 2. Place the RV in the RV holder on the synthesizer.

   Use the black, open-ended wrench to tighten the 3 mL RV caps.

3 Installing the Variable Measuring Loop

The ABI 433A is built using a 0.5-mL fixed measuring loop to deliver the solutions from Bottles 7 and 8. The Variable Measuring Loop kit contains the tubes required to change the fixed measuring loop to one that can deliver either 0.5 mL (for 0.10-, 0.25-, 0.50-, and 1.0-mmol cycles) or 0.125 mL (for 5-, 10-, or 20-µmol cycles). Perform the entire installation procedure to calibrate both measuring loops.

IMPORTANT	*Once you install the Variable Measuring Loop, you must always use the new cycles included on the floppy disk. The old cycles will not function properly with the Variable Measuring Loop hardware.*

Equipment Required

Wrenches: 5/16-inch, 3/8-inch, and 7/16-inch

New, single-edged razor blade

To prepare for installation:

WARNING	***CHEMICAL HAZARD. Chemicals in bottles under pressure can discharge dangerous liquids into eyes and onto skin. Always wear protective lab coat, gloves, and safety goggles when working with chemicals and bottles under pressure.***

1. Run Flow Tests 10 (NMP to the metering vessel) and 11 (NMP to cartridge) to verify proper reagent flow before doing any hardware installation. (See section on Flow Tests in 433A User's Manual.)

	Expected	Found
Flow Test 10	2.45 to 2.55 mL to RV position	
Flow Test 11	1.95 to 2.35 g to cartridge	

   If the flow test values are not correct, replace the inline filters, then repeat the tests.

   If the flow test values are still not correct, adjust the bottom regulator, then repeat the tests.

2. Clean the existing 0.50-mL measuring loop with NMP by using the following procedure:
   a. Add about 25 mL NMP to an empty 200-mL bottle and place the bottle in the Bottle 8 position.
   b. Switch the ABI 433A to manual control (use the manual control menu).
   c. Turn on Fxn 69 and let the ABI 433A run until all the NMP has been removed from Bottle 8.
   d. Turn off Fxn 69.
   e. Turn on Fxn 70 for about 10 seconds.
3. Clean the Bottle 7 tube with NMP by using the following procedure:
   a. Add about 25 mL NMP to an empty 200-mL bottle and place the bottle in the Bottle 7 position.
   b. Turn on Fxn 68 and let the ABI 433A run until all the NMP has been removed from Bottle 7.
   c. Turn off Fxn 68.
   d. Turn on Fxn 70 for about 10 seconds.
4. Add 25 mL NMP to Bottle 7 and 8, then run Flow Test 17 and 18.

	Expected	Found
Flow Test 17 & 18	0.515 to 0.554 g to cartridge	

5. Empty both Bottles 7 and 8 by turning on Fxn 68 and 69 until the Bottles are empty.
6. Turn on Fxn 70 for about 10 seconds.

Installation Procedure

Removing the existing 0.5-mL measuring loop tube

The existing 0.5-mL tube is located between Valve 13 on the 11-port Valve Block and the Teflon cross-fitting (Figure 3-2).

To remove the 0.5-mL measuring loop:

1. If you have not already done so, remove the right side panel from the instrument. Refer to Figure 3-1 to identify the correct panel to remove.

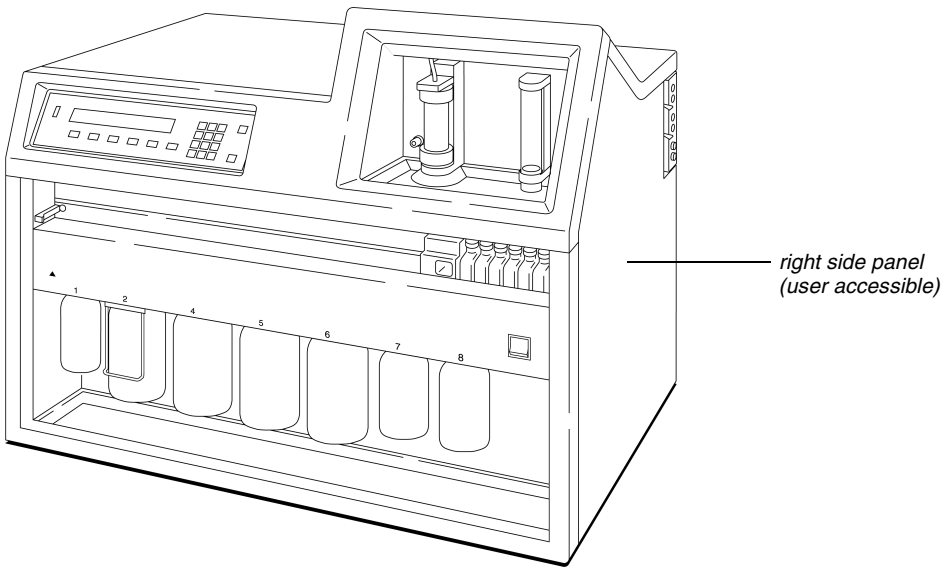


Figure 3-1. ABI 431/433 showing location of right side (user accessible) panel

2. Remove the end of the 0.5-mL loop from the cross-fitting (Figure 3-2).

   To find which tube to remove, locate valve 13 on the middle valve block, trace that tube back to the cross fitting, then remove the tube end from the cross fitting.

3. At the middle valve block, loosen the metal hex nut, then loosen the black bushing that secures the other end of the 0.5-mL loop.

4. Pull the 0.5-mL tube out of the valve block *very carefully*, making sure that the white ferrule does not pull loose from the tube.

   If it is difficult to pull out the tube and ferrule, use the following procedure for gas-assisted removal of the tube:

   a. Switch the ABI 433A to manual control (use the manual control menu).

   b. Turn on Fxn 10 (Gas B VB) for about 10 seconds.

   c. Turn off Fxn 10.

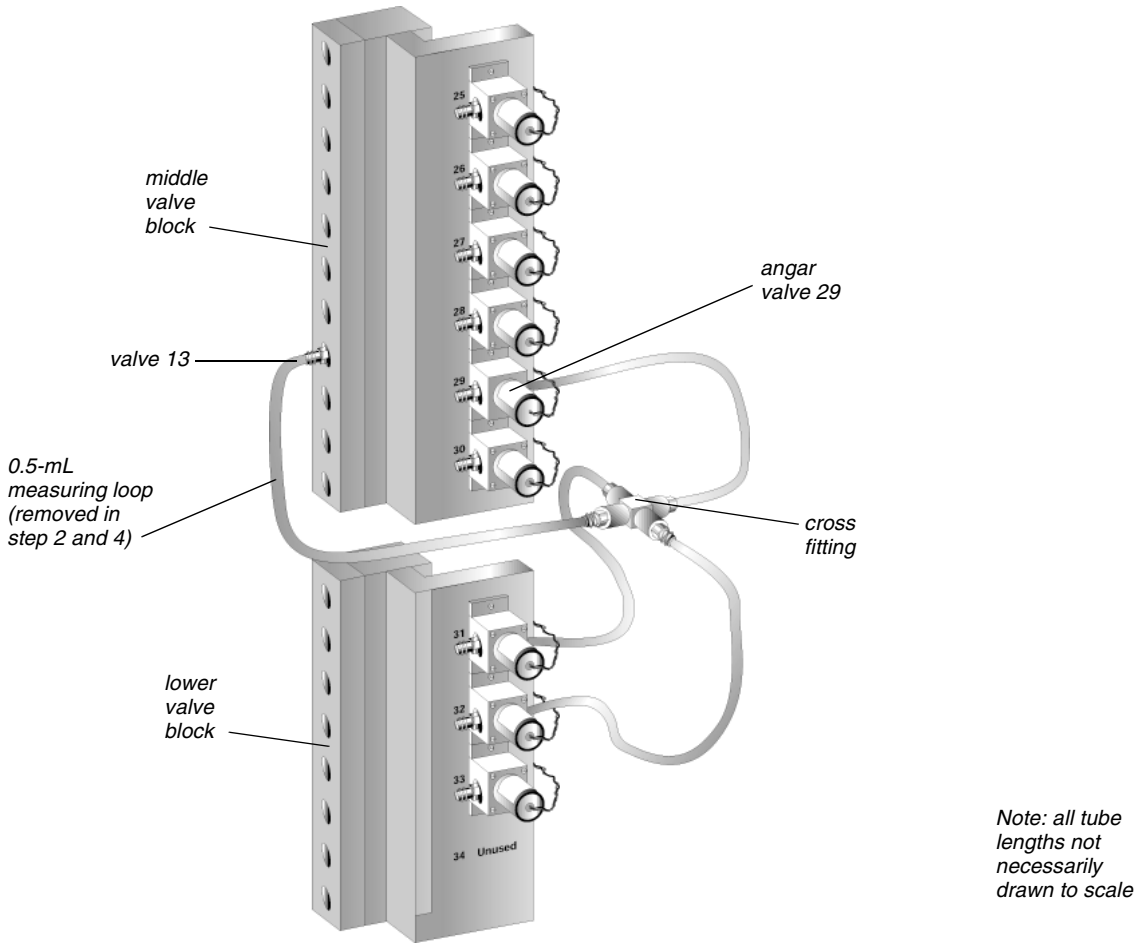


Figure 3-2. Original 0.5-mL measuring loop configuration

d. Open valves 17, 16 and 13 to send gas through the tube.

e. Place your fingertip over the cross-fitting end of the tube to cause the gas pressure inside the tube to build up.

f. Carefully remove the tube, which should now be easier to remove because of the gas pressure assistance.

g. Turn off the valves.

To connect the variable measuring loop tube:

1. Locate the short red tube labeled "ASSY, VALVE 13 TO CLPG" (P/N 604130) and install the metal hex nut removed in step 3 above.

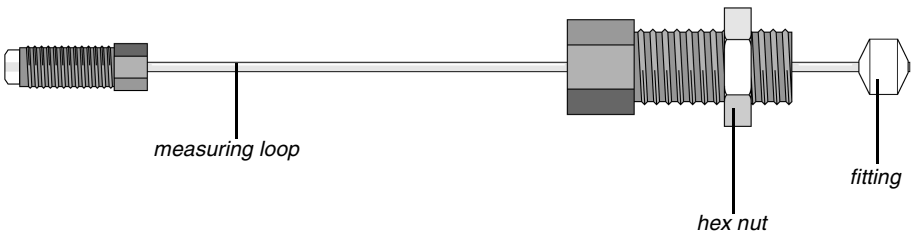


Figure 3-3. Measuring loop tube with hex nut installed

2. Attach this short red tube to Valve 13 on the 11-port Valve Block (Figure 3-4). Use only your fingers to tighten the fitting.

Caution	***Do not over tighten the measuring loop fitting. Damage to the ferrule from over tightening can cause leaks in the measuring loop.***

3. After the fitting is finger tight, use the 5/16-inch wrench to tighten the fitting a maximum of one quarter turn more. Tighten the metal hex nut finger tight.
4. Attach the new long red tube labeled "ASSY, TUBE CPLG TO X-FTG" (P/N 604129) to the cross-fitting (Figure 3-4), again using your fingers.

Note	*You will trim the long red piece of tube to calibrate the 0.125-mL measuring loop in step 3 below. A spare piece of tube is provided in the Tubing Kit.*

5. Connect the two red tubes (P/N 604129 and 604130) with a 1/4-28 flange coupling (P/N 110070) (Figure 3-4) using only your fingers until the connections are tight.
6. Remove the existing tube that connects the cross-fitting to Angar Valve 29 and replace it with the new yellow tube labeled "ASSY, TUBE X-FTG TO VALVE 29" (P/N 604128) (Figure 3-4).

   Be sure the fitting is straight as you screw it into the Angar valve to avoid cross threading.
7. After the fitting is finger tight, use the 5/16-inch wrench to tighten the fitting one quarter turn more.

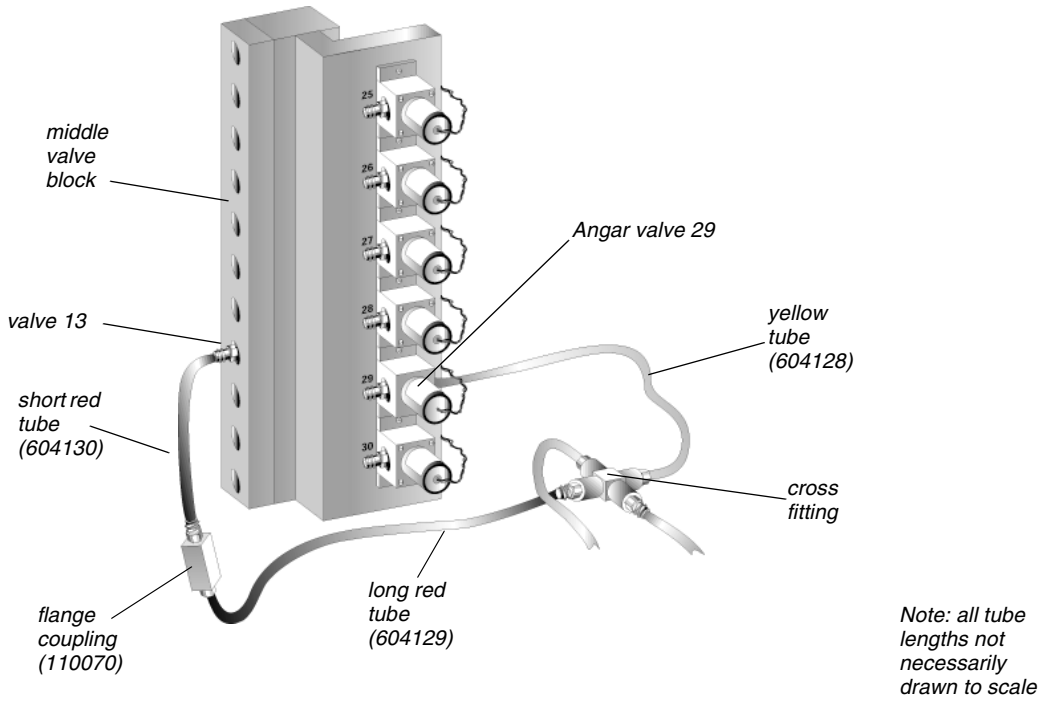


Figure 3-4. New 0.125-mL measuring loop configuration

To calculate the 0.125-mL Measuring Loop calibration:

The long red tube labeled "ASSY, CPLG TO X-FTG" (P/N 604129) must be trimmed so it will deliver between 0.123 mL and 0.127 mL.

1. Install a bottle of NMP at the Bottle 8 position.

   NMP has a density of 1.033 g/mL, which will be used for calculating the calibration later (Table 3-1 and Table 3-2).

2. Replace the cartridge inline filter with a flange coupling. (This is only for calibrating the measuring loop. You will replace the inline filter later in this section.)

3. Place an empty tared cartridge with a septum into the guideway under the needle assembly and place the pusher block against the cartridge.

4. If you have not already done so, load the new cycles provided on the Variable Measuring Loop Cycles disk. In the Flow Tests folder, open "Flow Tests (New VML)" and send to the ABI 433A.

5. Run flow test “a” (module “a”). This module fills the measuring loop 10 times from Bottle 8 and delivers the contents to the cartridge position. The steps in this module are listed on page 5-27.

   As flow test “a” runs, watch the flow at the waste port to verify that reagents are flowing quickly enough. You should see the NMP fill the tube within three seconds of the start of delivery. If the NMP does not fill the tube within the proper time, a tube restriction or fitting leak is the probable cause.

6. Check all the tube connections you have made to ensure that there are no leaks. If you find a leak on the valve block, tighten the fitting no more than one quarter turn. If the leak is on a fitting, tighten the fitting with your fingers.

7. Weigh the cartridge containing the NMP from the 11-step module. Consult Table 3-1 to determine whether the weight falls within the specified range.

Table 3-1. 0.125-mL measuring loop weight specification

Weight of NMP (10 loops)	Resulting Volume (1 loop)
1.27 - 1.31 g	0.123 - 0.127mL

8. Repeat steps 3 through 7 repeatedly until you achieve three successive results that are consistent in their weight measurements. After the results are consistent, then go on to step 9.

9. Determine how much the NMP is over the desired weight and use this information to determine approximately how much tube to trim. The example below shows how to calculate the amount of tube to cut. The linear volume of the red tube is about 0.002 mL per centimeter.

WARNING ***CHEMICAL HAZARD. The measuring loop and other tubes contain N-methylpyrrolidone (NMP). Always wear protective lab coat, gloves, and safety goggles when handling tubes that may contain even small amounts of reagents such as NMP.***

Example for calculating how much to cut 0.125 mL measuring loop

❶ weight of 10 loops of NMP delivery: 1.35 g

❷ weight of 1 loop of NMP delivery $= \frac{1.35\text{ g}}{10\text{ loops}} = 0.135\text{ g}$

❸ volume of 1 loop of NMP delivery $= \frac{\text{NMP weight}}{\text{NMP density}}$

$$= \frac{0.135\text{ g}}{1.033\text{ g/mL}} = 0.131\text{ mL}$$

❹ difference between the volume of NMP delivery and the upper volume specification (Table 3-1)

$$= 0.131\text{ mL} - 0.127\text{ mL} = 0.004\text{ mL excess}$$

❺ amount of tube to be cut

$$= \frac{\text{volume in excess}}{\text{linear vol of tube}} = \frac{0.004\text{ mL}}{0.002\text{ mL/cm}} = 2\text{ cm to cut}$$

To cut measuring loop to correct length:

1. Disconnect the long red tube from the flange coupling and cross fitting. Remove the long red tube to a secure work surface.
2. Separate the ferrule (white) from the fitting (black), then slide both the ferrule and fitting several inches farther onto the tube (Figure 3-5).

Note *When estimating how much tube to trim, it is better to cut too little than to cut too much.*

3. Using a new single-edged razor blade, trim the appropriate amount of tube, determined in step 9 above, using a perfectly straight cut (Figure 3-5).
4. Slide the ferrule and fitting to within $\frac{1}{4}$ inch of the trimmed end of the tube.
5. Press fit the ferrule and fitting together, leaving approximately $\frac{1}{4}$ inch of tube protruding from the ferrule.

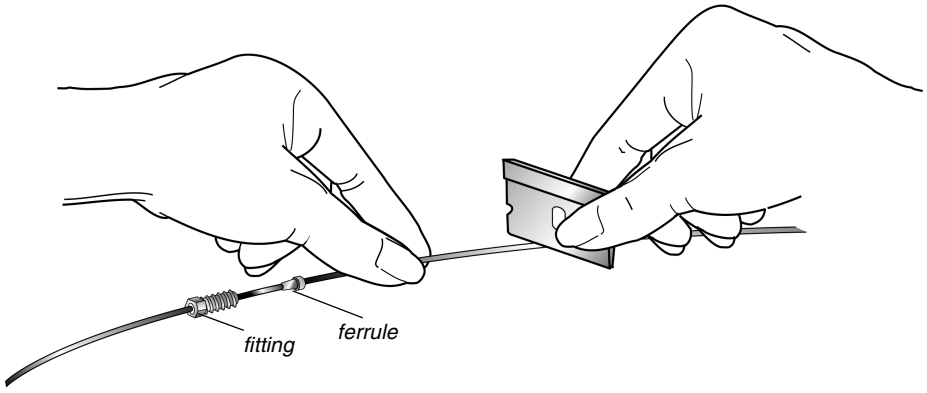


Figure 3-5. Trimming the long red tube

6. Holding the fitting/ferrule assembly between your fingers, press the trimmed end of the red tube down onto a hard surface to bring the ferrule flush with the end of the tube (Figure 3-6).

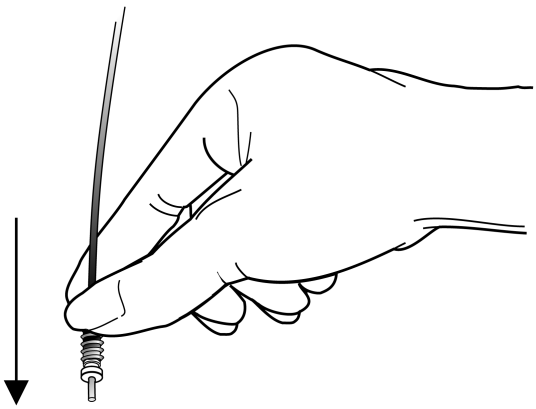


Figure 3-6. Pressing long red tube into fitting and ferrule

7. Reinstall the long red tube between the flange coupling and cross fitting.
8. Rerun flow test "a" to determine whether the measuring loop is within specification (Table 3-1). If further calibration is required, repeat the calculation procedure starting on page 3-6.

To calibrating the 0.5-mL Measuring Loop:

1. Connect the new blue tube labeled "ASSY, TUBE 0.5 ML LOOP" (P/N 604127) between the two pieces of red tube (P/N 604129 and 604130) using two flange couplings (P/N 110070) (Figure 3-7).
2. Place an empty tared cartridge with a septum into the guideway under the needle assembly and place the pusher block against the cartridge.

3. Run flow test “b” located in the file “Flow Tests (New VML).” The steps in this module are listed on page 5-28.
4. Check all the tube connections you have made to ensure that there are no leaks. If you find a leak, tighten the connection with your fingers.
5. Weigh the cartridge containing the NMP from the 11-step module. Consult Table 3-2 to determine whether the weight falls within the specified range.

Table 3-2. 0.5-mL measuring loop weight specification

Weight of NMP (4 loops)	Resulting Volume (1 loop)
2.037 - 2.095 g	0.493 - 0.507 mL

6. Run flow test “b” repeatedly until you achieve three successive results that are consistent in their weight measurements. After the results are consistent, then go on to step 7.
7. Determine how much the NMP is over the desired weight and use this information to determine approximately how much of the blue tube to trim. The example below shows how to calculate the amount of tube to cut. The linear volume of the blue tube is about 0.005 mL per centimeter.

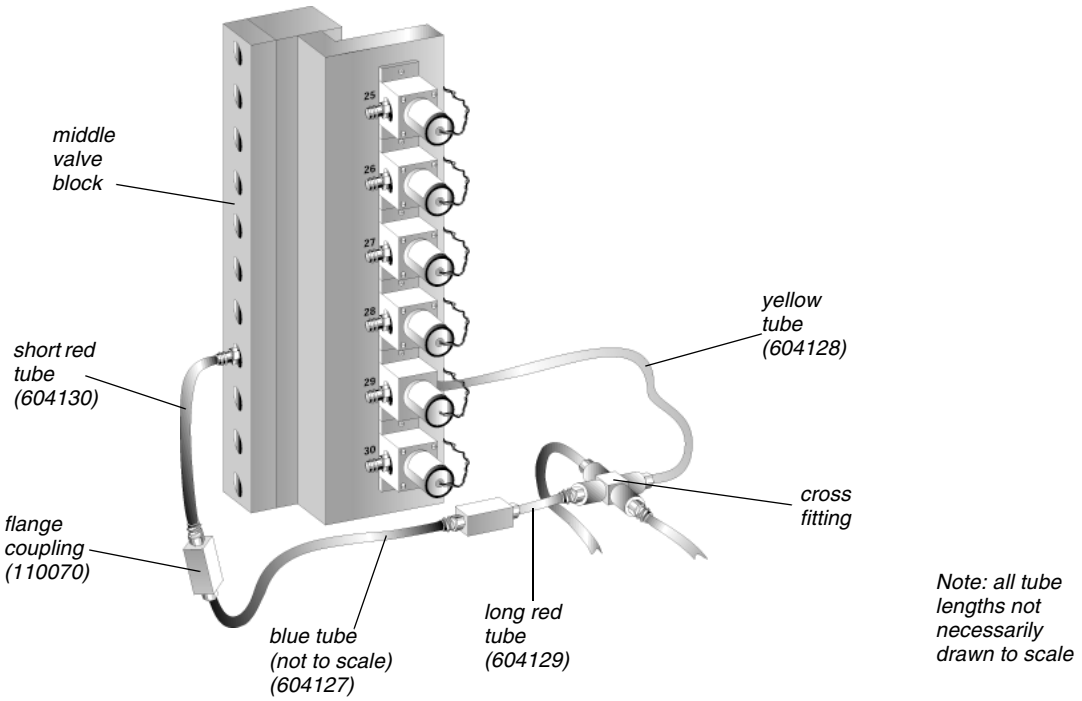


Figure 3-7. New 0.5-mL measuring loop configuration

WARNING	Wear protective gloves when handling tubes that may contain even small amounts of reagents such as N-methylpyrrolidone(NMP).

Example for calculating how much to cut 0.5 mL measuring loop

❶ weight of 4 loops of NMP delivery: 2.373 g

❷ weight of 1 loop of NMP delivery $= \frac{2.373\text{ g}}{4\text{ loops}} = 0.593\text{ g}$

❸ volume of 1 loop of NMP delivery $= \frac{\text{NMP weight}}{\text{NMP density}}$

$= \frac{0.593\text{ g}}{1.033\text{ g/mL}} = 0.574\text{ mL}$

❹ difference between the volume of NMP delivery and the upper volume specification (Table 3-2)

$= 0.574\text{ mL} - 0.507\text{ mL} = 0.067\text{ mL excess}$

❺ amount of tube to be cut

$= \frac{\text{volume of NMP}}{\text{linear vol of tube}} = \frac{0.067\text{ mL}}{0.005\text{ mL/cm}} = 13.4\text{ cm to cut}$

To cut measuring loop to correct length:

1. Disconnect the blue tube from the flange couplings. Remove the blue tube to a secure work surface.
2. Separate the ferrule (white) from the fitting (black), then slide both the ferrule and fitting several inches farther onto the tube (Figure 3-5 on page 3-9).
3. Using the single-edged razor blade, trim the appropriate amount of tube determined in step 7 above using a perfectly straight cut.
4. Slide the ferrule and fitting to within $\frac{1}{4}$ inch of the trimmed end of the tube.
5. Press fit the ferrule and fitting together, leaving approximately $\frac{1}{4}$ inch of tube protruding from the ferrule.
6. Holding the fitting/ferrule assembly between your fingers, press the trimmed end of the red tube down onto a hard surface to bring the ferrule flush with the end of the tube (Figure 3-6 on page 3-9).

7. Reinstall the long red tube between the flange coupling and cross fitting.
8. Rerun flow test “b” to determine whether the measuring loop is within specification (Table 3-2). If further calibration is required, follow this procedure again.
9. Check one more time all the tube connections you have made to ensure that there are no leaks.
10. Reinstall the inline filter you removed earlier (step 2 on page 3-6).
11. Run flow tests “c” and “d” to check the variable measuring loop. These tests will let you verify that the measuring loop fills within the correct time. The 0.125-mL loop should fill within three seconds and the 0.50-mL measuring loop should fill within five seconds. Module “c” is for Bottle 7 and module “d” is for Bottle 8 (see page 5-28 for details).

The Variable Measuring Loop is now installed and calibrated. Table 3-3 shows which tubes to use for each scale synthesis.

Table 3-3. Measuring Loop Configurations

	synthesis scale						
	5-µmol	10-µmol	20-µmol	0.1-mmol	0.25-mmol	0.5-mmol	1.0-mmol
short red tube	●	●	●	●	●	●	●
long red tube	●	●	●	●	●	●	●
blue tube	removed	removed	removed	●	●	●	●

● = use this tube for synthesis

Note: When you remove the blue tube for the 5-, 10-, and 20-µmol scales, also remove one of the flange couplings, then connect the two red tubes together.

Refer to Section 4 for Chemistry information or Section 5 for Cycle and Module information.

4 Chemistry

The cycles provided for the 5-, 10-, and 20-µmol scales use the HBTU activation strategy for both Fmoc and Boc chemistries. This approach is equivalent to Applied Biosystems *FastMoc* chemistry for the higher scales. However, we refer to these cycles as either Fmoc or Boc cycles because modules from both chemistries are present within a single chemistry file. This is useful when both Fmoc and Boc deprotections are required for one synthesis.

The 3 mL Reaction Vessel System uses five equivalents of amino acid monomer, which is predissolved in NMP before being added to an empty amino acid cartridge. Activation occurs through the use of HBTU located in Bottle 8 and DIEA located in Bottle 7. The measuring loop for Bottles 7 and 8 is 0.125 mL and the concentration of the DIEA solution in Bottle 7 and the HBTU solution in Bottle 8 varies according to the scale of synthesis. The amounts of the various reagents are summarized in Table 4-1 on page 4-2.

In the Boc cycle, neutralization and coupling are performed simultaneously. This process is often called *in situ* neutralization[†] and is possible because the HBTU activation uses excess DIEA. The only different Boc derivative that should be used with this approach is Boc-Asn(Xan) or Boc-Asn(Trt) instead of Boc-Asn.

[†] Schnölzer, M., Alewood, P., Jones, A., Alewood, D. and Kent, S.B.H. 1992. *In situ* neutralization in Boc-chemistry solid phase peptide synthesis. *International Journal of Peptide & Protein Research* 40:180-193

Table 4-1. Concentration and amounts of reagents

Resin	5 µmol	10 µmol	20 µmol	0.1 mmol
Monomer	25 µmol ~ 0.110 mL	50 µmol ~ 0.220 mL	100 µmol ~ 0.440 mL	
HBTU	0.19 M HBTU X 0.125 mL 23.0 µmol	0.38 M HBTU X 0.125 mL 47.5 µmol	0.38 M HBTU X 0.250 mL 95 µmol	
DIEA	0.40 M DIEA X 0.125 mL 50 µmol	0.80 M DIEA X 0.125 mL 100 µmol	1.60 M DIEA X 0.125 mL 200 µmol	
Coupling Volume	~ 0.35 mL	~ 0.47 mL	~ 0.69 mL	
Coupling Concent.	~ 0.068 M	~ 0.100 M	~ 0.138 M	(comparison) ~ 0.167 M

Cycle Times

The cycle time for both the Fmoc and Boc cycles is approximately 50 minutes. The cycles and their durations are listed in Table 4-2 and Table 4-3.

Table 4-2. Fmoc Cycle with 3 mL RV

Module	Total Time (min)
Piperidine Deprotection (2 times)	7.0
NMP Washes (6 times)	2.9
Activate monomer	0.3
Coupling	35
Capping	1.0
NMP Washes (3 times)	1.4
Cycle Time for Fmoc chemistry	Approximately 50 minutes

Table 4-3. Boc Cycle with 3 mL RV

Module	Total Time (min)
DCM Wash (1 time)	0.9
TFA Deprotection (2 times)	6.0
DCM Wash(1 time)	0.5
NMP Washes (6 times)	2.9
Activate monomer	0.3
Coupling	35
Capping	1.0
NMP Washes (3 times)	1.4
Cycle Time for Boc chemistry	Approximately 50 minutes

Solvent Consumption

One cycle consumes a total volume of approximately 60 mL of solvent. Each of the three scales of synthesis use the same quantity of solvent. One reason for this is that the quantity of solvent needed to wash the 3 mL RV and amino acid cartridge are the same for each scale. In addition, the quantities of resin used in the 5-, 10- and 20-µmol scale syntheses are so small that the solvent used to wash the RV and cartridge is more than enough to wash the resin.

Reagent and Bottle Positions

WARNING	***CHEMICAL HAZARDS. Chemicals used on the ABI 433A can be hazardous and cause injury, illness or death. Become completely familiar with the Material Safety Data Sheet (MSDS) for each hazardous chemical before attempting to operate the instrument or use the reagents. MSDSs are provided in the Safety Supplement of the ABI 433A User's Manual. When working with hazardous chemicals, wear all appropriate safety attire listed in the MSDSs. To minimize inhalation of the chemicals, do not leave any chemical bottles uncapped.***

IMPORTANT	*Cartridges swell after extended contact with solvents such as NMP and DCM. After only a single synthesis cycle, a cartridge can swell enough to exceed the recommended cartridge size. Reusing a cartridge can result in the cartridge becoming stuck in the autosampler and shutting down your synthesis.*

Bottle 1: Piperidine (P/N 401750)
Piperidine is used for Fmoc removal. Even if only Boc cycles are used, Piperidine (or DIEA) is needed in Bottle 1 because Flow Test 2 contains a step that uses Bottle 1 to neutralize the metering vessel.

Bottle 2: TFA (P/N 400137)
When synthesizing peptides with Boc-protected amino acids, use 100% TFA in Bottle 2. When synthesizing PNAs, use 95% TFA/5% m-cresol in Bottle 2. Use Bottle 2 bottle seal (P/N 400789) when using TFA in Bottle 2.

Avoid leaving TFA installed on an unused synthesizer for an extended period of time. Because an unused synthesizer does not backflush the TFA tubing with nitrogen, TFA fumes are in long-term contact with synthesizer valves. If you plan to not use TFA for an extended period of time (for example, two weeks or more), remove the TFA bottle from the instrument.

See *Waste Container* on page 4-6 for information about neutralizing TFA in the waste container.

Bottle 4: Capping solution

Two capping solutions have been used. The typical capping solution for peptides is 0.5 M acetic anhydride, 0.125 M DIEA and 0.015 M HOBt in NMP. This is made by combining the following components:

- 19 mL of acetic anhydride (P/N 400660)
- 9 mL of 100% DIEA (P/N 400136)
  or 26 mL of 2 M DIEA (P/N 401517)
- 6 mL of 1 M HOBt/NMP (P/N 400662)

and diluting to 400 mL with NMP (P/N 400580)

The other capping solution often used in Peptide Nucleic Acid (PNA) synthesis is a 1/25/25 mixture of acetic anhydride/pyridine/NMP.

Note *Make both capping solutions fresh each week.*

Bottle 7: DIEA solution

The concentrations of DIEA for different scales of synthesis, and their preparation using 2 M DIEA (P/N 401517), are shown in Table 4-4.

Table 4-4. DIEA solutions

Scale	DIEA	Preparation
5 µmol	0.40 M	40 mL 2 M DIEA diluted to 200 mL in NMP
10 µmol	0.80 M	80 mL 2 M DIEA diluted to 200 mL in NMP
20 µmol	1.60 M	160 mL 2 M DIEA diluted to 200 mL in NMP

Bottle 8: HBTU solution

Note	*Before running any synthesis, verify that the tube for Bottle 8 has the HBTU filter installed.*

The HBTU solution can be made with or without the additional HOBt. For peptide synthesis, it is usually made with the additional HOBt, according to the directions on the HBTU Activation kit (P/N 401132). This gives a 0.45 M HBTU/0.45 M HOBt solution, which should be diluted with NMP to give the desired solution as shown in Table 4-5 on page 4-6.

Table 4-5. HBTU solutions using 0.45 M HBTU/HOBt

Scale	HBTU	HBTU/HOBt Preparation	Volume
5 µmol	0.19 M	Dilute 84 mL 0.45 M HBTU/HOBt to 200 mL with NMP	1 X 0.125 mL = 0.125 mL
10 µmol	0.38 M	Dilute 169 mL 0.45 M HBTU/HOBt to 200 mL with NMP	1 X 0.125 mL = 0.125 mL
20 µmol	0.38 M	Dilute 169 mL 0.45 M HBTU/HOBt to 200 mL with NMP	2 X 0.125 mL = 0.250 mL

For PNA synthesis, the HBTU solution usually does not have the additional HOBt. The HBTU (mw 379.3) is dissolved in NMP as shown in Table 4-6.

Table 4-6. HBTU solutions using solid HBTU

0.19 M HBTU	14.4 g HBTU dissolved in NMP and diluted to 200 mL
0.38 M HBTU	28.8 g HBTU dissolved in NMP and diluted to 200 mL

WARNING	***RESPIRATORY HAZARD. Inhaling HBTU dust can cause bronchial irritation with coughing. Repeated or prolonged exposure may cause allergic respiratory system sensitization. Handle HBTU under a chemical fume hood.***

The cycles designed for the 3 mL RV do not contain a loading cycle. Loading Fmoc-amino acids on HMP resins requires DCC in Bottle 8. Therefore, use pre-loaded resins for syntheses in these micromole scales.

Bottle 9: DCM (P/N 400142)

Bottle 10: NMP (P/N 400580)

Waste Container

If you are using TFA, pour a bottle of Ethanolamine/Methanol (P/N 400230) into the waste container to neutralize the TFA in the waste.

Cartridge

Predissolve the Fmoc amino acids, Boc amino acids or Boc-PNAs in NMP, using five or ten equivalents of monomer for each coupling. Add the mixture to the cartridge.

The formula for calculating monomer quantity is

$$\text{monomer MW} \times \text{mmoles of AA} = \text{mg of AA per cartridge}$$

To calculate the quantity of monomer when five equivalents is required:

a. From Table 4-1 on page 4-2, find the quantity of monomer you need for your scale synthesis.

   If a 5-µmol scale synthesis is being performed, you need 25 µmol of monomer (5 equivalents).

b. If you use Fmoc-Ala (MW 311.3), then the calculation using the formula above is as follows:

$$311.3 \times .025 \text{ mmol} = 7.8 \text{ mg}$$

c. According to the calculation, 7.8 mg of Fmoc-Ala is needed for a 5-µmol scale synthesis.

The formula for calculating the quantity of NMP required to dissolve the monomer is:

$$\underset{\text{(in mmol)}}{\text{monomer quantity}} \times \text{Vol of solvent per mmol} = \text{Volume of NMP}$$

To calculate the quantity of NMP required:

a. Use an amount of NMP that equals 4 mL/1 mmol of monomer.

b. If you use Fmoc-Ala, the quantity of NMP required is determined as follows:

$$(0.025 \text{ mmol}) \left(\frac{4 \text{ mL}}{\text{mmol}} \right) = 0.10 \text{ mL}$$

c. According to the calculation, 0.10 mL of NMP is required for a 5-µmol scale synthesis using Fmoc-Ala.

If the same monomer is used several times, then a larger amount of material can be dissolved. The resultant monomer solution can be refrigerated and stored for a week. Details for this are given in the next section.

Bulk Amino Acid Solutions

When the same monomer is used several times per week, a larger amount of solution can be prepared. Two calculations are required, one for millimoles of amino acid and one for quantity of solvent.

To calculate the number of mmoles of amino acid in 0.25 g Fmoc-Ala.

$$\frac{\text{grams of monomer}}{\text{MW}} \times 1000 = \text{no. of mmol}$$

$$\left(\frac{0.25\ \text{g}}{311.3}\right) \times 1000 = 0.803\ \text{mmol}$$

To calculate the quantity of NMP required for 0.25 g Fmoc-Ala:

$$(\text{no. of mmol}) \times (\text{vol of solvent per mmol}) = \text{solvent required}$$

$$(0.803\ \text{mmol}) \left(\frac{4\ \text{mL}}{\text{mmol}}\right) = 3.21\ \text{mL}$$

The amount of this solution to pipet into the cartridge has been calculated for each Fmoc and Boc amino acid using a density of 1.04 g/mL for each solution. This information is shown in Table 4-7 for the three different scales of syntheses when five equivalents of monomer is used. For example, in Table 4-7, 107-µL of the Fmoc-Ala solution is used for the 5-µmol cycle.

Note	*The solution volumes given in Table 4-7 and Table 4-8 represent the* minimum *amount of amino acid to use.*

Table 4-7. Amino acid solutions when using five equivalents (1 mmol in 4 mL NMP)

amino acid	MW	weight (g) of mmol AA and 4 mL NMP	weight (g) of solution for 25 µmol	monomer: / resin:	volume (mL) of solution for: 25 µmol 5 µmol	50 µmol 10 µmol	100 µmol 20 µmol
Fmoc-Ala-OH	311.3	4.443	0.111		107	214	428
Fmoc-Cys(Trt)-OH	585.7	4.718	0.118		114	228	456
Fmoc-Asp(OtBu)-OH	411.4	4.543	0.114		110	220	440
Fmoc-Glu(OtBu)-OH	425.5	4.557	0.114		110	220	440
Fmoc-Phe-OH	387.4	4.519	0.113		109	218	436
Fmoc-Gly-OH	297.3	4.429	0.111		107	214	428
Fmoc-His(Trt)-OH	619.7	4.752	0.119		115	230	460
Fmoc-Ile-OH	353.4	4.485	0.112		108	216	432
Fmoc-Lys(Boc)-OH	468.6	4.600	0.115		111	222	444
Fmoc-Leu-OH	353.4	4.485	0.112		108	216	432
Fmoc-Met-OH	371.5	4.503	0.113		109	218	436
Fmoc-Asn(Trt)-OH	596.7	4.729	0.118		114	228	456
Fmoc-Pro-OH	337.4	4.469	0.112		108	216	432
Fmoc-Gln(Trt)-OH	610.7	4.743	0.119		115	230	460
Fmoc-Arg(Pmc)-OH	662.8	4.795	0.120		116	232	464
Fmoc-Ser(tBu)-OH	383.4	4.515	0.113		109	218	436
Fmoc-Thr(tBu)-OH	397.5	4.529	0.113		109	218	436
Fmoc-Val-OH	339.4	4.471	0.112		108	216	432
Fmoc-Trp-OH	426.5	4.558	0.114		110	220	440
Fmoc-Tyr(tBu)-OH	459.5	4.591	0.115		111	222	444
Boc-Ala-OH	189.2	4.321	0.108		104	208	416
Boc-Cys(Mob)-OH	341.4	4.473	0.112		108	216	432
Boc-Asp(OBzl)-OH	323.4	4.455	0.111		107	214	428
Boc-Glu(OBzl)-OH	337.4	4.469	0.112		108	216	432
Boc-Phe-OH	265.3	4.397	0.110		106	212	424
Boc-Gly-OH	175.2	4.307	0.108		104	208	416
Boc-His(Bom)-OH	375.4	4.507	0.113		109	218	436
Boc-His(DNP)-OH	421.4	4.553	0.114		110	220	440
Boc-Ile-OH (1/2 H2O)	240.3	4.372	0.109		105	210	420
Boc-Lys(Cl-Z)-OH	414.9	4.547	0.114		110	220	440
Boc-Leu-OH (H2O)	249.3	4.381	0.110		106	212	424
Boc-Met-OH	249.3	4.381	0.110		106	212	424
Boc-Asn(Xan)-OH	412.4	4.544	0.114		110	220	440
Boc-Pro-OH	215.3	4.347	0.109		105	210	420
Boc-Gln-OH	246.3	4.378	0.110		106	212	424
Boc-Arg(Mts)-OH	456.6	4.589	0.115		111	222	444
Boc-Ser(Bzl)-OH	295.3	4.427	0.111		107	214	428
Boc-Thr(Bzl)-OH	309.4	4.441	0.111		107	214	428
Boc-Val-OH	217.3	4.349	0.109		105	210	420
Boc-Trp-OH	304.4	4.436	0.111		107	214	428
Boc-Tyr(Br-Z)-OH	494.4	4.626	0.116		112	224	448

If you want to perform a synthesis in which some of the monomers are very expensive (such as glycosylated amino acids) and the remaining monomers relatively inexpensive, run the synthesis using five equivalents of the expensive monomer and ten equivalents of the inexpensive monomer. When you select the activation to use five equivalents of the expensive monomer, choose cycles that contain module A (for example, BD**A**Fd). When you select the activation to use ten equivalents of the inexpensive monomer, choose cycles that contain module E (for example, BD**E**Fd).

When you use ten equivalents of monomer, you can make the monomer solution more concentrated, for example, 1 mmol dissolved in 2 mL NMP. Table 4-8 gives the volumes for the three scales when using ten equivalents of monomers.

Table 4-8. Amino acid solutions when using ten equivalents (1 mmol in 2 mL NMP)

amino acid	MW	weight (g) of mmol AA and 2 mL NMP	weight (g) of solution for 50 µmol	monomer: / resin:	volume (mL) of solution for: 50 µmol 5 µmol	100 µmol 10 µmol	200 µmol 20 µmol
Fmoc-Ala-OH	311.3	2.377	0.119		115	230	460
Fmoc-Cys(Trt)-OH	585.7	2.652	0.133		128	256	512
Fmoc-Asp(OtBu)-OH	411.4	2.477	0.124		119	238	476
Fmoc-Glu(OtBu)-OH	425.5	2.492	0.125		120	240	480
Fmoc-Phe-OH	387.4	2.453	0.123		118	236	474
Fmoc-Gly-OH	297.3	2.363	0.118		114	228	456
Fmoc-His(Trt)-OH	619.7	2.686	0.134		129	258	516
Fmoc-Ile-OH	353.4	2.419	0.121		116	232	464
Fmoc-Lys(Boc)-OH	468.6	2.535	0.127		122	244	488
Fmoc-Leu-OH	353.4	2.419	0.121		116	232	464
Fmoc-Met-OH	371.5	2.438	0.122		117	234	468
Fmoc-Asn(Trt)-OH	596.7	2.663	0.133		128	256	512
Fmoc-Pro-OH	337.4	2.403	0.120		116	232	464
Fmoc-Gln(Trt)-OH	610.7	2.677	0.134		129	258	516
Fmoc-Arg(Pmc)-OH	662.8	2.729	0.136		131	262	524
Fmoc-Ser(tBu)-OH	383.4	2.449	0.123		118	236	472
Fmoc-Thr(tBu)-OH	397.5	2.464	0.123		118	236	472
Fmoc-Val-OH	339.4	2.405	0.120		116	232	464
Fmoc-Trp-OH	426.5	2.493	0.125		120	240	480
Fmoc-Tyr(tBu)-OH	459.5	2.526	0.126		121	242	484
Boc-Ala-OH	189.2	2.255	0.113		109	218	436
Boc-Cys(Mob)-OH	341.4	2.407	0.120		116	232	464
Boc-Asp(OBzl)-OH	323.4	2.389	0.120		116	232	464
Boc-Glu(OBzl)-OH	337.4	2.403	0.120		116	232	464
Boc-Phe-OH	265.3	2.331	0.117		113	226	452
Boc-Gly-OH	175.2	2.241	0.112		108	216	432
Boc-His(Bom)-OH	375.4	2.441	0.122		117	234	468
Boc-His(DNP)-OH	421.4	2.487	0.124		119	238	476
Boc-Ile-OH (1/2 H2O)	240.3	2.306	0.115		111	222	444
Boc-Lys(Cl-Z)-OH	414.9	2.481	0.124		119	238	476
Boc-Leu-OH (H2O)	249.3	2.315	0.116		112	224	448
Boc-Met-OH	249.3	2.315	0.116		112	224	448
Boc-Asn(Xan)-OH	412.4	2.478	0.124		119	238	476
Boc-Pro-OH	215.3	2.281	0.114		110	220	440
Boc-Gln-OH	246.3	2.312	0.116		112	224	448
Boc-Arg(Mts)-OH	456.6	2.523	0.126		121	242	484
Boc-Ser(Bzl)-OH	295.3	2.361	0.118		114	228	456
Boc-Thr(Bzl)-OH	309.4	2.375	0.119		115	230	460
Boc-Val-OH	217.3	2.283	0.114		110	220	440
Boc-Trp-OH	304.4	2.370	0.119		115	230	460
Boc-Tyr(Br-Z)-OH	494.4	2.560	0.128		123	246	492

Peptide Nucleic Acids: Recommended Modifications

Peptide Nucleic Acids (PNAs) are DNA analogues with a polyamide backbone consisting of an uncharged 2-aminoethylglycine (aeg) unit instead of the charged ribose-phosphate backbone of DNA. The first publication on PNAs was in 1991†. At the time of the writing of this manual (February, 1996) there have been over 75 papers on the synthesis and use of PNAs.

The disk contained in the 3 mL Reaction Vessel kit contains special cycles designed specifically for synthesizing PNAs. These cycles are based on modifications and improvements provided by PNA Diagnostics, Copenhagen, who have been synthesizing PNAs on the ABI 433A with the 3 mL RV since November, 1994. As new improvements are made in the PNA cycles, you should include these improvements in your syntheses.

PNA Diagnostics has recommended the following changes, which are included in the 5-µmol, 10–µmol, and 20-µmol PNA cycle files:

1. After you add the DIEA, allow for a 60-second activation period of the PNA monomer. To accomplished this, extend the time in module A, step 23 from 5 seconds to 60 seconds. (It may be that the activation of the PNA takes more time than amino acids.)
2. Additional DCM washes are necessary between the TFA deprotection and the NMP washes. To make this change, a module "G" was added to the cycle. (BDAFCd was changed to BGDAFCd.) You can change the number of loops in module "G" from five to two (step 3).
3. For capping, use a 1:25:25 solution of acetic anhydride, pyridine and NMP.
4. Use a resin with a loading of 0.20 mmol/g or less. If the resin you use has a higher substitution, you can lower the resin substitution. For more information on lowering the resin substitution, see *Lowering the Resin Substitution* on page 4-13.
5. Use 95% TFA/5% m-cresol instead of 100% TFA in Bottle 2.
6. Use a greater excess of PNA monomer over the uronium activator than when synthesizing peptides. For PNA synthesis, use 0.9 equivalent of uronium activator per 1.0 equivalent of PNA monomer. (For peptide synthesis, use 0.95 equivalent of uronium activator per 1.0 equivalent of amino acids.)
7. If you are using HBTU for activation, do not add any additional HOBt.

† Nielsen, P.E., Egholm, M., Berg R.F. and Buchardt, O. 1991. Sequence-Selective Recognition of DNA by Strand Displacement with a Thymine-Substituted Polyamide. *Science* 254: 1497-1500

Synthesizing PNAs can be more challenging (and more expensive) than peptides or DNA. For the first PNA synthesis you perform, choose a relatively simple oligomer, such as $(Taeg)_6$-Lys-NH_2. PNAs are often synthesized with an amino acid at the C- or N-terminus. The C-terminus is often started with a lysine amide to suppress aggregation of the PNA.

Lowering the Resin Substitution

The following procedure for lowering the substitution for MBHA resin is adapted from a procedure developed by PNA Diagnostics.

To lower the substitution of MBHA resin:

1. Wash 3.0 g MBHA resin (0.45 mmol/g, 1.35 mmol) twice in DCM.
2. Wash the resin in 5% DIEA in DCM for 3 minutes.
3. Wash the resin twice in DCM.

   The resin is now neutralized.
4. Dissolve 0.60 mmol PNA monomer in 7.5 mL NMP.
5. Add 1.2 mmol DIEA to the monomer solution.
6. Dissolve 224 mg (0.59 mmol) HBTU in 7.5 mL NMP, and add this to the monomer solution.
7. Activate the monomer for 2 minutes.
8. Add the activated monomer solution to the neutralized resin.
9. Allow the reaction to proceed for 1 hour.
10. Filter the resin.
11. Wash the resin with 1x NMP.
12. Make 50 mL of capping solution using a 1:2:2 ratio of acetic anhydride, pyridine, and NMP
13. Add the capping solution to the resin and allow the reaction to proceed for 1 hour.

    Successful capping will produce a negative Ninhydrin test.
14. Wash the resin with DMF.
15. Wash the resin with 4x DCM.
16. Wash the resin with 5% DIEA in DCM.
17. Wash the resin with 4x DCM.
18. Dry the resin in a vacuum.

To determine the new loading of the resin, couple Fmoc-Gly to about 20 mg of the resin. Follow the procedure for a 5 µmol Boc synthesis using the following cycles:

Table 4-9. Cycles for determining resin loading

Cycle	Modules
Boc Depro/Single	BDAFd
Final DCM Wash	c

To determine the substitution, follow the procedure located on page 3-18 of the ABI 433A User's Manual.

PNA Monomer Solutions

The Boc-PNA monomers are predissolved in NMP and the appropriate amount of the solution is pipeted into a cartridge. Table 4-10 gives the volume of each dissolved monomer for the 3 scales of syntheses when using 4 mL of NMP to dissolve 1 mmol of monomer. All the monomers dissolve at this concentration at room temperature except Boc-Gaeg(Z)-OH, which requires sonication or heating to 50 °C to dissolve the solid, but it will stay in solution once it has dissolved.

In Table 4-10, the actual density of 1.06 g/mL was used to calculate the volumes of the four PNA monomers. The density of NMP is 1.033 g/mL.

The reason 5.3 equivalents of PNA monomer is used, instead of the 5 equivalents used with the amino acids, is to use a greater excess of monomer over the uronium activator.

Table 4-10. PNA Monomer solutions in NMP when using 5.3 equivalents

		weight (g) of mmol AA and	weight (g) of solution for	monomer:	volume (µL) of solution for:		
					26.5 µmol	53 µmol	106 µmol
amino acid	MW	4 mL NMP	26.5 µmol	resin:	5 µmol	10 µmol	20 µmol
Boc-Aaeg(Z)-OH	527.5	4.660	0.123		116	232	464
Boc-Caeg(Z)-OH	503.5	4.636	0.122		115	230	460
Boc-Gaeg(Z)-OH	543.5	4.676	0.123		116	232	464
Boc-Taeg-OH	348.4	4.480	0.118		112	224	448

Synthesis Setup for the 3 mL Reaction Vessel

You can use the following checklist with the ABI 433A *FastMoc* Quick Start Card to help set up your synthesis. Much of the information on the Quick Start Card, however, is incorrect when you are doing a synthesis using the 3 mL RV cycles. For example, predissolved monomers, bottle position, HBTU concentration, DIEA concentration, and measuring loop volume are all different.

WARNING ***Always wear gloves, protective clothing and eye protection when working with chemicals and bottles under pressure.***

1. Check the barcode reader, nitrogen tank and waste container for proper connection and operation.
2. If necessary, change the in-line filters (top RV, bottom RV and cartridge).

Note *For the next step, weigh the HBTU in a fume hood.*

3. Prepare DIEA and HBTU solutions according to your synthesis scale, then place the solutions in Bottles 7 and 8. Install an HBTU line filter on the end of the tube in Bottle 8.

Reagent	Scale		
	5-µmol	10-µmol	20-µmol
DIEA (Bottle 7)	0.40 M	0.80 M	1.60 M
HBTU (Bottle 8)	0.19 M	0.38 M	0.38 M

4. Prepare the monomer solutions, using 1 mmol/4 mL NMP when using five equivalents and 1 mmol/2 mL NMP when using ten equivalents. These monomer solutions can be stored for up to one week when refrigerated. Add the appropriate amount of solution to the cartridges, according to Table 4-7, Table 4-8 and Table 4-10.
5. Prepare the capping solution for Bottle 4.

Note *Make the capping solution fresh each week.*

6. Check all the other solvents and reagents: Piperidine, TFA (if using Boc cycles), DCM, and NMP.
7. Install or remove the conductivity cell, depending on whether conductivity monitoring is used.
8. Check that the 0.125 mL variable measuring loop is installed.

9. Open Flow Test 1-18 (VML) and send to the synthesizer. Run the appropriate flow tests. Check for leaks after finishing flow tests.

Flow Test 10 (module A)	NMP to metering vessel.
Flow Test 11 (module B)	NMP to cartridge.
Flow Test 2 (module b)	TFA to metering vessel (only if using Boc cycles).
Flow Test 1 (module a)	Piperidine to metering vessel.
Flow Test 4 (module d)	Capping solution to metering vessel.
Flow Test 7 (module g)	DIEA to measuring loop (check that it fills loop in 3 sec or less)
Flow Test 8 (module h)	HBTU to measuring loop (check that it fills loop in 3 sec or less)

10. If the sequence is not already entered, open "New," then enter and save the sequence.
11. Open the appropriate 3 mL chemistry (5-, 10- or 20-µmol).
    a. Choose Boc or Fmoc in the Chemistry Information dialog box. (SynthAssist User's Manual, page 4-3)
    The possible cycles to choose from are described on pages 5-6 and 5-7. It is also possible to create your own cycles.
    b. Check Default Set and change if necessary. (SynthAssist User's Manual, page 4-14).
    c. Save and send chemistry to synthesizer.
12. Add resin to the 3 mL RV.

    The 3 mL RV must be closed and tightened completely before any synthesis is started. You will feel resistance when you tighten the caps of the RV. This resistance is the result of O-rings beginning to become compressed. Keep tightening the RV caps until the RV is completely closed. To completely tighten the RV caps, you may need to use the wrench included in the 3 mL RV kit. If the RV caps are not completely tight, resin will escape from the body of the vessel during the synthesis.

    For more information about assembling and closing the 3 mL RV, refer to page 2-5.
13. Open a New Run using the following steps:
    a. Choose sequence.
    b. Choose resin.
    c. Enter the resin substitution.

d. Enter the weight of resin.

e. Check cycles to make sure the correct cycles are entered.

   You may want some cycles to be different from the default.

f. Check amino acids (pop-up menu)

g. Save Run.

14. Send Run File to synthesizer.

15. Load the cartridges that contain the monomer solutions into the guideway.

16. Place RV on synthesizer and begin synthesis.

Reminder when using the 8 mL, 40 mL and 55 mL Reaction Vessels.
Always check that the 0.50 mL variable measuring loop is installed when you use the cycles written for the 8 mL, 40 mL and 55 mL RV. In addition, you must use the cycles containing the extending filling times for the measuring loop. The correct cycles are in the 0.50 mL Loop Folder and they have VML in the Information Box.

Note	*If you haven't already removed the original cycles in the Chemistry folder of SynthAssist, remove them from the computer now (drag them into the trash, then empty the trash).*

Test Synthesis Example

Before performing a synthesis using expensive monomers, make a simple peptide to verify that all the changes made to the measuring loop, cycles and reagents are correct. Table 4-11 shows an example of a 5-µmol ACP (65-74) synthesis using Fmoc cycles.

Table 4-11. 5-µmol ACP synthesis using Fmoc cycles.

ACP (65-74):	Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly		
Fmoc-Gly-HMP resin:	(0.0082 g)(0.65 mmol/g) = 0.0053 mmol		
Bottle 7:	0.40M DIEA		
Bottle 8:	0.19M HBTU		
Fmoc-amino acids:	Use the following volumes of amino acids solutions (1 mmol amino acid/4 mL NMP):		
	Fmoc-Asn(Trt)	114 µL	
	Fmoc-Tyr(tBu)	111 µL	
	Fmoc-Ala	107 µL	
	Fmoc-Val	108 µL	
	Fmoc-Ile	108 µL	
	Fmoc-Asp(OtBu)	110 µL	
	Fmoc-Gln(Trt)	115 µL	
Final weight of resin:	0.0142 g (Theory: 0.0155 g)		
Cycle information:	Cycle:	5 µmol	
	Type:	Fmoc (see *SynthAssist User's Manual*, p. 4-3)	
Default set:	Default:	Fmoc Depro/Single	bDAFd
	Preload:	NMP Wash	D
	Load:	None	
	End:	Final Fmoc Depro	bDc
Cycles:	Amino Acid	Cycle	Modules
	1 Gly	NMP Wash	D
	2 Asn	Fmoc/Depro/Single	bDAFd
	3 Ile	Fmoc/Depro/Single	bDAFd
	4 Tyr	Fmoc/Depro/Single	bDAFd
	5Asp	Fmoc/Depro/Single	bDAFd
	6 Ile	Fmoc/Depro/Single	bDAFd
	7 Ala	Fmoc/Depro/Single	bDAFd
	8 Ala	Fmoc/Depro/Single	bDAFd
	9 Gln	Fmoc/Depro/Single	bDAFd
	10 Val	Fmoc/Depro/Single	bDAFd
	11	Fmoc Final Depro	bDc

Cleavage

6.8 mg of resin was treated with 200 µL of 90% TFA, 5% EDT, 2.5% thioanisole and 2.5% H2O for 2 hours. Filter resin, precipitate peptide in 10 mL methyl t-butyl ether, centrifuge, decant, redissolve peptide in 0.5 mL TFA, reprecipitate in 10 mL ether, centrifuge, decant, dissolve peptide in 10% acetic acid and lyophilize. Weight = 1.8 mg (theory 2.5 mg)

HPLC

Performed on an ABI 130A Micro Separation System equipped with an Aquapore® OD-300, C18, 300-Å pore size, 7-µm particle size reverse phase column. Flow rate of 250 µL/min. with a gradient of 5%-60% B in 45 minutes, where buffer A is 0.10% TFA/H2O and buffer B is 0.08% TFA/ acetonitrile. Detection was at 214 nm.

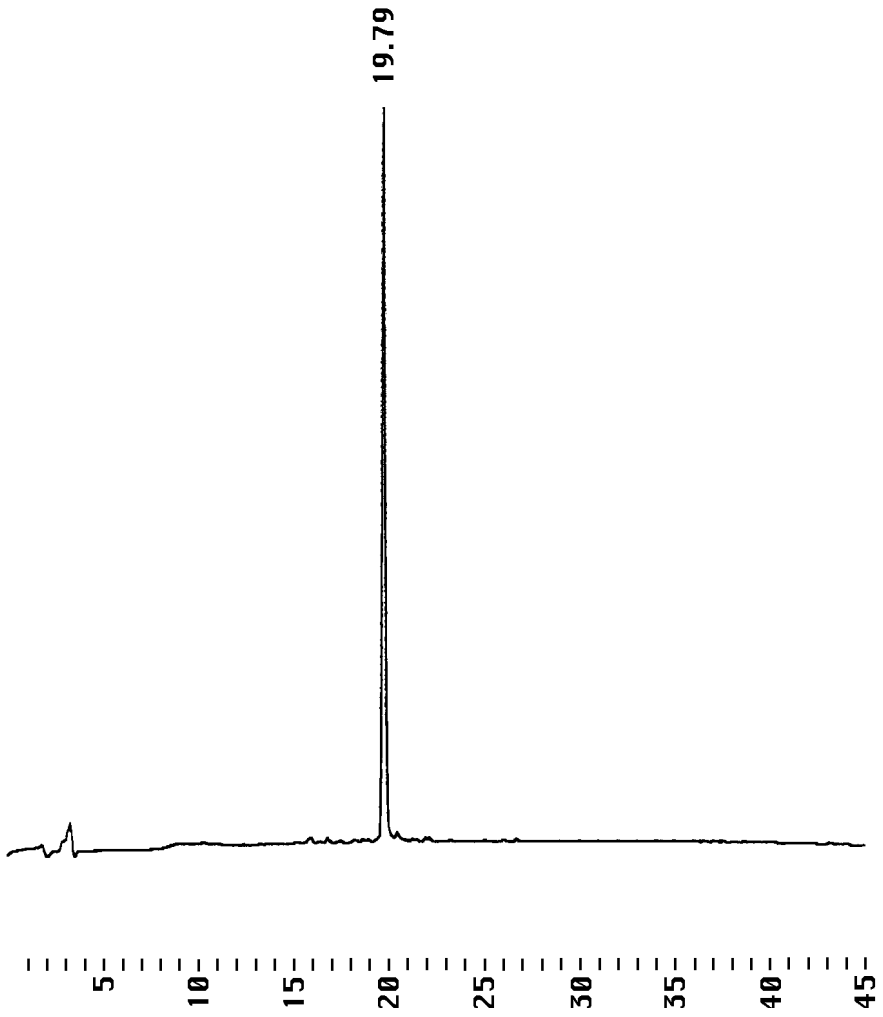


Figure 4-1. HPLC results

5 Cycles and Modules

Cycles in SynthAssist 2.0 Software

The disk provided with the 3 mL Reaction Vessel Kit contains new cycles, written in SynthAssist software, that use the Variable Measuring Loop. Figure 5-1 shows the folder structure. The 0.125 mL Loop folder contains the new 0.125 mL cycles. The 0.5 mL Loop folder contains all the old cycles previously on the SynthAssist Chemistry disk, with some steps modified as described in Table 5-5 on page 5-24.

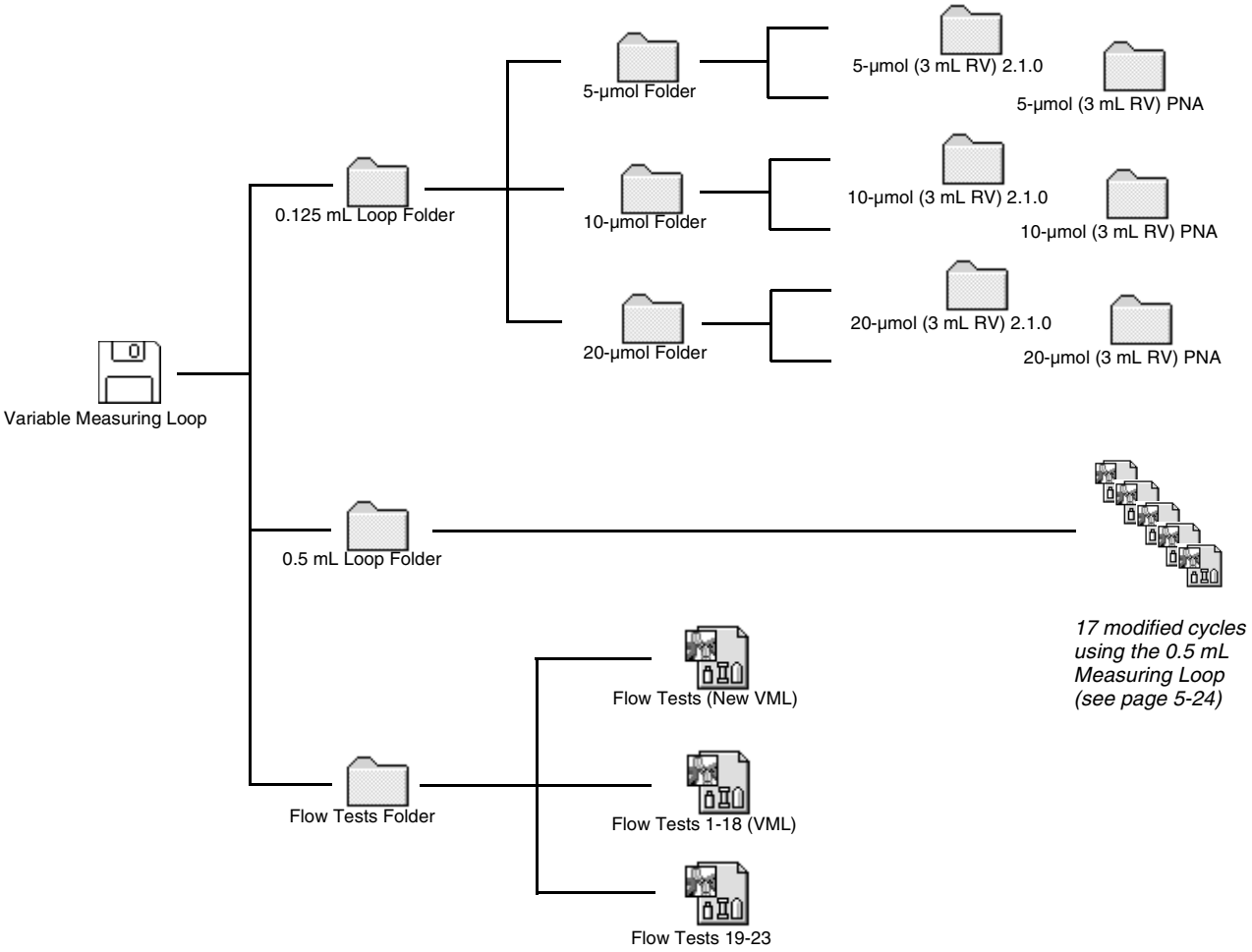


Figure 5-1. Arrangement of folders on Variable Measuring Loop disk

The Variable Loop Folder also contains four new flow tests for the Variable Measuring Loop, located in the Flow Tests Folder (Figure 5-1). This folder also contains the original flow tests from the ABI 433A with minor modifications made to Flow Tests 7, 8, 17, and 18 (modules g, h, H, and I).

The 3 mL RV cycles are provided with the Fmoc chemistry selected. If you are using Boc resins and Boc amino acids, select Boc in the information window (refer to the *SynthAssist 2.0 User's Manual*, page 4-3).

Module Descriptions for ABI 433A

The modules described in this section are used for both Boc and Fmoc syntheses. The TFA deprotection for the Boc removal is written in module "B." The piperidine deprotection for the Fmoc removal is written in module "b" when there is no monitoring and in modules "H" and "h" when there is conductivity monitoring.

Module A - Read Cartridge and add HBTU and DIEA
Total time = 1.2 minutes

The amino acid cartridge name is read, the old cartridge is ejected and the new cartridge is advanced. HBTU (0.95 equiv.) is added to the cartridge. For the 5-µmol cycles, 0.125 mL of 0.19 M HBTU is added; for the 10-µmol cycles, 0.125 mL of 0.38 M HBTU is added; for the 20-µmol cycles, 0.250 mL (2 loops) of 0.38 M HBTU is added. Next, the DIEA solution is added to the cartridge to initiate activation of the carboxylic acid group. The concentration of the DIEA is 0.40 M for the 5-µmol scale synthesis, 0.80 M for the 10-µmol scale synthesis, and 1.6 M for the 20-µmol scale synthesis. When synthesizing PNAs, the 5-second activation period on step 23 is extended to 60 seconds.

Module B - TFA Deprotection
Total time = 8.6 minutes

The resin is washed one time with DCM, then treated for 1 minute with TFA. After draining, the resin is treated again with TFA, this time for 4.1 minutes. At the end of the module there is a single DCM wash.

Module C - Capping
Total time = 1.6 minutes

The resin is drained, the capping solution from Bottle 4 is added and the reaction vessel is vortexed for 1 minute.

Module D - NMP Washes
Total time = 2.9 minutes

The resin is drained and washed six times with NMP.

Module E - Read Cartridge and Add Double the Amount of HBTU and DIEA
Total time = 2.0 minutes

This module is identical to module A, except twice the amount of HBTU and DIEA is added. This module is used with the less expensive amino acids. Module A uses five equivalents of amino acids and module E uses 10 equivalents of amino acids.

Module F - Transfer, Clean Cartridge and Couple
Total time = 35.1 minutes

At the beginning of this module, the activated monomer is transferred to the reaction vessel and the cartridge is washed two times with NMP. This NMP is transferred to the Activator Vessel and is used later in module "d." After the cartridge is washed, the coupling is continued. You can modify the coupling time by changing the number of loops in step 29.

Module G - DCM Washes
Total time = 2.4 minutes

The resin is drained and washed five times with DCM. This is an optional module that you might use in the Boc cycles after the TFA deprotections.

In the PNA cycles, the resin is drained and washed only twice (see page 5-19).

Module H - Piperidine Deprotection - Prev. Peak
Total time:

if 3 loops: 12.4 minutes

if 4 loops: 16.3 minutes

The resin is washed three times with NMP. A 20% piperidine/NMP solution is introduced and allowed to deprotect for 2 minutes. The conductivity of the deprotection solution is measured and the resin is drained. This process is continued for at least three deprotections. At the end of the third deprotection, the value of the second and third deprotections are compared to see if they are within the limits defined by step 54 (End loop monitoring). A value of 25 in step 54 means that if the third deprotection is within 2.5% of the second deprotection, then no more deprotections will occur. Three to four deprotections will occur.

Module I - Vortex
Total time = 5.0 minutes

The reaction vessel is vortexed for 5 minutes. Use this module to extend the coupling or capping.

Module a - Read Cartridge
Total time = 0.9 minutes

The cartridge name is read, the old cartridge is ejected and the new cartridge is advanced. Use this module when the material is already in its activated form, such as when using fluorescein isothiocyanate. The material must be dissolved in at least 0.36 mL of solvent. The number of equivalents is user determined and may depend on the expense and availability of the material, as well as the efficiency of the reaction.

Module b - Piperidine Deprotection - no monitoring
Total time = 8.7 minutes

The resin is washed one time with NMP. A 20% piperidine/NMP solution is introduced and allowed to deprotect for 2 minutes. The RV is drained and a second treatment with 20% piperidine/NMP is performed for 5 minutes. You can change the time in step 24 to extend the second treatment. At the end of the module, the resin is drained.

Module c - Final DCM Washes
Total time = 5.1 minutes

The resin is drained and washed six times with DCM. At the end of the cycle, the resin is drained for 30 seconds and the Activator Vessel is washed with DCM and drained. Use this module at the end of a synthesis.

Module d - NMP Wash from Activator
Total time = 1.4 minutes

The NMP that was used in Module "F" to wash the cartridge is used in this module to wash the resin after the coupling is completed.

Module f - DIEA Neutralization
Total time = 1.5 minutes

The resin is washed two times with NMP and 0.125 mL of the DIEA solution.

Module h - Conditional Piperidine Deprotection
Total time = 10.8 minutes

This module is used only when the maximum deprotection loops have been used in module "H." When this module is active, a 20% piperidine/NMP solution is introduced and allowed to deprotect for 10 minutes. The conductivity of the solution is measured at the end of the deprotection.

Module i - Conditional Vortex
Total time = 5.0 minutes

This module is used only when the maximum deprotection loops have been used in module H. When this module is active, the reaction vessel is vortexed for 5 minutes. Use this module to conditionally extend the coupling or the capping.

Fmoc and Boc Cycles

(PrPk = previous peak monitoring)

Boc Depro/Single	BDAFd
Boc Depro/Single/cap	BDAFCd
Boc Depro/Single (10 eq.)	BDEFd
Boc Depro/Single (10 eq.)/cap	BDEFCd
Boc Depro/Single (no 7 & 8)	BDfDaFIIId
Boc Final Depro	BDc
Boc Final Depro & Acetylation	BDCCIDc
Fmoc Depro/Single	bDAFd
Fmoc Depro/Single/cap	bDAFCd
Fmoc Depro/Single (10 eq.)	bDEFd
Fmoc Depro/Single (10 eq.)/cap	bDEFCd
Fmoc Depro/Single (no 7 & 8)	bDaFIIId
Fmoc Final Depro	bDc
Fmoc Final Depro & Acetylation	bDCCIDc
PrPk Fmoc Depro/Single/cap	HhDAFiiiCidD
PrPk Fmoc Depro/Single (10 eq.)/cap	HhDEFiiiCidD
PrPk Fmoc Depro/Single (no 7 & 8)	HhDaFIIIiiidD
PrPk Fmoc Final Depro	HhDc
PrPk Fmoc Final Depro & Acetylation	HhDCCIiDc
NMP Wash	D
Final DCM Wash	c

Cycles (grouped by coupling)

The following cycles are single coupling with five equivalents of monomer.

Boc Depro/Single	BDAFd
Fmoc Depro/Single	bDAFd

The following cycles are single coupling with five equivalents of monomer followed by capping with a pre-mixed solution of acetic anhydride.

Boc Depro/Single/cap	BDAFCd
Fmoc Depro/Single/cap	bDAFCd
PrPk Fmoc Depro/Single/cap	HhDAFiiiCidD

The following cycles are single coupling with 10 equivalents of monomer.

Boc Depro/Single (10 eq.)	BDEFd
Fmoc Depro/Single (10 eq.)	bDEFd

The following cycles are single coupling with five equivalents of monomer followed by capping with a pre-mixed solution of acetic anhydride.

Boc Depro/Single (10 eq.)/cap	BDEFCd
Fmoc Depro/Single (10 eq.)/cap	bDEFCd
PrPk Fmoc Depro/Single (10 eq.)/cap	HhDEFiiiCidD

The following cycles are single treatment with a material that is already in an activated form (for example, a solution containing fluorescein isothiocyanate). The cycle with the TFA deprotection requires a neutralization step.

Boc Depro/Single (no 7 & 8)	BDfDaFIIId
Fmoc Depro/Single (no 7 & 8)	bDaFIIId
PrPk Fmoc Depro/Single (no 7 & 8)	HhDaFIIIiiidD

The previous cycles do not include capping. The following cycles may be written if capping is desired.

Boc Depro/Single (no 7 & 8)/cap	BDfDaFIIICd
Fmoc Depro/Single (no 7 & 8)/cap	bDaFIIICd
PrPk Fmoc Depro/Single (no 7 & 8)/cap	HhDaFIIIiiiCdD

The following cycles are final deprotection cycles. The final deprotection is usually not performed when using the Boc protecting group. The Boc group is left on the resin and is removed during the cleavage. If one does not want to remove the final Boc group, then the syntheses is finished with a final DCM wash (module c) instead of modules BDc.

Boc Final Depro	BDc
Fmoc Final Depro	bDc
PrPk Fmoc Final Depro	HhDc

The following cycles are final deprotection and acetylation cycles. After the deprotection, there are two treatments with the capping solution. After the second treatment, the acetylation is continued for an additional 5 minutes.

Boc Final Depro & Acetylation	BDCCIDc
Fmoc Final Depro & Acetylation	bDCCIDc
PrPk Fmoc Final Depro & Acetylation	HhDCCIiDc

Fmoc Cycle, no monitoring

Table 5-1. Fmoc Cycle described by modules

Module b	Piperidine Deprotection	8.7 min.
	1st treatment = 2 min.	
	2nd treatment = 5 min.	
Module D	NMP Wash	2.9 min.
Module A	Read Cart, Add 7 & 8	1.2 min.
Module F	Transfer & Coupling	35.1 min.
Module C	Capping	1.6 min.
	1 min. of capping	
Module d	NMP Wash from Act.	1.4 min.
	Total Fmoc Cycle Time	**50.9 min.**

Boc Cycle

Table 5-2. Boc Cycle described by modules

Module B	TFA Deprotection	8.6 min.
	1st treatment = 1 min.	
	2nd treatment = 4.1 min.	
Module D	NMP Wash	2.9 min.
Module A	Read Cart, Add 7 & 8	1.2 min.
Module F	Transfer & Coupling	35.1 min.
Module C	Capping	1.6 min.
	1 min. of capping	
Module d	NMP Wash from Act.	1.4
	Total Boc Cycle Time	**50.8 min.**

Cycle Modifications

Cycles often need to be modified to change times, number of washes, or other cycles parameters. Refer to Table 5-3 for a list of common cycle modifications.

Table 5-3. Common Cycle Modifications

If you want this:	Then do this:
Longer or shorter TFA deprotections for the Boc deprotections	Change the time in module B, step 71 (page 5-14)
Longer or shorter coupling times	Change the loop count in Module F, step 29 (page 5-4)
Longer or shorter capping times	Change the time in Module C, step 8
To change the number of NMP washes	Change the loop count in Module D, step 3
To change the amount of NMP in each wash	Change the time listed in Module D, step 7

Deprotection Monitoring

Difficulties arise when using monitoring at the small scales made possible by the 3 mL RV. Synthesis reagents and system electronics combine to generate a "background" conductivity value, which is referred to as a "noise baseline." For example, a noise baseline of 700 units is typical for the 5-µmol scale. The initial Fmoc deprotection of such a small quantity of resin will generate only slight additional conductivity, for a total conductivity of perhaps 1000 units during the initial deprotection (Figure 5-2). The conductivity from the initial deprotection, which in this example is less than 50% above the background of 700, is difficult to discriminate from the background conductivity.

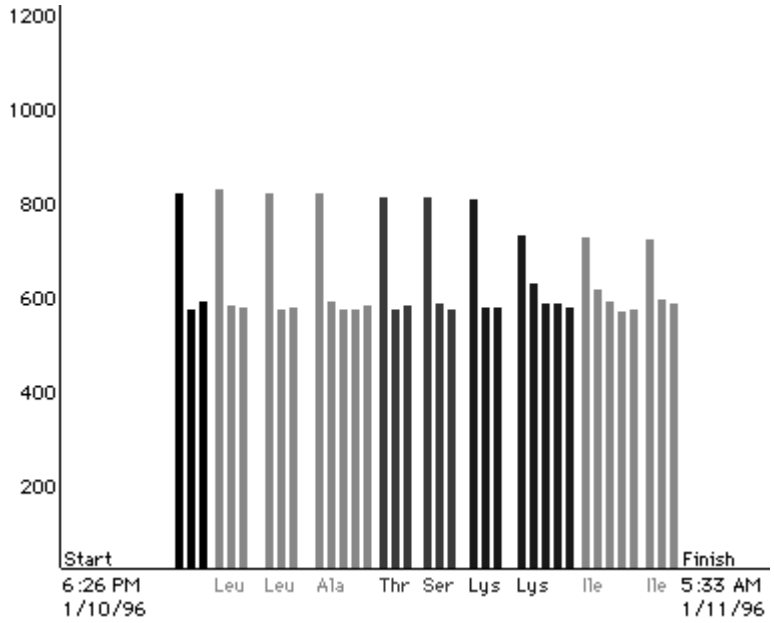


Figure 5-2. Background and initial deprotection conductivity using 3 mL RV

In larger syntheses, such as those using the 0.1-mmol scale, the signal-to-noise ratio is much higher. As deprotection continues, the conductivity above the noise baseline becomes progressively smaller and it becomes more difficult to detect changes, to the point that the system may be effective at detecting only very poor deprotections at the small scales.

Because the signal-to-background ratio is small for the 5-, 10- and 20-µmol cycles, the Previous Peak Deprotection monitoring cycles have capping and extra NMP washes (Module D). The capping is included because uncoupled amino groups seem to cause an ion-exchange effect. The extra washing is included to make sure most of the conductive species from the coupling is removed.

When you use the Previous Peak (PrPk) Fmoc Deprotection cycles, connect the conductivity cell between the bottom in-line filter and valve 10. If you use only the non-monitoring cycles, (Boc cycles and non-monitoring Fmoc Cycles), remove the conductivity cell and the small connecting tube to help minimize the volume between the valve block and the reaction vessel.

When using a combination of Boc and Fmoc cycles, we recommend that you use non-monitoring Fmoc cycles. This is because the long-term effect of TFA on the conductivity cell is not known.

Only the Previous Peak algorithm cycles are included on the disk contained in the 3 mL Reaction Vessel Kit. Before you use the 1st Peak–X algorithm, you must perform enough syntheses to obtain a confident estimate of the X value to use. For example, if the baseline is steady at 700, the X value to use could be 68 or 69. If the base line is not steady, then do not use the 1st peak–X algorithm.

To change Module "H" from Previous Peak to 1st Peak–X, make the following changes:

Step	Function	Description	Change
step 3	Fxn 110	Begin lower loop	change time 2 to 1
step 24	Fxn 130 change to	Monitor previous peak	time = 1
	Fxn 128	Monitoring first peak - X	time = X value
step 50	Fxn 130 change to	Monitor previous peak	time = 1
	Fxn 128	Monitoring first peak - X	time = X value
step 57	Fxn 2	Vortex reaction vessel on	time 5 to 120

To change Module "h" from Previous Peak to 1st Peak–X, make the following change:

Step	Function	Description	Change
step 15	Fxn 130 change to	Monitor previous peak	time = 1
	Fxn 128	Monitoring first peak - X	time = X value

Example of a Deprotection Calculation

Module H (Piperidine Deprotection - Previous Peak) determines the number of deprotections based on the measured conductivity monitoring values. At the end of the third deprotection, the value of the second and third deprotections are compared to see if they are within the limits defined by step 54 (End loop monitoring).

SynthAssist uses the following formula to calculate whether three or four deprotections are used:

$$\frac{\text{Value of 2nd deprotect} - \text{Value of 3rd deprotect}}{\text{Value of 2nd deprotect}} = \text{percentage}$$

If the percentage calculated is lower than 2.5%, then no more deprotections will occur.

For example, if the values of the three deprotections are 995, 775, and 766,

$$\text{then} \quad \frac{775\text{-}766}{775} = \frac{9}{775} = 0.012 = 1.2\%$$

And only three deprotections will occur.

However, if the values of the three deprotections are 903, 792, and 771,

$$\text{then} \quad \frac{792\text{-}771}{792} = \frac{21}{792} = 0.026 = 2.6\%$$

And four deprotections will occur in Module H.

If four deprotections occur in Module H, then Module h will become active.

Note	*You can modify the value in step 54 to change the sensitivity of the monitoring. A lower value will provide greater sensitivity.*

SynthAssist Dictionary

You may use compounds with these cycles that are not in the SynthAssist Dictionary. For information on creating new amino acids or protecting groups in the Dictionary, refer to Chapter 5 of the *SynthAssist 2.0 User's Manual*.

For peptide nucleic acid monomers, enter into the Dictionary the formula and suggested code shown in Table 5-4.

Table 5-4. Peptide Nucleic Acid Monomers

Monomer	Formula	Code
Aaeg	C_{11} H_{15} N_7 O_3	One Letter Code = 1
Caeg	C_{10} H_{15} N_5 O_4	One Letter Code = 2
Gaeg	C_{11} H_{15} N_7 O_4	One Letter Code = 3
Taeg	C_{11} H_{16} N_4 O_5	One Letter Code = 4

If you use benzyloxycarbonyl protection for monomers "A," "C," and "G," remember to choose Z side-chain protection. Using the one-letter codes shown in Table 5-4 puts the four PNA monomers at the start of the palette rather than mixing them with the amino acids.

Modules for 433A Peptide Synthesizer

The modules described in this section are used for both Boc and Fmoc syntheses. The TFA deprotection for the Boc removal is written in module "B." The piperidine deprotection for the Fmoc removal is written in module "b" when there is no monitoring and in modules "H" and "h" when there is conductivity monitoring.

Module A: Read Cartridge and Add HBTU and DIEA

			μmol		
			5	10	20
Step	Fxn	Name	Time (sec)		
1	1	Wait	1	1	1
2	4	Read cartridge	10	10	10
3	6	Needle up	10	10	10
4	7	Eject cartridge	10	10	10
5	8	Advance cartridge	10	10	10
6	5	Needle down	10	10	10
7	14	Flush bottom valve block with NMP to waste	1	1	1
8	9	Flush top valve block with gas to waste	2	2	2
9	10	Flush bottom valve block with gas to waste	3	3	3
10	70	Flush bottom valve block with loop to waste	2	2	2
11	78	Pressurize manifold	5	5	5
12	98	Begin Loop UPPER	1	1	**2**
13	69	Deliver HBTU to measuring loop (open)	3	3	3
14	10	Flush bottom valve block with gas to waste	2	2	2
15	63	Transfer measuring loop to cartridge	10	10	10
16	99	End Loop UPPER	1	1	1
17	60	Mix cartridge	2	2	2
18	98	Begin Loop UPPER	1	1	1
19	68	Deliver DIEA to measuring loop (open)	3	3	3
20	10	Flush bottom valve block with gas to waste	2	2	2
21	63	Transfer measuring loop to cartridge	10	10	10
22	99	End Loop UPPER	1	1	1
23	60	Mix cartridge	5/60*	5/60*	5/60*

* For PNA cycles, step 23, Mix Cartridge is 60 seconds

Module B: TFA Deprotection

Step	Fxn	Name	Time
1	1	Wait	1
2	12	Flush bottom valve block with DCM to waste	1
3	55	Deliver DCM to reaction vessel	4
4	40	Mix reaction vessel	2
5	2	Vortex reaction vessel on	1
6	10	Flush bottom valve block with gas to waste	6
7	3	Vortex reaction vessel off	1
8	73	Vent TFA without gas	2
9	75	Vent TFA with gas	2
10	76	Pressurize TFA	15
11	11	Flush top valve block with DCM to waste	2
12	9	Flush top valve block with gas to waste	3
13	42	Drain reaction vessel to waste	10
14	49	Flow DCM through reaction vessel to waste	10
15	42	Drain reaction vessel to waste	10
16	41	Vent reaction vessel	2
17	72	Deliver TFA to reaction vessel	15
18	40	Mix reaction vessel	2
19	1	Wait	2
20	41	Vent reaction vessel	2
21	12	Flush bottom valve block with DCM to waste	2
22	55	Deliver DCM to reaction vessel	1
23	41	Vent reaction vessel	2
24	11	Flush top valve block with DCM to waste	2
25	45	Deliver DCM to reaction vessel top	1
26	2	Vortex reaction vessel on	1
27	9	Flush top valve block with gas to waste	3
28	10	Flush bottom valve block with gas to waste	10
29	73	Vent TFA without gas	5
30	74	Back-flush TFA	3
31	73	Vent TFA without gas	2
32	13	Flush top valve block with NMP to waste	1
33	14	Flush bottom valve block with NMP to waste	1
34	12	Flush bottom valve block with DCM to waste	2
35	11	Flush top valve block with DCM to waste	2
36	9	Flush top valve block with gas to waste	6
37	10	Flush bottom valve block with gas to waste	6
38	76	Pressurize TFA	15
39	3	Vortex reaction vessel off	1
40	41	Vent reaction vessel	3
41	42	Drain reaction vessel to waste	10
42	41	Vent reaction vessel	2
43	72	Deliver TFA to reaction vessel	15
44	40	Mix reaction vessel	2
45	1	Wait	2

Step	Fxn	Name	Time
46	41	Vent reaction vessel	2
47	12	Flush bottom valve block with DCM to waste	2
48	55	Deliver DCM to reaction vessel	1
49	41	Vent reaction vessel	2
50	11	Flush top valve block with DCM to waste	2
51	45	Deliver DCM to reaction vessel top	1
52	2	Vortex reaction vessel on	1
53	9	Flush top valve block with gas to waste	3
54	10	Flush bottom valve block with gas to waste	10
55	73	Vent TFA without gas	5
56	74	Back-flush TFA	6
57	73	Vent TFA without gas	2
58	75	Vent TFA with gas	4
59	74	Back-flush TFA	4
60	73	Vent TFA without gas	3
61	13	Flush top valve block with NMP to waste	2
62	14	Flush bottom valve block with NMP to waste	2
63	98	Begin loop UPPER	3
64	12	Flush bottom valve block with DCM to waste	2
65	11	þFlush top valve block with DCM to waste	2
66	10	þFlush bottom valve block with gas to waste	2
67	9	þFlush top valve block with gas to waste	2
68	99	End loop UPPER	1
69	9	Flush top valve block with gas to waste	6
70	10	Flush bottom valve block with gas to waste	6
71	2	Vortex reaction vessel on	180
72	3	Vortex reaction vessel off	1
73	41	Vent reaction vessel	3
74	42	Drain reaction vessel to waste	5
75	12	Flush bottom valve block with DCM to waste	1
76	55	Deliver DCM to reaction vessel	4
77	40	Mix reaction vessel	2
78	2	Vortex reaction vessel on	5
79	3	Vortex reaction vessel off	1
70	41	Vent reaction vessel	2
81	42	Drain reaction vessel to waste	10
82	49	Flow DCM through reaction vessel to waste	10
83	42	Drain reaction vessel to waste	10

Module C: Capping (1 minute)

Step	Fxn	Name	Time
1	1	Wait	1
2	77	Pressurize Cap Solution	10
3	42	Drain reaction vessel to waste	10
4	17	Flush bottom valve block with Cap Sol. to waste	2
5	10	Flush bottom valve block with gas to waste	2
6	52	Deliver Cap Solution to reaction vessel	8
7	40	Mix reaction vessel	2
8	2	Vortex reaction vessel on	60
9	3	Vortex reaction vessel off	1

Module D: NMP Washes

Step	Fxn	Name	Time
1	1	Wait	1
2	3	Vortex reaction vessel off	1
3	98	Begin loop UPPER	6
4	41	Vent reaction vessel	2
5	50	Flow NMP through reaction vessel to waste	2
6	42	Drain reaction vessel to waste	5
7	56	Deliver NMP to reaction vessel	4
8	40	Mix reaction vessel	2
9	2	Vortex reaction vessel on	3
10	40	Mix reaction vessel	2
11	3	Vortex reaction vessel off	1
12	42	Drain reaction vessel to waste	7
13	99	End loop UPPER	1
14	42	Drain reaction vessel to waste	5

Module E: Read Cartridge and Add Two Times HBTU and DIEA

			µmol		
			5	10	20
Step	Fxn	Name	Time		
1	1	Wait	1	1	1
2	4	Read cartridge	10	10	10
3	6	Needle up	10	10	10
4	7	Eject cartridge	10	10	10
5	8	Advance cartridge	10	10	10
6	5	Needle down	10	10	10
7	14	Flush bottom valve block with NMP to waste	1	1	1
8	9	Flush top valve block with gas to waste	2	2	2
9	10	Flush bottom valve block with gas to waste	3	3	3
10	70	Flush bottom valve block with loop to waste	2	2	2
11	78	Pressurize manifold	5	5	5
12	98	Begin Loop UPPER	2	2	**4**
13	69	Deliver HBTU to measuring loop (open)	3	3	3

			µmol		
			5	10	20
Step	Fxn	Name	Time		
14	10	Flush bottom valve block with gas to waste	2	2	2
15	63	Transfer measuring loop to cartridge	10	10	10
16	99	End Loop UPPER	1	1	1
17	60	Mix cartridge	2	2	2
18	98	Begin Loop UPPER	2	2	2
19	68	Deliver DIEA to measuring loop (open)	3	3	3
20	10	Flush bottom valve block with gas to waste	2	2	2
21	63	Transfer measuring loop to cartridge	10	10	10
22	99	End Loop UPPER	1	1	1
23	60	Mix cartridge	5	5	5

Module F: Transfer, Clean Cartridge, and Couple

Step	Fxn	Name	Time
1	1	Wait	1
2	5	Needle down	10
3	98	Begin loop UPPER	8
4	41	Vent reaction vessel	2
5	96	Transfer cartridge to reaction vessel (top closed)	6
6	2	Vortex reaction vessel on	3
7	3	Vortex reaction vessel off	1
8	99	End loop UPPER	1
9	40	Mix reaction vessel	1
10	2	Vortex reaction vessel on	1
11	62	Drain cartridge to waste	10
12	98	Begin loop UPPER	3
13	67	Deliver NMP to cartridge small needle	2
14	62	Drain cartridge to waste	5
15	99	End loop UPPER	1
16	98	Begin loop UPPER	2
17	65	Deliver NMP to cartridge	22
18	60	Mix cartridge	10
19	24	Transfer cartridge to activator	20
20	62	Drain cartridge to waste	10
21	99	End loop UPPER	1
22	98	Begin loop UPPER	2
23	67	Deliver NMP to cartridge small needle	2
24	62	Drain cartridge to waste	10
25	99	End loop UPPER	1
26	62	Drain cartridge to waste	10
27	60	Mix cartridge	5
28	61	Vent cartridge	2
29	98	Begin loop UPPER	60
30	2	Vortex reaction vessel on	15
31	3	Vortex reaction vessel off	13
32	41	Vent reaction vessel	2
33	99	End loop UPPER	1

Module G: DCM Washes

Step	Fxn	Name	Time
1	1	Wait	1
2	3	Vortex reaction vessel off	1
3	98	Begin loop UPPER	5 (PNA = 2)
4	41	Vent reaction vessel	2
5	49	Flow DCM through reaction vessel to waste	2
6	42	Drain reaction vessel to waste	5
7	55	Deliver DCM to reaction vessel	4
8	40	Mix reaction vessel	2
9	2	Vortex reaction vessel on	3
10	40	Mix reaction vessel	2
11	3	Vortex reaction vessel off	1
12	42	Drain reaction vessel to waste	7
13	99	End loop UPPER	1
14	42	Drain reaction vessel to waste	5

Module H: Piperidine Deprotection, Previous Peak

Step	Fxn	Name	Time
1	1	Wait	1
2	135	Monitoring reset	1
3	110	Begin loop lower	2
4	42	Drain reaction vessel to waste	7
5	98	Begin loop UPPER	3
6	56	Deliver NMP to reaction vessel	3
7	40	Mix reaction vessel	2
8	2	Vortex reaction vessel on	2
9	40	Mix reaction vessel	2
10	3	Vortex reaction vessel off	1
11	42	Drain reaction vessel to waste	7
12	41	Vent reaction vessel	2
13	50	Flow NMP through reaction vessel to waste	3
14	42	Drain reaction vessel to waste	7
15	99	End loop UPPER	1
16	56	Deliver NMP to reaction vessel	2
17	79	Pressurize piperidine	10
18	51	Deliver piperidine to reaction vessel	3
19	56	Deliver NMP to reaction vessel	2
20	40	Mix reaction vessel	2
21	2	Vortex reaction vessel on	120
22	3	Vortex reaction vessel off	1
23	42	Drain reaction vessel to waste	2
24	130	Monitor previous peak	1
25	1	Wait	3
26	131	Monitoring stop	1
27	132	Read monitoring peak	1

Step	Fxn	Name	Time
28	111	End loop lower	1
29	133	Begin loop monitoring	2
30	42	Drain reaction vessel to waste	7
31	98	Begin loop UPPER	3
32	56	Deliver NMP to reaction vessel	3
33	40	Mix reaction vessel	2
34	2	Vortex reaction vessel on	2
35	40	Mix reaction vessel	2
36	3	Vortex reaction vessel off	1
37	42	Drain reaction vessel to waste	7
38	41	Vent reaction vessel	2
39	50	Flow NMP through reaction vessel to waste	3
40	42	Drain reaction vessel to waste	7
41	99	End loop UPPER	1
42	56	Deliver NMP to reaction vessel	2
43	79	Pressurize piperidine	5
44	51	Deliver piperidine to reaction vessel	3
45	56	Deliver NMP to reaction vessel	2
46	40	Mix reaction vessel	2
47	2	Vortex reaction vessel on	120
48	3	Vortex reaction vessel off	1
49	42	Drain reaction vessel to waste	2
50	130	Monitor previous peak	1
51	1	Wait	3
52	131	Monitoring stop	1
53	132	Read monitoring peak	1
54	134	End loop monitoring	25
55	10	Flush bottom valve block with gas to waste	3
56	40	Mix reaction vessel	2
57	2	Vortex reaction vessel on	5
58	3	Vortex reaction vessel off	1
59	42	Drain reaction vessel to waste	5
60	41	Vent reaction vessel	2
61	50	Flow NMP through reaction vessel to waste	5
62	42	Drain reaction vessel to waste	10

Module I: Vortex, 5 minutes

Step	Fxn	Name	Time
1	1	Wait	1
2	2	Vortex reaction vessel on	300
3	3	Vortex reaction vessel off	1

Module a: Read Cartridge

Step	Fxn	Name	Time
1	1	Wait	1
2	4	Read cartridge	10
3	6	Needle up	10
4	7	Eject cartridge	10
5	8	Advance cartridge	10
6	5	Needle down	10
7	14	Flush bottom valve block with NMP to waste	1
8	9	Flush top valve block with gas to waste	2
9	10	Flush bottom valve block with gas to waste	5
10	60	Mix cartridge	5

Module b: Piperidine Deprotection, no monitoring

Step	Fxn	Name	Time
1	1	Wait	1
2	56	Deliver NMP to reaction vessel	3
3	40	Mix reaction vessel	2
4	2	Vortex reaction vessel on	5
5	40	Mix reaction vessel	2
6	3	Vortex reaction vessel off	1
7	42	Drain reaction vessel to waste	5
8	41	Vent reaction vessel	2
9	50	Flow NMP through reaction vessel to waste	5
10	42	Drain reaction vessel to waste	10
11	56	Deliver NMP to reaction vessel	2
12	79	Pressurize piperidine	10
13	51	Deliver piperidine to reaction vessel	3
14	56	Deliver NMP to reaction vessel	2
15	40	Mix reaction vessel	2
16	2	Vortex reaction vessel on	120
17	3	Vortex reaction vessel off	1
18	42	Drain reaction vessel to waste	10
19	56	Deliver NMP to reaction vessel	2
20	79	Pressurize piperidine	5
21	51	Deliver piperidine to reaction vessel	3
22	56	Deliver NMP to reaction vessel	2
23	40	Mix reaction vessel	2
24	2	Vortex reaction vessel on	300
25	3	Vortex reaction vessel off	1
26	42	Drain reaction vessel to waste	5
27	41	Vent reaction vessel	2
28	50	Flow NMP through reaction vessel to waste	5
29	42	Drain reaction vessel to waste	10

Module c: Final DCM Washes

Step	Fxn	Name	Time
1	1	Wait	1
2	12	Flush bottom valve block with DCM to waste	1
3	9	Flush top valve block with gas to waste	2
4	10	Flush bottom valve block with gas to waste	2
5	98	Begin loop UPPER	6
6	55	Deliver DCM to reaction vessel	5
7	40	Mix reaction vessel	2
8	2	Vortex reaction vessel on	1
9	40	Mix reaction vessel	2
10	1	Wait	5
11	3	Vortex reaction vessel off	1
12	42	Drain reaction vessel to waste	5
13	41	Vent reaction vessel	2
14	49	Flow DCM through reaction vessel to waste	3
15	42	Drain reaction vessel to waste	10
16	99	End loop UPPER	1
17	42	Drain reaction vessel to waste	30
18	29	Flow DCM through activator to waste	5
19	22	Drain activator to waste	30
20	11	Flush top valve block with DCM to waste	1
21	12	Flush bottom valve block with DCM to waste	1
22	10	Flush bottom valve block with gas to waste	10
23	9	Flush top valve block with gas to waste	10

Module d: NMP Wash from Activator

Step	Fxn	Name	Time
1	1	Wait	1
2	98	Begin loop UPPER	3
3	3	Vortex reaction vessel off	1
4	28	Pressurize activator	4
5	42	Drain reaction vessel to waste	7
6	38	Transfer activator to reaction vessel (top open)	6
7	40	Mix reaction vessel	1
8	2	Vortex reaction vessel on	3
9	40	Mix reaction vessel	2
10	99	End loop UPPER	1
11	22	Drain activator to waste	5
12	3	Vortex reaction vessel off	1
13	42	Drain reaction vessel to waste	7

Module f: DIEA Neutralization

Step	Fxn	Name	Time
1	1	Wait	1
2	3	Vortex reaction vessel off	1
3	98	Begin loop UPPER	2
4	42	Drain reaction vessel to waste	7
5	56	Deliver NMP to reaction vessel	4
6	40	Mix reaction vessel	2
7	78	Pressurize manifold	5
8	68	Deliver DIEA to measuring loop (open)	3
9	43	Transfer measuring loop to reaction vessel	10
10	56	Deliver NMP to reaction vessel	2
11	40	Mix reaction vessel	2
12	2	Vortex reaction vessel on	3
13	40	Mix reaction vessel	2
14	3	Vortex reaction vessel off	1
15	99	End loop UPPER	1
16	42	Drain reaction vessel to waste	7

Module h: Conditional Deprotection, Previous Peak

Step	Fxn	Name	Time
1	137	Do module if condition not met	1
2	56	Deliver NMP to reaction vessel	2
3	79	Pressurize piperidine	10
4	51	Deliver piperidine to reaction vessel	3
5	56	Deliver NMP to reaction vessel	2
6	40	Mix reaction vessel	2
7	2	Vortex reaction vessel on	1
8	13	Flush top valve block with NMP to waste	2
9	14	Flush bottom valve block with NMP to waste	2
10	9	Flush top valve block with gas to waste	5
11	10	Flush bottom valve block with gas to waste	5
12	1	Wait	600
13	3	Vortex reaction vessel off	1
14	42	Drain reaction vessel to waste	2
15	130	Monitor previous peak	1
16	1	Wait	3
17	131	Monitoring stop	1
18	132	Read monitoring peak	1
19	42	Drain reaction vessel to waste	10

Module i: Conditional Vortex, 5 minutes

Step	Fxn	Name	Time
1	137	Do module if condition not met	1
2	2	Vortex reaction vessel on	300
3	3	Vortex reaction vessel off	1

Cycles using the new 0.5 mL measuring loop

Because the new 0.5 mL measuring loop is made of smaller diameter tubing, the cycles using this new 0.5 mL measuring loop need to have the times in some functions changed, as compared to the cycles using the original 0.5 mL measuring loop. These functions are:

- Fxn 23 - Transfer measuring loop to Act
- Fxn 43 - Transfer measuring loop to RV
- Fxn 63 - Transfer measuring loop to Cart
- Fxn 68 - Deliver #7 to measuring loop
- Fxn 69 - Deliver #8 to measuring loop
- Fxn 70 - Flush bottom valve block to waste

Table 5-5 shows the modifications that are made to the cycles that use the 0.5 mL Measuring Loop. These new cycles are included in the Variable Measuring Loop disk. The cycles that have been modified are from the SynthAssist Chemistry disk, version 2.0.3.

Check to see if your 0.5 mL measuring loop fills in 6 seconds with Bottle 7 or 8 (Flow Tests, VML, modules “c” and “d”).

This test should be done with the solution that is being used in the synthesis because the viscosity of the solutions affect the flow rate. If it is difficult to see the liquid as it fills the measuring loop, check for the appearance of the liquid at the waste tube (valve position 6, top of the 11-port valve block). If the time is less than 6 seconds, you might want to reduce the time of delivery for Fxn 68 and/or Fxn 69. Table 5-5 will provide the information on where Fxn 68 and 69 appear.

In addition, if you have already modified the cycles previously provided in the SynthAssist Chemistry disk, then you need to modify the cycles to match the specifications shown in Table 5-5.

Table 5-5. Changes to cycles on ABI 433A chemistry disk (version 2.03)

***FastMoc* Cycles (0.10 & 0.25 mmol)**

Module E: Transfer			Time (sec)	
Step	Fxn	Name	Old	New
5	70	Flush bottom valve block with loop contents to waste	2	6
7	68	Deliver #7 to measuring loop	2	6
8	63	Transfer measuring loop to Cart	4	10

Module H: Load and Cap			Time (sec)	
Step	Fxn	Name	Old	New
27	69	Deliver #8 to measuring loop	3	6
28	43	Transfer measuring loop to RV	4	10

Module a: Activation & Transfer (Conditional cycles only)			Time (sec)	
Step	Fxn	Name	Old	New
29	70	Flush bottom valve block with loop contents to waste	2	6
31	68	Deliver #7 to measuring loop	2	6
32	63	Transfer measuring loop to Cart	4	10

FastMoc Cycles (1.0 mmol)

Module E: Transfer			Time (sec)	
Step	Fxn	Name	Old	New
4	70	Flush bottom valve block with loop contents to waste	2	6
6	68	Deliver #7 to measuring loop	2	6
7	23	Transfer measuring loop to Act	2	10

Module H: Load and Cap			Time (sec)	
Step	Fxn	Name	Old	New
27	69	Deliver #8 to measuring loop	3	6
28	43	Transfer measuring loop to RV	4	10

Module H: Load and Cap (monitoring cycles)			Time (sec)	
Step	Fxn	Name	Old	New
28	69	Deliver #8 to measuring loop	3	6
29	43	Transfer measuring loop to RV	4	10

Fmoc/HOBt/DCC (0.10 mmol)

Module a: Activation			Time (sec)	
Step	Fxn	Name	Old	New
15	68	Deliver #7 to measuring loop	3	6
17	63	Transfer measuring loop to Cart	6	10
61	69	Deliver #8 to measuring loop	3	6
62	23	Transfer measuring loop to Act	3	10
64	23	Transfer measuring loop to Act	3	5

Module h: Loading			Time (sec)	
Step	Fxn	Name	Old	New
45	69	Deliver #8 to measuring loop	3	6
46	23	Transfer measuring loop to Act	3	10
48	23	Transfer measuring loop to Act	3	5

Fmoc/HOBt/DCC (0.25 mmol)

Module a: Activation			Time (sec)	
Step	Fxn	Name	Old	New
16	68	Deliver #7 to measuring loop	3	6
19	63	Transfer measuring loop to Cart	6	10
21	63	Transfer measuring loop to Cart	6	5
54	69	Deliver #8 to measuring loop	3	6
55	23	Transfer measuring loop to Act	3	10
57	23	Transfer measuring loop to Act	3	5

Module h: Loading			Time (sec)	
Step	Fxn	Name	Old	New
45	69	Deliver #8 to measuring loop	3	6
46	23	Transfer measuring loop to Act	3	10
48	23	Transfer measuring loop to Act	3	5

Boc/HOBt/DCC (0.10 mmol)

Module a: Activation			Time (sec)	
Step	Fxn	Name	Old	New
21	68	Deliver #7 to measuring loop	3	6
23	63	Transfer measuring loop to Cart	6	10
61	69	Deliver #8 to measuring loop	3	6
62	23	Transfer measuring loop to Act	3	10
64	23	Transfer measuring loop to Act	3	5

Boc/HOBt/DCC (0.50 mmol)

Module a: Activation			Time (sec)	
Step	Fxn	Name	Old	New
34	70	Flush bottom valve block with loop contents to waste	2	6
37	68	Deliver #7 to measuring loop	3	6
40	63	Transfer measuring loop to Cart	5	10
73	70	Flush bottom valve block to waste	2	6
76	69	Deliver #8 to measuring loop	3	6
78	23	Transfer measuring loop to Act	2	10
80	23	Transfer measuring loop to Act	2	5
82	23	Transfer measuring loop to Act	2	5

Flow Tests Folder

The Flow Test folder contains three flow test files:

- Flow Tests (New VML)
- Flow Tests 1-18 (VML)
- Flow Tests 19-23

Flow Tests (New VML)
The Flow Tests (New VML) file contains 4 flow tests that are needed to install and check the variable measuring loop (VML) system.

Flow Test a: Calibrate 0.125-mL VML
This flow test is used when calibrating the 0.125-mL measuring loop, as described on page 3-6. When running this flow test, Bottle 8 is filled with NMP and the in-line filter to the cartridge is replaced with a flange coupling (P/N 110070). After the calibration of the 0.125-mL measuring loop is completed, calibrate the new 0.50-mL measuring loop. However, before starting a synthesis, remove the flange coupling and re-attach the in-line filter.

This flow test uses an empty, tared cartridge, with the septum installed.

The flow test fills the 0.125-mL measuring loop with NMP and delivers this NMP to the cartridge 10 times.

Step	Fxn	Name	Time
1	5	Needle down	10
2	78	Pressurize manifold	15
3	98	Begin Loop UPPER	10
4	69	Deliver #8 to measuring loop (open)	5
5	10	Flush bottom valve block with gas to waste	2
6	63	Transfer measuring loop to cartridge	10
7	99	End Loop UPPER	1
8	6	Needle up	10
9	7	Eject cartridge	10
10	70	Flush bottom valve block with loop to waste	5
11	10	Flush bottom valve block to waste	5

Flow Test b: Calibrate 0.50-mL VML

This flow test is used when calibrating the new 0.50-mL measuring loop, as described on page 3-9 of this manual. Perform this calibration immediately after calibrating the 0.125 mL loop. When running this flow test, Bottle 8 is filled with NMP and the in-line filter to the cartridge is replace with a flange coupling (P/N 110070). After the calibration of the measuring loop is complete and before a synthesis is started, remove the flange coupling and re-attach the in-line filter.

This flow test uses an empty, tared cartridge, with the septum installed.

The flow test fills the 0.50-mL measuring loop with NMP and delivers this NMP to the cartridge 4 times.

Step	Fxn	Name	Time
1	5	Needle down	10
2	78	Pressurize manifold	15
3	98	Begin Loop UPPER	4
4	69	Deliver #8 to measuring loop (open)	8
5	10	Flush bottom valve block with gas to waste	2
6	63	Transfer measuring loop to cartridge	10
7	99	End Loop UPPER	1
8	6	Needle up	10
9	7	Eject cartridge	10
10	70	Flush bottom valve block with loop to waste	5
11	10	Flush bottom valve block to waste	5

Flow Test c: Test Bottle 7 VML

Use this flow test after the 0.125-mL and 0.50-mL measuring loops are calibrated. They can be used with either of the two measuring loops. The objective of the flow test is to insure that the measuring loop can be filled when Bottle 7 is used. The flow test is very similar to Flow Test 7, which is described in the Model 433A User's manual, except there is an additional step added (step 3, Fxn 1, Wait), which lets you check the waste line to make sure the liquid has completely filled the measuring loop. It does not matter what liquid is in Bottle 7. When the 0.125-mL measuring loop is attached, the loop should fill in 3 seconds. When the 0.50-mL measuring loop is attached, the loop should fill in 5 seconds.

Step	Fxn	Name	Time
1	78	Pressurize manifold	15
2	68	Deliver #7 to measuring loop (open)	6
3	1	Wait	5
4	70	Flush bottom valve block with loop to waste	10
5	10	Flush bottom valve block with gas to waste	2
6	14	Flush bottom valve block with #10 to waste	2
7	10	Flush bottom valve block with gas to waste	10

Flow Test d: Test Bottle 8 VML

This flow test is identical to flow test c, except it is to check the measuring loop when using Bottle 8.

Step	Fxn	Name	Time
1	78	Pressurize manifold	15
2	69	Deliver #8 to measuring loop (open)	6
3	1	Wait	5
4	70	Flush bottom valve block with loop to waste	10
5	10	Flush bottom valve block with gas to waste	2
6	14	Flush bottom valve block with #10 to waste	2
7	10	Flush bottom valve block with gas to waste	10

Flow Tests (1-18 VML)

The Flow Tests (1-18 VML) file contains the 18 flow tests described in the 433A User's Manual. These flow tests are the identical, except for:

- Module g: Flow Test 7
- Module h: Flow Test 8
- Module H: Flow Test 17
- Module I: Flow Test 18

In modules g and h, the time to fill the loop has been increased from 4 to 6 seconds. In modules H and I, the time to fill the loop has been changed from 3 to 6 seconds, and the time to deliver the loop contents to the cartridge has been increased from 5 to 10 seconds.

Once the variable measuring loop has been installed, Flow Tests (1-18 VML) should be used instead of Flow Test 1-18.

When Flow test 17 and 18 are used with the 0.125 mL measuring loop, only one quarter of the amount of solution shown in the ABI 433A User's Manual (pages 6-31 through 6-32) will be obtained.

Flow Tests 19-23

The Flow Tests 19-23 file is identical to the Flow Test 19-23 file that is described in the ABI 433A User's Manual. This file is included on the disk for your convenience.

A Appendix

Plumbing Schematics

Installing the Measuring Loop for the 3 mL RV involves changing some of the tubes (also known as “plumbing”). The schematics on pages A-2 and A-3 show what the plumbing should look like after modifications are made.

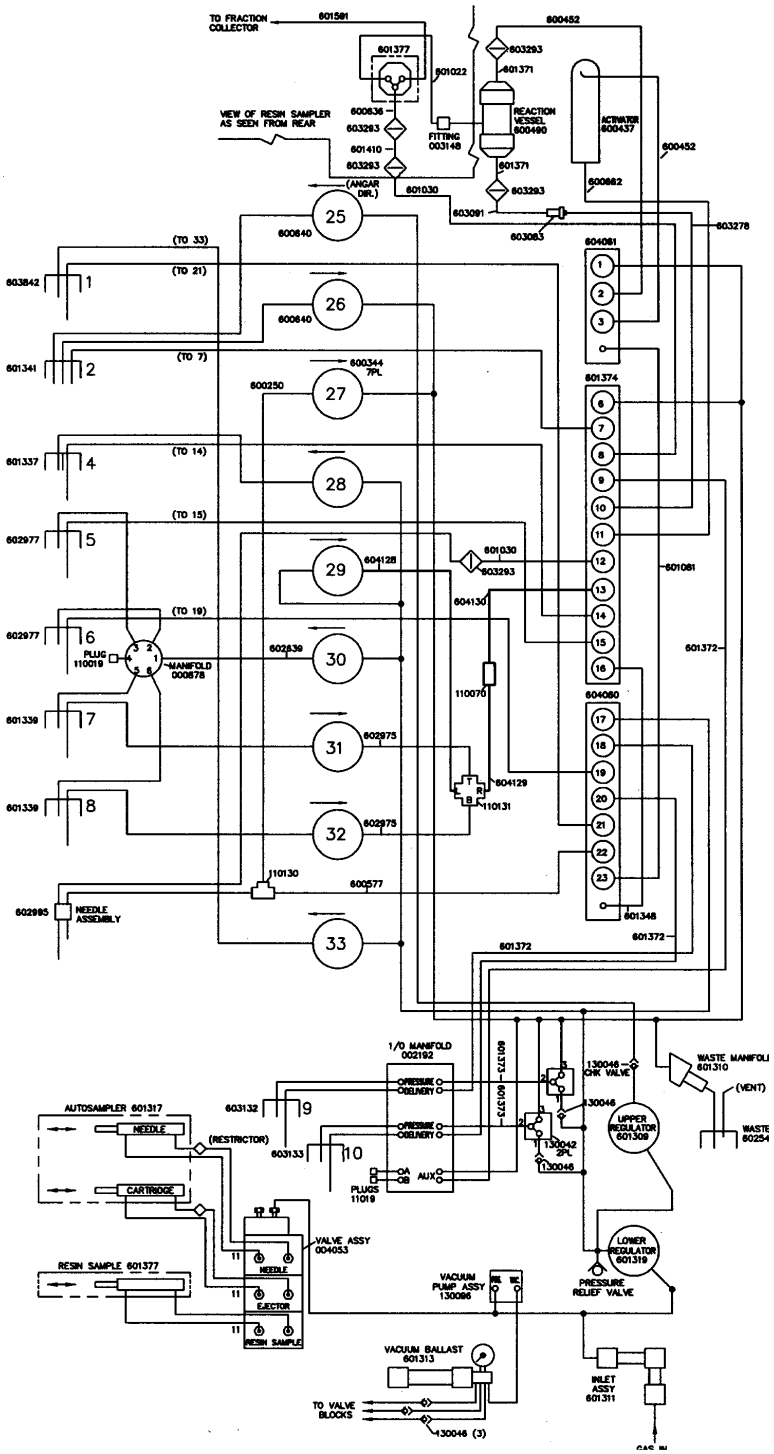


Figure A-1. Plumbing diagram for 0.125 mL configuration

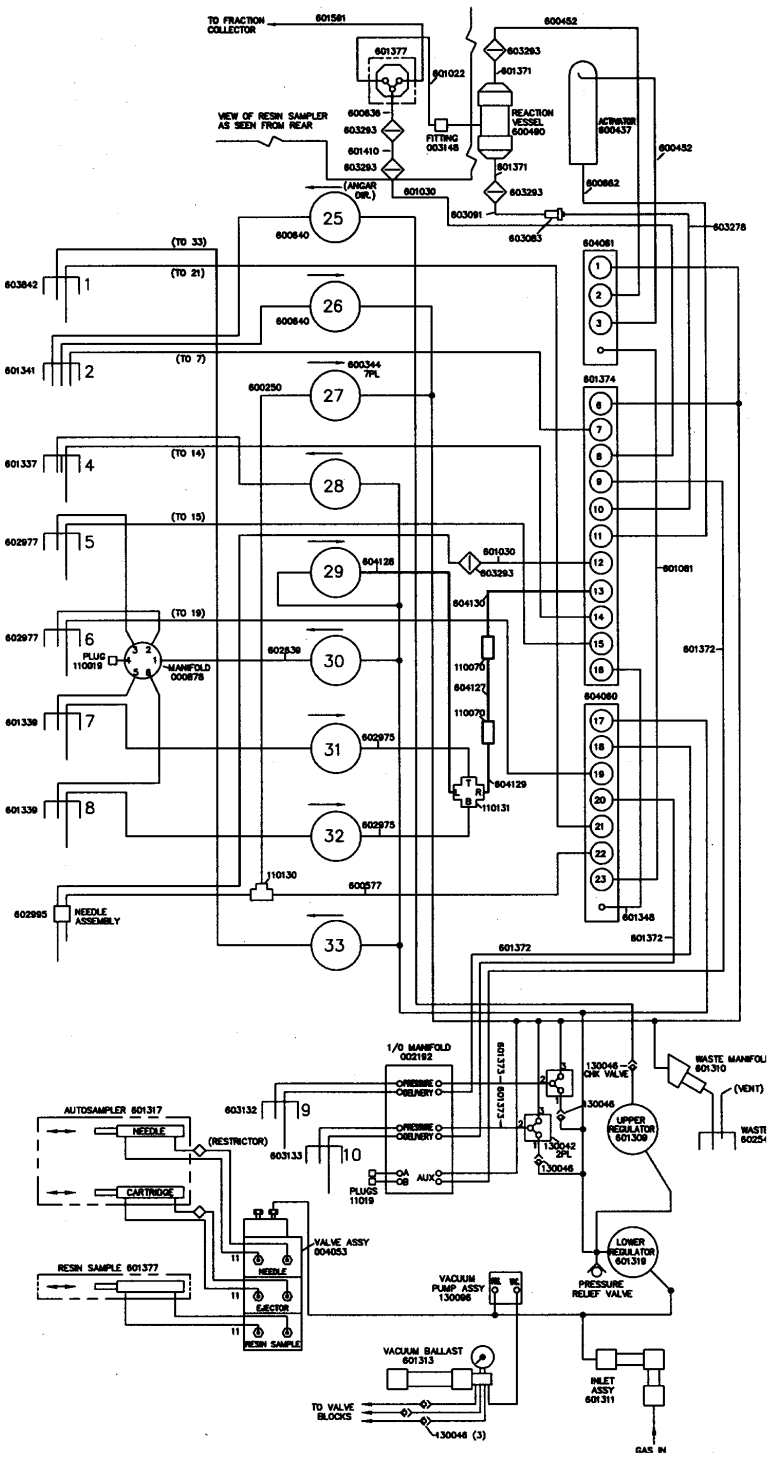


Figure A-2. Plumbing diagram for 0.500 mL configuration

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