I want to use this communication to highlight some important point of the system's operation.

A. I installed five **microwave methods** into the Discover microwave system. They are:

1. Deprotection - It is named DPRTC 75*C 3 Min: This method is to be used for all deprotection steps. It can be used with all resins and for all residues (with the exception of sequences that have a phospho or glyco amino acid present - in this case you will need to perform a room temperature (rt) deprotection step so you do not hydrolyze off the phospho or sugar group).

2. Coupling - There are two coupling methods

a. CPLNG 75*C 6 Min - This is for all resins with the exception of Chlorotrityl resins and for all amino acids couplings with the exception of Arg, Cys and His.

b. For Chlorotrityl resin use the CPLNG 50*C 10 Min so you do not self cleave the peptide from the resin

c. For Arg, you will couple at rt for 20 minutes before putting in the microwave using the CPLNG 75* 6 Min method. After this initial coupling, you will want to drain the coupling reagents, add a fresh batch and couple a second time using the CPLNG 75*C 6 Min (no need for the rt step for the second coupling

d. Cys/His - Use the CPLNG 50*C 10 Min method so you can prevent racemization

e. CPLNG 50*C 10 Min - Use this method for couplings on a

Chlorotrityl resin and for all Cys/His couplings

3. Adding First Residue to an unloaded Chlorotrityl Resin –

It is named CHLR 50*C 30 Min - If you need to add the first residue to an unloaded Chlorotrityl Resin, you will use this method (see the guidelines in the attachment below).

4. Cleavage - It is named CLVG 38*C 30 Min: This method is used to cleave the peptide from the resin. You can use the system to cleave the peptide from the resin but it is requires you to perform added clean up steps(putting DCM in place of the DMF bottle, cleaning out the reaction vessel after use) that negate the benefit of using the microwave for this step. I suggest you do your cleavages at room temperature.

5. Nitrogen bubbling - This is somewhat touchy - you need to manipulate the right side valve carefully to obtain the proper level of bubbling (mixing).

I suggest you turn the Nitrogen bubbling on after the deprotection base and coupling reagent additions and leave them on during the microwave heating step.

6. The 'Add' Function- When you add the coupling reagents through the injection port, you want to have the syringe attached to the port when the left hand valve is set to the 'Add' position. Before you remove the syringe, make sure the left hand valve is set to the 'Stop' position. The instructions for the 'Add' step should read:

a. place a reagent filled syringe onto the Injection port,
b. put the left hand valve to the 'Add' position
c. pushing the syringe barrel down to add reagents to the reaction vessel

d. Turn the left hand valve to stop

e. remove the syringe from the 'add' port

f. fill the syringe with air and return to the 'add' port

g. turn the left hand valve to 'add'

h. push down the syringe barrel to force any reagents in the line into the reaction vessel.

i repeat steps d-h 2 more times to insure there is no reagents left in the line.

j. Turn the left hand valve to stop and remove the syringe from the injection port

7. Nitrogen Valve on Wash Station - The nitrogen valve has 3 positions. Each is

described below

1. Off - The nitrogen supply is isolated from the work station. Any pressure in the bottles will remain in the bottles

2. N2 - Nitrogen is actively being supplied to the work station. When a valve is turned to a position that consumes pressure (N2 Bubbling, Wash Solvent, Deprotect, Purge), the nitrogen used will be replaced by the supply.

3. Vent - This vents the work station of all nitrogen pressure (it puts the work station at atmospheric conditions which is necessary if you need to change a reagent bottle).