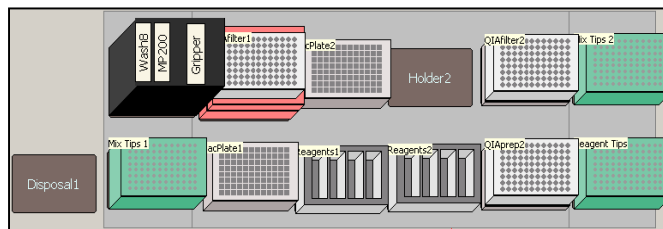


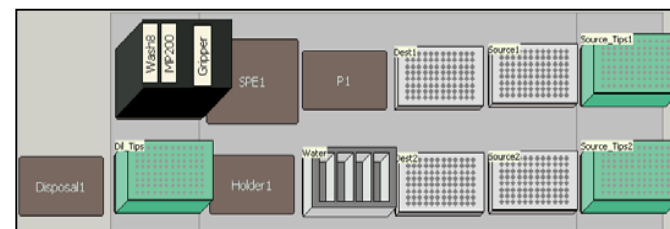
# Molecular Biology Suite Automation: Upfront Processing of Plasmid DNA for Sequencing

Matthew Cu, Keith Roby and Graham Threadgill  
Biomedical Research Division, Beckman Coulter, Inc.

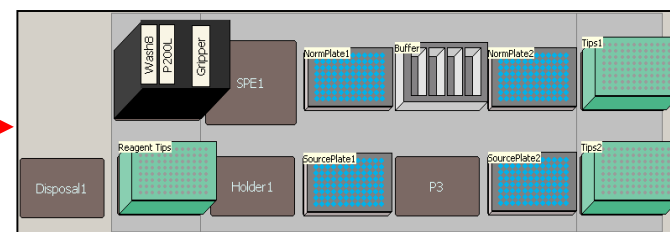
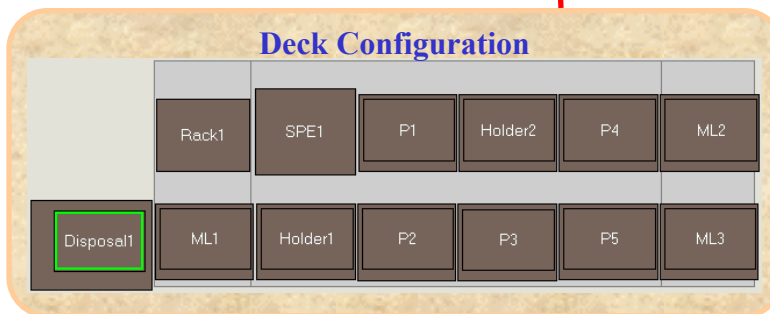
The Molecular Biology Suite encompasses a series of automated methods on the Biomek 3000 Laboratory Automation Workstation for plasmid purification, quantitation and normalization, sequencing reaction setup and sequencing reaction cleanup. Since the CEQ™ 8800 Genetic Analysis System utilizes two plates and sample tracking technology, the Suite is designed to handle batches of two 96-well microtiter plates with all the methods conveniently using the same deck configuration enabling faster and easier transitions from one method to another. In addition, each method allows the user the option to process anything less than two plates full in multiples of eight with the start and stop on desired columns.



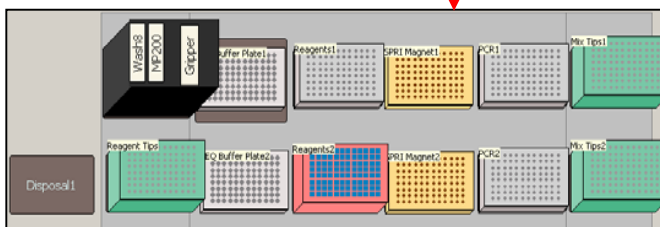
1. Plasmid Purification



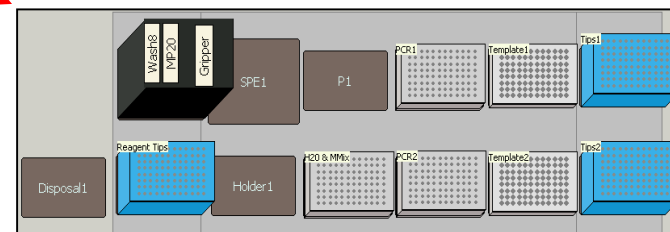
2. Quantitation



3. Normalization



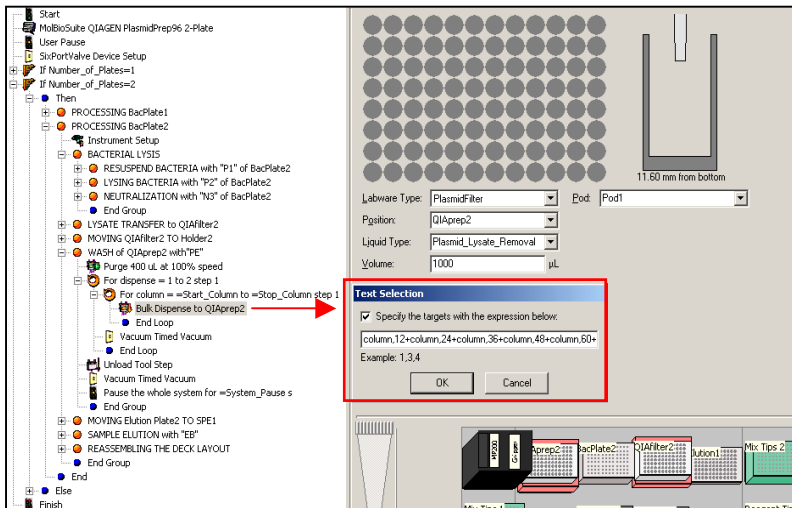
5. Sequencing Reaction Cleanup



4. Sequencing Reaction Setup

The same deck configuration used by all the methods.

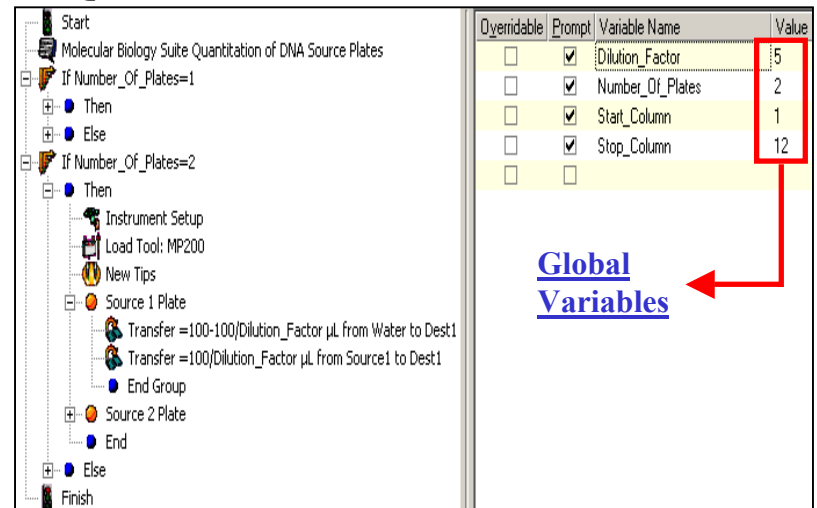
# 1. Plasmid Purification Method



## Specify Selection as Text Values

“column,12+column,24+column,36+column,48+column,60+column,72+column,84+column”

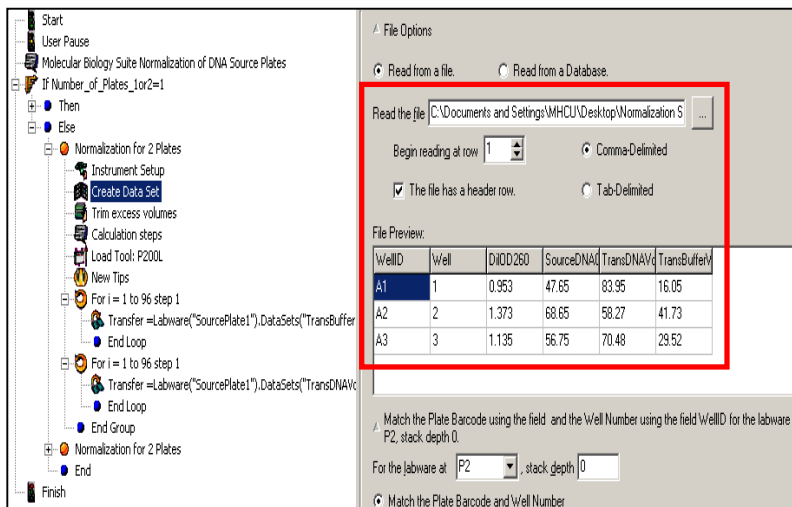
# 2. Quantitation Method



## Global Variables

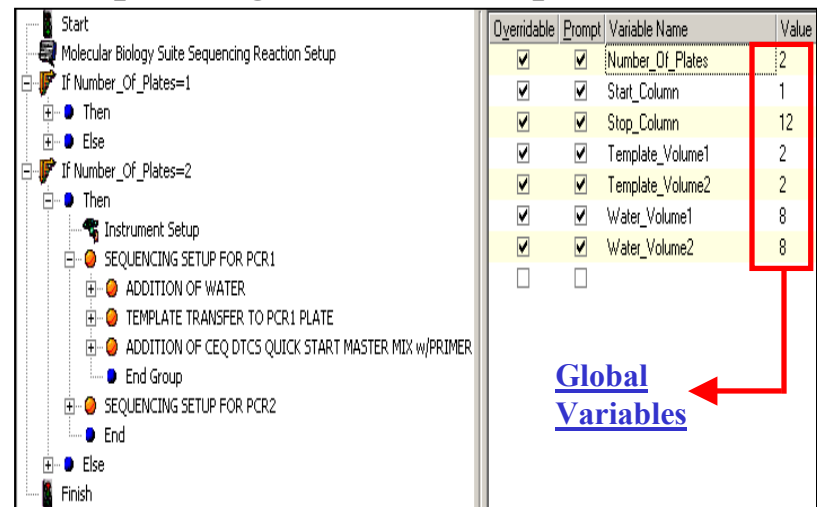
If dilution factor is anything other than 5, then enter the desired value when prompted. This method will calculate all relevant factors.

# 3. Normalization Method



The source plate is read offline using a plate reader and that the conversion from an OD260 value to a concentration is done offline also. Browse for the generated spreadsheet to use when creating data sets.

# 4. Sequencing Reaction Setup Method



## Global Variables

Accept these values or change as necessary. To determine the amount of DNA template used in the sequencing reaction, follow the instructions provided in the CEQ DTCS Quick Start Kit.

## 5. Sequencing Reaction Setup Method

Generate a CEQ import file for the bottom labware at position Holder2

named  with the sample ids coming from the data set

**Set the import file options for the samples below:**

Column Name	Use contents of the Data Set	Use this value for all samples
Method	<input type="radio"/> Method	<input type="radio"/>
Automatic Analysis	<input type="radio"/> Automatic Analysis	<input type="radio"/>
Analysis Parameter Set	<input type="radio"/> Analysis Parameter Set	<input type="radio"/>
Analysis Type	<input type="radio"/> Analysis Type	<input type="radio"/>
Note	<input type="radio"/> Note	<input type="radio"/>
Properties	<input type="radio"/> Properties	<input type="radio"/>

Automatic Report  Automatic Export

Export Filename:

Export Format:   Remove CEQ Tracking Suffix:

Header  Raw Data  Resolve Filename Conflicts

Result Data  Result Output  Apply Trimming

Quality Parameters  Alignment Results  Export Only If Sequence >

Select the designated deck position where the sequencing reaction plate is located (i.e. Holder2). Click the six radio buttons under the *Use contents of the Data Set*. From the pull down menu at the right of each category select the corresponding text

## Molecular Biology Suite Method Summary

Method Name (.bmf)	Labware	Reagents	Run Time
<b>Plasmid Purification</b>	AP250 Tips (3) 96 Deep-Well Square Plates (2) Reservoir Holders (2) Quarter Reservoirs (7) 96-Well Microtiter Plates (2)	QIAGEN QIAprep 96 Turbo Miniprep Kit	~ 171 minutes
<b>Quantitation</b>	AP250 Tips (3) Reservoir Holder (1) Quarter Reservoir (1) 96-Well Microtiter Plates (4)	Nuclease-Free Water	~ 15 minutes
<b>Normalization</b>	AP250 Tips (3) Reservoir Holder (1) Quarter Reservoir (1) 96-Well Microtiter Plates (4)	Nuclease-Free Water	~ 81 minutes
<b>Sequencing Reaction Setup</b>	AP20 Tips (3) 96-Well PCR Plates (3) 96-Well Microtiter Plates (5)	CEQ DTCS Quick Start Kit	~ 17 minutes
<b>Sequencing Reaction Cleanup</b>	AP250 Tips (3) 96-Well PCR Plates (3) 96-Well Microtiter Plates (5) 12-Column Reservoir Plate (1)	Agencourt CleanSEQ Kit	~ 90 minutes

## RESULTS

### Spectrophotometric Determination of Plasmids

Plate Name pGEM-3Zf(+)	Bacterial Density (OD600)	Average Concentration ( $\mu\text{g/mL}$ )	Average Purity (A260/280)
Plate 1	2.197	42.266	1.658
Plate 2	2.197	33.169	1.776
Plate 3	2.087	50.338	1.603
Plate 4	1.889	56.076	1.676
<b>Average</b>	<b>2.093</b>	<b>45.462</b>	<b>1.678</b>

Average values of pGEM-3Zf(+) for bacterial density, concentration and purity from the four plates processed.

### Sequencing Data Summary

Plate	Percent Accuracy (%) at 700 base pairs	Read Length in base pairs	98% Cutoff	Sample Number
Plate 1	98.77	841	769	96
Plate 2	98.89	814	748	96
<b>Average</b>	<b>98.83</b>	<b>828</b>	<b>759</b>	<b>96</b>

Sequencing results from two 96-well plasmid DNA plates. The average percent accuracy at 700 bases was excellent at approximately 99%. The average read length and 98% cutoff in base pairs were 828 and 759, respectively.