

Bauer Core Standard Protocol		
Title: AutoGenprep 965 Plasmid Protocol		
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1. Purpose

This protocol describes how to run the Plasmid/BAC DNA isolation protocol on the AutoGenprep 965. The protocol is designed as a reference and is not a substitute for training. Users must complete a training session before using any of the Bauer Core's instrumentation.

2. Materials

- 2.1. 0.2-1.8 ml *E.coli* liquid culture (up to 384 samples in the deep well plates below)
- 2.2. 2ml 96 Deep Well Plate(s) (Corning # 3960)
used as source plates for the starting samples and destination plates for the purified DNA.
- 2.3. Plasmid & BAC Extraction Kit (Autogen # AGKT965PB)
- 2.4. Plate sealers (eg Hypertask # MPCAL096)

3. Instrumentation - AutoGenPrep 965

4. Reagent preparation

- 4.1 Load 0.2-1.8 ml liquid culture into each well of the 2ml 96 deep well plates.
 - 4.1.1. Plates will be centrifuged and therefore must be balanced. If the plates are not full, split the samples into the upper rows of two plates.

5. Procedure

- 5.1 Load the Instrument
 - 5.1.1. Make sure the waste containers are empty.
 - 5.1.2. Place the deep well plates containing the samples onto the sample rack.
The sample rack is on the right hand side of the deck
Well A1 should be in the back left corner.
 - 5.1.3. Place an empty deep well plates to the left of each sample plate.
 - 5.1.4. Place tip boxes to the right of the sample plates.
Load one box for each sample plate (front) plus one for reagent dispensing (rear).
 - 5.1.5. Load the Reagent Reservoirs according to the table below:

Reagent	Reservoir	Vol/Sample (ml)	Sample #			
			96	192	288	384
R1 Resuspension	1	0.24	48	72	95	118
R2 Lysis	2	0.24	48	72	95	118
R3 Deproteinization	3	0.15	40	54	69	83
R4 DNA Precipitation	4	0.27	51	77	103	129
R5/6/7 DNA Wash	5,6,7	0.45	69	112	155	198
R9 DNA Resuspension	9	0.05	30	35	40	45

- 5.1.5.1. See table for reagent volumes for 96, 192, 288 or 384 samples.

- 5.1.5.2. For other numbers of samples, multiply Vol/Sample by the number of samples and add 25 ml of “dead” volume.
- 5.1.5.3. Reagent 5/6/7 can be used for up to 3 DNA washes
Fill one reservoir for each wash desired (default = 2).

5.2. Run the Software

- 5.2.1. Turn on the Autogen instrument.
- 5.2.2. Open the Autogen software.
 - 5.2.2.1. Select On Line and hit Ok.
- 5.2.3. Choose to run a Standard Protocol.
 - 5.2.3.1. Check all the items in the checklist to make sure they are in place.
 - 5.2.3.2. Select the protocol to run - either Plasmid or BAC.
 - 5.2.3.3. Select Automated Run.
- 5.2.4. Choose either the Standard protocol or create a User protocol.
 - 5.2.4.1. Standard protocols already have most parameters defined.
The user can only change the starting sample volume and number.
 - 5.2.4.2. Custom protocols can be edited – use to change # of washes or resuspension vol
Commonly, users change the # of washes or resuspension vol.
 - 5.2.4.3. Click OK when the protocol is edited as desired.
- 5.2.5. Click Start to run the System check.
- 5.2.6. Click OK to begin.

5.3. Clean Up

- 5.3.1. Dispose of all tips as biohazard waste.
- 5.3.2. Add 10% bleach to waste bin 1 for 5 minutes and then empty in the drain.
- 5.3.3. Empty leftover reagents in the reagent trays into waste bin 2.
 - 5.3.3.1. Rinse the reagent trays with water and add rinse water to waste bin 2
- 5.3.4. Empty waste bin 2 and dispose of the contents as hazardous waste.
 - 5.3.4.1. Label the waste as toxic and flammable.
 - 5.3.4.2. Record the following waste composition:

Isopropanol	13%
Ethanol	41%
Phenol	<1%
Chloroform	<1%
- 5.3.5. Dispose of source plates as chemical waste
 - 5.3.5.1. Label the waste as toxic.
 - 5.3.5.2. Record the following waste composition:

Chloroform	11%
Phenol	5%
- 5.3.6. Shut down the software and turn off the power to the instrument.
- 5.3.7. Log off the computer.