Sample Growth and Collection Methods

Preparation and Sterilization of Media

One liter of media was prepared in a 2 L Erlenmeyer flask by adding 22 g of Brodo Mueller Hinton II Media (BMHII) and filling to 1 L with Millipore water. The flask was capped with aluminum foil and autoclave tape. The solution was heated and stirred on a hot plate with a stir bar for 10-15 min until homogeneous. The media was autoclaved. After the media was autoclaved, sterile procedures were used every time the aluminum foil lid was removed.

Sterile Technique

The hood was sprayed with 70% ethanol while the glass door was opened 1 foot or less. Anything coming into or near the hood was sprayed with 70% ethanol. No lids were removed during this process. All equipment and supplies were sprayed with 70% ethanol and/or autoclaved before they were placed in the hood. After the ethanol had fully evaporated inside the hood, a Bunsen burner was lit. All flasks were autoclaved and capped with aluminum foil. The rim of each flask was passed through the flame before and after pouring media. The lids on all containers were closed for as much as time as possible.

Overnight Culture Preparation

Sterile technique was used to pour 80 mL of BMHII media into four 250 mL Erlenmeyer flasks. Two 80 mL cultures were started per type of *E. coli* from stored stock samples. All equipment and supplies were sterilized before removing the stock *E. coli* from the freezer. The stock *E. coli* was kept on ice and was not allowed to thaw. The sterile pipette tip was used to scrape the frozen bacterial stock and injected into the media. The culture flasks were placed into the incubator overnight at 37 ºC and 250 rpms.

Starting the Culture

After 12 hours, the overnight cultures were checked for cloudiness. Four sterile 1 L Erlenmeyer flasks were prepared by adding 500 mL of sterile BMHII media to each 1 L flask using the sterile technique. The Optical Density at 600 nm (OD_{600}) of each flask was brought up to about 0.1 by adding the overnight culture. After the OD_{600} of each culture was taken, the flasks were placed back in the 37 ºC incubator at 250 rpms.

OD_{600} Readings

A sterile capped cuvette was filled with sterile media to use as a blank. For the 1:5 dilution blank, 0.20 mL of sterile media and 0.80 mL of sterile Millipore water was used. SpectraMax plus ® SoftMax Pro 5 ® Molecular Devices Spectrometer was used to collect the OD_{600} readings. The spectrometer was set to a wavelength of 600 nm. The blank cuvette was wiped down with a Kimwipe and inserted into the spectrometer. The blank spectrum was acquired, and the cuvettes were filled with 1 mL of each culture. Each sample was individually wiped down and inserted into the spectrometer. The OD_{600} was recorded. The OD_{600} was plotted versus time in minutes to generate a growth plot.

Culture Growth

The cultures were kept in the incubator at 37 ºC and 250 rpms except for as short of a time as possible during sample collection. The OD_{600} was taken every 0.5 h with the first few cultures grown and every hour with the last set of cultures grown. The 1:5 dilution of the samples in the cuvettes was implemented to keep the OD_{600} readings in the linear phase. When the cultures reached mid log phase (half of the ending OD_{600}) and stationary phase, samples were collected. After the cultures reached stationary phase, the cultures were left for another 6 to 8 h before the OD_{600} readings were taken again. The new OD_{600} readings were compared to the ones 6-8 h prior to ensure that the culture was in stationary phase when the samples were obtained.

Sample Collection

The mid log phase samples were collected when the OD_{600} reached half of the stationary phase OD_{600}. Three 50 mL aliquots per culture were poured into 50 mL screw-cap conical centrifuge tubes. The samples were centrifuged at 2,500 rpms for 12 min. The supernatant was discarded, and 5 mL cold sterile 1x PBS was added. The samples were transferred to glass centrifuge tubes, and OD_{600} readings of each tube were taken with a 1:10 dilution. Each tube was centrifuged at 2,500 rpms for 12 min. The supernatant was discarded, and the pellets were frozen at –80 ºC. The mutated samples did not centrifuge down as fast as the wild type. The mutated samples had to be centrifuged for twice as long (24 min).