METHODS
The CRISPR/Cas9 gene editing system is a very popular tool for studying gene function due to its relative ease of use and accuracy. Additionally, the system has enormous potential for treating hereditary diseases. Validation of CRISPR/Cas9 gene editing is necessary to ensure that genes of interest are successfully knocked down or knocked out. Here, we demonstrate how Molecular Devices’ family of instruments can be utilized in gene editing experiments by using CRISPR/Cas9 to knockdown autophagy-related protein 5 (ATG5) in HEK293 cells. Cells successfully transfected with the CRISPR/Cas9 plasmid expressed GFP, and a 13% transfection efficiency was calculated automatically using the SpectraMax™ i3x Multi-Mode Microplate Reader with MinMax™ 300 imaging Cytometer. The same platform was used to quantify DNA and protein concentrations for subsequent validation experiments. We observed about a 70% decrease in ATG5 protein expression in CRISPR-edited cells compared to non-edited cells using the ScanLater™ Western Blot Detection System. Finally, autophagosome formation was measured in CRISPR-edited vs. non-edited cells via high-content imaging using the ImageXpress® Micro Confocal High-Content Imaging System.

DNA and Protein Purification and Quantitation
Edited cells and non-edited cells were lysed and genomic DNA and total protein were collected. Afterwards, the SpectraMax i3x reader was used to quantify DNA concentration and protein concentration using ultraviolet (UV) absorbance quantitation and the BCA protein assay respectively.

CRISPR/Cas9 Gene Edit Validation
library (not shown). A protein ladder, 5 µg and 10 µg total protein from edited and unedited cells were loaded onto a 4–20% TSD gels, and 30% PAGE was performed. Proteins were then transferred to a PVDF membrane and separated into 3 parts: Scan-Later ladder, loading control, and the ATG5 region. The ladder was treated with 1:10000 europium-bound secondary antibodies. Blots were washed three times before being reassembled. The blots were scanned using the SpectraMax i3x reader with the ScanLater Western Blot detection system. Afterwards, the scanned western blot data was exported to SoftMax Pro software, and relative ATG5 protein expression was calculated from western blot band densities.

Autophagosome Quantitation
Edited and non-edited cells were plated into a 96-well cell culture-treated plate, and they were subsequently treated with a dilution series of chloroquine for 18 hours. Afterwards, autophagosomes were stained using Enzo’s CYTO-ID® Autophagy detection kit, and nuclei were stained with Hoechst. Images were acquired with a 10x objective in the FITC and DAPI channels on the ImageXpress Micro Confocal High-Content Imaging System. The number of autophagosomes per cell was quantified using MetaXpress® Software and a pre-configured module for detecting granularity. Data was exported to SoftMax Pro software for generating graphs.

CONCLUSION
• Molecular Devices’ family of instruments can effectively be used to perform experiments ensuring the success of CRISPR/Cas9 gene edits.
• The SpectraMax™ i3x Multi-Mode Microplate Reader can be used to assess transfection efficiency, monitor cell growth, quantitate DNA & protein, and validate CRISPR/Cas9 edits through ScanLater Western Blot analysis.
• High quality images of autophagosomes can be acquired using the ImageXpress Micro Confocal System.
• MetaXpress® Software was able to identify and quantify individual autophagosomes from every cell allowing us to analyze phenotypic changes occurring from the CRISPR/Cas9 gene edits.
• Unexpectedly, there was no difference in autophagosome formation between the edited and non-edited cells. This could be due to compensation from a parallel autophagic pathway.[1]