Researchers at the University of South Florida have developed a novel method of cloning using the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 technique combined with the homologous recombination technique. This invention can be used to directly clone a fragment into a large vector both seamlessly and in a shorter length of time.

Cloning is the essential tool for genetic engineering and in recent years has been subjected to intensive investigations with the development of various cloning techniques. Many of these techniques rely on cutting DNA by restriction enzymes. These restriction enzymes have six or eight basepair (bp) recognition sequences that have an occurrence of one in every 4096 or 65536 bp in a random sequence. This represents two major limitations for current cloning procedures: the restriction enzymes cannot cleave at any desired location in a DNA sequence and may not cleave uniquely within a DNA sequence especially in large vectors. In most cases, unique sites are necessary for cloning. When dealing with large vectors, such as BAC, cosmid, baculoviral and adenoviral vectors, direct cloning is impossible unless a unique cut site has been engineered into these vectors. Such requirements make traditional cloning techniques largely ineffective. Additionally, modifying existing constructs with current cloning techniques is a difficult and time-consuming task, further hindering studies that depend on cloning.

Our researchers have devised a procedure that can be used to directly clone a fragment into a large vector seamlessly, as well as modify an existing construct when there are no other methods available. This invention uses CRISPR/Cas9, which has a cleavage frequency of 1 in every 8 bp in a random dsDNA sequence. However, a specific CRISPR/Cas9 cleavage can happen only once in a random sequence larger than the human genome. The entire process can be completed in one week as compared to current protocol, which can take one to several months to get positive clones for recombinant adenovirus and baculoviruses.

**ADVANTAGES:**

- Efficiently shortens cloning process time length
- Seamlessly cloning with one basepair accuracy
- Can be used to modify an existing construct