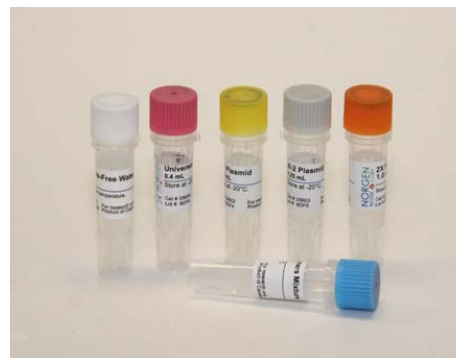
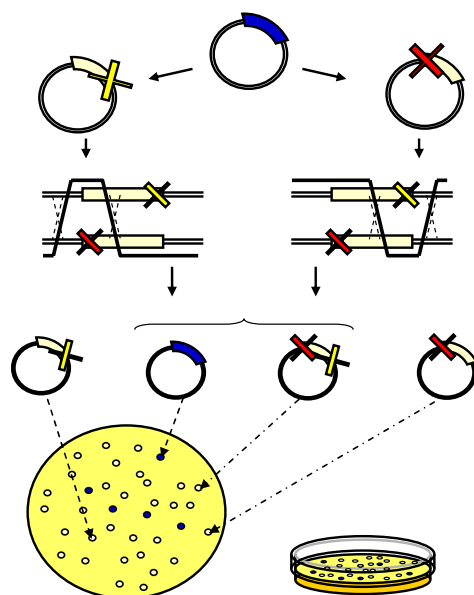


## Homologous Recombination Assay Kit

Norgen's Homologous Recombination Assay Kit provides a rapid and sensitive tool for measuring the efficiency of homologous recombination in both bacterial and mammalian cells. The kit consists of 5 components: (1) dl-1 plasmid, (2) dl-2 plasmid, (3) positive control plasmid, (4) assay primers mixture, and (5) universal primers mixture. The assay is based on the co-transformation of the two dl plasmids into bacterial cells followed by the isolation of plasmid DNA and quantification of the homologous recombination efficiency through qPCR. In addition, PCR can be used for qualitative analysis of the recombination efficiency. The primer mixture provided with the kit is designed to amplify only the homologous recombination product but not the dl plasmids (dl-1 or dl-2). The two plasmids can be co-transfected into mammalian cells, then the DNA can be isolated and the homologous recombination efficiency can be assayed by qPCR (or PCR).



In addition, the efficiency of homologous recombination can be assessed in bacteria by blue/white screening using X-gal. The provided positive control contains the functional LacZ sequence and can be used as a positive control for the plasmid DNA isolation efficiency (after transformation or transfection) or PCRs. The use of PCR-based homologous recombination assay enhances the sensitivity and eliminates the need for labor intensive screening by using traditional protein expression and staining techniques and is suitable for high throughput screening studies. The kit is designed to perform 200 transformations or 24 transfections.



**Figure 1. Schematic representation of homologous recombination assays.** The lacZa coding region of the positive control plasmid was mutated and two plasmids with different mutations were generated. Therefore, the two plasmids containing different defective lacZa cassettes can form a functional lacZa cassette through intermolecular homologous recombination. The two defective plasmids, once transformed into bacterial cells, will form white colonies. Only the plasmid that has undergone homologous recombination and generated the functional lacZa can form blue colonies. The homologous recombination efficiencies can be determined by calculating the percentages of the blue colonies among all the blue and white colonies.

### Homologous Recombination Assay Kit Benefits

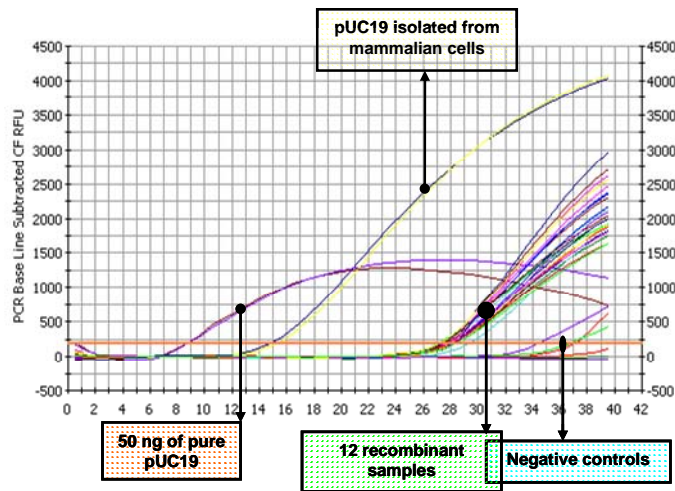
Rapid and Sensitive Tool

All primers included to allow for measuring the efficiency of homologous recombination through PCR or qPCR

For use with all cell types

Kit can be used to measure efficiency of homologous recombination in both prokaryotes and eukaryotes

## Homologous Recombination Assay Kit



**Figure 1: Real-time PCR amplification of plasmid DNA isolated from HEK 293 cells.** The genomic DNA was isolated from each well of a 6-well plate using Norgen's RNA/DNA/Protein Purification Kit. Fifty nanograms of the positive control plasmid was transfected into each well of the HEK 293 cells, and the total DNA was isolated 12 h later. For the recombinant samples, 25 ng of dl-1 plus 25 ng of dl-2 were transfected and then isolated. For the negative controls, 50 ng of dl-1 or 50 ng of dl-2 were transfected and then isolated. The experiment was carried out in duplicate to avoid the reading error of the PCR machine. The melt curve is shown in the top-left.

### Homologous Recombination Assay Kit Contents

1. dl-1 Plasmid
2. dl-2 Plasmid
3. Positive Control Plasmid
4. Assay Primers Mixture
5. Universal Primers Mixture
6. Product insert

### Shelf Life and Handling

All solutions should be kept tightly sealed and stored at -20°C or lower for up to 1 year without any reduction in kit performance.

Cat #	Description	Quantity
35600	Homologous Recombination Assay Kit	200 Transformations or 24 Transfections