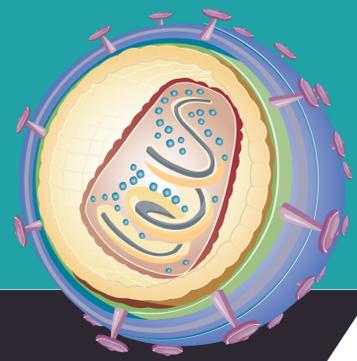


Lentiviral Integration Site Sequencing

– Eliminate the Uncertainty

The rapid development in gene and cell therapies has created breakthroughs in the treatment of numerous devastating diseases. Many of these therapies involve gene delivery. Under normal conditions, the target genes are usually difficult to integrate directly into most eukaryotic cells. To solve this problem, one common approach is to package the target gene into a viral vector to infect the cell, from which the gene can be expressed for research or treatment needs. Lentivirus-based gene transfer vectors are the choice of many researchers.

Lentivirus is a genus of retroviruses. A lentiviral vector can be constructed and amplified to replace the transient expression vector. After the lentiviral vector clone is packaged, it can be used to infect cells that are difficult to transfect with traditional transfection reagents, such as primary cells, suspension cells, and cells in a non-dividing state, and then integrated into the genome of infected cells for long-term stable expression.

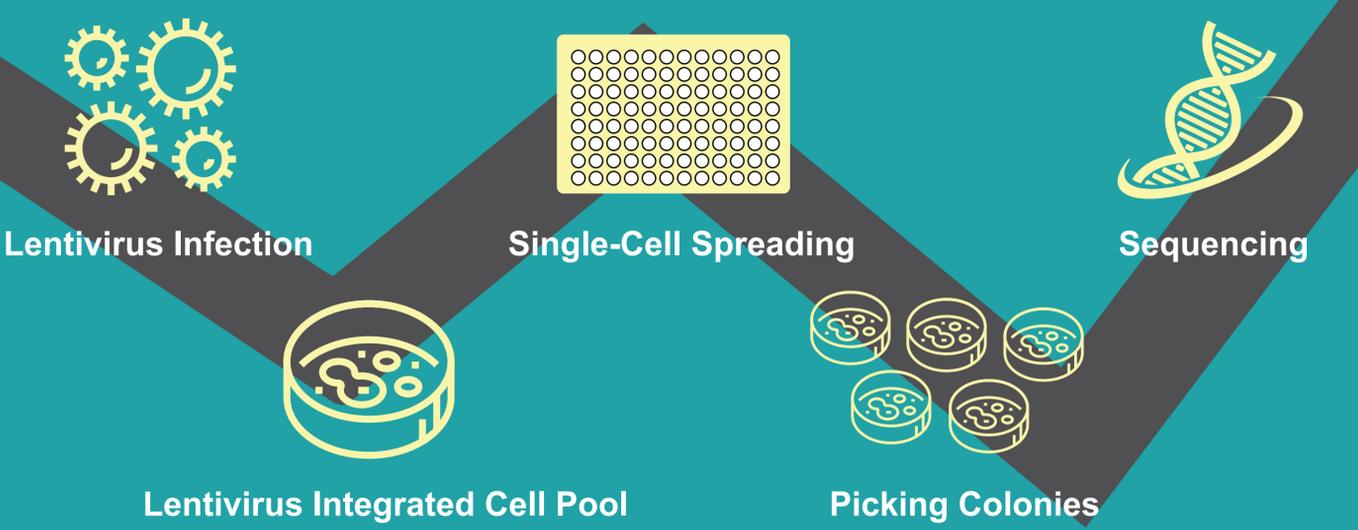


Why is integration site analysis so important?

Lentiviral vector-mediated gene therapy has great potential in the treatment of genetic diseases and infectious diseases. Lentiviral vectors integrate into the host genome with usually unpredictable integration sites, which may increase the uncertainty of their applications in gene therapy, and also lead to dysregulation of nearby genes, commonly referred to as genotoxicity. Lentiviral integration site analysis can be used not only for retroviral mutagenesis screening but also for the evaluation of genotoxicity in clinical trials of gene therapy and the development of improved vectors in preclinical research. Therefore, integration site analysis is a major safety and quality control checkpoint for lentiviral applications.

Using next-generation sequencing for integration site analysis

Ligation-mediated PCR and linear amplification-mediated PCR have been the most frequently used systems to identify retroviral provirus or vector integration sites. Both techniques involve ligating a splice DNA cassette to fragmented genomic DNA, allowing PCR amplification between the viral LTR and a known sequence in the splice. The DNA of the host integration site between the LTR and the flanking restriction site is then depicted by next-generation sequencing (NGS).

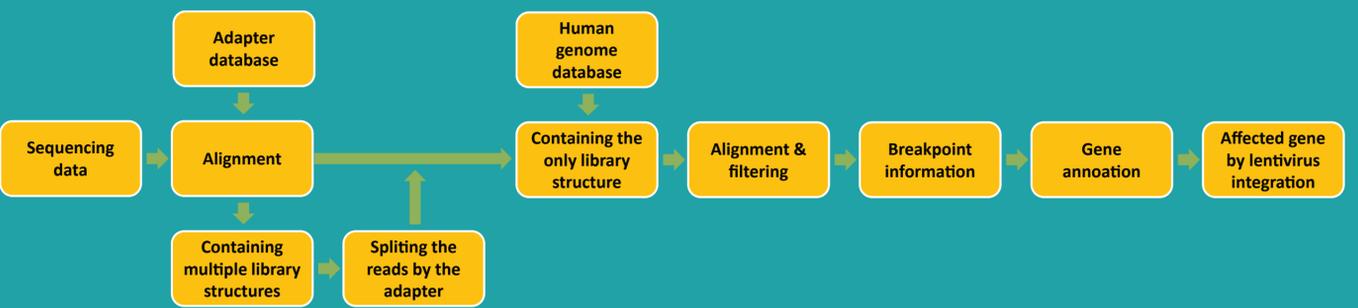


How to conduct integration site sequencing and data analysis?

Targeted sequence capture and NGS are performed on the Illumina platform to identify lentiviral integration sites in infected cells. This NGS-based approach has been proven to be specific and sensitive to lentiviral vectors. The following steps describe the overall workflow:

DNA Preparation > Illumina Library Preparation > Targeted Capture and Sequencing > Data Analysis

The sequencing data generated from NGS can be analyzed according to the flow chart below:



Analysis contents of lentiviral integration site sequencing:

ANALYSIS CONTENTS	DETAILS
Read mapping	Sequence quality filtering, trimming of DNA sequences, align sequence data to the genome of interest, identification of junction reads.
Integration site calling	Identification and annotation of lentiviral integration sites, visualization of integration site datasets.
Estimation of clonal abundance	Estimation of clonal abundance using the IntSiteDB database that contains genomic locations of integration sites, PCR breakpoints, and their counts.
Other analyses	Other bioinformatics analyses such as the comparison with oncogene annotation.

Conclusion

Targeted sequence capture of unique regions is a novel technique that can be applied to a variety of genomic applications. Sequence capture enrichment is an amplification-free method that hybridizes the region of interest with labeled, complementary DNA probes, and the captured DNA is then sequenced. Lentiviral vector integration sites can be captured using targeted sequence capture technology for the lentiviral vectors to have improved safety properties verified by NGS technology, enabling an accurate, time-saving, and cost-effective process.

CD Genomics is a preeminent service provider specialized in sequencing and bioinformatics analysis. We provide comprehensive services with accuracy and supreme data quality based on cutting-edge platforms. Our microbial sequencing platform provides one-stop services from primer design and library construction to sequencing and bioinformatics analysis. We can analyze and annotate the integration sites and the frequency of each site based on our cutting-edge integration site analysis platform.

