Creative Biogene

Linear-amplification mediated PCR (LAM-PCR)

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creative biogene BIOTECHNOLOGY

The application of viral vectors in gene therapy is rapidly developing and the therapeutic results are highly promising. However, actively and passively integrating (viral) vectors may fail to deliver, or trigger severe side effects by undirected or unintended integration into the genome of the target cell.

Thus, the analysis of vector integration sites in target cells is extremely important to address both biological and safety issues. The linear-amplification mediated (LAM) PCR can be used to analyze the viral vector insertion/integration site.

An Overview of LAM-PCR

- The LAM-PCR technique allows identifying unknown DNA sequences that flank a known DNA region. Because of the high sensitivity resulting from preamplification of the junctions with specific primers hybridizing in the known DNA sequence, it is possible to amplify and detect even rare junctions down to the single cell level. Contrary, in a polyclonal situation LAM-PCR is able to amplify thousands of different junctions in one single reaction.

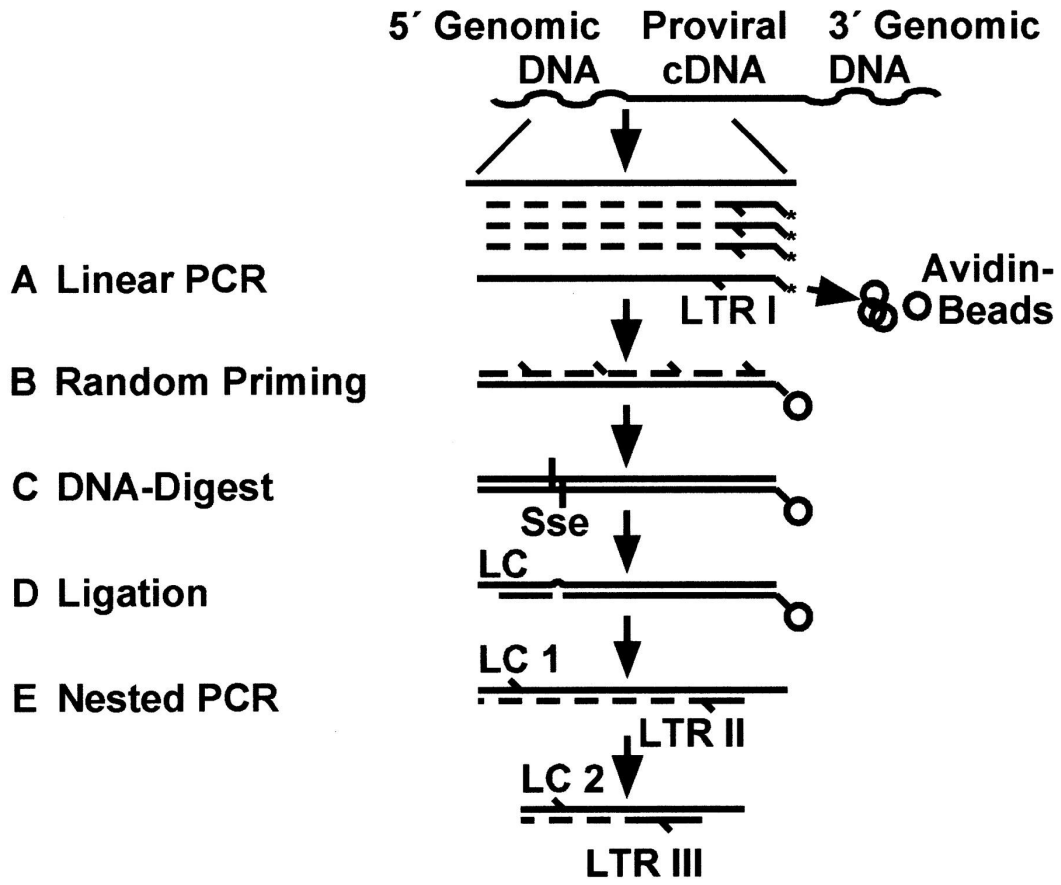
- However, due to the use of restriction enzymes, only a subfraction of the integrome can be analyzed by LAM-PCR for the presence of junctions with every particular restriction enzyme. Thus, repeated analysis of the same sample with different enzymes is recommended. If no LAM-PCR amplicons are present on the gel, most likely the distance between the location of the known DNA fragment and the closest recognition site of the chosen restriction enzyme is too large to result in LAM-PCR products. In this case other enzymes should be used to amplify the junction.

An Overview of nrLAM-PCR

- non-restrictive LAM-PCR (nrLAM-PCR) is independent of the use of restriction enzymes and therefore represents a highly valuable method to comprehensively characterize sequences flanking a known DNA sequence.

- Omitting restriction digest from the protocol results in the loss of specific restriction fragment length polymorphism characterizing each amplified junction. Instead every amplified junction is represented by PCR products of various sizes resulting in a smear on the gel after electrophoresis, independent of the diversity of amplified junctions.

The Outline of LAM-PCR



(A) Linear PCR with a long terminal repeat (LTR)–specific biotinylated primer is performed by repeated primer extension. Subsequently, the amplified fragments of target DNA are enriched by magnetic tag selection of extension primers.

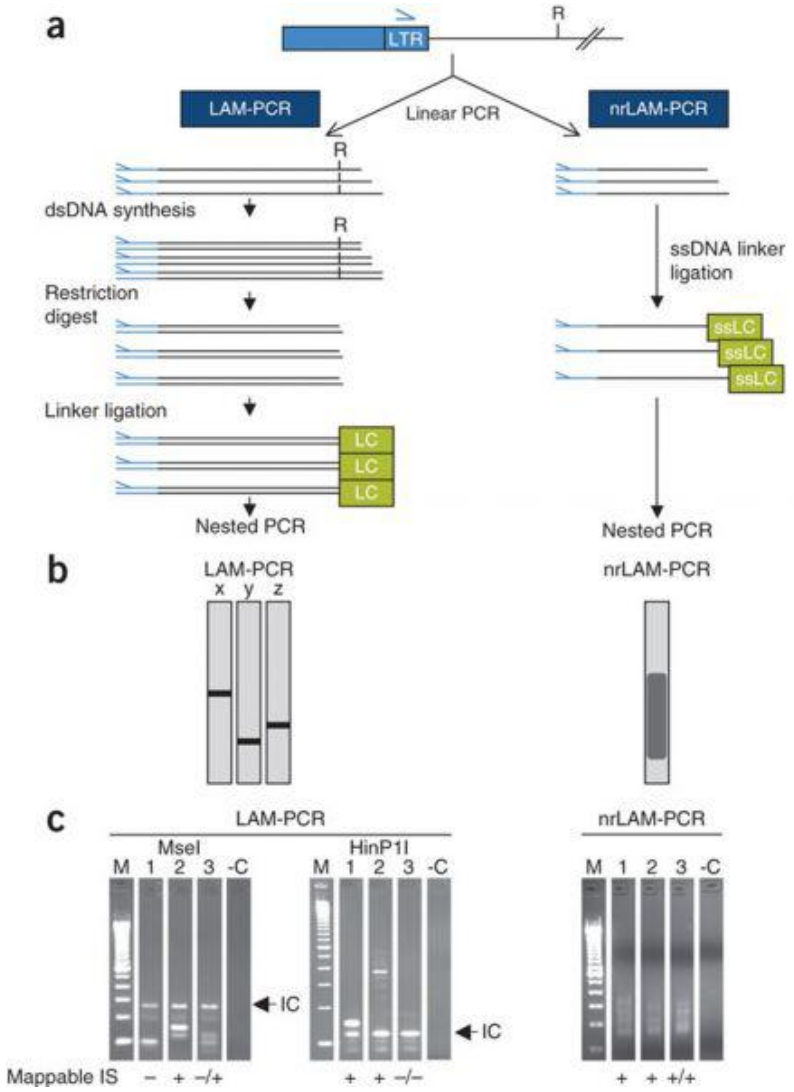
(B) A second DNA strand of each enriched target sequence is synthesized by random hexanucleotide priming.

(C) Resulting double-stranded DNA is specifically digested with the restriction enzyme Sse9I, which cuts within genomic DNA approximately every 256 bp.

(D) An asymmetric oligonucleotide ligation cassette (LC) is ligated to the end of the Sse9I-digested fragments.

(E) Nested exponential PCR amplifications are then performed with LC-specific forward primers (LC 1 followed by LC 2) and LTR-specific reverse primers (LTR II followed by LTR III).

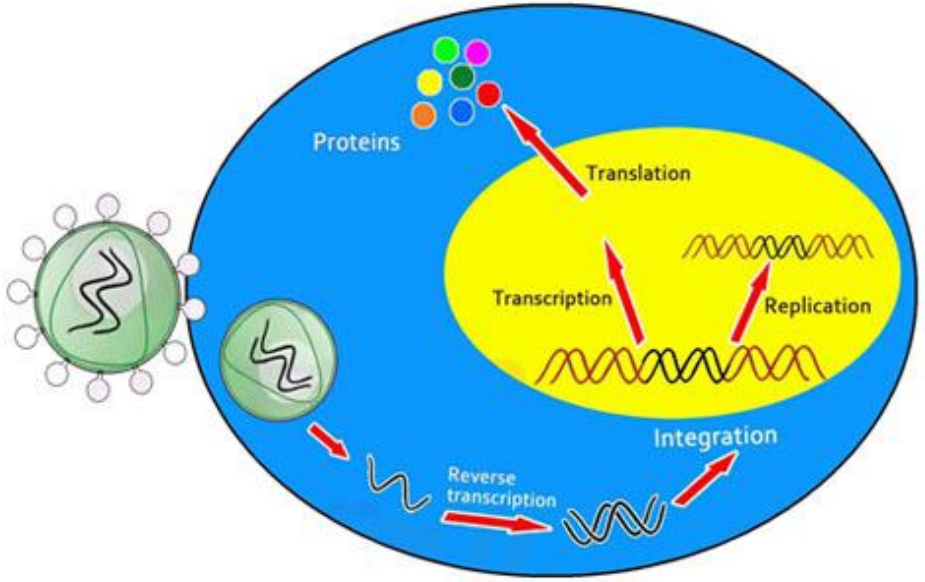
LAM-PCR and nrLAM-PCR



For LAM-PCR, flanking sequences are amplified by linear PCR using biotinylated primers hybridizing to vector sequences. Subsequent steps involve magnetic capture of the biotinylated PCR products, hexanucleotide priming by Klenow polymerase for double-strand DNA synthesis and restriction digest. After digestion, a double-stranded sequence adaptor (linker cassette) carrying a molecular barcode is ligated to the restricted DNA.

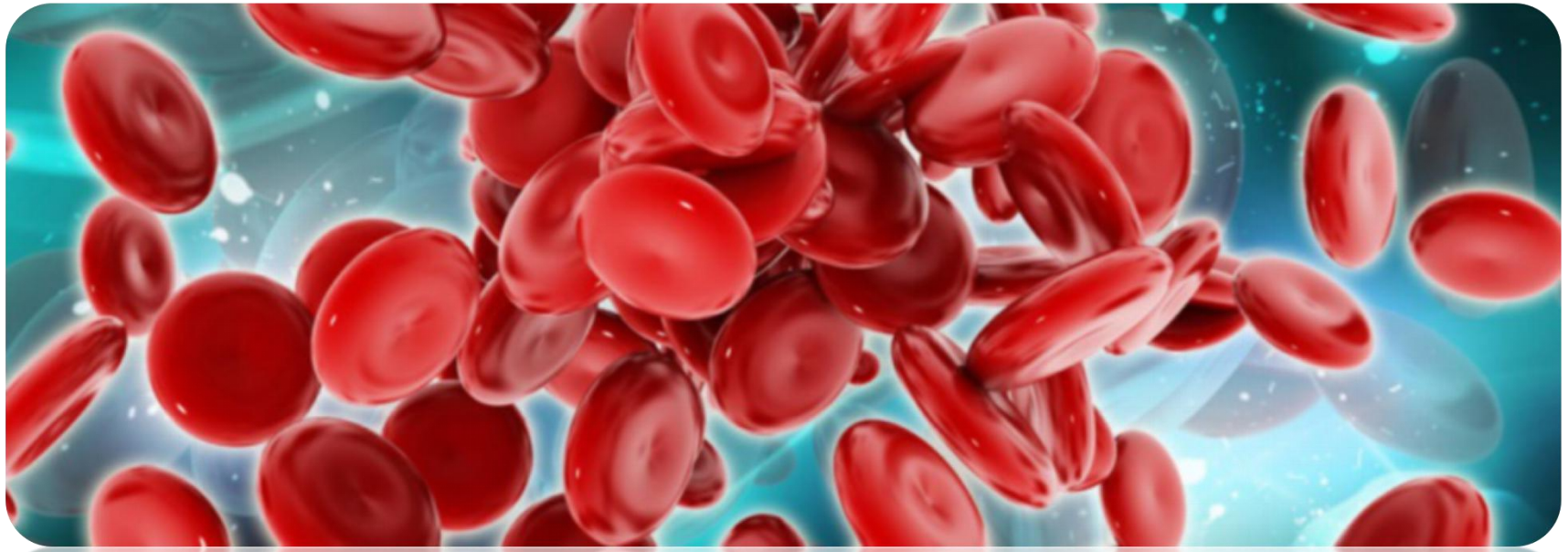
Also for nrLAM-PCR, two linear PCR amplification steps with a vector specific biotinylated primer are used. Subsequent steps involve magnetic capture of the biotinylated PCR products and ligation of a single-stranded linker cassette carrying a molecular barcode.

The Applications of LAM-PCR



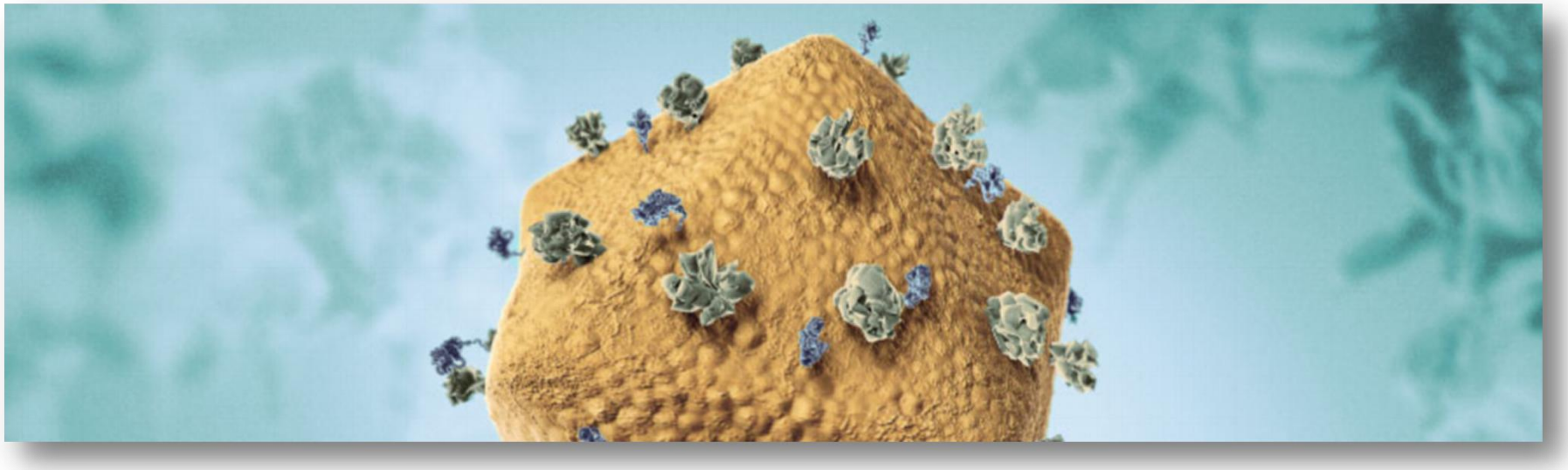
LAM-PCR has been adapted to identify IS from other integrating vectors (lentiviral vectors, transposons) and also to identify integration patterns of passively integrating vectors like adeno-associated vectors (AAV) or integrase-defective lentiviral vectors (IDLV). Applications of LAM-PCR are widespread: traditionally, the technique is widely used to study the clonal composition of gene-modified cells in patients that have undergone gene therapy or to assess the biosafety of novel vector systems by unraveling their integration behavior. Recently, LAM-PCR enabled determining specificity and off-target activity of designer nucleases by an IDLV trapping assay.

The Applications of LAM-PCR



Moreover, LAM-PCR allows to easily follow the fate of a transduced cell over time in an organism. This allows to identify proto-oncogenes as well as tumor suppressor genes and also to study hematopoiesis or cancer stem cell biology. Last but not least, LAM-PCR is adapted to study T-cell receptor diversity in humans . The intrinsic power of the technology is reinforced by linking the method to deep sequencing technologies that allow characterizing millions of unknown flanking DNA with single nucleotide resolution in whole genomes.

LAM-PCR Services At Creative Biogene



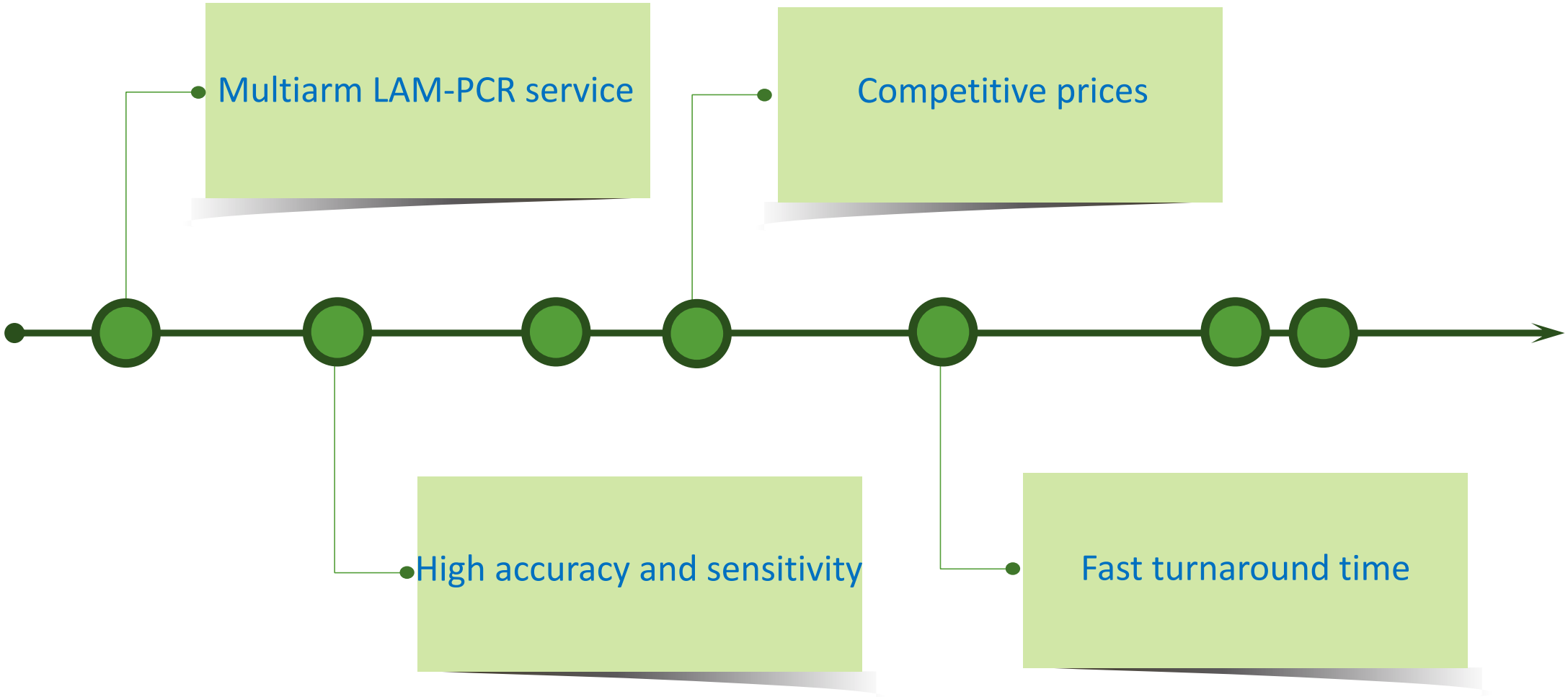
Creative Biogene, as a leading biotechnology company in the world, has extensive expertise and experience which are available to provide you with customer LAM PCR service to analyze the viral vector insertion/integration site. We have set up mature assays for several vectors, including lentiviral and most oncoviral vectors, sleeping beauty transposons, and some AAV-related vectors, *etc*..

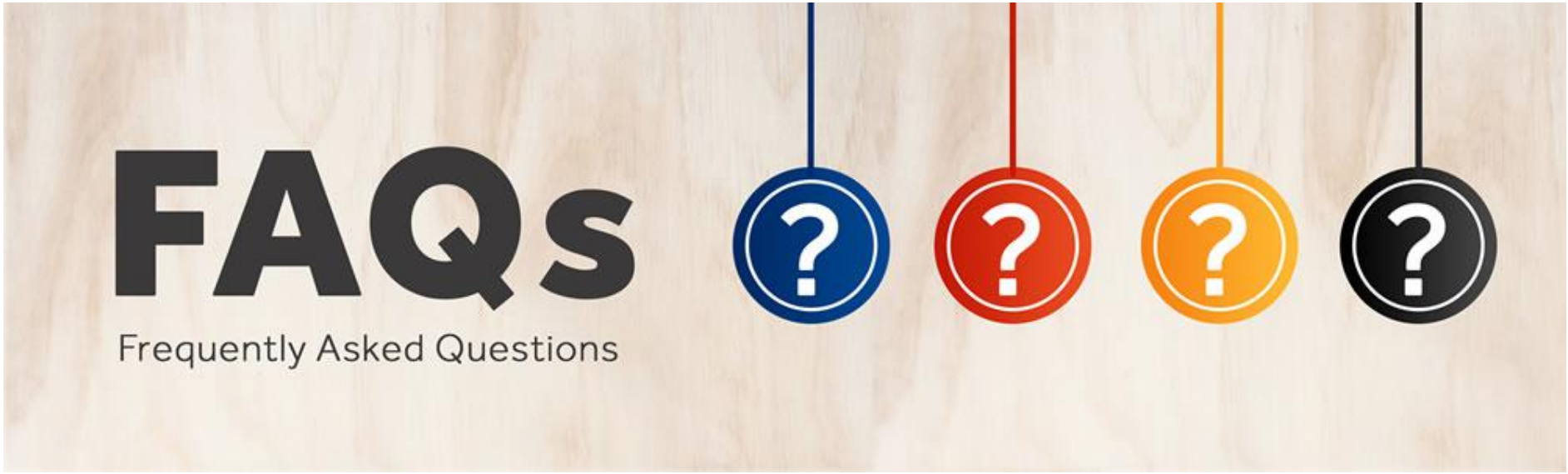
LAM-PCR Services At Creative Biogene

Creative Biogene offers custom LAM PCR services for your scientific research as follows:

- Linear PCR;
- Magnetic capture and double strand DNA (dsDNA) synthesis;
- Restriction digest and ligation of ds linker (LK);
- Denaturation of synthesized dsDNA and nested PCR;
- Purification the PCR products and sequencing.

LAM-PCR Services At Creative Biogene





1. What is the LAM PCR?
2. What are the advantages of multiarm LAM PCR compared to standard single-arm LAM-PCR?
3. How long does it take to finish the full service?
4. What forms of sample do you accept (frozen cells or purified DNA)?
5. Would it be possible to send you frozen cells and you isolated the DNA?

https://www.creative-biogene.com/Support/LAM-PCR-service.html

THANKS

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