



# Vector Atlas

**Necessary for Scientific Research**

This comprehensive vector atlas, meticulously compiled by Synbio Technologies, is to serve as a convenient reference tool for scientific researchers. This guide covers a wide range of vector types, ranging from classical plasmid vectors to cutting-edge viral vectors, as well as feature-rich cloning vectors and highly efficient expression vectors. We provide detailed information of each vector type, including the most important information such as its characteristics, advantages, limitations, and applications.



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# 1. VECTORS

Vectors are DNA molecules used as a vehicle to carry target genes (DNA fragments) into target cells, tissues, or organisms so that the genes can be replicated and expressed. A vector's ability to replicate in the host cell can prevent degradation and increase functional effectiveness. Among all types of vectors, plasmids are the most commonly used in genetic engineering.

# 2. PLASMID VECTORS

Plasmids are a class of nucleic acid molecules inherently present in biological cells, commonly found in prokaryotic bacteria and fungi, that can replicate autonomously and be stably inherited independently of the host's chromosome. The vast majority of plasmids are DNA-based, with a minority being RNA-based. Natural DNA plasmids mostly possess a covalent, closed, and circular molecular structure, characterized by their large molecular weights, low copy numbers, and scarce unique restriction enzyme cutting sites. Through modifications, such as the addition and optimization of components, natural plasmids can be transformed into the most commonly used vectors in genetic engineering - plasmid vectors.

## Functional Characteristics

- **Loading Capacity:** Possesses exogenous gene insertion sites that, when an exogenous gene is inserted, will not disrupt the functionality of the plasmid vector.
- **Transport Capability:** Enables the transfer of target genes into cells.
- **Replication or Integration Ability:** Provides replication or integration capabilities for the target gene.
- **Amplification or Expression Capability:** Offers the necessary conditions for the amplification or expression of the target gene.
- **Selection Marker:** Incorporates a selection marker.
- **Incompatibility:** Two different plasmids containing the same Ori cannot coexist within a single cell simultaneously.

## BASIC COMPONENTS



**1. Origin of Replication (Ori):** A specific sequence, rich in ATs and repetitive sequences, that initiates plasmid replication by recruiting replication-related proteins. The Ori determines the copy number of the plasmid in the host. High-copy plasmids (10-60 copies) are known as relaxed-replication plasmids, whereas low-copy plasmids (1-3 copies) are designated as stringent-replication plasmids and are often suited for cloning toxic genes and large gene fragments. A single Ori indicates a prokaryotic cloning or expression plasmid, while two Oris suggests a shuttle plasmid capable of replicating in both prokaryotic and eukaryotic systems. Note: Plasmids with identical Oris are incompatible and cannot be co-transfected.

**2. Resistance Selection Gene (R):** Also known as an antibiotic resistance gene, which facilitates subsequent selection of positive clones through antibiotic screening. Typically, cloning vectors have a single resistance selection marker, while some shuttle plasmids possess two. Note: Selection genes differ between prokaryotes and eukaryotes. Common in prokaryotes are Ampr, Camr, Kanr, Tetr, etc.; while Puro, G418, Hygr are prevalent in eukaryotes. Zeocin and Blastidicin can be used in both prokaryotes and eukaryotes.

**3. Multiple Cloning Site (MCS):** A region containing multiple restriction enzyme cleavage sites, and each site is unique within the entire plasmid vector. The MCS serves as the insertion site for foreign genes, typically located between the promoter and transcription termination signals. Different vectors have varying types and numbers of restriction enzymes in their MCS. Note: Inserting excessively long gene sequences can reduce plasmid vector transformation efficiency.

## BASIC COMPONENTS



**4. Promoter (P):** A DNA sequence that initiates downstream DNA transcription by specifically binding to RNAPol, without being transcribed itself. The promoter determines the cell type and expression level of the gene. Based on their expression patterns, promoters are classified into three types: constitutive/ubiquitous, tissue/cell-specific, and inducible promoters.

**5. Enhancer:** A cis-acting element that enhances the transcription of adjacent genes, with no directionality.

**6. Ribosome Binding Site (RBS):** Located upstream of the start codon (AUG) in mRNA, where ribosomes recognize and bind to initiate protein translation by searching downstream for ATG. In prokaryotes, the RBS on expression vectors is the SD sequence preceding AUG. In eukaryotes, reliance is primarily on the 5' cap structure of mRNA. Additionally, IRES elements and 2A polypeptide elements can act as RBSs to initiate protein expression.

**7. Transcription Terminator:** Located downstream of the gene to be transcribed, following the 3' regulatory elements. This region primarily contains polyadenylation (poly(A)) signals, which play a key role in terminating transcription. In prokaryotes, polyadenylation is the primary mechanism for transcription termination. In contrast, eukaryotes, including mammals, typically use specific terminators—such as SV40 polyA, hGH polyA, BGH polyA, and rbGlob—that contain the conserved sequence motif AAUAAA. This motif facilitates both the polyadenylation process and transcriptional termination.

**8. Primer Binding Site (PBS):** A short single-stranded DNA sequence that binds to PCR amplification or sequencing primers, primarily used for manual detection of plasmid sequences.

## 3. CLASSIFICATION OF PLASMID VECTORS

Plasmid vectors facilitate the introduction of genes of interest into host cells, enabling the expression of their functional capabilities. These vectors play a pivotal role in molecular biology research and serve as the foundation for advancing fields such as synthetic biology, biomedicine, gene therapy, target drug screening, and agricultural biotechnology. Given the diverse applications of plasmid vectors, their classification is equally varied, with the following primary categories based on their attributes:

**3.1 Viral Vectors vs. Non-Viral Vectors:** According to their nature, plasmid vectors can be classified into viral and non-viral vectors. Viral vectors utilize viruses as gene delivery agents, typically by inserting or replacing the viral genome with a foreign gene to transport the desired gene into target cells. Additionally, viral vectors exhibit high infectivity and specificity, enabling efficient delivery and expression of exogenous genes within host cells.

### Applications of Viral Vectors:

- **Gene Therapy:** Viral vectors are utilized to introduce therapeutic genes into target cells for the treatment of genetic disorders, cancers, and other diseases. This approach enables the delivery of corrective genetic material directly to affected cells.
- **Gene Editing:** Viral vectors serve as vehicles to transport gene-editing tools into target cells. This facilitates precise editing and modification of the genome, enabling scientists to correct genetic errors or introduce desired genetic changes.
- **Vaccine Development:** Viral vectors are employed as platforms for vaccine delivery, wherein they carry the gene encoding the target antigen into immune cells. This triggers an immune response, leading to the production of protective immune responses that can confer immunity against the targeted disease.
- **Genetic Research:** In the fields of gene function research, protein expression, and drug screening, viral vectors are used to transfer specific genes into cells or animal models. This approach enables the investigation of gene functions and mechanisms of action, facilitating a deeper understanding of biological processes and facilitating the discovery of new therapeutic targets and drugs.

## Lentiviral (LV) Vector

Definition	A gene delivery system based on lentiviruses (such as human immunodeficiency virus HIV-1). By removing viral replication and pathogenic genes, the lentiviral vector retains the characteristics of the virus to integrate genetic material into the genome of the host cell.
Characteristics	<ol style="list-style-type: none"><li>1. Integration Ability: Foreign genes can be integrated into the chromosome DNA of host cells.</li><li>2. The host range is wide and can infect both dividing cells and non-dividing cells.</li><li>3. Stable Expression.</li></ol>
Advantages	Large Load Capacity: suitable for carrying large gene fragments or multiple genes transduction.
Restrictions	Potential Carcinogenic Risk: Genetic integration may lead to insertional mutations that increase the risk of carcinogenesis.

## Adenovirus Vector

Definition	A gene transfer tool based on adenoviruses. Adenoviruses are a non-enveloped, double-stranded DNA virus that can efficiently express foreign genes in non-dividing cells. It is not integrated into the host cell genome and is suitable for short-term expression.
Characteristics	<ol style="list-style-type: none"><li>1. Non-Integration Ability.</li><li>2. Wide Range of Infection: It can infect a wide range of host cells including dividing cells and non-dividing cells.</li><li>3. Transient Expression.</li></ol>
Advantages	<ol style="list-style-type: none"><li>1. Efficient Infection: It can infect many cells in a short time.</li><li>2. High Safety: Adenovirus vectors are not integrated into the host cell chromosome, no insertional mutagenesis.</li><li>3. High Titer: Adenovirus vectors can produce high titer virus solutions, which is conducive to large-scale preparation and application.</li></ol>
Restrictions	<ol style="list-style-type: none"><li>1. Instantaneous Expression: The duration of exogenous gene expression is short, and multiple inoculations are required to achieve therapeutic effects.</li><li>2. Risk of Immune Response: Adenoviruses have strong immunogenicity, which may trigger the immune response and inflammatory response of host cells and affect the therapeutic effect.</li></ol>

# CLASSIFICATION OF PLASMID VECTORS

**3.2** Depending on the type of recipient cells they enter, vectors can be classified as **prokaryotic vectors**, **eukaryotic vectors**, and **shuttle vectors** (which can exist in both prokaryotic and eukaryotic cells).

**3.3** Based on the Ori element, vectors can be categorized as **high-copy-number plasmids** and **low-copy-number plasmids**.

**3.4** According to their nature, vectors can be divided into fusion vectors and non-fusion vectors. Fusion vectors are capable of integrating foreign genes into the genome of host cells, enabling stable expression of the foreign genes in the host cells. They are also known as **stable expression vectors**. In contrast, non-fusion vectors carry the target gene in a free-floating form within the cell, leading to transient expression of the target gene in the host cells. Hence, they are also referred to as **transient expression vectors**.

**The comparison between transient expression vectors and stable expression vectors is as follows:**

	<b>Transient Expression Vectors</b>	<b>Stable Expression Vectors</b>
<b>Working Principle</b>	It exists freely within the cell, promotes rapid expression of the target gene, reaches a peak within a short period of time, and is followed by a gradual decline.	By integrating the target gene into the genome of the host cell, it enables sustained and stable expression of the target gene.
<b>Methods of Introduction into Cells</b>	Virus Packaging, Chemical Reagents, Electroporation, and more.	Virus Packaging, Chemical Reagents, Electroporation, and more.
<b>Characteristics</b>	Screening Marker Genes as Non-Essential Components: Rapid Expression and Simple Operation	Screening Marker Genes as Essential Components: Continuous and Stable Expression
<b>Advantages</b>	The expression is fast, simple, flexible, and low in cost.	Long-term stable expression, controllable expression level, genetic stability, high safety and good repeatability.

# CLASSIFICATION OF PLASMID VECTORS

	Transient Expression Vectors	Stable Expression Vectors
<b>Restrictions</b>	<ol style="list-style-type: none"><li>1. May trigger cellular defense mechanisms, leading to gene silencing.</li><li>2. High-level expression may be toxic to cells.</li><li>3. Expression is neither permanent nor stable.</li></ol>	<ol style="list-style-type: none"><li>1. Low transformation efficiency.</li><li>2. Prolonged cell screening cycle with high costs.</li><li>3. Stringent transfection/transformation conditions.</li></ol>
<b>Applications</b>	Rapid analysis of gene function or protein activity.	It is widely used in genetic engineering, cell biology and medicine, such as recombinant protein production, gene function research, gene therapy drug development and so on.

**3.5** Based on their different functions, vectors can be divided into **cloning vectors**, **expression vectors**, **gene editing vectors**, **expression regulation vectors**, ***in vitro* transcription vectors**, and **function detection vectors**.



## 3.5.1 CLONING VECTORS

### Main Components:

Replication origin, multiple cloning site (MCS), and antibiotic resistance gene.

### Main Functions & Applications:

Used for DNA replication and amplification.

## 3.5.2 EXPRESSION VECTORS

**Main Components:** Besides the basic components of cloning vectors, it also requires DNA elements necessary for transcription and translation, such as promoters, ribosome binding sites, and transcription termination signals. The promoter drives the transcription of the target gene to produce RNA, which stops transcribing under the transcription termination signal.

### 1. Classification Based on Promoter Types:

#### Constitutive Expression Vectors

Characteristics	Can achieve continuous gene expression in multiple cell types or organisms.
Advantages	<ol style="list-style-type: none"><li>1. High expression levels.</li><li>2. Simple construction and usage.</li><li>3. Universal applicability regardless of cell type.</li></ol>
Restrictions	<ol style="list-style-type: none"><li>1. Lack of regulation.</li><li>2. Overexpression may be toxic to cells.</li><li>3. Continuous expression consumes excessive cellular resources, affecting normal physiological functions.</li></ol>
Applications	<ol style="list-style-type: none"><li>1. Studying gene functions through subcellular localization and overexpression.</li><li>2. Increasing gene expression to obtain large amounts of target proteins.</li></ol>

## Tissue-Specific Expression Vectors

Characteristics	Can selectively express the target gene in specific cell types, developmental stages, or environmental conditions.
Advantages	<ol style="list-style-type: none"><li>1. Specific expression in certain cell or tissue types</li><li>2. Specific regulatory elements, such as tissue-specific promoters, regulators, or response elements, for regulating foreign gene expression.</li></ol>
Restrictions	<ol style="list-style-type: none"><li>1. Complexity in designing and constructing specific regulatory elements.</li><li>2. Relatively complex and requires technical and resource support.</li><li>3. Fluctuations in expression levels.</li></ol>
Applications	Suitable for various research or application areas such as gene therapy, gene regulation, and biosensing.

## Inducible Expression Vectors

Characteristics	Contains specific inducible or responsive elements to regulate the expression of the target gene under specific external signals or inducers.
Advantages	<ol style="list-style-type: none"><li>1. Precise regulation of the time and intensity of gene expression.</li><li>2. Avoids nonspecific effects.</li><li>3. Cost-saving by inducing expression only when necessary.</li></ol>
Restrictions	<ol style="list-style-type: none"><li>1. Complex operation requiring precise control of inducer addition time and concentration.</li><li>2. Potential impact of inducers on cells.</li><li>3. Inducible expression usually limited by specific conditions.</li><li>4. Fluctuations in expression levels under different experimental conditions.</li></ol>
Applications	Suitable for studying gene functions and expressing proteins under specific conditions.

**2. Classification Based on Promoter Sources:** Mammalian expression vectors, yeast expression vectors, Escherichia coli expression vectors, etc.

**3. Classification Based on Target Gene Types:** Conventional gene expression vectors, recombinant protein expression vectors, non-coding RNA expression vectors, antibody protein expression vectors, etc.

Comparison of conventional gene expression vectors, recombinant protein expression vectors, non-coding RNA expression vectors, and antibody protein expression vectors is provided below:

## Conventional Gene Expression Vector

Target Gene	Genes of unknown function or specific sequences.
Characteristics	Screening marker or reporter gene elements: Subcellular localization of genes or overexpression or restoration of gene expression is achieved by screening markers or reporter genes.
Restrictions	Immunogenicity: Some expression vectors will cause the host cells to produce an immune response, resulting in the removal of the vector or cell death.
Applications	Functional study of genes.

## Recombinant Protein Expression Vector

Target Gene	Gene sequence encoding the target protein.
Characteristics	<ol style="list-style-type: none"><li>1. The gene sequence designed according to the target protein is not necessarily natural.</li><li>2. Specific tag elements containing target protein screening and purification.</li><li>3. It has a variety of protein expression systems such as E.coli, yeast, mammalian, insect cells and so on.</li><li>4. Most of the promoters are inducible expression promoters.</li><li>5. Good Scalability: From the experimental level to the industrial level.</li></ol>
Restrictions	<ol style="list-style-type: none"><li>1. High Cost: From vector construction to protein purification.</li><li>2. Difficulties in Amplification: Mainly trapped in cell growth conditions, product stability maintenance, etc.</li><li>3. Some proteins cannot be efficiently expressed due to their complex structure, difficult to fold correctly or easy to degrade by host cells.</li></ol>
Applications	<ol style="list-style-type: none"><li>1. Protein Production</li><li>2. Gene Therapy</li><li>3. Vaccine Development</li></ol>

## Non-Coding RNA Expression Vector

Target Gene	There are many types of miRNAs, siRNAs, lncRNAs, or circRNAs.
Characteristics	High Specificity: RNA sequence selection and design for specific genes or RNA molecules.
Restrictions	<ol style="list-style-type: none"><li>1. Initiate Immune Response</li><li>2. Non-Specific Regulation</li><li>3. Poor Stability: The stability of non-coding RNA in vivo is low.</li></ol>
Applications	<ol style="list-style-type: none"><li>1. Regulate Gene Expression.</li><li>2. Can be used as a useful tool to study the biological function of non-coding RNA.</li><li>3. Can treat some diseases caused by abnormal gene expression.</li></ol>

## Antibody Protein Expression Vector

Target Gene	A gene sequence that encodes a specific antibody, including DNA fragments that encode antibody heavy and light chains.
Characteristics	Flexibility: Can express a variety of types of antibodies, including monoclonal antibodies, polyclonal antibodies, chimeric antibodies, etc. By modifying and optimizing the vector, advanced applications such as multi-gene co-expression and fusion protein expression can also be achieved.
Restrictions	<ol style="list-style-type: none"><li>1. Instability of Expression Efficiency: The expression efficiency of the vector is unstable due to factors such as host cell type, culture conditions, and gene sequence.</li><li>2. Activity Not Easy to Control: Antibody aggregation and misfolding lose activity.</li><li>3. High Purification Cost: Expensive purification reagents and equipment increase the cost of antibody production.</li><li>4. Host Cell Safety: Some host cells, such as mammalian cells, may carry viruses, bacteria, or other pathogens when expressing antibodies. Strict biosafety measures are required to ensure the safety of the production process.</li></ol>
Applications	Antibody Drug Development

### Main Functions and Applications

**1. Gene Expression and Function Study:** The target gene is expressed in specific host cells, and the function of the gene in host cells can be studied. This technology is of great significance for understanding the mechanism of gene action in organisms and gene function discovery.

**2. Protein Production:** For the production of proteins with specific functions. By selecting the appropriate host cells and expression vectors, the target protein can be efficiently expressed and purified for biopharmaceuticals, diagnostic reagents, industrial enzymes, and other fields.

**3. Gene Therapy:** In the field of gene therapy, expression vectors can introduce normal genes into the patient's cells to replace or repair defective genes. Through this expression-vector-mediated gene transfer technology, long-term and stable expression of genes in patient cells can be achieved to provide new disease treatments.

**4. Vaccine Development:** The expression vector introduces the antigen gene into a specific host cell for expression and induces an immune response to prepare an antigen protein with immunogenicity. These antigenic proteins can be used to prepare vaccines to prevent and control the occurrence of infectious diseases.

## 3.5.3 EDITING VECTORS

Editing vectors are molecular tools for gene editing. They are usually based on plasmids or other DNA vector systems and carry specific gene editing elements, such as DNA nucleases, recognition sequences, and template DNA, for precise DNA sequence modification in the genome of the target organism. The design and construction of editing carriers require a high degree of professional knowledge and skills to ensure the accuracy and efficiency of editing. With the continuous development of gene editing technology, the application prospect of editing vectors in biomedical research, agricultural biotechnology, gene therapy, and other fields continues to broaden.

### Main Functions and Applications:

**1. Gene Knockout:** DNA is cleaved at a specific location by DNA nuclease, and then the gene is deleted or destroyed by the DNA repair mechanism of the cell (such as non-homologous end ligation).

**2. Gene Knock-In:** After DNA cleavage, the cells are guided to homologous recombination by providing template DNA, thereby inserting a new DNA sequence at a specific location.

**3. Gene Repair:** Similar to gene knock-in, but used to repair existing mutations or defects.

Depending on the target molecule, CRISPR-based editing vectors are primarily categorized into CRISPR-Cas9 knockout (KO) vectors and CRISPR-Cas13 knockout vectors. The comparison is as follows:

## CRISPR-Cas9 Knockout Vector

Definition	A gene knockout tool based on the CRISPR-Cas9 system. The Cas9 protein on the vector is derived from the type II CRISPR / Cas system of <i>Streptococcus pyogenes</i> , which can accurately identify and cut the target DNA sequence to achieve targeted gene knockout.
Characteristics	<ol style="list-style-type: none"><li>1. Flexibility: The vector construction technology is easy to operate, and the cleavage of different DNA sequences can be achieved by changing the Cas9 protein sequence.</li><li>2. Wide Applicability: Vector construction technology can be widely used in a variety of biological systems, including bacteria, yeast, plants, animals, and more.</li></ol>
Editing Object	DNA
Restrictions	<ol style="list-style-type: none"><li>1. Off-Target Effect: There is a certain off-target effect.</li><li>2. Genome Instability: DNA breakage and repair may lead to genomic instability and increase the risk of mutation.</li><li>3. Carcinogenic Risk: Studies have shown that CRISPR-Cas9 KO vectors may cause certain types of cancer.</li></ol>

## CRISPR-Cas13 Knockout Vector

Definition	CRISPR-Cas13 is a CRISPR-associated protein capable of cutting RNA with a high degree of RNA recognition and cutting ability, which can accurately identify and cut the target RNA sequence.
Characteristics	<ol style="list-style-type: none"><li>1. Flexibility: The Cas13 KO vector can be flexibly edited for different RNA sequences and only the corresponding crRNA needs to be replaced.</li><li>2. Safety: The Cas13 KO vector is edited at the RNA level and does not change the genomic DNA sequence, thus avoiding the risk of off-target effects and gene mutations that may be caused by DNA editing. In addition, the Cas13 KO carrier produces fewer by-products during the editing process and has lower cytotoxicity that further improves its safety.</li></ol>
Editing Object	RNA
Restrictions	<p>Off-Target Effect: Although CRISPR-Cas13 has high specificity in RNA recognition, there is still a certain risk of off-target effect.</p> <p>Long-Term Efficacy and Safety: The long-term efficacy and safety of the CRISPR-Cas13 KO vector in RNA editing still needs to be further studied and evaluated.</p>

## 3.5.4 EXPRESSION REGULATORY VECTORS

Expression regulatory vectors usually contain the necessary cis-regulatory elements such as promoters, enhancers, silencers, and one or more cloning sites for inserting the target gene. By designing these regulatory elements, researchers can accurately control the expression level of the target gene at a specific time, in a specific tissue, or under specific conditions.

### ■ Main Functions and Applications:

- 1. Basic Scientific Research:** By constructing expression regulation vectors, the expression of the target gene can be accurately controlled at a specific time or in a specific cell type to further study the function of the gene in organisms.
- 2. Metabolic Engineering:** By precisely controlling the expression level of key enzymes, the yield of the target product is increased or the proportion of its metabolites is changed.
- 3. Pharmaceutical Research and Development:** Gene therapy aims to correct or compensate for the function of defective genes by regulating the expression of therapeutic genes, thereby treating diseases at the genetic level.
- 4. Crop Improvement:** By increasing the expression level of drought resistance, disease resistance, or insect resistance genes, the stress resistance of crops is enhanced.

Based on the mode of gene expression regulation, CRISPR-based vectors are primarily classified into CRISPRi (interference) vectors and CRISPRa (activation) vectors. Details are as follows:



## CRISPRi Vector

Characteristics	<ol style="list-style-type: none"><li>1. The CRISPR-Cas system is used to suppress or silence the expression of specific genes by preventing the initiation of gene transcription.</li><li>2. CRISPRi-mediated knockdown is inducible and reversible.</li></ol>
Key Components	dCas9, sgRNA, KRAB
Working Principle	It mainly depends on the complex of dCas9 (Cas9 without cleavage activity) and sgRNA (single-stranded guide RNA). When this complex is directed to the transcription start site (TSS) of the target gene, dCas9 physically blocks the passage of RNA polymerase, leading to gene silencing. At the same time, dCas9 can fuse a gene inhibition domain, such as the KRAB domain, to further improve the efficiency of transcriptional inhibition. This domain can prevent the binding of transcription factors to DNA, thereby inhibiting gene expression.

## CRISPRa Vector

Characteristics	Gene expression is activated by the CRISPR-Cas9 system.
Key Components	dCas9, sgRNA, transcriptional activation domain (e.g. VP64 or p65AD)
Working Principle	The dCas9 is fused with the transcriptional activation domain (such as VP64 or p65AD) to form a Cas9-activation domain fusion protein with transcriptional activation function. When this fusion protein binds to sgRNA, it can be directed to the promoter or enhancer region of the target gene, and promotes the recruitment of RNA polymerase and the formation of transcription initiation complex through the role of transcriptional activation domain, thereby activating the target gene.

### 3.5.5 *IN VITRO* TRANSCRIPTION VECTORS

*In Vitro* Transcription (IVT) vectors, also known as cell-free transcription vectors, is an RNA synthesis process that uses purified DNA as a template in a test tube or other container under laboratory conditions. It is mainly used for the production and research of RNA molecules.

**☐ Main Functions and Applications:**

**1. mRNA Vaccines and Immunotherapy:** mRNAs encoding specific antigens can be prepared by *in vitro* transcription techniques. These mRNAs can be taken up by cells in the body and translated into proteins, thereby inducing an immune response in the body. This technology has been successfully applied to the development of vaccines for a variety of infectious diseases and tumors.

**2. Gene Function Research:** *In vitro* transcription is an important experimental tool in the field of basic research and teaching of molecular biology and biotechnology. *In vitro* transcription experiments can help students and researchers better understand the synthesis, processing, and functional mechanisms of RNA.

**3. Biosynthesis and Metabolic Engineering:** For the production of RNA molecules with specific functions, such as RNA enzymes, RNA aptamers, etc., these RNAs can be used to produce specific bioactive substances or regulate metabolic pathways.

**4. Diagnosis and Detection:** IVT vectors can be used to produce RNA molecules labeled with radioisotopes or other probes. By detecting the expression level or structural changes of specific RNA molecules, the pathogenesis and progression of the disease can be assessed.

According to the different *in vitro* transcription products, they are mainly divided into RNA *in vitro* transcription vectors and mRNA *in vitro* transcription vectors. Details are as follows :

Vector Type	Necessary Components	IVT Production
<b>RNA <i>In Vitro</i> Transcription Vectors</b>	T7 promoter	RNA
<b>mRNA <i>In Vitro</i> Transcription Vectors</b>	T7 Promoter, Kozark Sequence, 5'UTR, 3'UTR, polyA.	mRNA

## ☐ Advantages:

- 1. Efficient and Fast:** Many RNA/mRNA molecules can be synthesized in a short time to meet the experimental needs.
- 2. High Purity:** RNA/mRNA molecules synthesized by the *in vitro* transcription system are of high purity and do not require complex separation or purification steps.
- 3. Flexibility:** RNA/mRNA molecules of various lengths and sequences can be synthesized, which is suitable for different experimental requirements.
- 4. Strong Controllability:** The *in vitro* transcription process, reaction conditions, and time can be easily adjusted according to the experimental requirements.
- 5. Suitable for Large-Scale Production:** Due to the high efficiency and controllability of the *in vitro* transcription system, it is suitable for large-scale production of RNA molecules - such as the production of mRNA vaccines.

## ⊘ Restrictions:

- 1. Limited Stability:** RNA/mRNA molecules transcribed *in vitro* may be affected by environmental factors such as temperature and humidity during storage and transportation, resulting in decreased stability.
- 2. Non-Specific Products May Be Produced:** During the process of *in vitro* transcription, some non-specific RNA products may be produced, affecting the accuracy of the experimental results.
- 3. Not Applicable to All Types of RNA Synthesis:** Although *in vitro* transcription systems can synthesize many types of RNA molecules, effective synthesis may not be achieved for specific RNA structures or functions.

## 3.5.6 PROMOTER FUNCTION DETECTION VECTORS

Promoter function detection vectors connect the target promoter sequence to the reporter gene (fluorescent protein, enzyme, etc.) and introduces it into the target cell or tissue. When the promoter is activated, it drives the expression of the reporter gene, and the activity of the promoter is evaluated by observing the expression of the reporter gene.

### **Key Components:**

A promoter insertion site and a reporter gene for promoter-driven expression, as well as necessary regulatory sequences and marker genes.

### **Features:**

**Reporter Gene Diversity:** Usually contains one or more reporter genes, such as luciferase or green fluorescent protein (GFP), used to measure the activity of the promoter.

### **Application Advantages:**

- 1. Rapid Screening:** It can quickly identify and evaluate the activity of the promoter.
- 2. Quantitative Analysis:** The expression level of the reporter gene can be quantified to facilitate the comparison of the efficiency of different promoters.
- 3. Diversity Assessment:** Suitable for a variety of research applications, including promoter identification, functional analysis, and optimization.
- 4. Technical Maturity:** Experimental design ideas and details such as transcriptional regulatory element activity and binding site prediction using a dual luciferase reporter system are very mature.

### **Restrictions:**

- 1. Background Noise:** Some vectors may have non-specific expression, resulting in background noise.
- 2. Host Restriction:** The activity of the promoter may be affected by the type of host cells.
- 3. Cost:** It may be costly to purchase or construct a highly specific detection carrier.

## 3.5.7 PROTEIN INTERACTION DETECTION VECTORS

Protein interaction detection is usually achieved by constructing a fusion protein expression vector, in which the target protein sequence is fused with a reporter gene or a tag sequence, and the two fusion proteins are co-expressed in host cells. When these two fusion proteins interact, they can activate or inhibit the reporter genes that are linked to them, thereby triggering measurable signal changes, such as enhanced fluorescence intensity or color changes. By detecting these signal changes, researchers can assess and quantify protein-protein interactions.

### **Key Components:**

Target gene insertion sites, reporter system elements for reporting protein interactions.

### **Features:**

- 1. Dual Reporter Gene System:** Usually contains two reporter genes to monitor the interaction between proteins.
- 2. Fusion Tag:** The vector design contains a fusion protein tag, such as GST, His, or Flag, to facilitate protein purification and detection.
- 3. Signal Amplification:** Some carriers improve the sensitivity of detection by signal amplification mechanisms.

### **Advantages:**

- 1. Real-Time Monitoring:** The dynamic process of protein interaction can be monitored in real time.
- 2. Quantitative Analysis:** A method for quantitative analysis of protein interaction intensity is provided.
- 3. High-Throughput Screening:** Suitable for large-scale screening experiments and helps to quickly identify interacting proteins.
- 4. Widely Used:** Can be used for drug screening, disease mechanism research, and signal transduction pathway analysis.

### **Restrictions:**

- 1. False Positive / False Negative:** May be due to non-specific binding or improper experimental conditions lead to wrong results.
- 2. Limitations:** Protein interaction detection vectors usually can only detect the interaction between known proteins and may not be able to detect unknown protein interactions. In addition, some low-affinity or transient protein-protein interactions may not be detectable.

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