At the statistical level, multivariate regression, structural equation modeling (SEM), and the animal model can be used to decompose phenotypic variance into genetic and non-genetic components, jointly assessing indicators of genetic stability (such as integration site consistency, single-copy targeting, and expression variance) and phenotypic uniformity. Cross-species evidence also suggests a significant correlation between the stability of gene expression in founder generations and the conservation of phenotypes in progeny — lineages with more stable expression tend to exhibit lower intergenerational phenotypic diversity.

In animal populations, this relationship often manifests as a negative correlation between phenotypic variance and genotypic consistency. For example, in transgenic pig lines, if the copy number and expression level of an exogenous growth hormone gene vary greatly among individuals, the coefficients of variation (CV) for weight gain rate and fat ratio increase significantly. Conversely, when integration sites are fixed and expression is stable, the phenotypic distribution becomes more concentrated. Furthermore, environmental factors (E) can be incorporated into a three-dimensional relationship model:

$$P = G + E + G \times E$$

where G represents genetic stability factors and G × E represents gene-environment interaction effects.

By analyzing variance components across generations, the contribution of genetic instability to phenotypic inconsistency can be quantified, allowing the prediction of trait performance trends of exogenous genes in breeding populations.

6.2 Effects of different transgenic integration sites on phenotypic consistency

The chromatin environment and local regulatory network of the integration site determine expression robustness and inter-individual consistency. A single, well-defined integration site with single-copy expression usually corresponds to more stable recombinant protein yields and lower variability among individuals (Van Cott et al., 1997). Conversely, random integration often causes position effects. If located near coding regions or key regulatory elements, it may cause insertional mutations or expression drift, thereby increasing phenotypic uncertainty.

By inserting exogenous genes into genomic safe harbors and using insulator sequences, position effects can be mitigated, neighboring chromatin influence reduced, and phenotypic consistency improved.

6.3 Molecular links between gene expression regulation and phenotypic stability

At the transcriptional regulation level, selecting appropriate tissue-specific or host-compatible promoters and optimizing the arrangement of regulatory elements can reduce spatiotemporal fluctuations in gene expression, improving the predictability and stability of transgene expression.

At the epigenetic level, DNA methylation status and histone modification patterns determine how gene expression is maintained over time. During generational transmission, accumulation of methylation may cause gene silencing, while the inclusion of insulator or barrier sequences during construct design can effectively block the influence of unfavorable chromatin environments, thereby delaying or preventing silencing.

At the post-transcriptional and translational regulation level, interactions between miRNAs and mRNAs, as well as RNA-binding protein (RBP)-mediated regulation, affect mRNA stability and protein yield. By optimizing sequences and adjusting codon usage, the risk of transgene sequences being targeted by endogenous miRNAs can be reduced, minimizing expression fluctuations and ensuring stable product output.

Genomic structural variations (CNVs and SVs) also play a key role in phenotypic consistency. These variations alter gene dosage and regulatory network structures, directly influencing key economic traits such as growth rate, muscle development, and reproductive performance. Within regulatory networks, redundant elements such as shadow enhancers can maintain target gene expression when disturbances occur, enhancing overall phenotypic robustness.