For transgenic livestock, genetic stability not only determines the reproducibility and controllability of experimental outcomes but also directly affects the feasibility and safety of breeding and industrial application (Yum et al., 2018; Yum et al., 2024). Long-term population tracking has indicated that livestock produced via non-viral integration strategies such as transposon systems exhibit no significant accumulation of somatic mutations, abnormal copy number variations, or telomere anomalies after years of breeding and multigenerational propagation. This suggests that such methods maintain high levels of genomic integrity and physiological health (Yum et al., 2018; Yum et al., 2024).

3.2 Effects of insertion site and copy number on stability

The insertion site and copy number are critical determinants of genetic stability and predictable gene expression (Table 2). Random integration methods—such as pronuclear microinjection or certain viral vectors—are prone to position effects. When exogenous genes are inserted into heterochromatic regions, repetitive sequences, or areas near active transposable elements, they often experience epigenetic silencing, expression drift, or integration rearrangements. In contrast, integration into genomic safe harbors can substantially reduce insertional mutagenesis risks and enhance expression stability.

Regarding copy number, while high copy numbers may initially increase expression levels, they also elevate the likelihood of homologous recombination or tandem repeat-induced instability and silencing. Conversely, low copy numbers, particularly single-copy targeted insertions, favor long-term stable expression and predictable inheritance. Studies on transgenic goats and cattle have demonstrated that expression levels are not linearly correlated with copy number, indicating that local chromatin environment, promoter selection, and epigenetic modifications are equally crucial in maintaining genetic stability (Yum et al., 2018; Yum et al., 2024).

Table 2 Comparison of the effects of insertion site and copy number on genetic stability and expression

Factor	Favorable Conditions	Unfavorable Conditions	Main Risks	Countermeasures and Recommendations
Insertion Site	Euchromatin regions, genomic safe harbors, distant from key regulatory areas	Heterochromatin, repetitive sequences, regions near active transposons	Position effect, gene silencing, structural variation	Select genomic safe harbors; perform breakpoint sequencing; combine with homologous recombination repair
Copy Number	Single-copy or low-copy targeted integration	High-copy tandem insertions	Recombination, silencing, expression drift	Use single-copy knock-in (KI); perform ddPCR/qPCR quantification; optimize expression control
Regulatory Elements	Species-matched promoters, use of insulators/barriers	Heterologous strong promoters without protective elements	Epigenetic silencing, abnormal histone modifications	Introduce insulators; apply site-specific enhancer strategies

3.3 Molecular mechanisms of exogenous gene inheritance and potential variation risks

The inheritance of exogenous genes in livestock populations follows Mendelian laws, and their stability is influenced by multiple factors such as integration mechanisms, epigenetic regulation, and the host genomic environment. Transposon systems represented by Sleeping Beauty and PiggyBac integrate through a “cut-and-paste” mechanism, tending to insert into non-coding or low-risk regions, thereby reducing the potential hazards of insertional mutagenesis and ensuring the stable transmission of exogenous genes in the germline. Whole-genome sequencing and copy number variation analyses have shown that this type of integration has minimal impact on global genomic stability indicators such as SNP profiles, CNV, and telomere length (Yum et al., 2018).

The potential risks are mainly reflected in three aspects. In terms of structural variation, if the integration site is close to coding regions or key regulatory elements, it may lead to large fragment deletions, rearrangements, or functional disturbances. In terms of epigenetic silencing, changes in promoter methylation or histone modifications may cause intergenerational decreases in expression levels or even gene inactivation. Regarding