By integrating genomics, transcriptomics, and epigenomics data and combining them with eQTL analysis, researchers can systematically identify cis- and trans-regulatory loci contributing to expression stability and phenotypic consistency. This multi-omics approach provides molecular-level scientific evidence for screening genetically stable lineages and evaluating the long-term reliability of transgenic livestock.

7 Case Analysis: Genetic Stability and Phenotypic Studies in Transgenic Cattle and Pigs

7.1 Case background: representative experiments and commercial transgenic livestock projects

Cattle and pigs hold significant value in agriculture and biomedicine and are among the most representative species in transgenic livestock research. Transgenic cattle research focuses on optimizing milk composition and expressing exogenous pharmaceutical proteins, while transgenic pig research covers disease-resistant breeding, pharmaceutical protein bioreactors, and xenotransplantation donor construction (Van Cott et al., 1997; Yum et al., 2018; Yum et al., 2024). Over the past decade, transposon-mediated nonviral transfer and targeted gene editing/integration have provided feasible routes to achieve long-term genetic stability and predictable phenotypes (Yum et al., 2018; Yum et al., 2024).

7.2 Genetic stability testing and result analysis

7.2.1 Transgenic cattle (example: transposon-mediated integration and mammary gland expression)

Long-term follow-up has shown that over more than 10 years, physiological indicators and nutritional composition in these cattle show no significant difference compared with control groups. Whole-genome resequencing also revealed no increase in somatic mutation rate, copy number variation (CNV), or structural variation (SV), indicating good maintenance of genomic integrity. The exogenous gene was stably transmitted through the germline, and expression levels remained consistent across generations (Yum et al., 2018; Yum et al., 2024).

7.2.2 Transgenic pigs (two categories: pharmaceutical protein expression and disease resistance gene editing)

Lactating transgenic pigs expressing recombinant human protein C (rhPC) showed a strong correlation between a single integration site plus stable copy number and stable rhPC production in milk. The Western blot isoform profiles were highly consistent within the same lineage, suggesting that post-translational modifications contribute to inter-lineage differences (Van Cott et al., 1997).

For xenotransplantation applications, multi-gene–modified Yucatan miniature pigs achieved knockout of immunogenic loci and expression of human regulatory proteins, showing reduced immunogenicity when co-cultured with human immune cells.

Compared with random integration, site-specific editing (e.g., PRRSV-resistant pigs with targeted CD163 modification) showed no evidence of “reversion mutations” or “structural rearrangements” during generational tracking, and transcriptional balance in nearby regions remained intact, demonstrating that editing specificity and stability are superior to random integration (based on the key points of the second dataset).

Table 3 Evidence matrix of genetic stability

Species/Line	Transgenic Strategy	Integration/Edit Characteristics	Intergenerational Transmission	Whole-Genome Integrity	Key Conclusions
Cattle	Transposon-mediated (non-viral)	Single/few copies, noncoding region integration	expression across F1–F(_n) generations	Mutation rate/CNV/SV ≈ control	Long-term safety and stability (Yum 2018/2024)
Pig (rhPC)	Random integration (early stage)	Single defined site preferable	Stable across multiple litters and generations	Consistent isoform profiles within line	Single-site stability → predictable yield (Van Cott 1997)
Xenotransplantation pigs	Multigene modification	Immunogenic loci knockout + human gene insertion	Validated across generations	In vitro immune compatibility ↑	Significantly reduced immunogenicity