positional effect variation, changes in local chromatin plasticity may lead to expression differences among individuals or generations.

To address these issues, researchers have proposed a variety of optimization strategies, including using safe harbor targeting and breakpoint sequencing to ensure integration site safety, adopting single-copy targeted knock-in techniques to reduce structural interference risks, introducing insulator or barrier sequences to prevent epigenetic silencing, selecting promoters that match the host species, and conducting long-term population tracking to verify genetic stability. Long-term follow-up results show that livestock lines established following these design principles generally achieve stable inheritance and reproducible expression of exogenous genes, and no significant negative effects on animal health or genomic integrity have been observed (Yum et al., 2018; Yum et al., 2024).

4 Theoretical Framework and Indicator System for Phenotypic Evaluation

4.1 Significance of phenotypic evaluation in transgenic research

Phenotypic evaluation is the core link connecting genotype, expression, and functional traits, and it is a key method for verifying the biological effects and application value of exogenous genes. The goal of transgenesis is not only to achieve stable integration and expression of exogenous genes but also to continuously and reproducibly achieve the expected trait improvements (such as growth performance, disease resistance, and product quality). Therefore, it is necessary to conduct systematic, quantitative, and long-term phenotypic tracking of individuals and populations to identify expected effects and possible unintended effects (off-target/pleiotropy), and to provide evidence for safety and ethical assessments.

With the development of high-throughput and digital technologies, phenotypic evaluation is evolving from traditional single-trait observation toward multimodal integration (imaging phenomics, metabolomics/proteomics, wearable/environmental sensing, and behavioral monitoring), and combined modeling with genomic information, thereby improving the accuracy of breeding selection and enhancing the depiction of environmental adaptability and animal welfare.

4.2 Common phenotypic indicators: growth rate, physiological metabolism, reproductive performance, disease resistance

The phenotypic indicators of transgenic livestock vary according to target traits and application needs, typically covering five aspects: growth, metabolism, reproduction, health and disease resistance, and welfare and environmental adaptability (König and May, 2019).

For growth rate and body conformation, common indicators include body weight, average daily gain (ADG), feed conversion ratio (FCR), skeletal development, and body composition. For genetic modifications related to the GH/IGF axis and muscle development, the focus should be on verifying energy utilization efficiency and tissue development characteristics (Pinkert, 2014). The introduction of imaging measurement and automatic weighing technologies makes dynamic monitoring more accurate and objective, effectively reducing human error.

In terms of physiological metabolism and endocrinology, energy metabolism indicators such as blood glucose, insulin, cholesterol, and lipid profiles can be monitored, as well as endocrine levels including GH, IGF, and the thyroid axis. Combined with physiological parameters of organs such as liver enzymes and kidney function, and metabolomic and proteomic analyses, these help assess the remodeling of metabolic pathways and potential health risks caused by transgenic manipulation (Pinkert, 2014).

For reproductive performance and offspring traits, indicators such as age at first mating, estrous cycle, semen quality, conception rate, embryo survival and implantation rate, litter size, and lactation ability are examined to assess the impact of exogenous genes on the reproductive axis and their intergenerational inheritance effects.

In terms of disease resistance and immune response, for transgenic modifications aimed at improving disease resistance (such as antiviral, immune regulation, or pathogen recognition receptors), immunological tests