Phenotypic consistency is a crucial indicator for assessing the practical value of transgenic livestock. Even if the insertion and expression of an exogenous gene are relatively stable, inconsistent phenotypic manifestations across different environments, sexes, or genetic backgrounds can undermine both the scientific significance and application potential of the transgenic line (Van Cott et al., 1997; Evangelou et al., 2018). Phenotypic consistency encompasses multiple aspects, including physiological metabolism, reproductive capacity, immune response, and behavioral characteristics. Through systematic phenotypic assessment, researchers can determine whether the introduced gene functions as intended and whether it exerts unintended effects on animal health or growth. For instance, although certain transgenic cows maintain stable gene expression, their milk composition fluctuates abnormally, suggesting that environmental factors and gene network regulation play critical roles in phenotype formation (Yum et al., 2018; Yum et al., 2024). Establishing a standardized phenotypic evaluation system is therefore essential for promoting the transition of transgenic livestock from laboratory research to industrial application (Hryhorowicz et al., 2020; Park, 2023).

This study aims to systematically investigate the methods for evaluating genetic stability and phenotypic performance in transgenic livestock lines, elucidating their intrinsic relationship and implications for breeding practices. By summarizing mainstream detection technologies, analyzing stability variations under different transgenic strategies, and integrating genetic and phenotypic data from representative cases (such as transgenic cattle and pigs), this study seeks to construct a scientific and reproducible evaluation framework. Through this systematic analysis, the study aspires to advance transgenic livestock research toward greater scientific rigor and application sustainability, thereby providing robust support for the modernization of animal husbandry and innovation in life sciences.

2 Transgenic Livestock Technologies

2.1 Common transgenic methods

The construction of transgenic livestock relies on various efficient and stable technologies for introducing and expressing foreign genes. Among them, pronuclear microinjection was the first method to be widely used. This technique involves directly injecting target DNA into the pronucleus of a fertilized egg, allowing the exogenous gene to integrate randomly into the host genome and thereby producing transgenic individuals (Robl et al., 2007).

This approach is relatively simple to perform and applicable to a wide range of species, laying the foundation for the creation of early transgenic animal models such as pigs, cattle, and sheep (Niemann and Kues, 2003). However, due to its uncontrolled integration sites, high mosaicism rates, and significant variability in gene expression, its transgenic efficiency remains low—only about 1%-2%—which limits its suitability for modern commercial breeding applications (Robl et al., 2007).

To overcome these limitations, researchers developed viral vector–mediated transduction. This method employs vectors such as lentiviruses or retroviruses to deliver target genes into the host genome. Viral vectors exhibit a high integration rate and broad host range, allowing for tissue-specific expression of transgenes.

In the past decade, the advent of genome editing technologies has brought revolutionary progress in transgenic livestock production. Among these, the CRISPR/Cas9 system has emerged as the mainstream tool due to its simplicity, low cost, and ability to perform multi-site genome editing. This technique uses an RNA-guided nuclease to induce double-strand breaks at target sites, enabling precise insertion of exogenous genes or knockout of endogenous genes through homology-directed repair (HDR) (Table 1).

Additionally, somatic cell nuclear transfer (SCNT) is often used in combination with the CRISPR system to clone embryos that have been successfully edited (Robl et al., 2007). This integration of techniques significantly improves transgenic efficiency and provides a more reliable model for studying phenotypic stability and genetic consistency in transgenic livestock.