CRISPR Stable Knockout Cell Line Generation (Cat No. C208)

Case Study: IFITM2 Gene Knockout in A549 Cells

Target	Human IFITM2 gene in A549 lung carcinoma cells
Deliverables	Two validated knockout clones with complete documentation
Timeline	3 Months

The Challenge

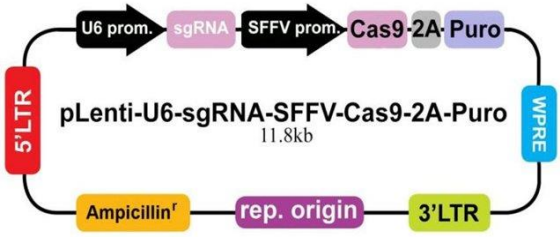
The goal was to generate a complete knockout of IFITM2 in A549 cells, a gene involved in antiviral defense. Key requirements included confirmed frameshift mutations, validated clones, and comprehensive quality control to ensure reproducibility and downstream functionality. Challenges included maintaining cell health during editing and ensuring clonal purity while avoiding residual wild-type alleles.

abm's Approach

1. Cell Line Validation: Parental A549 cells were thoroughly evaluated for viability, morphology, and growth characteristics prior to gene editing to ensure a robust starting population. abm assessed multiple delivery strategies (lentiviral transduction, plasmid transfection, electroporation) to determine the optimal approach for achieving efficient editing while maintaining cell health. Ultimately, lentiviral delivery was selected as the most effective method for stable CRISPR integration and minimal cytotoxicity in A549 cells.

2. sgRNA Design: Two high-specificity sgRNAs targeting IFITM2 were designed using abm's optimized CRISPR design pipeline to maximize on-target efficiency and minimize off-target effects. The sgRNAs were cloned into lentiviral CRISPR vectors, and high-titer lentiviral particles were produced under rigorous quality control to ensure consistent delivery and reproducible editing outcomes.

Figure 1. Lentiviral knockout vector map



3. CRISPR Editing: A549 cells were transduced with two lentiviral particles containing Target 1 and Target 2, respectively. Both populations were confirmed to be edited using the CRISPR Genomic Cleavage Detection Kit (G932) prior to selection.

4. Clonal Selection: Puromycin selection and monoclonal isolation were then performed to obtain individual clones. Selected clones were expanded and screened using Sanger sequencing with ICE analysis. A total of 35 clones were screened to identify the final validated clones.

5. Quality Control: Validated knockout clones were expanded, tested for quality attributes, cryopreserved, and prepared for shipment.

Validation Criteria

- Complete absence of wild-type sequence
- Frameshift mutations confirmed
- Mycoplasma negative
- Post-thaw viability >70%
- No contamination detected

Results

abm successfully generated two independent, fully validated IFITM2 knockout A549 clones with confirmed biallelic frameshift mutations and no detectable wild-type sequence.

Both clones demonstrated stable expansion and preserved morphology, providing reliable models for downstream virology and immunology studies.

ICE Analysis Results

Wild-type A549 IFITM2

Clone 1: Complete biallelic frameshift

Clone 2: Mixed frameshift alleles

Deliverables Included:

- 2 cryopreserved vials (>1 × 10^6 cells/ml)
- Full sequence data (Sanger + ICE analysis)
- Detailed culture and recovery protocols
- Comprehensive QC and sterility report

Summary

The outcome of this project provided the client with two fully validated, independent IFITM2 knockout clones that expand reliably, maintain normal morphology, and are ready for downstream virology or immunology applications. By leveraging abm's optimized sgRNA design, efficient lentiviral delivery, multi-step validation, and rigorous QC workflows, the client avoided months of internal optimization, mitigated technical risk, and received publication-ready data along with reproducible experimental models.

This case study illustrates abm's expertise in generating stable CRISPR knockout cell lines. Through precise engineering, comprehensive validation, and meticulous quality assurance, abm delivers reliable, reproducible, and ready-to-use models that accelerate research and reduce development timelines.

Key Benefits & ABM's CRISPR Knockout Service Excellence

Time Savings – Rapid, turnkey development of custom knockout lines—eliminating months of internal optimization.
Proven Expertise – Benefit from abm's extensive experience across thousands of successful CRISPR engineering projects.
Reliability – Multi-step validation ensures accurate and reproducible gene disruptions.
Quality Assurance – Each clone is accompanied by complete QC documentation, including microbiology reports, sequencing data, and viability metrics.
Risk Mitigation: Off-target predictions considered to ensure specificity.