CRISPR Workflow Guide

How to Use Your CRISPR Knockout Plasmid or Virus

A step-by-step guide to enrich for edited cells and detect successful knockout.

> ⚠ **The three most common reasons KO experiments fail:**
> (1) not enriching or selecting for transfected cells before preliminary screening,
> (2) screening too early or too late after transfection, and
> (3) not performing clonal selection before final screening and validation.

Recommended Workflow

1 Delivery: Transfect or transduce your cells

Deliver the plasmid using your preferred method: lipofection, electroporation, or viral transduction. Follow your standard protocol for the cell type. Optimize transfection efficiency beforehand if you haven't already — this directly impacts editing yield.

2 Critical Step: Enrich or select for transfected cells

This step is essential and is the most commonly skipped. Only a fraction of cells will take up the plasmid — if you screen the whole population, signal from unedited cells will mask your results.

Use the selection marker included in the vector:
GFP marker: Sort and collect top 10-20% of GFP+ cells by FACS 24–72 hours post-transfection.
Antibiotic resistance marker: Select at 24–48 hours post-transfection at validated concentration.
Puromycin: Typical selection at 1–5 µg/mL; kill curve validation is recommended for cell line.

3 Recovery: Allow editing and recovery time

After enrichment, cells need time to complete NHEJ or HDR and for protein turnover to occur. Residual wild-type protein must be degraded before a functional KO phenotype is detectable. Screening too early is a frequent source of false negatives.
Do not assay during this window — let cells recover, expand, and allow protein levels to fall.

4 Validation: Screen for knockout efficiency

Assess editing at the DNA and/or protein level using methods appropriate to your downstream application. For population-level screens, use the enriched pool. For clonal analysis, single-cell sort into 96-well plates after enrichment, then expand clones before screening. **Clonal selection is highly recommended** as polyclonal or pooled populations may show residual expression or incomplete editing.

- **Genomic confirmation:** Sanger sequencing or T7E1/SURVEYOR assay at the target locus
- **Protein confirmation:** Western blot or immunofluorescence for target protein loss
- **Functional readout:** Assay relevant to your biological question

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Common Pitfalls & How to Avoid Them

⚠ Screening without clonal selection
Assaying the bulk transfected population dilutes signal from edited cells. Unedited cells dominate and produce false-negative readouts.

Fix: Always sort GFP+ cells by FACS or apply antibiotic selection before proceeding to clonal selection and screening.

⚠ Screening too early
Residual wild-type protein persists for days after editing. Functional or protein-level assays performed <7 days post-transfection will not reflect true KO status.

Fix: Wait at least 7–10 days post-transfection after enrichment before protein-level validation.

⚠ Poor transfection efficiency
If transfection efficiency is <20–30%, even FACS-sorted pools may contain insufficient edited cells for robust analysis.

Fix: Optimize delivery conditions for your cell line before your KO experiment. Run a pilot GFP-only transfection.

Typical Timeline Reference

Timepoint	Action	Notes
Day 0	Transfect / transduce	Plate cells at appropriate density 24 h before transfection if using lipofection.
Day 1–2	Assess transfection efficiency	Check GFP signal under fluorescence microscope or by flow cytometry before proceeding.
Day 2–3	Enrich for transfected cells	FACS sort GFP+ population or begin antibiotic selection. Allow 24 h before sorting to ensure peak GFP expression.
Day 3–7	Recovery & expansion	Expand enriched cells. Avoid phenotypic or protein-level assays during this window.
Day 7–10	Genomic & protein validation	Sanger sequencing, T7E1 assay, Western blot, or IF for target protein.
Day 10–14+	Functional assays	Perform clonal selection, confirm editing of isolated clones, then perform downstream biology experiments. For clonal lines, allow additional time for expansion after single-cell sorting.

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Pro Tips

- **Cryopreserve enriched pools**
  Freeze down a portion of your enriched, sorted population at Day 3–4 before proceeding. This preserves your work if downstream experiments fail.
- **Use positive & negative controls**
  Include a non-targeting guide RNA control alongside your KO construct to distinguish guide-specific effects from delivery or selection artifacts.
- **Perform clonal selection**
  Perform clonal selection before validating KO at the genomic level, indel confirmation, or the protein level, Western blot or IF.
- **Cell-line-specific protocols**
  Transfection conditions, selection doses, and protein half-lives vary significantly. Pilot experiments in each new cell line are always worthwhile.

Related Technical Resources

LEARNING RESOURCES	CRISPR Cas9 Knowledge Base
PROTOCOL	CRISPR Knockout Manual
CASE STUDY	CRISPR Cas9 Case Studies
WEBINAR	Essential Guide to Becoming a CRISPR Cas9 Expert
VIDEO	CRISPR Video Tutorial Series on our Youtube Channel

Still have questions?
Our technical support team (technical@abmgood.com) is happy to help with experiment design, troubleshooting, or cell-line-specific recommendations.