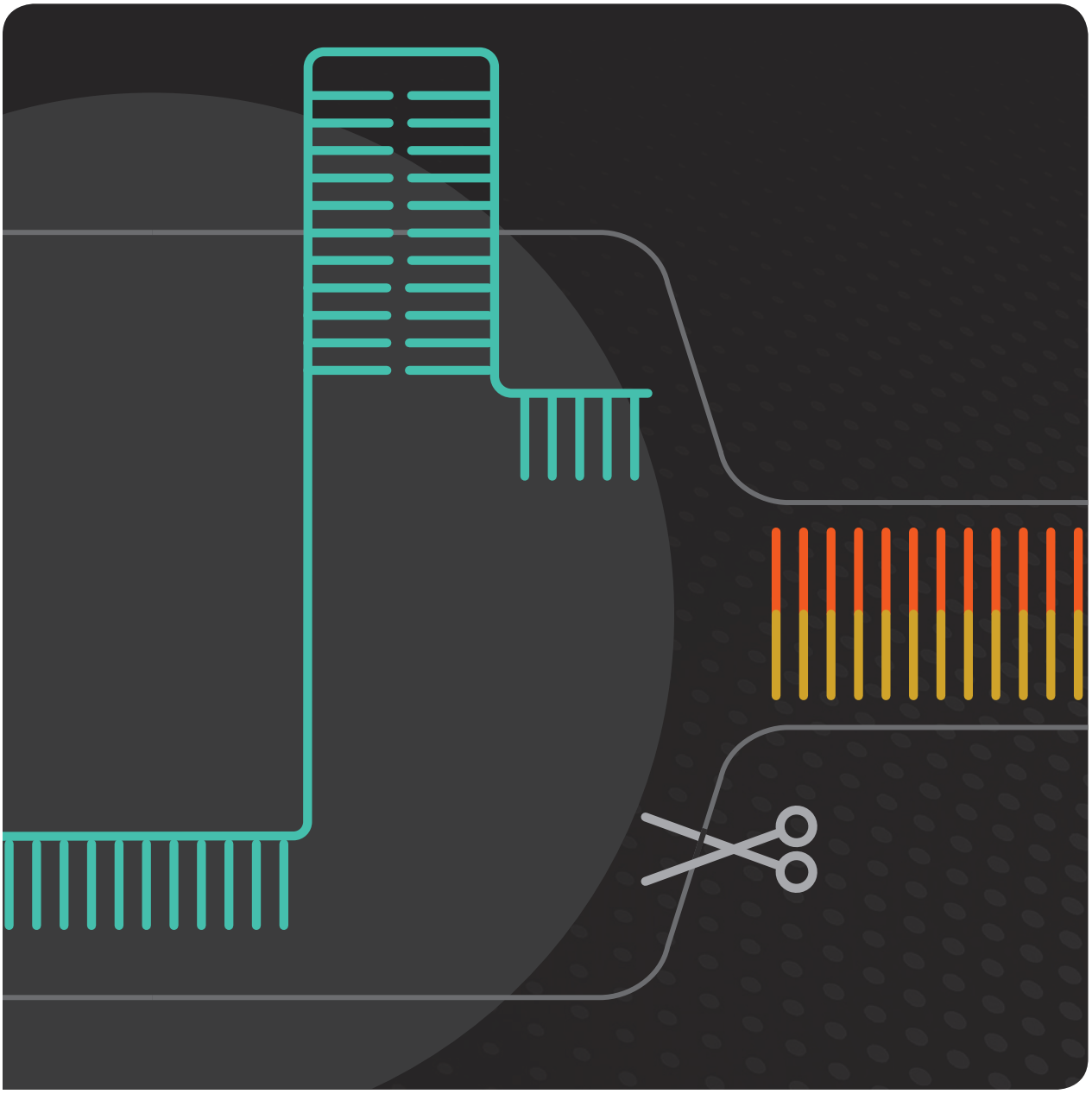
Pharma Starts with You



CRISPR Gene Editing Workflow

A toolbox for successful gene editing
in drug discovery and development

BIO-RAD

A VERSATILE AND EFFICIENT WORKFLOW

The power to edit a gene is the power to change its function, and with it the biology of a cell. From generating novel cell lines and better animal models for the discovery and preclinical phases of therapeutic research to actually creating a therapeutic itself, such as CAR T-cell therapies, CRISPR gene editing is allowing science to advance rapidly. The downstream applications are countless and are changing the way we perform R&D and the way we think about treating and curing disease.

We present a workflow that guides you through the four main steps of gene editing using the cancer cell line PC3. We designed a gene knockout strategy that uses knock-in of *GFP* and the puromycin resistance gene to disrupt *ICAM-1*, a gene known to play a role in cell adhesion and cell signaling. GFP and puromycin resistance serve as easy to follow markers for successful gene disruption. Although we chose a knock-in strategy, this workflow can be applied to most other gene editing experiments. Follow us through the generation and downstream analysis of *ICAM-1* knockout cell lines and see how Bio-Rad products support and accelerate gene editing at each stage.

BIO-RAD'S CRISPR GENE EDITING WORKFLOW: GENERATING AND ANALYZING *ICAM-1* KNOCKOUT CELL LINES

1 TRANSFECTION

GENE PULSER Xcell™ ELECTROPORATOR

TransFectin™ LIPID REAGENT

2 ENRICHMENT AND SINGLE-CELL ISOLATION

S3e™ CELL SORTER

ZOE™ CELL IMAGER

3 CONFIRMATION OF EDITS

V3 WESTERN WORKFLOW™

CFX REAL-TIME PCR SYSTEM

QX200™ DROPLET DIGTAL™ PCR SYSTEM

4 DOWNSTREAM ANALYSIS

CFX AUTOMATION SYSTEM II

BIO-PLEX® 200 SYSTEM

TRANSFECTION

TRANSFECT WITH TRUSTED TOOLS

GENE PULSER Xcell™
ELECTROPORATOR

ZOE™ CELL IMAGER

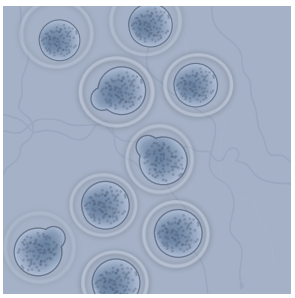
TransFectin™
LIPID REAGENT

TC20™ CELL COUNTER

After you have designed your gene editing strategy, the first step in the CRISPR gene editing workflow is to identify the best method for delivering the CRISPR-Cas9 system into your cells of interest, be they animal or human, tumor-derived or wild type. Transfer efficiency and subsequent cell viability are very important when considering which method to use. Bio-Rad provides several transfection options.

The industry-leading Gene Pulser Xcell Electroporation System has been used in over 700 peer-reviewed studies and has supported a wide range of research for more than 20 years. If lipid-mediated transfer proves most efficient, TransFectin Lipid Reagent provides a gentle, effective option even for primary cell lines. The ZOE Fluorescent Cell Imager can be used to monitor transfection efficiencies and cell health right on your bench, and the TC20 Automated Cell Counter automates cell counting at multiple stages of transfection, improving accuracy in cell counting across all stages of the experiment.

We designed a knockout strategy that uses knock-in of a donor template containing the GFP and puromycin resistance genes to disrupt *ICAM-1.* PC3 cells were transfected using either TransFectin Reagent or the Gene Pulser Xcell System. GFP fluorescence was used as an initial readout for successful transfection.



SUCCESS STORY CRISPR RNP Electroporation of Zygotes (CRISPR-EZ) uses the Gene Pulser Xcell System and enables highly efficient high-throughput in vivo genome editing (Chen S et al. 2016). CRISPR-EZ is a simple, economical, high-throughput, and highly efficient method with the potential to replace microinjection for in vivo genome editing.

Chen S et al. (2016). Highly efficient mouse genome editing by CRISPR ribonucleoprotein electroporation of zygotes. J Biol Chem 291, 14,457–14,467.

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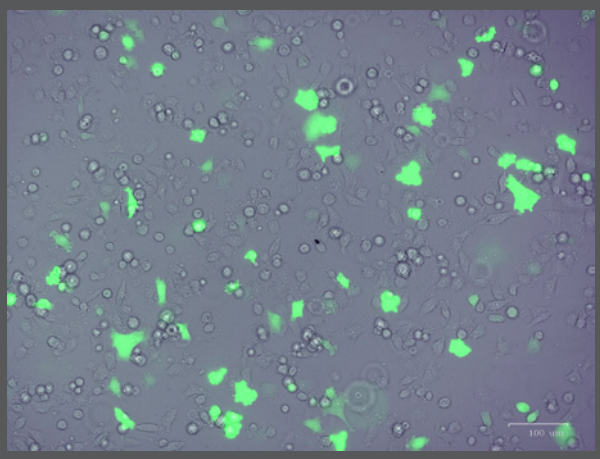

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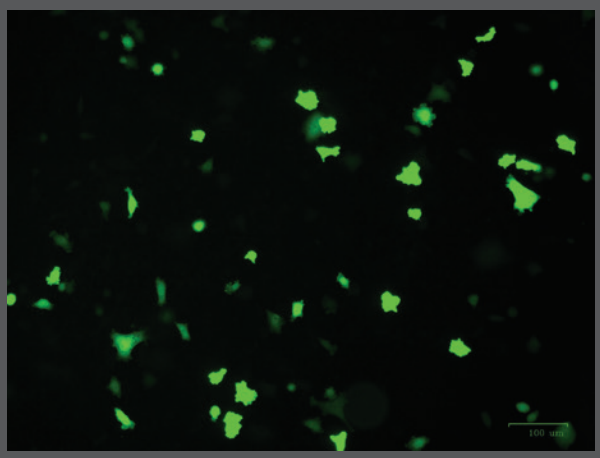


Optimizing transfection efficiency. PC3 cells (ATCC) were cultured in Ham's F-12 Nutrient Mix (GIBCO) with 10% HyClone FBS (GE Healthcare), 1% Penicillin-Streptomycin (GIBCO) and maintained at 37°C and 5% CO_2 for 7 days. Prior to transfection, cells were counted using the TC20 Cell Counter. One of two different CRISPR-Cas9 plasmids targeting *ICAM-1* (OriGene) and a donor vector containing left and right homologous arms as well as a GFP-Puro functional cassette (OriGene) were transfected into PC3 cells using either the Gene Pulser Xcell Electroporation System or lipid-mediated transfection with TransFectin Lipid Reagent. Media of both electroporated and TransFectin treated cells was replaced with fresh growth media 24 hours after treatment. The ZOE Fluorescent Cell Imager was used to assess GFP expression in transfected cultures and showed that the plasmids had been successfully transferred into cells. Merged (**A**) and GFP channel (**B**) view of PC3 cells 24 hours after transfection using the Gene Pulser Xcell System captured using the ZOE Fluorescent Cell Imager. TransFectin and the Gene Pulser Xcell System provided similar levels of transfection efficiency (data not shown).

ENRICHMENT AND SINGLE-CELL ISOLATION

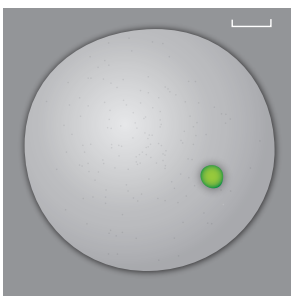
SORT CELLS AND SAVE 30 DAYS

S3e™ CELL SORTER

ZOE™ CELL IMAGER

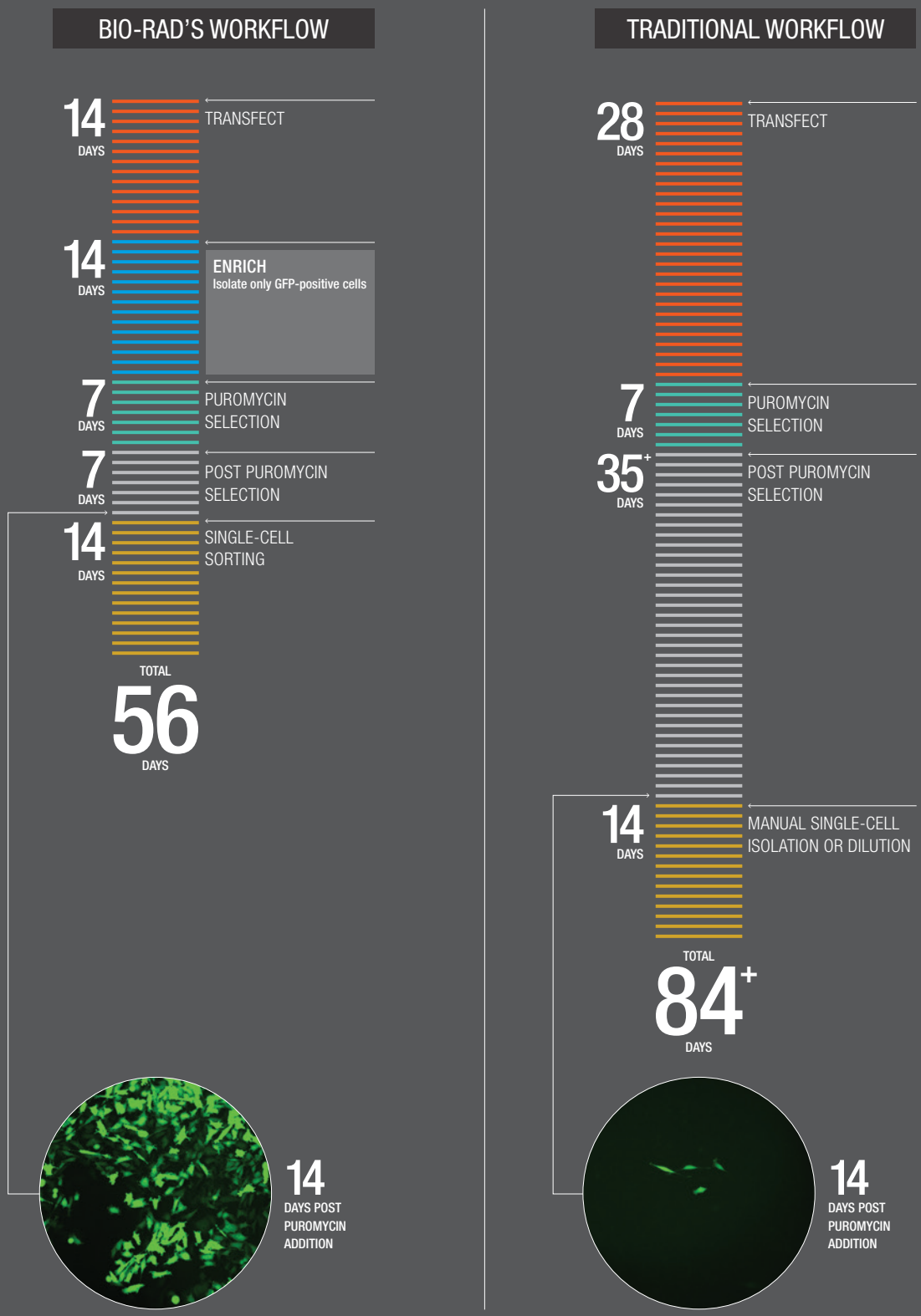
Once you have transfected your cells you need to enrich for your cells of interest. This step speeds up your workflow and reduces unnecessary spending on reagents and consumables by allowing identification and expansion of only those cells that carry desired edits. Enrichment also reduces the number of passages cells undergo, ensuring healthy cells for downstream analysis. With the S3e Cell Sorter and ZOE Fluorescent Cell Imager, enrichment is easily performed at your bench without the need for a core facility and your workflow can be shortened by as much as 30 days.

We enriched for successfully transfected cells by sorting GFP-positive PC3 cells using the S3e Cell Sorter. After enrichment and subsequent puromycin selection, the S3e Cell Sorter was used to isolate single GFP-positive, puromycin resistant cells for subsequent expansion. The ZOE Cell Imager allowed early confirmation of successful enrichment pre- and post-puromycin treatment.



SUCCESS STORY Whether you are analyzing difficult-to-interpret heterogeneous cell populations or are trying to isolate edited cells for expansion and creation of homogeneous cell lines, reliable single-cell sorting is a powerful and important first step. Bio-Rad bulletin 6853 demonstrates a simple four-step workflow that uses the TC20™ Cell Counter and ZOE Cell Imager to verify the reliability of single-cell sorting on the S3e Cell Sorter.

Visit **bio-rad.com/CRISPR1** to read more.



Accelerated target cell enrichment. Cells transfected either by electroporation or by lipid-mediated TransFectin Reagent were cultured for 14 days. Live GFP-positive cells were sorted from a mixed cell culture using the S3e Cell Sorter. Fourteen days later enriched cells were subjected to puromycin selection to further select for edited cells. Post-treatment, single GFP-positive cells were sorted using the S3e Cell Sorter. Single-cell isolation and GFP fluorescence were confirmed using the ZOE Cell Imager. Cells were then passaged for 2 weeks until 80% confluent for further analysis. This accelerated workflow allowed generation of confluent cultures of clonal *ICAM-1* knockout cell lines in 56 days instead of the more than 84 days.

CONFIRMATION OF EDITS

CONFIRM EDITS WITH CONFIDENCE

V3 WESTERN WORKFLOW™

ZOE™ CELL IMAGER

QX200™ DROPLET DIGITAL™ PCR SYSTEM

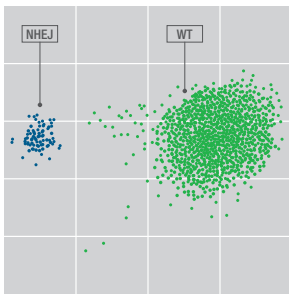
CFX REAL-TIME PCR DETECTION SYSTEM

Once you have enriched your cells of interest, you need to confirm that you have successfully edited your target cells before moving on to downstream assays. This can be accomplished through direct detection of edits using genomic methods or through indirect detection using cellular or proteomic methods.

PCR-based methods provide precise and sensitive detection and quantification of edits. High-fidelity PCR, Droplet Digital PCR (ddPCR™), real-time PCR, and high resolution melt analysis (HRMA) allow analysis of NHEJ and HDR edits. The CFX Real-Time PCR Detection System is a versatile platform for quantitative PCR and HRMA. For standard PCR and sequencing validation, iProof™ High-Fidelity PCR Reagents minimize the incorporation of errors. When editing efficiencies are low, ddPCR technology allows identification of allele changes with unmatched precision; mutations present at frequencies ≤0.5% can be identified.

The ZOE Fluorescent Cell Imager allows confirmation of knocked in and knocked out protein expression in your newly edited cells right at your bench. The V3 Western Workflow quantitatively measures protein expression faster and with more accuracy than other western blot procedures. By incorporating validated PrecisionAb™ Western Blotting Antibodies, you can further remove uncertainty from your experiments.

We used the ZOE Cell Imager and V3 Western Workflow to confirm successful gene edits. These orthogonal methods, along with direct methods such as Droplet Digital PCR or HRMA using qPCR (data not shown), give you the confidence to move forward with downstream analysis of your edited cells.



SUCCESS STORY Bio-Rad bulletin 6712 describes a rapid, cost-effective, and high-throughput Droplet Digital PCR method that provides convenient readout for the detection of edited alleles and for the optimization of CRISPR and TALEN editing protocols. This method is especially useful for those working with primary and pluripotent stem cells, where editing frequency is often low.

Visit **bio-rad.com/CRISPR2** to read more.

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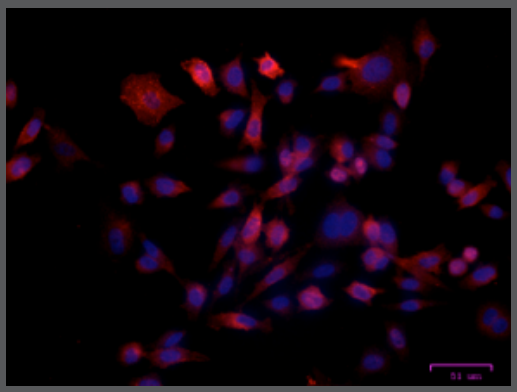

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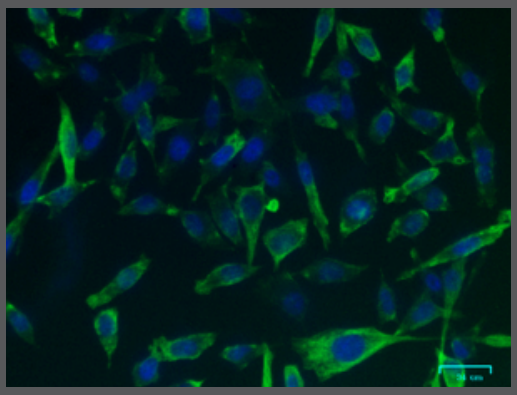
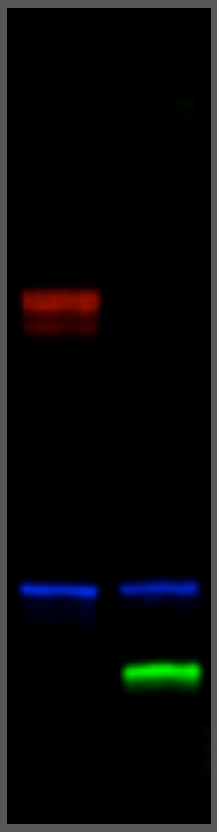


Confirmation of successful gene edits by immunocytochemistry. Control and edited cells were plated onto microscope slides 48 hours prior to staining with anti-*ICAM-1* antibody (red), and DAPI (blue)-containing mounting medium. Slides were imaged at the bench using the ZOE Cell Imager. Edited cells show expression of *GFP* but no *ICAM-1* expression. **A**, immunostaining of control PC3 cells reveals abundant *ICAM-1* expression. **B**, CRISPR-edited PC3 cells have lost *ICAM-1* expression and gained stable *GFP* expression.



Confirmation of successful gene editing by western blot. Control and edited cells were lysed on ice in RIPA Buffer (VWR) containing protease inhibitor cocktail (VWR), sonicated, and spun down to remove particulates. Lysates were centrifuged again and the supernatant was diluted in 4x Laemmli Sample Buffer (cat. #1610747) containing β-mercaptoethanol (cat. #1610710) and run on a 12% acrylamide precast stain-free gel (cat. #3451012). Proteins were transferred to nitrocellulose for 7 minutes at 100 V using the Trans-Blot® Turbo™ Transfer System (cat. #1704150). The blot was blocked with 1x Tris buffered saline (TBS) with 1% casein and then rinsed with 1x phosphate buffered saline Tween 20. CD54 PrecisionAb™ Primary Antibody recognizing ICAM-1 (cat. #VPA00014K), Goat Anti-Rabbit IgG StarBright™ Blue 700 (cat. #12004161) (red), Anti-GAPDH hFAB™ Rhodamine Labeled Primary Antibody (cat. #12004167) (blue), and Anti-turboGFP Primary Antibody (OrigGene) (green) were applied. The blot was imaged on the ChemiDoc™ MP Imaging System (cat. #17001402).

DOWNSTREAM ANALYSIS

ANALYZE AND MAKE DISCOVERIES

ZE5™ CELL ANALYZER

CFX AUTOMATION SYSTEM II

BIO-PLEX® 200 SYSTEM

V3 WESTERN WORKFLOW™

QX200™ DROPLET DIGITAL™ PCR SYSTEM

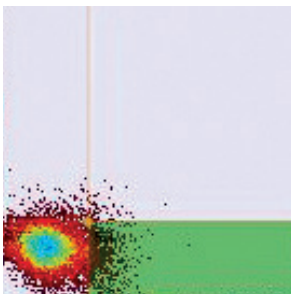
Now that you have confirmed that your cells are correctly edited, you can begin to investigate their phenotype. You may even wish to further perturb your system with a drug as part of a cell-based assay during target or lead discovery and validation. Bio-Rad provides tools to study your newly generated cells lines at the genomic, cell, and proteomic levels.

We provide predesigned and validated, as well as customizable, tools for high-throughput gene expression analysis, including the CFX Automation System, PrimePCR™ Gene Expression Assays and Panels, and CFX Maestro™ Software for Bio-Rad real-time PCR detection systems. For more sensitive absolute quantitation, use predesigned or custom ddPCR™ Genome Edit Detection Assays and our Droplet Digital PCR Systems.

The ZE5 Cell Analyzer allows you to study the effects of your edits at the cellular level. This flexible analyzer can be run by novices and experts alike. It accommodates up to 30-parameter experiments. This high-level multiplexing allows you to more fully characterize your edited cells and their response to different assay conditions.

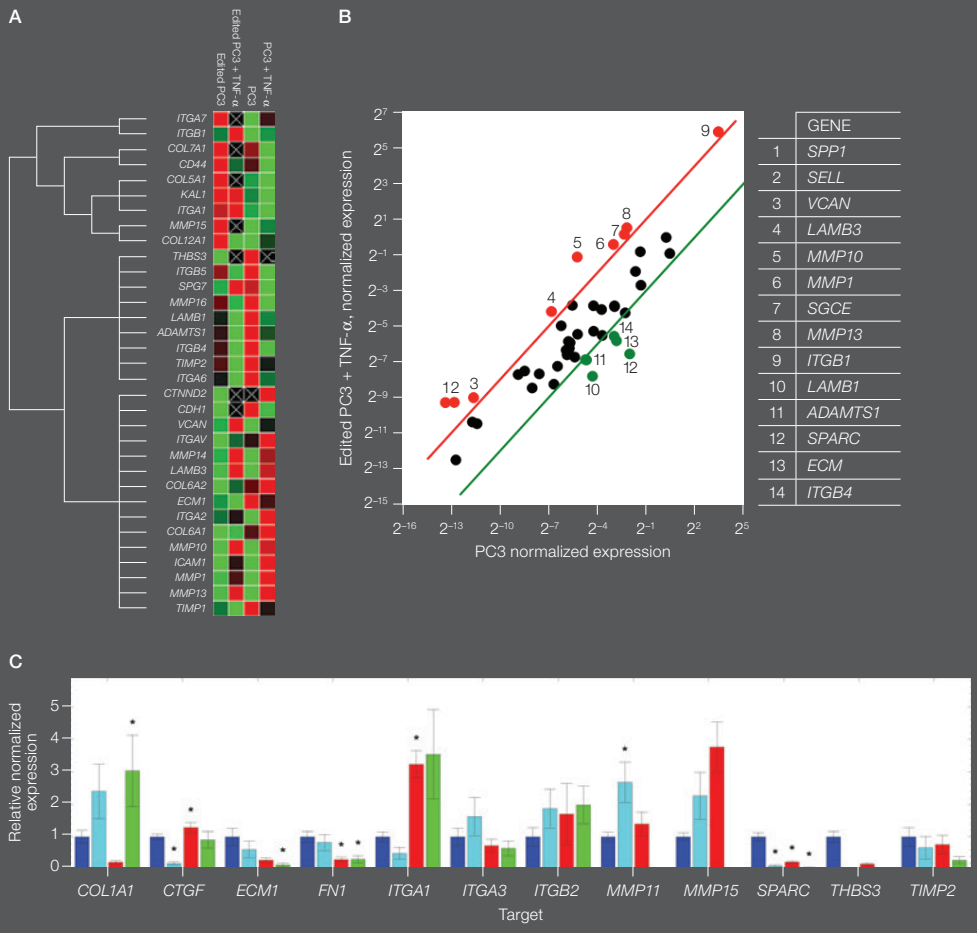
Multiplex proteomic analysis using the V3 Western Workflow and validated Bio-Plex Multiplex Immunoassays gives you a complete picture of the proteomic profile of your new cell lines. Validated PrecisionAb™ Western Blotting Antibodies and disease-specific or customizable multiplex Bio-Plex Assays ensure sensitive and reliable results.

To characterize our *ICAM-1* knockout cells we used PrimePCR Gene Expression Panels and CFX Maestro Software to assess changes in gene expression.



SUCCESS STORY In this brochure we have shown that incorporating flow cytometry into our CRISPR Gene Editing Workflow speeds time to results. Bio-Rad bulletin 6961 provides expanded experimental details, as well as additional results from our *ICAM-1* knock out study.

Visit **bio-rad.com/CRISPR3** to read more.



Gene expression analysis in control and edited cells in response to TNF-α treatment. Control and edited cells were treated with TNF-α to stimulate *ICAM-1* expression. Cells were lysed using the SingleShot™ Cell Lysis Kit (cat. #1725080), which allows preparation of gDNA-free RNA directly from cell culture. SingleShot Cell Lysates were added to three different PrimePCR Predesigned 384-Well Pathway Plates: Male Urogenital Diseases Tier 1, Extracellular Matrix, and Neoplasm Metastasis. We screened 962 targets by real-time PCR to look for changes in gene expression using a CFX384 Touch™ Real-Time PCR Detection System (cat. #1855485) and analyzed resulting data using CFX Maestro Software (cat. #12004110). **A**, relative normalized expression plot shows little or no change in expression of *ICAM-1* in edited cells compared to wild-type PC3 in response to TNF-α treatment. These results also show that edited cells retained a low level of *ICAM-1* expression. **B**, a scatter plot provides an alternate view of changes in expression. The red line represents a 4-fold increase and the green line a 4-fold decrease in expression compared to control cells (unstimulated wild-type PC3 cells); **C**, interestingly other cell adhesion markers showed altered expression between wild-type and edited PC3 cells before and after stimulation with TNF-α, suggesting that cells are using these gene products to compensate for lack of a *ICAM-1*. PC3 (—); PC3 + TNF-α (—); edited PC3 (—); edited PC3 + TNF-α (—).

WHY BIO-RAD?

Bio-Rad has over 60 years experience supporting life science research and clinical diagnostics. We are the leader in digital PCR, transfection, and in gene and protein expression analysis technologies. Our innovative products are available as stand-alone solutions or combined into workflows that are optimized to minimize downtime and to provide the right answer the first time, every time. The CRISPR Gene Editing Workflow is only one of many Bio-Rad workflows designed to shorten discovery and development time lines for novel therapeutics.

As part of our commitment to your research, we provide:

- Products and reagents that are reliable and validated
- Responsive, trusted global service and technical support teams
- Technical expertise across multiple workflows and applications

	Transfection	Enrichment	Confirmation	Analysis
Cells				
S3e Cell Sorter	•	•	•	•
ZOE Fluorescent Cell Imager	•	•	•	•
Gene Pulser Xcell Electroporation Systems	•			
TransFectin Lipid Reagent	•			
Helios® Gene Gun Systems	•			
TC20 Cell Counter	•	•	•	•
Antibodies for Flow Cytometry and Immunohistochemistry	•	•	•	•
Genes				
QX200 Droplet Digital PCR System			•	•
PrimePCR Assays for qPCR and ddPCR			•	•
CFX Real-Time PCR Detection Systems			•	•
CFX Maestro Sofware			•	•
Hard-Shell® PCR Plates			•	•
C1000 Touch™ Thermal Cycler			•	•
Ready Gel® Precast Agarose Gels			•	•
SsoAdvanced™ Universal Supermixes			•	•
Precision Melt Supermix			•	•
iProof High-Fidelity PCR Reagents			•	•
Precision Melt Analysis™ Software			•	•
Proteins				
Bio-Plex Multiplex Immunoassay Systems				•
Bio-Plex Multiplex Immunoassays				•
Stain-Free Mini and Midi Protein Gels			•	•
Trans-Blot Turbo Transfer System			•	•
ChemiDoc MP Imaging System			•	•
PrecisionAb Validated Western Blotting Antibodies			•	•
Antibodies for Flow Cytometry and Immunohistochemistry			•	•
Protein Sample Preparation Beads and Kits			•	•

Visit **bio-rad.com/web/CRISPRinfo** to learn more about our CRISPR toolkit.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

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