CRISPR-CAS SYSTEMS VALIDATION USING A FLUORESCENT REPORTER CELL LINE

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CRISPR-Cas systems are molecular mechanisms in bacteria and archaea that are responsible for protecting these prokaryotes from mobile genetic elements. Owing to their programmable nuclease activity, these systems have become widely used tools for genome editing not only in prokaryotic cells, but also in mammalian cells. In bacteria, CRISPR-Cas systems are validated using plasmid interference, PAM depletion, and/or phage assays [1,3]. In mammalian cells, validation is performed by RT-qPCR, Western blot and sequencing [2,3]. These methods are time consuming and require specific equipment and skills, which is why a more straightforward way of validating new CRISPR-Cas systems is needed.

The aim of this study was to apply the mouse neuroblastoma Neuro-2a KI: EGFP-mRUBY2-PURO cell line, which was derived in our laboratory, for the validation of CRISPR-Cas systems. These cells stably express EGFP (green fluorescent protein) in their nuclei and mRuby2 (red fluorescent protein) in the membrane. To assess whether these cells can be used for screening new gene editing tools and evaluating their efficiency, Neuro-2a cells were transfected with a plasmid encoding SaCas9 and *EGFP*-targeting gRNA. *EGFP* knock-out efficiency was evaluated by fluorescence microscopy. EGFP fluorescence was analysed on days 3, 5, 7, and 10 after transfection to determine the optimal time point for *EGFP* knock-out analysis.

The results demonstrated that SaCas9 efficiently disrupted the *EGFP* gene at all analysed time points. On day 5 post-transfection, less than 10% of transfected cells remained EGFP-positive, compared to 91.78% in the control group. These findings indicate that fluorescent reporter cell lines may serve as a simple and effective platform for screening and validating novel CRISPR-Cas systems in mammalian cells.

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