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Title: Genome-Wide CRISPR Screen for Unveiling Radiosensitive and Radioresistant Genes

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   If **Yes**, how far apart are the locations? Within 500 meters.

Current Protocol Length
Number of Steps: 22
Number of Shots: 48

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Ziyu Jiang:** This research focuses on genome-wide CRISPR screen and radiotherapy. We provide a protocol to unveil radio-sensitive and radio-resistant genes using a genome-wide CRISPR screen in lung cancer cells after irradiation **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.2*

What are the current experimental challenges?

1.2. **Ziyu Jiang:** The current experimental challenges include the off-target effects, which is caused by the vast complexity of genome, and potential difficulties in exploring the underlying mechanisms **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.2*

What advantage does your protocol offer compared to other techniques?

1.3. **Ziyu Jiang:** Compared with traditional screening methods, CRISPR achieves permanent genetic modifications and shows superior precision, which makes it especially valuable in functional genomic research and target discovery **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.4.2*

What research questions will your laboratory focus on in the future?

1.4. **Ziyu Jiang:** In the future, our team will focus on studying in-vivo CRISPR screen to solve the problems that this research left behind, and will be committed to optimizing CRISPR technology **[1]**.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.7.2*

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Culture Preparation and Radiation Treatment

Demonstrator: Yu Yuan

2.1. To begin, adjust the adherent cell density to 5 × 10^5 cells per milliliter **[1]**. Using a pipette, distribute 2 milliliters of the cell suspension into each 3.5-centimeter culture dish for radiation treatment at different doses **[2]**. Place the dishes in an incubator set to 37 degrees Celsius with 5 percent carbon dioxide and incubate overnight **[3]**.

2.1.1. WIDE: Talent mixing the cells using a pipette.

2.1.2. Talent pipetting 2 milliliters of cell suspension into 3.5-centimeter dishes.

2.1.3. Talent placing culture dishes into an incubator.

2.2. Number each 3.5-centimeter culture dish from 1 to 5 using a marker **[1]**. Using a radiation source, administer radiation doses of 2, 4, 6, and 8 gray, respectively, to dishes 2 through 5 **[2]**.

2.2.1. Talent labeling dishes 1 to 5 with a marker.

2.2.2. Talent placing a dish under the radiation chamber.

2.3. Adjust the irradiated cell density to 1 × 10^5 cells per milliliter **[1]**. Seed 10 microliters per well, corresponding to 1,000 cells per 100 microliters, into 6-well plates with 3 replicates per radiation dose **[2]**.

2.3.1. Talent mixing a new cell suspension by pipetting up and down.

2.3.2. Talent pipetting 10 microliters into each well of a 6-well plate.

2.4. Then, seed 30 microliters per well, corresponding to 3,000 cells per 100 microliters, into 96-well plates with 5 replicates per radiation dose **[1]**.

2.4.1. Talent pipetting 30 microliters into each well of a 96-well plate.

2.5. Now, mix the CCK8 reagent with RPMI 1640 *(R-P-M-I-sixteen forty)* medium without FBS in a 1 to 9 ratio **[1]**. Add the mixture to the 96-well plate **[2]** and incubate the plate in the dark for 1 hour **[3]**. Then, use a microplate reader to measure the optical density at 450 nanometers **[4]**. NOTE: VO is added for the added shot, and sentence numbers are adjusted.

2.5.1. Talent mixing CCK8 reagent and RPMI medium in a tube by pipetting up and down.

Added Shot: Talent adding the mixture to the 96-well plate.

2.5.2. Talent placing the 96-well plate in a dark storage container or cupboard.

2.5.3. Talent placing the sample in the microplate reader.

3. Lentiviral Infection and Puromycin Selection

3.1. To begin the infection process, set up a logarithmic concentration gradient for lentivirus from 0 to 800 units per milliliter **[1]**. Add the corresponding volume of lentivirus to 2 microliters of polybrene per dish **[2]** and let it equilibrate at room temperature for 5 minutes **[3]**. Slowly drip the lentivirus-polybrene mixture into each well **[4]**.

3.1.1. Talent preparing labeled tubes for different lentivirus concentrations.

3.1.2. Talent adding lentivirus to polybrene.

3.1.3. Talent placing the sample aside.

3.1.4. Talent pipetting the virus-polybrene mixture into the culture wells.

3.2. Adjust the parental cell density to 3×10^5 cells per milliliter **[1]** and inoculate 1 milliliter into each well of a 12-well plate **[2]**. Add puromycin in a concentration gradient to the wells **[3-TXT]**.

3.2.1. Talent mixing the cell tube by inversion.

3.2.2. Talent pipetting 1 milliliter of cell suspension into each well of a 12-well plate.

3.2.3. Talent sequentially adding different puromycin concentrations into the corresponding wells. **TXT: Puromycin: 0, 0.1, 0.2, 0.5, 1, 2, 4, and 8 µM**

3.3. After 72 hours of infection, replace the medium in each well with complete medium containing the minimum puromycin concentration for cell killing **[1]**. Calculate the multiplicity of infection for each well based on surviving cells **[2]**.

3.3.1. Talent aspirating the old medium and adding fresh medium with puromycin.

3.3.2. Talent looking at the cell counter display and noting down.

4. Genome-Wide CRISPR Lentiviral Library Infection

4.1. Adjust the adherent cell density to 1×10^7 cells per milliliter **[1]**.

4.1.1. Talent mixing the tube with cells by inverting.

4.2. Add lentivirus at a multiplicity of infection equal to 0.3 into 30 microliters per dish of polybrene **[1]** and allow it to equilibrate at room temperature for 5 minutes **[2]**.

4.2.1. Talent pipetting lentivirus into polybrene.

4.2.2. Talent setting the tube aside.

4.3. Slowly drip the lentivirus and polybrene mixture into the 15-centimeter culture dish **[1]**, mix well, and incubate it overnight at 37 degrees Celsius with 5 percent carbon dioxide **[2]**.

4.3.1. Talent carefully pipetting and dispensing mixture into large culture dish.

4.3.2. Talent placing dish in an incubator.

4.4. On the second day post-infection, aspirate the medium from the culture dish **[1]** and replace it with 15 milliliters of RPMI-1640 complete medium containing 10 percent FBS **[2]**.

4.4.1. Talent aspirating medium from the culture dish.

4.4.2. Talent adding fresh complete medium to the dish.

4.5. Repeat the same treatment for the uninfected parental cells as a negative control and continue culturing for 72 hours **[1]**.

4.5.1. Talent placing all dishes back into the incubator.

4.6. Now, digest the cells from one 15-centimeter culture dish using 0.25 percent trypsin **[1]**. Resuspend the cells in RPMI 1640 complete medium with 10 percent PBS **[2]** and count the number of cells **[3]**.

4.6.1. Talent adding trypsin to a dish and gently swirling.

4.6.2. Talent pipetting up and down to resuspended cells into a tube.

4.6.3. Talent loading the sample into a cell counter.

4.7. After extracting the genomic DNA for Day 0, use a nanodrop UV spectrophotometer to measure DNA concentration and purity **[1]**.

4.7.1. Shot of nanodrop interface showing DNA concentration and purity.

5. Radiation Treatment for Screening, Genome Extraction, and Sequencing

Demonstrator: Jiawen Tang

5.1. Administer an appropriate dose of radiation to cells in the treatment group **[1]**, and leave control group cells untreated to propagate normally **[2]**.

5.1.1. Talent positioning treatment dish for radiation exposure.

5.1.2. Talent labeling and setting control dish aside in the incubator.

5.2. After 14 days of treatment, digest both the treatment and control group cells using 0.25 percent trypsin **[1]**. Resuspend the cells in RPMI 1640 complete medium with 10 percent FBS **[2]**.

5.2.1. Talent adding trypsin to treated and control dishes.

5.2.2. Talent pipetting and resuspending cells into labeled tubes.

5.3. Centrifuge the cells at 300 *g* for 5 minutes and discard the supernatant **[1]**. Resuspend the pellet in 1 milliliter of PBS **[2]**.

5.3.1. Talent loading tubes into centrifuge and starting spin.

5.3.2. Talent adding PBS to the pellet and pipetting up and down.

5.4. After repeating the centrifugation step, extract day 14 genomic DNA from the pellet **[1]** and determine the DNA concentration **[2]**.

5.4.1. Talent displaying the clear DNA solution in a tube.

5.4.2. Shot of nanodrop reading for Day 14 DNA sample.

5.5. Next, prepare the required primers and dilute them to 10 micromolar **[1]**. After adding the components to set up a 20-microliter reaction system, centrifuge the tube briefly at 300 *g* for 5 seconds **[2]**.

5.5.1. Talent pipetting and diluting primers into labeled tubes.

5.5.2. Talent placing the reaction mix tubes in centrifuge.

5.6. For agarose gel electrophoresis, prepare the gel **[1],** remove the comb from it **[2],** and fill the electrophoresis tank with sufficient buffer to cover the gel **[3]**.

5.6.1. Talent placing the gel in the tank.

5.6.2. Talent carefully removing comb.

5.6.3. Talent pouring buffer into electrophoresis tank.

5.7. Add loading buffer to the DNA sample and mix well **[1].** Finally, load the mixture into the wells and start the electrophoresis **[2]**.

5.7.1. Talent mixing DNA sample with loading dye by pipetting.

5.7.2. Talent adding sample into gel wells.

Results

6. Results

6.1. Colony formation after 14 days revealed that exposure to 2 Gray of radiation significantly reduced the number of surviving colonies compared to 0 Gray **[1]**.

6.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the 0 Gray and 2 Gray images*

6.2. The CCK-8 assay showed a substantial decline in cell viability at 2 Gray, with further decrement at higher radiation doses **[1]**.

6.2.1. LAB MEDIA: Figure 2B and C.

6.3. Treatment with increasing concentrations of puromycin for 72 hours showed that 1 micromolar was the minimum concentration required to eliminate A549 cells **[1]**.

6.3.1. LAB MEDIA: Figure 3. *Video editor: Highlight the bar or data point at 1*.

6.4. PCR validation showed distinct bands at 231 base pairs, confirming the expected length of sgRNA *(S-G-R-N-A)* sequences in the CRISPR *(crisper)* library **[1]**.

6.4.1. LAB MEDIA: Figure 4. *Video editor: Zoom in on the bright gel bands in both RT and NC lanes*.

6.5. Sequencing analysis revealed that approximately 60 percent of the reads successfully mapped to the reference genome **[1]**. sgRNA read counts followed a Poisson distribution, matching theoretical expectations for a genome-scale screen **[2]**.

6.5.1. LAB MEDIA: Figure 5A. *Video editor: Focus on the dark blue bar graph parts*.

6.5.2. LAB MEDIA: Figure 5B.

6.6. PCA and heatmap analysis showed high inter-group variability and low intra-group variation, validating experimental consistency **[1]**. Gene Ontology analysis identified DNA damage response as a top-enriched pathway among the top 15 results **[2].**

6.6.1. LAB MEDIA: Figure 5C.

6.6.2. LAB MEDIA: Figure 5C and D. *Video editor: Highlight the label "cellular response to DNA damage " in C*.