Experiment time: 2025-04-16, 18:00 - 2025-04-19, 17:00

Sample Preparation for Whole Genome Sequencing

I. Objective of the experiment

To prepare whole-genome sequencing (WGS) samples from the T24 wild-type cell line and low-, medium-, and high-drug-resistant T24-RC48 cell lines. The samples will be shipped on dry ice to the sequencing company for subsequent genomic variation analysis to investigate drug resistance-related genomic alterations.

II. Experimental content

2.1 Experimental design

Sample type: T24 wild-type cell line, low/medium/high drug resistance T24-RC48 cell line.

Number of repetitions: 3 samples per group.

2.2 Measurement principle

Whole Genome Sequencing (WGS) involves randomly fragmenting genomic DNA, constructing a library from these fragments, and then performing massive parallel sequencing using next-generation sequencing (NGS) technology. Short reads are subsequently assembled into a complete genome sequence using bioinformatic tools. The specific workflow includes: extracting high-quality DNA; randomly shearing long DNA strands into fragments of 200–500 bp via physical or chemical methods; constructing a sequencing library through steps including end repair, adapter ligation, and PCR amplification; sequencing the library using technologies such as sequencing by synthesis (SBS) or single-molecule real-time sequencing, which determines the sequence by detecting fluorescent signals during base extension; and finally, performing sequence alignment, variant detection (e.g., SNPs, CNVs), and functional annotation to reveal genomic structural variations and functional characteristics. WGS provides single-base resolution, high coverage, and unbiased whole-genome detection, making it widely applicable in fields such as cancer genomics and evolutionary studies.

III. Materials and reagents

3.1 Materials

Pipette tips and 15ml centrifuge tubes were purchased from Axygen

Serological pipettes were purchased from a domestic company

Micropipettes: Eppendorf

T25 culture flasks were purchased from Corning

10-cm cell culture dishes (Corning)

Liquid nitrogen, dry ice

Human bladder transitional cell carcinoma cell line (T24)

Low, medium, and high drug-resistant cell lines T24-RC48

3.2 Reagents

PBS and trypsin were purchased from Gibco, USA

McCoy's 5A medium from Shanghai Yuanpei Biotechnology Co., Ltd., China

Fetal bovine serum (FBS, Gibco)

IV. Experimental instruments

Clean bench: Suzhou purification equipment factory

Benchtop low-speed centrifuge at room temperature: Eppendorf

V. Experimental steps

5.1 Low, medium, high drug-resistant and wild-type T24 cell lines were seeded in 10-cm cell culture dishes until the cells grew to more than 80%.

5.2 The culture medium was discarded, and the cells were washed with 4°C pre-chilled PBS. The dish was gently rocked for 1 minute, and the PBS was aspirated. This washing step was repeated twice more.

5.3 The culture dish was placed on ice. Then, 1 mL of ice-cold PBS was added. Cells were quickly scraped off using a sterile cell scraper and pooled to one side of the dish by tilting it on ice.

5.4 Use a pipette to aspirate the cell suspension into a pre-cooled 1.5ml centrifuge tube and and centrifuged. The supernatant was then carefully discarded.

5.5 The tubes were labeled, rapidly frozen in liquid nitrogen, and subsequently stored at -80°C.

5.6 Samples were packaged on dry ice, accompanied by a completed sample submission form, and shipped to the sequencing company.

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VI. Photo of experimental operation

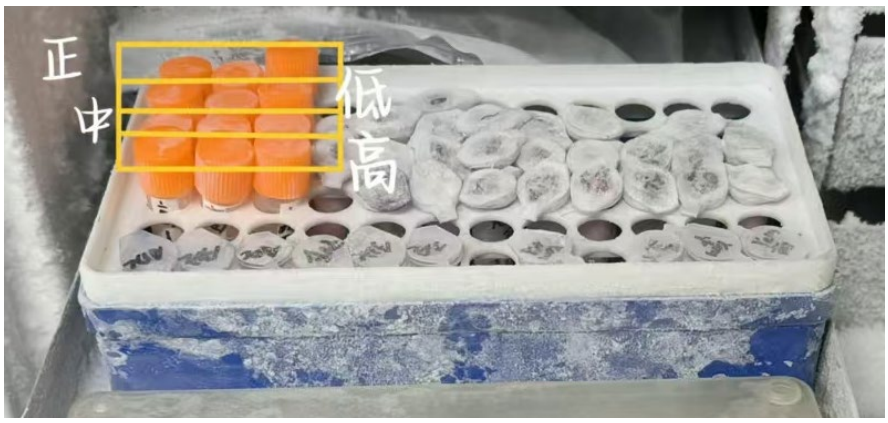

Figure 1 Samples prepared for whole genome sequencing

VII. Experimental results

3.1 检测结果总表

序号	样本名称	样本编号	文库类型	核酸编号	浓度(ng/ul)	体积(ul)	总量(ug)	检测结论	检测结果备注	剩余量	电泳原液上样量(ul)
1	正1-M-GSGC0447672	SKDO250000415-1A	DNA小片段文库	SZTD250000415-1A	414.516	60	24.87096	Pass	None	0	2
2	正2-M-GSGC0447672	SKDO250000416-1A	DNA小片段文库	SZTD250000416-1A	263.162	60	15.78972	Pass	None	0	2
3	正3-M-GSGC0447672	SKDO250000417-1A	DNA小片段文库	SZTD250000417-1A	399.944	60	23.99664	Pass	None	0	2
4	低1-M-GSGC0447672	SKDO250000418-1A	DNA小片段文库	SZTD250000418-1A	387.704	60	23.26224	Pass	None	0	2
5	低2-M-GSGC0447672	SKDO250000419-1A	DNA小片段文库	SZTD250000419-1A	386.781	60	23.20686	Pass	None	0	2
6	低3-M-GSGC0447672	SKDO250000420-1A	DNA小片段文库	SZTD250000420-1A	21.76	60	1.3056	Pass	None	0	2
7	中1-M-GSGC0447672	SKDO250000421-1A	DNA小片段文库	SZTD250000421-1A	585.067	60	35.10402	Pass	None	0	2
8	中2-M-GSGC0447672	SKDO250000422-1A	DNA小片段文库	SZTD250000422-1A	355.123	60	21.30738	Pass	None	0	2
9	中3-M-GSGC0447672	SKDO250000423-1A	DNA小片段文库	SZTD250000423-1A	155.131	60	9.30786	Pass	None	0	2
10	高1-M-GSGC0447672	SKDO250000424-1A	DNA小片段文库	SZTD250000424-1A	137.64	60	8.2584	Pass	None	0	2
11	高2-M-GSGC0447672	SKDO250000425-1A	DNA小片段文库	SZTD250000425-1A	73.875	60	4.4325	Pass	None	0	2
12	高3-M-GSGC0447672	SKDO250000426-1A	DNA小片段文库	SZTD250000426-1A	49.492	60	2.96952	Pass	None	0	2

VIII. Results analysis

This experiment successfully completed whole-genome sequencing library construction and quality control for 12 samples, including wild-type and low-, medium-, and high-drug resistance gradient groups. All DNA fragment libraries passed standardized quality control procedures with a "Pass" rating (all test notes indicated "None"). Library concentrations (ranging from 263.166 to 414.516 agu/μL) were highly consistent with the total amounts (ranging from 23.20 to 24.87μg), and a uniform loading volume of 2μL for electrophoresis was used, indicating standardized sample preparation and controllable batch variations. The complete set of nucleic acid identifiers and volume data (all samples had a volume of 60μL) confirmed that the samples met the requirements for 50X

whole-genome sequencing coverage, providing a high-reliability data foundation for subsequent SNP/CNV/SV analysis.