

## Karyotype Analysis

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**[Abstract]** A chromosome is the structure that organizes DNA and protein in cells. It is a single piece of coiled DNA containing coding and non-coding sequences. Human cells have 23 pairs of chromosomes including 22 pairs of autosomes and one pair of sex chromosome, giving a total of 46 per cell. In tumor cells, chromosomal instability has been considered to be one of the hallmarks of tumor formation. Here we use the karyotype analysis to separate the chromosomes and observe the chromosomes in tumor cells with a microscope.

### Materials and Reagents

1. Cell lines: FaDu (ATCC, catalog number: HTB-43™)
2. Trypsine (Life Technologies, Gibco®, catalog number: 15400-054)
3. 10% fetal bovine serum (Thermo Fisher Scientific, catalog number: SH30071.03)
4. 1% Penicillin-Streptomycin (10,000 U/ml; 10,000 µg/ml) (Life Technologies, Gibco®, catalog number: 15140-122)
5. Colcemid (10 µg/ml) (Life Technologies, Gibco®, catalog number: 15212-012)
6. Hypotonic solution (0.075 M KCl) (J.T.Baker®, catalog number: 7447-40-7)
7. Glacial acetic acid (J.T.Baker®, catalog number: 64-19-7)
8. Fixative (see Recipes)
9. Growth medium (see Recipes)

### Equipment

1. 10 cm cell culture dishes (Trueline Valve Corporation, catalog number: TR4002)
2. Centrifuge tube
3. Microscope (OLYMPUS, model: DP71)
4. CO<sub>2</sub> incubator
5. Centrifuge at room temperature (using “relative centrifugal force, rcf”)
6. Water bath

## Procedure

### Day 1

1. Seed  $5 \times 10^5$  cells on 10 cm cell culture dishes for attachment.
2. Cells were incubated at 37 °C, 5% CO<sub>2</sub> for 48 h.

### Day 3

1. Add 0.1 ml colcemid, which can collapse mitotic spindles and prevent the completion of mitosis, to each dish and mix gently. Incubated at 37 °C, 5 % CO<sub>2</sub> for 2 h.
2. Transfer medium to centrifuge tubes from the cell culture dishes. Use PBS to wash the dishes, and remove PBS. Then, add 1 ml 0.1% Trypsine into the dishes at 37 °C, 5% CO<sub>2</sub> for 2 min. Also, transfer the mixture (Trypsine and cells) into the centrifuge tubes and mix with the medium which is transferred to centrifuge tubes before we use PBS to wash the dishes. Further, centrifuge at 100 rcf for 10 min at room temperature.
3. Discard the supernatant and leave 0.5 ml medium to mix the pellet gently.
4. Resuspend the pellet in 5-7 ml 37 °C hypotonic solution and mix thoroughly. Incubate in water bath at 37 °C for 10 min.
5. Centrifuge at 100 rcf for 10 min at room temperature.
6. Discard the supernatant and leave 0.5 ml solution to mix the pellet gently.
7. Resuspend the pellet in 5 ml cold fixative (drop by drop and flick the tube between drops to prevent cell clumping).
8. Put the centrifuge tube on ice at least 20 min.
9. Centrifuge at 100 rcf for 10 min at room temperature.
10. Discard the supernatant and add 3-5 ml cold fixative.
11. Repeat steps 9-10 three times or until the supernatant is clear and the pellet become white.
12. After the final centrifugation, suspend the cells in a few drops of cold fixative to give a slightly opaque suspension.
13. Drop 1-2 drops onto wet and clean slides (Before that, the slides have to be rinsed by 1-2 drops of cold fixative.)
14. Dry the slides at room temperature.
15. Observe the chromosomes with the microscope (Olympus DP71 with 200x magnification).
16. Count the number of chromosomes about 50 cells (A normal human cell have 23 pairs of chromosomes.).

*Note: Chromosomes center in a scope, we define that "one cell".*

**Representative data**



**Figure 1. Representative pictures of karyotype analysis in FaDu cell line (see Chou *et al.*, 2013)**

**Recipes**

1. Fixative  
Methanol and glacial acetic acid (3:1) to be made fresh and chilled before using
2. Growth medium  
RPMI 1640 supplemented with:  
10% fetal bovine serum  
1% Penicillin Streptomycin (10,000 U/ml; 10,000 µg/ml)

**Acknowledgments**

This protocol has been adapted from Chou *et al.* (2013).

**References**

1. Chou, C. H., Yang, N. K., Liu, T. Y., Tai, S. K., Hsu, D. S., Chen, Y. W., Chen, Y. J., Chang, C. C., Tzeng, C. H. and Yang, M. H. (2013). [Chromosome instability modulated by BMI1-AURKA signaling drives progression in head and neck cancer](#). *Cancer Res* 73(2): 953-966.