A complete removal of genomic DNAs from cell lysate enhances the quality of protein research

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Abstract

The availability of cells in culture provides cell and molecular biologists an invaluable research platform to effectively study signal transduction pathways, protein-expression regulation, and protein-protein interactions. These cells can either be lysed under non-denaturing conditions leading to inefficient recovery of proteins from certain cellular compartments, or with denaturing lysis buffers that efficiently isolates proteins from all cell compartments, but also results in highly viscous genomic DNA (gDNA) contamination. This gDNA contamination can significantly interfere with downstream applications such as western blot (WB) and Immunoprecipitation (IP). However, up until now, no efficient method has been devised to remove a significant portion of this gDNA. Even though the gDNA can be sheared by either passing through a syringe needle or by sonication, small fragments of gDNA can still interfere and/or distort down-stream analyses. Additionally, both syringe and sonication methods are highly variable, time consuming, and may even damage protein in the lysate. Moreover, a massive amount of gDNA contamination may obstruct the light transmission in the spectrophotometer, which results in an inaccurate OD reading when obtaining protein concentrations. This study demonstrates a simple and fast method to remove gDNA from the cell lysate completely. A431 cell lysate was purified using this method and more than 98% of gDNA was removed while still recovering 90% of cell lysate without any effect on the protein population. Dynamic viscosities (N s/m^2 x10^3, at room temperature) of the crude cell lysate containing 50ug/ml and 100ug/ml of gDNA were reduced from 114 and 200 to ~1, which is the level of water. Importantly, removal of gDNA resulted in the reliable measurement of the cell lysate protein concentration using a spectrophotometer. We expect that this new method will contribute to the cell biology community by allowing scientist to obtain cell lysates that have a complete protein profile and is free of gDNA contamination .

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Immunoprecipitation (IP)

1. An easy and fast preparation of cell lysate for IP experiments

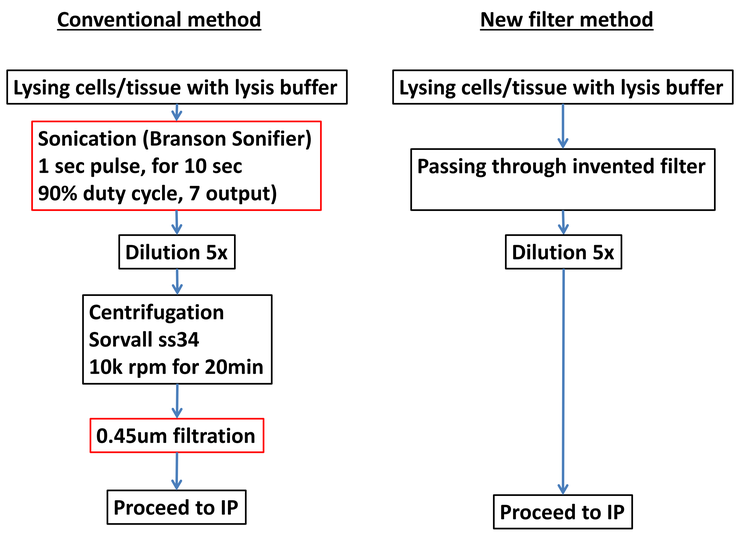
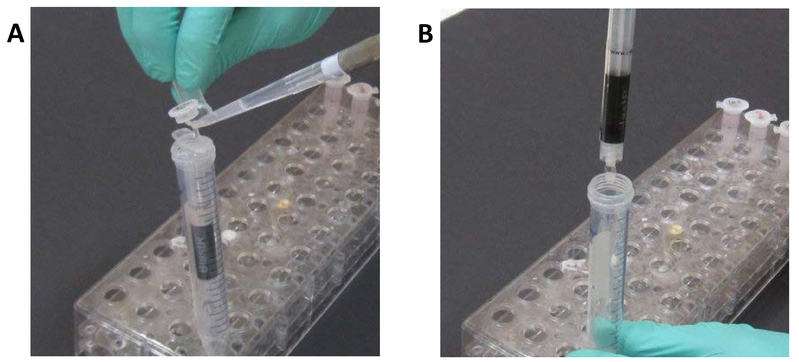


Fig. 1. A comparative schematic diagram between the conventional method and the new filter method for the preparation of total cell lysate for an IP experiment. In order to reduce the gDNA contamintion of total cell lysate, the conventional method shears gDNA with either sonication or passing through syringe needle, while the new filter method removes gDNAs with a patented filter system.

2. Filtering reduces the viscosity (gDNA contamination) of cell lysates



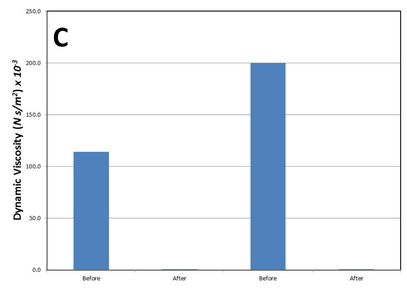


Fig. 2 50ug/ml and 100ug/ml of unsheared genomic DNA solutions were prepared by lysing 8 x 10^6 and 16 x 10^6 A431 cells with 600ul of a denaturing lysis buffer, respectively. Cell lysate was placed in the filter system (A) and clarified by applying force and passing through filter(B). Dynamic viscosities of filtered and non-filtered lysate were measured by Falling Sphere Viscometer at room temperature. Glycerol solutions (60%, 70%, 80%, 90% and 95% wt) were used for standards.

3. New filtering method can remove 98% of the gDNA contamination

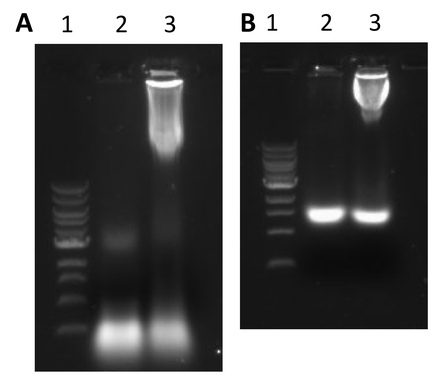
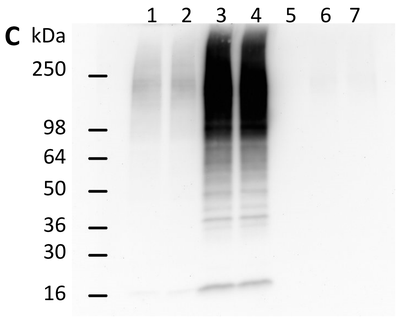


Fig. 3. Gel electrophoresis staining of A431 cell lysate before and after filtering
A) 16 x 10^6 of A431 cells were lysed with 600ul of denaturing lysis buffer. 2% of lysate was analyzed by ethidium bromide agarose gel electrophoresis. (1. DNA ladder, 2. Lysate after filtering and 3. Lysate before filtering)
B) E. Coli cells were grown in LB media. 9x10^9 cells were pelleted and lysed with 1ml of lysis buffer. (1. DNA ladder, 2. Lysate after filtering and 3. Lysate before filtering)



IP: 11G2, IB: 12F3

C) Cell lysate was prepared by lysing 16 x 10^6 of A431 cells with a denaturing lysis buffer. 1mg of lysate was incubated with 30ul of SUMO-2/3 affinity beads or 30ul of control beads at 4C for 2hrs and the immuno-precipitated proteins were analyzed by Western Blot. The blot was probed by anti-SUMO-2/3 antibody.
1. Input 0.1% w/o filter, 2.Input 0.1% w/ filter
3. Sumo IP w/o filter , 4. sumo IP w/ filter
5. Beads w/o lysate
6. control beads w/o filter
7. control beads w/ filter

Western Blotting (WB)

4. Filtering provides a simple way to obtain high quality cell lysate for WB analysis

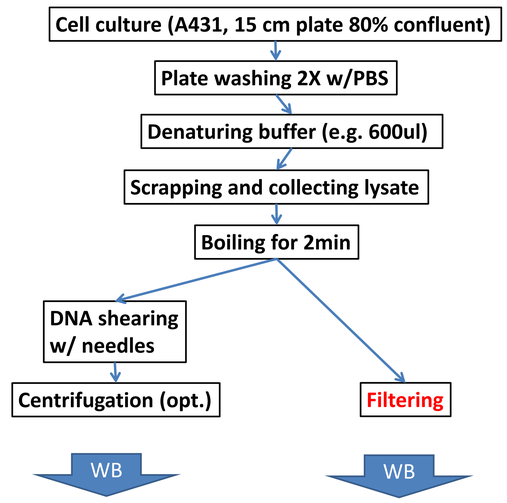


Fig. 4. A comparative schematic diagram between conventional method and new filter method for the preparation of total cell lysate for WB experiment.

5. Filtering of gDNA enhances sample quality and analysis by Coomassie staining and western blot

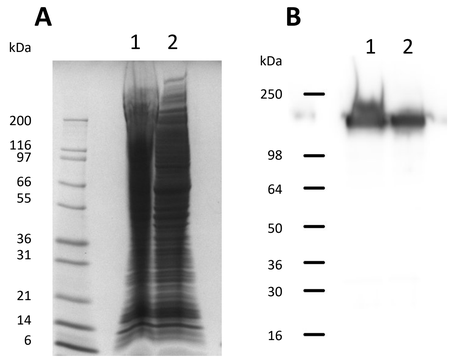


Fig. 5. 16 x 10^6 of A431 cells were lysed with 600ul of denaturing buffer and filtered with filter method or sheared with syringe needle. After boiling for 5 minutes, 3% of each sample was loaded on SDS-PAGE and stained with Coomassie Blue (A) and immuno-blotted with EGFR antibody (B)
1: gDNAs were sheard by syringe needle. 2: gDNAs were removed by new filter.

Conclusions

1. **New filtering method provides a fast, reliable and convenient method to obtain total cell lysate using denaturing buffers that are convenient for IP and WB experiment**
2. **This method removes 98% of genomic DNAs from mammalian cell lysate.**
3. **Dynamic viscosities (N s/m^2 x10^3, at room temperature) of the crude cell lysate containing 50ug/ml and 100ug/ml of gDNAs were reduced from 114 and 200 to ~1, which is the level of water**
4. **When used with BlastR denaturing buffer, samples can be quantitated using conventional protein quantitation assays**