State-of-the-art in human cell synchronization

Synchronization protocols for human cells from:
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I.) Double Thymidine block (early S-phase block)

1. At 25-30% confluency of HeLa cell culture, wash twice with 1X PBS and add DMEM (10%FCS, 1% Pen-Strep, 1% Glutamine) + 2mM Thymidine for 18h (first block).
2. After first Thymidine block: remove Thymidine by washing with 1X PBS; add fresh DMEM (10%FCS, 1% Pen-Strep, 1% Glutamine) for 9h to release cells.
3. After the release: add DMEM (10%FCS, 1% Pen-Strep, 1% Glutamine) + 2mM Thymidine for 17 h (second block).
4. After second block: remove Thymidine by washing with 1xPBS; release cells by adding fresh DMEM (10%FCS, 1% Pen-Strep, 1% Glutamine).

⇒ Cells progress synchronously through G2- and mitotic phase

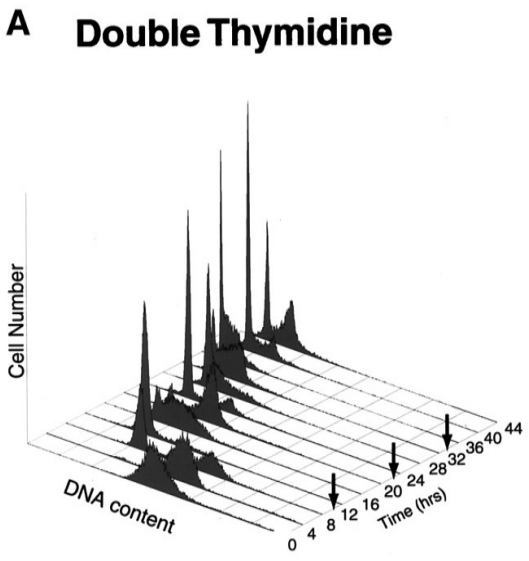

Figure A. Synchrony of HeLa cells. (A) Cells were arrested at the beginning of S phase by using a double thymidine block, and cell synchrony was monitored by flow cytometry of propidium iodide-stained cells. Flow cytometry data were collected for each of the three independent double thymidine blocks performed in this study; data are shown only for the second double thymidine arrest (Thy-Thy2), although equivalent synchrony was obtained in each of the three experiments. The number of cells (arbitrary units) is plotted against DNA content for time points at 4-h intervals for 44 h; an arrow indicates the time of mitosis, as estimated from the flow cytometry data. Upon release from the thymidine block, >95% of the cells progressed into S phase (0-4 h), entered G2 phase (5-6 h), underwent a synchronous mitosis at 7-8 h, and reentered S phase after completing one full cell cycle at 14-16 h. Typically two to three additional synchronous cell cycles were obtained.

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II.) Thymidine-Nocodazole block (mitotic block)

1. At 40% confluency of HeLa cell culture add DMEM (10%FCS, 1% Pen-Strep, 1% Glutamine) + 2mM Thymidine for 24 h (S-phase block)
2. After Thymidine block: remove Thymidine by washing with 1xPBS; add fresh DMEM (10%FCS, 1% Pen-Strep, 1% Glutamine) for 3h to release cells
3. After the release, add 100ng/ml Nocodazole to the media for 12h (mitotic block)
4. Remove Nocodazole by washing with 1X PBS and add fresh DMEM (10%FCS, 1% Pen-Strep, 1% Glutamine) to release cells.

⇒ Cells progress synchronously through G1- and S-phase

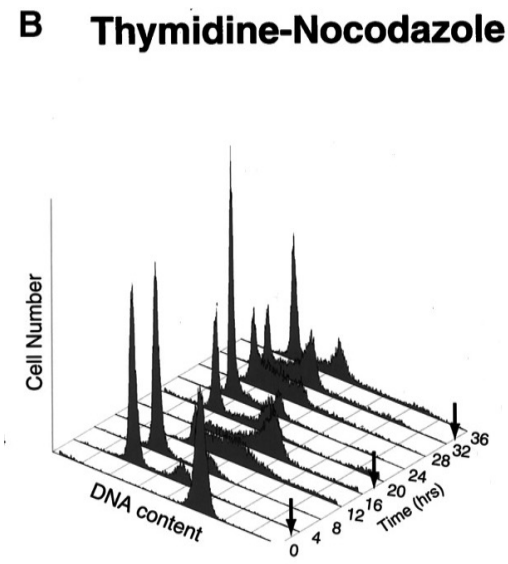

Figure B. Cells were arrested in mitosis by blocking first in thymidine followed by release and then blocking in nocodazole. After release from the nocodazole block, most of the cells (>75%) divided synchronously within 2 h of release from the arrest, entered S phase by 10-12 h after release, and completed the next synchronous mitosis by 18-20 h, ultimately completing two full cell cycles.

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III.) Serum starvation (G0/G1 block)

1. At 30-40% cell confluency wash twice with 1X PBS and add DMEM (1% Pen-Strep, 1% Glutamine) w/o Serum.
2. After 72h, re-stimulate cells with 10-15% Serum.

C Serum starvation

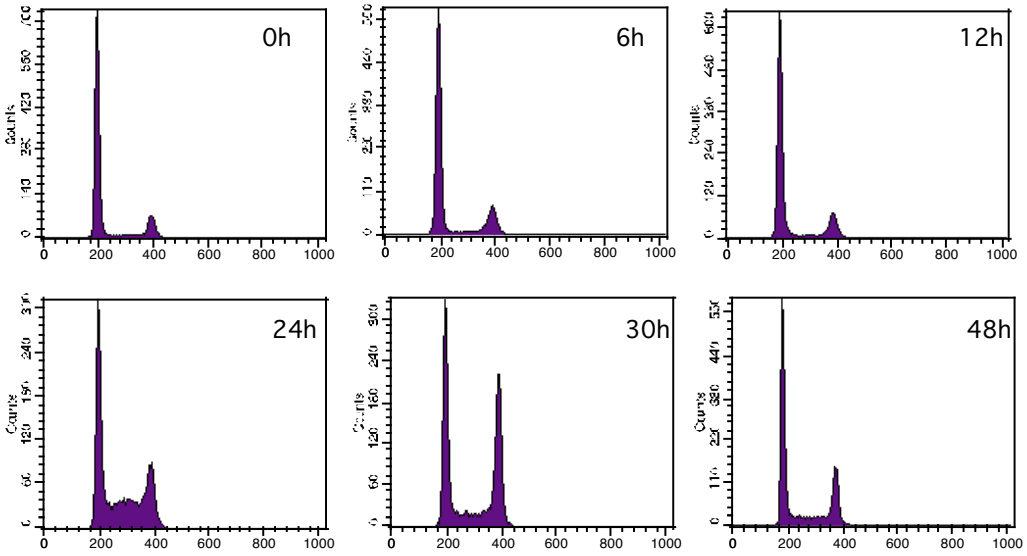

Figure C. Human diploid fibroblast (Tig3) were arrested in G1-phase by serum starvation. After release from G1 block, cells entered in S phase by 10-12 hours and completed the next synchronous mitosis by 30-48 hours. Cell Synchrony was monitored by flow cytometry of BrdU and propodium-iodide-stained cells.