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Cell Cycle Synchronization

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Procedure

- Use fresher “new” HeLa cells. They have a shorter doubling time and look “happier” i.e. rounder, less sickly looking, stick better to plastic).
- All steps in DMEM cosmic media, in 10 cm plates.
- All solutions should be made fresh.
- You can start with up to 500,000 cells per plate the day before you perform the first block, otherwise the cells will get too confluent and the blocks will not be as efficient.

First block: cells were placed in DMEM cosmic media (10% cosmic calf serum (Hyclone), plus Penn/Strep, L-Glu and NEAA) containing 2.5 mM thymidine (From 100 mM stock, sterile filtered) for 18 hours, followed by a fresh media release for 6 hours (Rinse once with fresh media, making sure sides of dish get rinsed).

The second block, in media containing 450 μ M L-mimosine (from 40 mM stock, sterile filtered), was also for 18 hours (You can go to 20 Hr for almost 100% block, but that is pushing the limit of whether cells will be able to release from the block), after which the cells were placed in fresh media (Rinse twice with fresh media. Rinse well or the release will not be as efficient). Samples were taken at time 0, and every 2.5 hours for 20 hours.

To synchronize a population of HeLa cells at G2/M to have maximum levels of H4K20 monomethylation, you cannot use nocodazole (H4K20 monomethylation levels will decrease despite the block). You must synchronize at G1/S using the above protocol, and then release and then collect cells at 10-12.5 hours post release.