APPENDIX 1. CRITERIA FOR A GOOD MICROARRAY/SYNCHRONY EXPERIMENT

Criteria for successful synchronization of cells

1) If newborn cells are produced by the synchronization method, there should be a minimal increase in cell number for a period of time covering a significant fraction of the interdivision time.
2) The rise in cell numbers during division should occur over a relatively small fraction of the total interdivision time. It may be as small as 10% for 90% of the final rise in cell number, or it may be as large as 20-25%. Knowing this value is important in judging a synchronization procedure.
3) At the time of synchronous division, the cell number should double. If cell number does not double, that means some cells are dead or altered; this minority of cells could be giving results that obfuscate the results emanating from the majority of dividing cells.
4) There should be at least two successive cycles available for analysis. If only one cycle is analyzed, the results may merely reflect artifacts or perturbations resulting from synchronization. Presumably, but not necessarily, these artifacts would be eliminated in the second cycle.
5) Successive generations (i.e., the time between rises in cell number) should be of equal length and equal to the doubling time of cells in exponential growth.
6) Data points should show synchrony without any need to connect points or draw a suggestive line indicating synchrony. The data should speak for itself.
7) The DNA distribution of cells should be narrow in the synchronized cells and these distributions should then reflect the movement of cells through the division cycle. Thus, newborn cells should be essentially pure cells with a G1-phase amount of DNA, the DNA content should then move through S-phase contents, there should be a period of time when cells have only G2-phase DNA contents, and then there should be a return to essentially pure G1-phase DNA contents.
8) The size distribution of newly synchronized cells should be narrower than the size distribution of the original population, cell size should increase as the cells move through the cell cycle, and during the period of cell division there should be a bi-modal distribution of cell sizes.
9) Cell numbers should be determined by a method that eliminates investigator bias. For example, electronic cell counting is to be preferred to microscope counting chambers.
10) Only selection methods can give synchrony. Whole-culture methods, using inhibition or starvation, cannot synchronize cells. This is not so much a criterion, as a theoretical rule regarding synchronization in general.
11) Alignment of cells so that cells all have a particular property in common (e.g., all cells have a G1-phase DNA content) does not mean that the cells are synchronized. Synchronized divisions are the *sine qua non* of synchrony.

Criteria for successful analysis of gene expression during the division cycle

12) Gene expression results should be replicated (with allowance for normal synchrony decay) in successive cycles. If data does not repeat over two or more cycles, the cells are very likely perturbed by the synchronization method.

13) Peaks in gene expression should decay when expression is studied over more than one cycle. This is because synchrony, if normal and unperturbed, should decay.

14) If a selection method is used, a mock selection should be performed where the selection procedure is performed but the cells are all recombined together and analyzed. These combined cells should not give a variable pattern of gene expression. This controls for perturbation of the culture by the selection method.

15) Results using different synchronization methods should give the same results. Different experiments should be reproducible in cyclicity and in phasing, and thus independent of synchronization methods. That is, the results should not depend on the particular synchronization method used.

Criteria for successful use of microarrays to analyze cycle-related gene expression

16) Analyses should be performed more than once, and the results should be "reproducible". The qualification on reproducible is related to the acceptance of some degree of statistical variation.

17) The data should be published in accordance with the MIAME (Minimum Information About a Microarray Experiment) or MAGE (microarray gene expression object model) standards, so that the public data can be analyzed [58-62].

18) Microarray results should be compared to randomized data to show that the observed cyclicities are not the result of random noise or experimental variation. Satisfaction of this criterion, however, does not mean the results are necessarily related to the cell cycle, as perturbations of cells by a synchronization procedure may still be present.

19) Criteria for successful identification of cyclicity should be determined before microarray analysis.

20) Both false positives and false negatives should be considered in the analysis. Just because a particular gene result fits pre-existing data collected by classical means, one must not consider this a support of the results unless the previous synchronization method was different. Otherwise the microarray experiment just repeats the same experiment, with a repetition of the artifacts of synchronization in two independent experiments.

21) If some genes are expressed differently in two successive cycles this should invalidate the entire experiment—even for those genes that are expressed similarly in two successive cycles—because the non-repeating patterns indicate that there are artifacts produced by the synchronization.