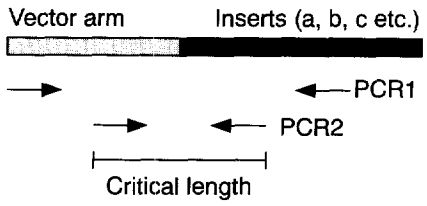

Figure 2. *A nested polymerase chain reaction (PCR)-based method for determining the 5′ end of cDNAs. The PCR product from the first reaction (PCR1) is isolated and then subjected to a second PCR reaction (PCR2) to increase the specificity. The PCR products from several clones (a, b, c, etc.) are isolated and the longest products are sequenced to give the complete 5′ end. The critical length defines the maximum length consistent with its genomic sequence.*

Cancer – an excellent example

Attempts to address the oncogenes and anti-oncogenes associated with particular tumours have been hindered by the basic genetic instability of neoplastic cells. With full-length sequence information, it is possible to identify the mechanism(s) responsible for instability and model the observed mutations into yeast, fly and worm systems. Scientists have begun both to classify tumours according to their drug susceptibility (reflection of the mutant proteins) and to design therapies. Several groups [Hartwell, L.H. *et al. Science* (1997) 278, 1064–1068; Weinstein, J.N. *et al. Science* (1997) 275, 343–349] have been classifying tumours and tumour cell lines by their drug responses. These studies have defined constellations of tumours as a function of such properties as *p53* alterations and cell-cycle abnormalities. Furthermore, they can predict the mutated genes by the pattern of drugs to which an unknown cancer (or cell line) shows heightened sensitivity.

A new potential-target-selection strategy, described by Hartwell and coworkers, using so-called synthetic lethal mutations, has resulted from the availability not only of complete expressed sequences but also of a complete genome sequence for yeast. A synthetic lethal mutation is one that compromises a gene in a parallel or a related pathway, rendering cells bearing the primary mutation (and only such cells) inviable. The secondary lethal mutation can be found by testing candidate loci, based upon a knowledge of biochemical pathways. Alternatively, a genome-wide scan of secondary sites can be performed to identify lethal mutations specific to yeast, fly or worm cells with the primary defect. Once such a lethal mutation is observed and the corresponding gene identified, a treatment can, theoretically, be developed in five further steps:

- Selection of pathways and alternative targets in yeast, flies or worms by introducing a homologous (to a putative human tumour locus) mutation and searching for a synthetic lethal mutation.
- Identification of the homologue to the alternative target, indicated by the synthetic lethal mutation, in mammals.
- Verification that the analogous mutations are synthetic lethal in mammalian cells. This will require a delayed knockout of the second target to demonstrate effectiveness against a growing tumour.
- Simultaneous evaluation of the pharmacological feasibility of inactivating the second gene. Generally an enzyme with a well-defined substrate is the target of choice.
- Initiation of high-throughput screening. Putative ligands for the chosen (synthetic lethal) target can first be designed by molecular modelling.

Summary

Genomics is presently undergoing a switch in emphasis from sequencing and mapping to the analysis of gene function – from 'structure' to 'function' of the genome [Hieter, P. and Boguski, M. *Science* (1997) 278, 601–602]. This reflects a more general move in biology to use pathology as an indication of gene function, rather than identifying genes to better understand and classify pathology. Thus, we are moving from a technology that classifies data to one that mines that data for valuable insights and extending the information based upon the biological hypotheses that evolve. This move has been made possible by the completion of the genomic sequence of the budding yeast, *S. cerevisiae*, and those of about a dozen prokaryotes. Short of the complete sequence of the human genome we can gain tremendous insight from studying complete expressed sequences of human genes and comparing them with homonyms in other eukaryotes.

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High-throughput screening

Managing the HTS laboratory

The role of screening in drug discovery has undergone enormous change. Twenty years ago, an academic postdoctoral scientist entering the industry might have considered the acceptance of a one-day-per-week screening commitment to be a necessary compromise in order to be able to do 'real science' during the other four days of the week (without the necessity of writing proposals for research grants).

By contrast, the modern screening laboratory is at the apex of the drug discovery process. Biomolecular screening is recognized as a scientific discipline in its own right, situated at the point of convergence of the many diverse technologies and sciences involved in generating a 'hit' and converting it into a lead compound.

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And today it is not 'screening', but 'high-throughput screening' (HTS) – a highly visible operation requiring an entire company division directed by managers at the Director or Associate Director level.

Screening environment

What characteristics are needed to manage an HTS facility? The immediate response of Dr Carol Homon, Associate Director of Biomolecular Screening at Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT, USA) is that an HTS manager has to be 'tough as nails because the HTS laboratory is often in a no-win situation'. She explains that, because so many different people within the organization are customers of the HTS laboratory and because each customer has a slightly different set of priorities, it is not always possible to satisfy everyone completely. According to Homon, the first principle of HTS management is that it requires a person who can function effectively and set priorities under less than ideal (and, in fact, sometimes chaotic and ambiguous) conditions.

John Babiak of Wyeth-Ayerst Research (Princeton, NJ, USA), writing in *High Throughput Screening* [Devlin, J.P., ed. (1997) Marcel Dekker] makes similar observations when he discusses the expectation and perceptions of HTS by senior management as compared with the current realities of the discipline. According to Babiak, senior management frequently have expectations regarding the number of assays that are possible based upon idealized demonstrations by equipment vendors. 'Generous verbal estimates of capacity and throughput assume 24-hour/365-day operation', writes Babiak. The manager of the HTS laboratory must be able to deal with these very high expectations from senior management – most of whom have no real experience in working with a centralized HTS operation – on the expected return from the considerable funds invested in the screening facilities. At the same time, the HTS manager must deal with the demands and conflicting priorities of research colleagues, who are the customers of a centralized HTS operation. Such demands are invariably limited by insufficient resources compared with demand for services.

Assessing new technologies

The effective manager of an HTS operation, according to Homon, must also be an exceptional judge of new technology, have the ability to discern fact from fiction, and be willing to take a chance on a new technology when warranted. Obtaining this level of judgment comes only with experience, explains Homon. It makes sense that the person in charge of screening should be a veteran of the organization, and who knows the drug discovery process very well from first-hand experience.

Dealing with burn-out

The inherent value of an HTS screening assay – the sameness from day to day – is just what makes it difficult for humans, who naturally crave change and creativity in their work, to run the same HTS screening assay over long periods of time. Babiak recognizes that an HTS manager must also be effective in dealing with staff 'burn-out' from such repetitious activity. The manager, notes Babiak, should motivate staff; for example, by ensuring that key staff members are not assigned the most repetitious work of actually running the assays on a day-to-day basis. 'Entry-level staff within the robotics group and technicians who work with the scientist customer are two obvious sources of people to operate robots during a high-throughput screen', writes Babiak.

Boredom and burn-out can be combated by occasionally rotating the staff to different assays and activities in the laboratory. Whenever possible, the staff who run the screening assay should be involved in the development of the assay or at least apprised of the scientific nature of the assay. This makes their job much more intellectually satisfying. Moreover, if they understand the assay, they will be more attuned to a problem that may develop or pick up on an unusual finding while conducting the assay. Finally, people with certain personality types (an ISTJ type on the Myers–Briggs Personality Inventory for example) are more capable of handling routine activities than are other personality types. It would fall to the manager of the HTS facility to become an expert in selecting staff and in putting together work teams with the optimal mix of personality types to fit the nature of the screening activity.

Meeting customer needs – a juggling act

Babiak acknowledges that a further major challenge facing the HTS manager when working with scientist customers is that it can be extremely difficult and frustrating. Such tension is understandable. Each scientist customer wants to get their assay on line as quickly as possible, frequently does not understand the limitations on assay complexity posed by use of centralized robotic screening facilities, and may not understand the competing priorities for limited equipment. The HTS manager must anticipate these reactions from the scientist and retain him or her as an enthusiastic customer even if it is not always possible to provide services to the desired timetable. Babiak notes that the HTS managers that do this successfully are those that 'think creatively and holistically to visualize a clear integrated solution to current and future assay needs...while possessing the intuition and interpersonal skills to work constructively with scientist customers with focused needs'. Thus, a collaborative style between the HTS manager and the customer scientist frequently works best. In this relationship, the HTS manager is fully aware of, and sensitive to, the organizational pressures imposed upon the scientist and gives the scientist 'serious attention, honesty and frequent communication'. This should provide an atmosphere of effective compromise in the face of conflicting priorities.

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