

Automated colony counting for molecular biologists

How it can improve productivity when selecting recombinant clone

Introduction

Transformation efficiency is a measure of the amount of cells within the bacterial culture that are able to take up DNA molecules. Some applications such as the construction of genomic libraries require that the bacteria have a high transformation efficiency. Counting of recombinant colonies is one criteria used to calculate transformation efficiency.

A common method for selecting recombinants involves altering the antibiotic resistance, eg, resistance to ampicillin or tetracycline, cells that contain the vector (plasmid) will survive and cells which do not contain the vector (plasmid) will not survive. Another method is to use a vector (plasmid) such as pUC which has a LacZ gene present. By inserting DNA within the LacZ gene this will inactivate this gene and colonies will remain white. Plasmids which do not contain the inserted DNA will have an active LacZ gene present producing blue colonies.

Manual counting of recombinant clones – the drawbacks

Manual methods of enumeration require users to count colonies or plaques using a light box and pen and then transfer the results into a computer. This is not only a time consuming and tedious task but can leave the way open for misinterpretation with plate reading (especially where trying to distinguish coloured from white colonies). Computer keying errors can also occur. In addition, because this method produces no computerised image of the plate alongside the count there is no means of carrying out an independent audit of the results to check for accuracy.

Automated counting of antibiotic resistant recombinants

Enumerating recombinants that have antibiotic resistance involves a total count of all colonies or plaques on the plate because only those recombinants that have antibiotic resistance will grow. Synbiosis recommends the aCOLyte or ProtoCOL systems for any types of total count because both systems can count colonies and plaques on spiral, pour and spread plates.

For molecular biologists with a limited budget that have to perform very straightforward counts of recombinant colonies or plaques, aCOLyte is the perfect choice. aCOLyte is a cost-effective, yet accurate alternative to using a traditional light box and pen.

The system comes complete with software, which can be quickly installed on virtually any laboratory PC. It also has two arrays of LEDs allowing incident,

transmitted and darkfield illumination, enabling colonies and plaques to be counted on a range of media types.

With aCOLyte Click'n'Count, enumeration is at least as fast as any light box counter because counting involves pointing at each colony and clicking to count. However, since the results are automatically transferred to PC, the time consuming data keying step is eliminated. For researchers wanting to increase sample throughput, Synbiosis offers a fully automated version of aCOLyte, SuperCount. The system can count up to 10^3 colonies in less than two seconds, while also automatically correcting for background variations and different media types and can provide time savings of up to 90 per cent on a manual count. This greatly increased laboratory productivity allows molecular biologists to make instant decisions about whether to discard plates or continue working with the recombinants they have.

For molecular biologists that have plates where the colony counting is complicated by having for example touching or non-recombinant satellite colonies present, Synbiosis recommends the ProtoCOL systems. These are fully automated colony counters, which integrate a CCD camera with extremely sophisticated software and each comes complete with its built-in own PC. The software features a unique auto-separation algorithm, which means touching colonies can be automatically, enumerated as separate ones, thus reducing the need to manually correct counts. For researchers experiencing problems with small non-recombinant satellite colonies, the software allows differentiation based on definable size limits so small colonies can be excluded from the final count. In addition, because the software allows users to set the size of the round frame placed on the plate image, it means that edge effects such as bubbles or pen markings can also be eliminated. Other problem areas within the plate, for instance, agar lumps or fungal contamination can also be removed from the count by manually drawing around them on screen. All these features ensure the count is highly accurate.

Both the aCOLyte and ProtoCOL systems produce live, full-colour on-screen images that can be saved with a time and date for GLP compliance. Images can be archived for future reference, if necessary, or can be printed out for reports or presentation material. This feature is important to companies involved in using recombinants for drug discovery research because it provides secure records that are compliant with the information required by external regulatory auditors.

Differentiating and automatically enumerating different colour colonies

Differentiating and counting of recombinants based on their colour are more demanding tasks to perform manually. This is because they rely on molecular biologists being able to accurately define by eye different coloured colonies, which in the case of the blue white selection system can vary from one researcher to another. To overcome this subjectivity in manual colour differentiation, Synbiosis recommends the colour option for its ProtoCOL systems. This enables automatic discrimination and counting of, for example, blue and white colonies on the same plate. This means the colour discrimination can automatically be set at an agreed level by all the users in the laboratory thus eliminating the need to spend time and effort deciding if a colony is white or coloured before counting it.

The ability to save full colour plate images is particularly useful for ensuring that the colour discrimination application is set at the correct sensitivity for automatically distinguishing and counting recombinants from non-recombinants.

Conclusions

The flexibility of the aCOLyte and ProtoCOL systems means that they can be used to rapidly count antibiotic resistant recombinant colonies on spread or spiral plates, as well as colonies and plaques on pour plates. The aCOLyte is excellent for simple total counts, whereas when the count is more complicated with different sized or touching colonies involved the ProtoCOL is a better choice. For the added sophistication of being able to discriminate and count recombinant plaques or colonies of different colours from the same plate, Synbiosis's ProtoCOL with the colour option is ultimately the superior system to use.

In summary, the use of automated colony counting for enumerating recombinants can eliminate a substantial part of an unpopular task, as well as take a fraction of the time it would take to perform this task manually. The knock-on effect of this increased productivity is it can ensure that making decisions about whether to continue working with or discarding recombinants becomes virtually instant. In some cases, this makes it possible for molecular biologists to undertake further processing of clones much sooner in the working day, thereby allowing results to be generated up to an entire day earlier.

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06.08.09

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