The Challenges of Managing New Biological Entities (NBEs)

As high throughput technology is implemented across laboratories and research facilities on an ever-increasing scale, the generation of new biological entities (NBEs) continues to rise. While expanding collections of NBEs hold significant and exciting potential, for example in terms of therapeutic applications, the ability to manage such a volume of new agents is critical. This article reviews the typical requirements and demands that are placed on sample management teams when coordinating NBEs management at a moderate scale. From practical experience implementing several systems within Pharma companies, the authors have learnt that there is more to the world of NBE management than just the tracking of containers in-and-out of -140 °C dewars. Below we have created 7 points points of guidance for managing NBE's.

The expansion of Pharma research into genomics, and subsequently proteomics, has fuelled the surge in the numbers of NBEs and, in contrast to bio-banking, the value of samples is extremely high. Primarily, this is because they are usually the result of complex engineering; requiring multiple skilful and time-consuming steps to yield a small amount of pure product. Furthermore, in some cases, their biological potency and toxicity are not quantified, which can cause concern (particularly if live strains are used), as potentially these can be hazardous to human health. Therefore it is rational that the use of these samples is managed conservatively and with great care.

1. Diversity rules

In the world of NBEs there may be a wealth of different sample or entity types that need to be managed. For each of these there will be different sets of associated properties or metadata - some properties may be common between sample types, others unique to that type (**Table 1** lists some typical entity types, sub-types and examples of associated properties). Although currently there are standard ways of representing nucleic acids and proteins at the sequence level, beyond this, the characterisation information becomes complex and variable. This diversity means that it is of paramount importance that a sample-management system for NBEs can be flexibly adapted to understand new sample types and manage any arbitrary number of their associated properties.

Furthermore, in contrast to small molecule collections, there is often little consistency in the registration systems used to record information about samples. It is common to encounter multiple registration systems in use, and some or all of these may need to be retained and integrated with an inventory management system. A sample management system flexible enough to integrate seamlessly with existing systems is therefore advantageous.

Entity class	Example types/sub-types	Example properties
Proteins	Hybridoma Supernatant Antibody - Therapeutic antibody - Reagent antibody - Bispecific antibody Antibody drug conjugate Antigen - Screening target - Assay reagent	Owner, Primary ID, Secondary ID, Name, Storage temperature, Species, Gene ID (e.g. Entrezgene Id, HUGO name), Parent expression cell line ID, Tag Name, Sequence, Endotoxin, % pure, Isotype, Affinity, Size Exclusion Chomatography, Epitope Binning, Thermal stability, Thermal reversibility, Epitope mapping, Molecular Weight, Concentration, Volume, Formulation Buffer, % purity, ELN reference
Nucleic Acids	Total RNA Plasmid DNA Genomic DNA shRNA - Library - shRNA clone - microRNA	Owner, Primary ID, Secondary ID, Name, Storage temperature, Species, Gene ID (e.g. Entrezgene Id, HUGO name), Parent Id, Vector Name, Sample Buffer, % pureVector Resistance Marker, Concentration, Volume or quantity, 260/280 ratio, Sequence, , ELN reference
E. coli cells	cDNA clone expression strain shRNA library shRNA clone	Owner, Primary ID, Secondary ID, Name, Storage temperature, Vector Resistance Marker, Host strain, Construct identifier, ELN reference
Mammalian cells	Expression cell-line Hybridoma Transfected Primary cells Cell paste Membrane preparation	Owner, Primary ID, Secondary ID, Name, Storage temperature, Species, Vector Resistance Marker, Source (external, in-house), Tag, Vector Name, Mycoplasma, Concentration, Volume, Sample Buffer, ELN reference

Table 1 - Illustration of the diversity of NBE-related entity types and properties

2. Parental guidance

It is important to track and record the inter-relationship between different entities and entity types – specifically their precursors. Using recombinant proteins as an example, it is beneficial to be able to track the parent-child relationships between, and capture data relating to, any intermediate entities (created through the steps of transfection, cloning, expression and passage) and the eventual product. The end-product may be the highest-value part of the chain, but being able to reference unambiguously to the parent expression clone, the host cell type used and to information about growth and expression conditions, is vital for reproducing the production process.

At each step of the process many inventory items (vials, cryotubes, flasks) will be created, which may be stored in temporary fridges and freezers or committed to longer-term storage. Keeping track of this inventory "explosion" is an increasing challenge with rising throughput, but critical due to the high value of these engineered products. A system that can automatically track movements between diverse locations and understand 1D or 2D barcoding makes this task much simpler and less error-prone.

Figure 1 provides an illustration of this typical workflow- the key entities and inventory produced. In this case the primary entity is an expression construct, but could be a DNA fragment of known sequence. This entity should be recorded, along with various property values and known inventory. It is likely that the host cell line itself is maintained as inventory, and therefore information on the cell line, quality, etc. will already be recorded. Upon transfection this expression cell line may also be managed as inventory, and its derivation via the transfection event must be recorded. Furthermore, resulting individual pools and clones will need to be tracked and key analysis results recorded against them.

Following initial screening, some clones will not progress any further but tracking their disposal is helpful for reducing inventory 'clutter'. Ultimately, the expressed protein will have its own set of properties but the ability to track this protein's relationship to parent DNA and to all other entities (tubes, plates and locations) is of great value.

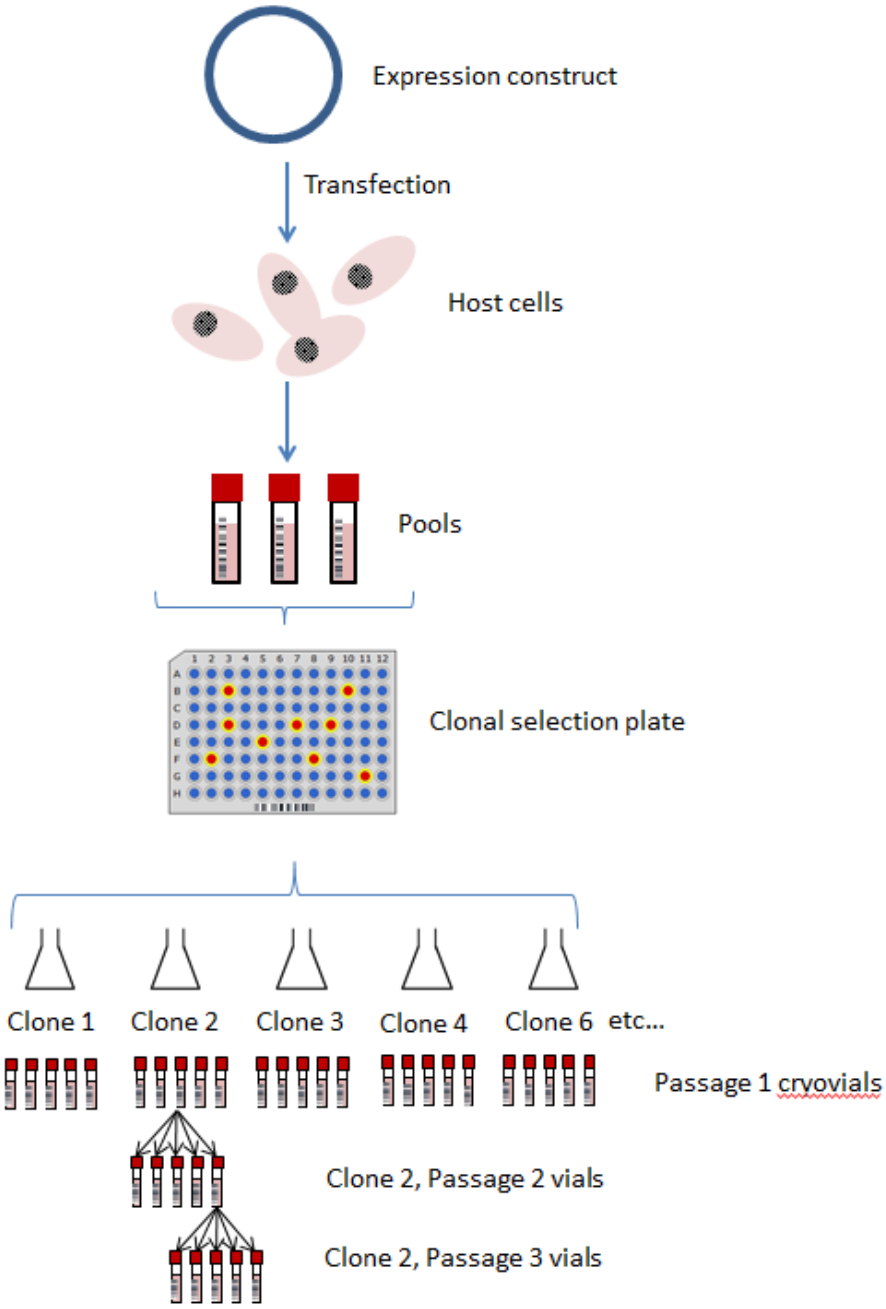

Figure 1 – Entities and associated inventory in a typical NBE workflow

3. Collection Management

Once the biological entities can be represented, they will be stored and held in containers deemed most suitable for storage, downstream handling, material containment and volume. It is important to check that when items are placed in containers, or moved to another location, that the container and conditions are compatible with the samples therein. Ultimately it is necessary to have mechanisms that not only record the container and storage properties, but also record compatibilities, restrictions and limitations, raising error warnings if these rules are breached.

Typically, users want to manage sample collections in useful 'sets'. Perhaps a particular area of study will want to use NBEs with specific characteristics, and may want to have exclusive access to such items. This highlights the realm of restriction requirements, which are typically based around restricting the use of individual containers or sample sets to a particular team or project, limiting the use of stock should it fall below a defined threshold level, or enforcing a timed restriction on sample use (e.g a quarantine period).

4. Workflows make history

The management of NBEs has to support the preparation steps and entity relationships not just sample inventory. In most environments, the steps to create an NBE are not automated, but manually intensive and confirmatory tracking is not deployed. Ideally, from a quality perspective, all steps leading to the provision of the desired biological entity would be planned, all of the related substances and biological entities would have barcode identification and the manipulation events would automatically be recorded. In essence, some form of workflow management would underpin a high quality audit trail.

Equally for the maintenance of NBE stocks, workflows could be triggered to replenish stocks, for example if quantities fall below threshold, or if entity expiration dates are approaching.

Workflow, when used in conjunction with sample identification and tracking, brings quality and consistency, and typically there will be an automatic chain of custody recorded in the audit trail. The choice of identification system is broad, from microMEMS tags to RFID; however, the most commonly used technique is 2D-barcoding, and simple devices are now available for bulk barcode reading of many container types.

Example picture of 2D rack barcode reader with rack of tubes, courtesy of Ziath UK Ltd

5. Organisation plays a part

The best return on investment in a sample management system may require some organisational changes. For a number of companies, centralising some of their NBE facilities has proven successful, and the pooled fulfilment capabilities can also justify the investment in automated instruments e.g. for cell culture and biological analysis tools.

To compensate for centralisation there needs to be a mechanism to provide access to the NBE samples, which supports timely turn-around and allows the end users to request the samples in the familiar formats. Samples can then be prepared and forwarded to requestors in a service-like operation. Typically there are request requirements that provide the requestor with many choices, perhaps to select samples by their characteristics, and these will differ according to the different types of sample (e.g. for cells there may be selection rules around the passage number and for proteins there may be purity criteria). In addition, there are a wide range of choices for delivery format (Table 2).

Delivery Choice	Format Details
Labware type	Vial and vial type, tube and tube type, microtitre plate (96/384) and choice of plate type
Quantities	Mass, volume/concentration (mg/ml, or mM), number of cells etc.
Layout	Empty wells/control/standards/NBE
Profile	Dilution series (typically from a list of different series), or common concentration
Options	Capping and seal types, project ownership, set membership, labelling choices
Distribution	For storage (with location) for delivery (e.g. requestor location)
Priority	Date Time required, High/Medium/Low
Shipment	Shipment documentation choice, despatch notes

Table 2: Sample Delivery Format - Choice and Details

All of these requirements need to be taken into account by the central fulfilment site if they are to provide a service equally as flexible as the traditional, locally managed collections.

It is appreciated that once centralisation is available, there are economies of scale that can be realised when maintaining the inventory. In the same way that requests are used to distribute the NBEs, the inventory can be checked for low stock levels and internal replenishment requests can be made, probably triggering re-stock or replenishment workflows. These steps require a convenient enquiry and report mechanism that can help with the replenishment decisions.

6. The Prevalence of Automation

There are many bench-top systems for sample preparation, and a variety of options for automated sample storage. Where automation is needed to handle high capacity operations, or where high sample integrity is needed, it is usually preferred that this can be easily integrated to respond directly to preparation criteria. Importantly for storage systems, this means they can respond to large numbers of requests while requiring minimal human attention.

With modern sample management systems it is expected that they can support a broad variety of NBE workflows with a high degree of autonomous operation, generating high quality audit data, and providing confirmation of transactions at critical handling steps.

7. Provider considerations

As well as the functional requirements that have been described already, there are non-functional requirements that should be taken into consideration when considering systems to manage NBEs. These typically focus on the supplier, its support services and application knowledge. Companies are increasingly reluctant to develop in-house solutions, and turn more frequently to off-the-shelf applications. While off-the-shelf applications have the benefit of long-term product development, maintenance and support, some products will require significant configuration effort that can only be done by experts, usually aligned to the provider company. Nowadays, many users expect to have access to configuration mechanisms to support new NBE workflows and be able to make such changes through the user interface.

Successful NBE Management

Providing a successful NBE management system requires consideration of informatics, workflows, business rules and operational considerations. An affirmative account of these is vital to fully support the needs of progressing research and be cost effective.

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