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The Manufacture and Setup of Single Use (SUB) Bubble Column Reactors (BCR): Litre-Scale Expression of Recombinant Proteins for Structural Biology and Drug Design (SBDD).

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ASAP Discovery

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

Structure-Based Drug Discovery (SBDD) is an approach to drug design which involves crystallising disease target proteins unbound and with candidate small-molecule binders, to observe and analyse the binding interaction, or to identify novel binders that can be developed into lead-like compounds.

Obtaining sufficient protein quantity and crystal forms amenable to soaking with candidate binders remains a bottleneck. The Centre for Medicines Discovery's (CMD) approach to this problem, is to design multiple constructs to explore protein expression and crystallisation behaviour. Protein crystallography (PX) has a high demand for protein quantities, a limiting factor in this workflow is the production of sufficient quantities of the multiple proteins for our experimental needs. This burden increases rapidly with the addition of each construct.

Existing shake-flask workflows for protein expression do not scale adequately to meet these requirements. To address this bottleneck a novel, single-use Bubble-Column Bioreactor (suBCR) array was developed, that allows for parallel 1 L E. coli batch cultivation. This bioreactor array will be incorporated into a Gene-to-Product workflow aimed at increasing protein production efficiency by increasing throughput whilst reducing labour burden.

This protocol covers materials, manufacture, and general system set-up. For a more complete protocol addressing system operation, the reader is referred to: "Setup and Operation of Single Use (SUB) Bubble Column Reactors (BCR): Litre-Scale Expression of Recombinant Proteins for Structural Biology and Drug Design (SBDD)."

Guidelines

The reader is referred to the following protocols, from which this protocol was developed:

- Parallel rapid expression and purification of proteins for crystallography (PREPX): large scale 1 L cultures. Fairhead, M. dx.doi.org/10.17504/protocols.io.4r3l22jkxl1y/v1
- Parallel rapid expression and purification of proteins for crystallography (PREPX): 48× 100 mL cultures V.2. Fairhead, M. dx.doi.org/10.17504/protocols.io.yxmvm35zbl3p/v2

Materials

Water bath:
Any sturdy container capable of holding 20-25 litres of water can be used. A 60×40cm EuroBox/KLT Box works well e.g. Ref: BK-SV64/42, **https://www.plastor.co.uk/**

Temperature Control:
Submersible aquarium heaters work well from ambient to 34-36°C, their typical limit. A 200-300w device is more than adequate e.g. https://www.tetra-fish.com/products/heaters/ht-submersible-heaters.aspx. Never remove these heaters from water until they are completely cold.

An aquarium recirculating pump must be installed in the water bath to stop temperature gradients.
Programmable Sous Vide catering immersion circulators have been used to great effect (>>37°C). Cell culture is not a use intended by manufacturers.

Metabolic processes will cause self-heating of the culture to 25-27°C. Protein expression below this will require a recirculating lab water chiller.

Air Pump:
An aquarium diaphragm air pump can be used for air supply, e.g. https://www.tetra.net/en-gb/products/tetra-aps-aquarium-air-pumps-anthracite
A small pump should be able to supply >6 bags. Larger models are available.
These pumps run at mains electrical frequency which varies at times.

Consumable Materials:
Air line:
Use a semi-rigid food or lab grade airline or pneumatic tubing of 4-4.5mm internal diameter eg:
Legris 1025U Series Polyether PUR compressed air pipe
Silicone tubing will be too flexible to stay in place during use. Excessively stiff polymers may be difficult to fit airstones into.

Air stones:
Use 25-30mm cylindrical air stones. They are widely available from aquarium supply retailers, e.g:
https://www.tetra.net/en-gb/products/tetra-as-air-stone

Flow Control Valves:
Expensive variable area flow meters are not required. Push-fit metering valves can be used, but must be selected with care; even precision valves are extremely sensitive to minor adjustments, chose a model with an indexable display and snap-locking cap e.g. SMC's AS1211FS-M5-06.

Single-Use Bubble Column Reactor (Bags):
Source 3-inch (75mm) wide layflat tubing (LFT) from a packaging supplier. When filled, they will form a turgid cylinder of 48-50mm diameter, so the foam stopper must be matched for size.

LFT is readily available from packaging suppliers, e.g:
https://www.kitepackaging.co.uk/scp/polythene-tubing/lay-flat-polythene-tubing/

However, there are many similar types not all of which will be suitable. Polythene (PE) is the most common type, but is can soften and stretch at 37°C, which can leak to pin-hole leaks. Therefore, choose a heavy (125 micron / 500 gauge), or extra heavy duty (250 micron / 1000 gauge) thickness. PE cannot be autoclaved, so should only be used in conjunction with an antibiotic.

The bag can tear if not handled carefully. Improper storage or repeated folding causes cracks in the plastic, especially at the creases that are formed when the LFT is on the roll.
Alternatively, Polypropylene (PP) can be used. Some grades can be autoclaved for example BOPP (Biaxially-Oriented Polypropylene) or 'Autoclavable Layflat Polypropylene Tubing':

- Item: 27-4-53, **https://www.associatedbag.com/**

Foam stopper:
The diameter should be somewhat larger than the bag, so that the stopper gently grips the inside of the bag, helping to secure the airline.

- Indenti-Plug L800-E Plastic Foam Stopper, 46-65mm Openings
- Item: WZ-06298-82, https://www.coleparmer.co.uk/b/indenti-plug

Injection ports:
Self-healing, adhesive injection ports for inoculation and sampling are made by 3M, and are available from on-line retailers, and mushroom growing supply stores.

Hanging Rail and Optional Enclosure:
Any hanging rail, or even a wire shelving rack can be used to suspend the bags over the water bath. A rail height of 1.2-1.5m is a convenient working height.

For the prototype apparatus, and rail was constructed from aluminium t-slot extrusion, such that the rail, water bath and bags would fit inside a laboratory incubator.

Before start

The reader should be familiar with the companion protocol "The Manufacture and Setup of Single Use (SUB) Bubble Column Reactors (BCR): Litre-Scale Expression of Recombinant Proteins for Structural Biology and Drug Design (SBDD)."

Preparing the Airline:

1 Measure out enough tubing to run from the control valve to bottom of the bag, plus a small allowance, 1.5-1.6m. Cut cleanly and squarely with a tubing cutter.

2 Insert an airstone in one end.

3 Make a small cut in the foam stopper through its centre, and thread the airline through. The foam should gently grip the airline keeping it in place.

3.1 A pipette tip or similar pointed implement can be used. It need not be very sharp but should be smoothly tapered and of a similar dimension to the tubing being inserted.

3.2 Slide the foam stopper down the airline to about 70cm above the airstone.

Making the Single Use (SUB) Bubble Column Reactors (BCR)

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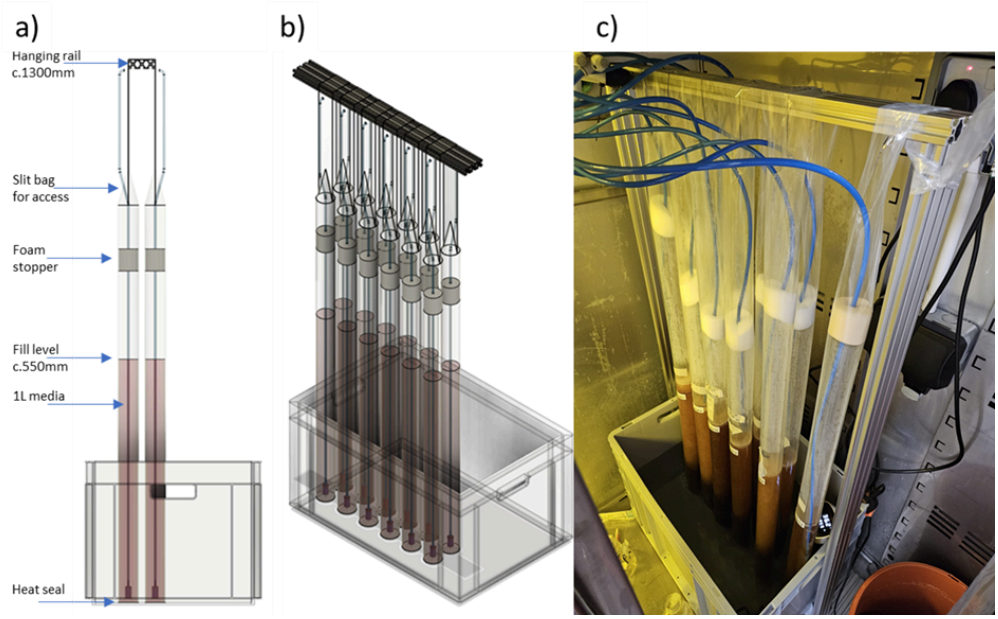

Bag setup: a) Guidance for Bag manufacture and assembly, b) Arrangement of the Bags over the hanging rail, c) A Bag Array in operation.

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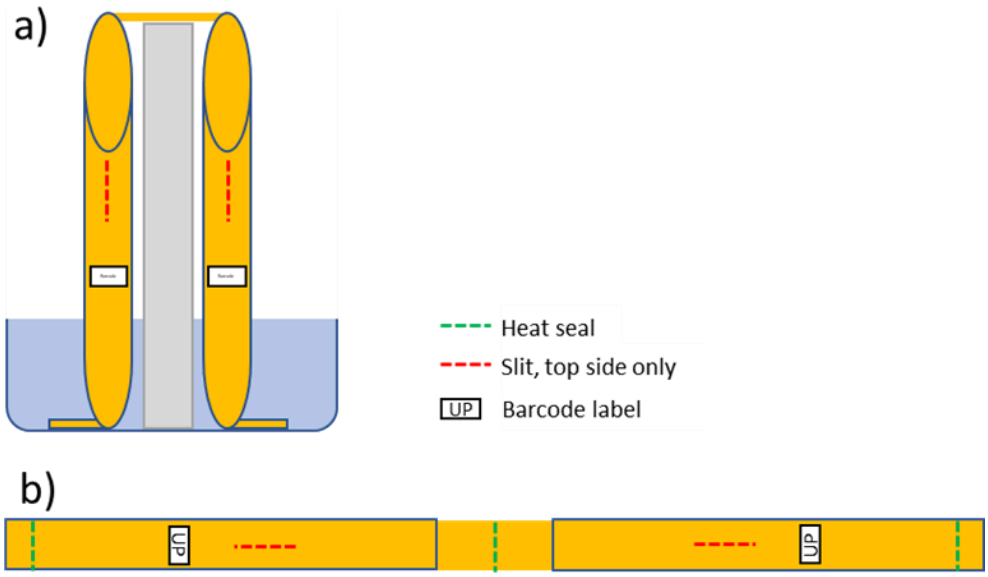
Note

Bags are made in pairs and hung over a rail such that they provide counter balance for each other.
Exact bag dimensions will depend on the height of the hanging rail used. Size the bags so that the bottom of each bag can rest on the bottom of the water bath without sagging over. This way the bags remain perfectly upright with less strain on the upper portion of the bag, or on the bottom seal .

6



A schematic of a) the how the bags are used, and b) how the bags should be marked-out and manufactured, when laid out.

7 Assuming a 1.2m tall rail a total c.2.7-2.8m of LFT should measured out per bag pair. Cut the end squarely.

8 A seam allowance should be added at the base of each bag for sealing (20-30mm), plus enough to span the hanging rail.

9 Heat seal the end of each bag with an impulse sealer. Exact heat sealing and dwell times will depend on material type and thickness, and sealer.

10 Make a c.100mm vertical slit in the centre of the outer face of each bag, below the point at which the bag hangs free of the rail. This is for airline and filling access.

11 Once made, inspect bags carefully for splits and other potential sources of leaks. Avoid folding or creasing the bags, and store them hanging up.

Labels and Marking the BCRs

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> Note
>
> PEs and PPs are low surface energy polymers that take inks and adhesives poorly. Adhesive tapes and labels specific for plastics/wet conditions/low temperature are available from suppliers such as LabTAG.com.

13 Place a self-healing, self-adhesive injection port above the liquid fill level as the highly curved bags cause leaks from behind the port.

14 If marking sample identifiers directly on the bags Sharpie brand permanent markers work well. Some paint-based markers can also be used. Surface primers help ink adhesion but aren't essential.