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Medium throughput protein expression and purification for protein design using heat lysis

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We use this protocol and it's working

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Abstract

This protocols describes medium throughput protein expression in *E. coli* using heat-lysis as a built in purification step. It is intended for preparation of heat-stable *de novo* designed proteins for screening *in vitro*.[1-3] It provides a small but relatively pure lysate sample of the protein within 2 working days.

Guidelines

Remember to always keep track of the samples when transferring between plate types.

Gene of interest should be in a pET system or another IPTG inducible expression vector.[4]

Materials

Equipment:

Suggested products will be in parantheses

- Flowbench, appropriate for working with bacteria
- Water bath
- Shaking incubator, preferably with sticky surface for securing plates
- Centrifuge with adaptor for multiwell plates
- Multichannel pipettes taking 1-1000 µL volumes
- Heatblock that can evenly heat 96 wells to 95°C (Eppendorf® ThermoMixer® with deepwell plate smartblock)
- Platereader for absorption at 600nm
- SDS-page setup

Materials:

Suggested products will be in parantheses

- Plasmid DNA at ≈ 100 ng/µL . (We use synthetic genes cloned into the NdeI-XhoI sites of pET28a+ and stored in 96-well plates)
- Chemically competent *E. coli* BL21 (DE3) strain
- Lennox Broth medium (Pr. liter: 10.0 g Tryptone, 5.0 g Sodium Chloride, 5.0 g Yeast Extract)
- Appropriate antobiotic (e.g. kanamycin for pET28a+)
- ZYM-5052 autoinduction medium[5]
- Sterile reservoirs for multichannel pipettes.
- Sterile 96-well plates (UNIPLATE Collection and Analysis Microplate, 96-well, 2 ml, natural polypropylene, round well bottom)
- Sterile 24-well plates (UNIPLATE Collection and Analysis Microplate, 24-well, 10 ml, natural polypropylene, round well bottom)
- Breathable film (AeraSeal™ film)
- 96-well plate able to withstand 95°C (Eppendorf® Deepwell Plate 96/1000µL)
- 96-well filter plate (AcroPrep™ Advance filter plates for Lysate Clearance - 1 mL, 3.0 µm Glass Fiber/0.2 µm Supor membrane)
- 96-well clear plate for the plate reader
- Lysis buffer (pH 7.4 phosphate buffer + 300 mM NaCl or similar)
- Polyacrylamide gels, loading dye, appropriate size marker and running buffer
- BCA Protein Assay Kit

If going for chemical lysis:

- Bacterial protein extraction reagent (B-PER™ Complete Bacterial Protein Extraction Reagent)

If going for purification:

- 96-well IMAC plate (Cytiva His MultiTrap FF)
- Imidazole stock for buffers

Before start

If you have not bought your 96- and 24-well plates, medium and reservoirs sterile, make sure to autoclave them or sterilize in another way before starting.

If keeping the medium in the fridge, bring it to room temperature before adding to the cells.

Prepare the lysis buffer before starting day 2.

Transformation (day 1):

1h 53m

1 Before starting: 15m

- Thaw competent *E. coli* BL21 On ice
- Thaw plasmid DNA Room temperature
- Cool a sterile 96-well plate On ice
- Preheat the water bath 42 °C

2 Add 10 µL *E. coli* BL21 per well in the 96-well plate. 10m

3 Add 1 µL plasmid DNA solution to the bacteria. Stir with pipette tip to connect droplets, do not pipette up and down. 10m

Note

Alternatively, spin the plate after adding the DNA to make sure that cells and DNA come into contact.

4 Incubate the plate 00:15:00 On ice . 15m

5 Heat-shock bacteria in water bath by holding the plate half submerged in the water 42 °C 00:00:30 . Put directly back on ice afterwards. 1m

6 Add 50 µL sterile LB medium to each well. 2m

7 Incubate transformation mixtures 400 rpm, 37°C, 01:00:00 . 1h

Expression (day 1)

12h 22m

8 Prepare autoinduction medium with appropriate antibiotic in a sterile reservoir. 2m

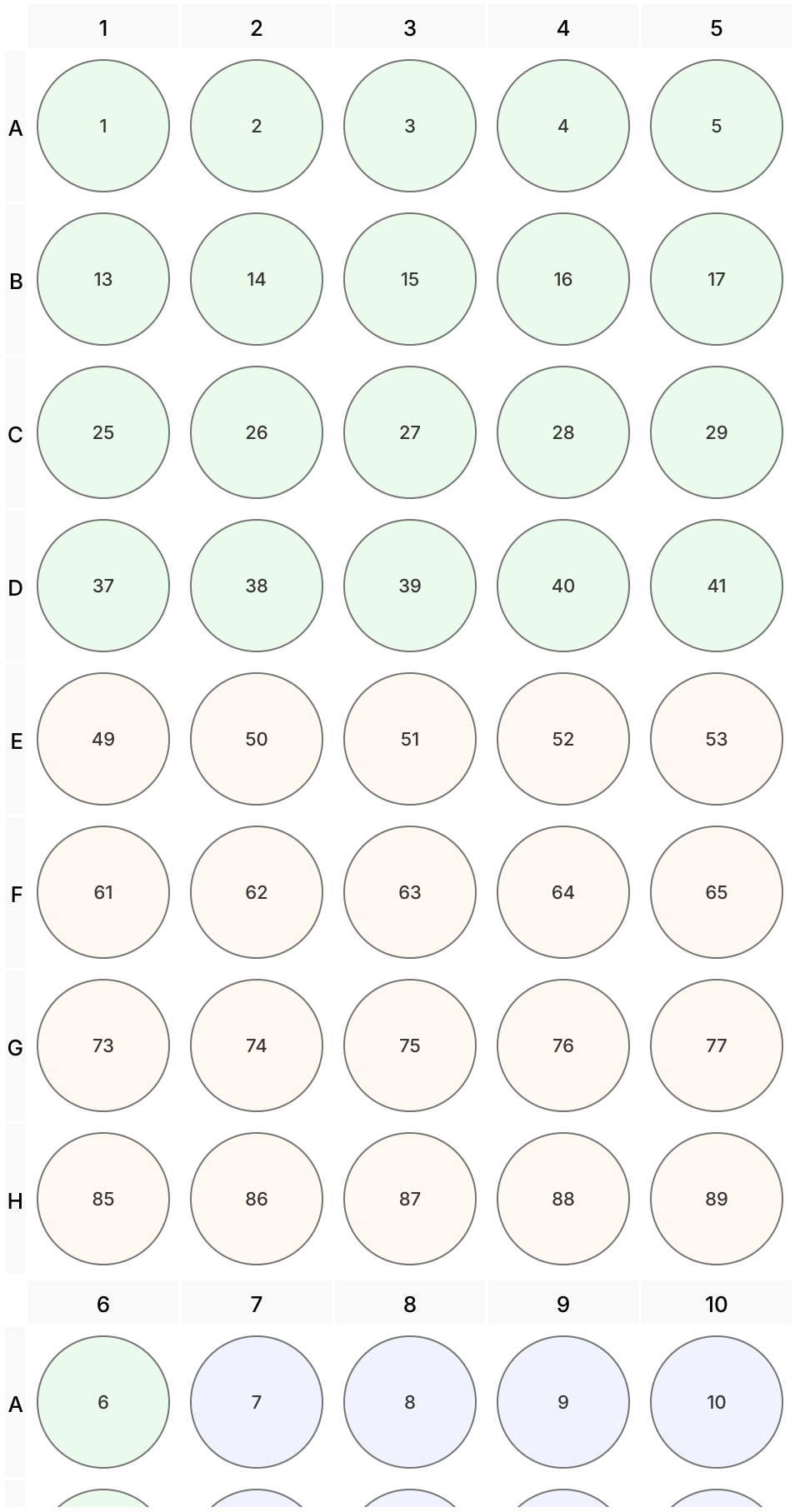
9 Add 2 mL autoinduction medium to each well in 4 sterile 24-well plates. 5m

10 Add 50 µL of the transformation mixture to each well in the 24-well plates. Resuspend any pelleted bacteria before transferring. 15m

Note

To keep the layout compatible with a multichannel pipette, this template can be used (assuming samples 1-96)

96 well plate: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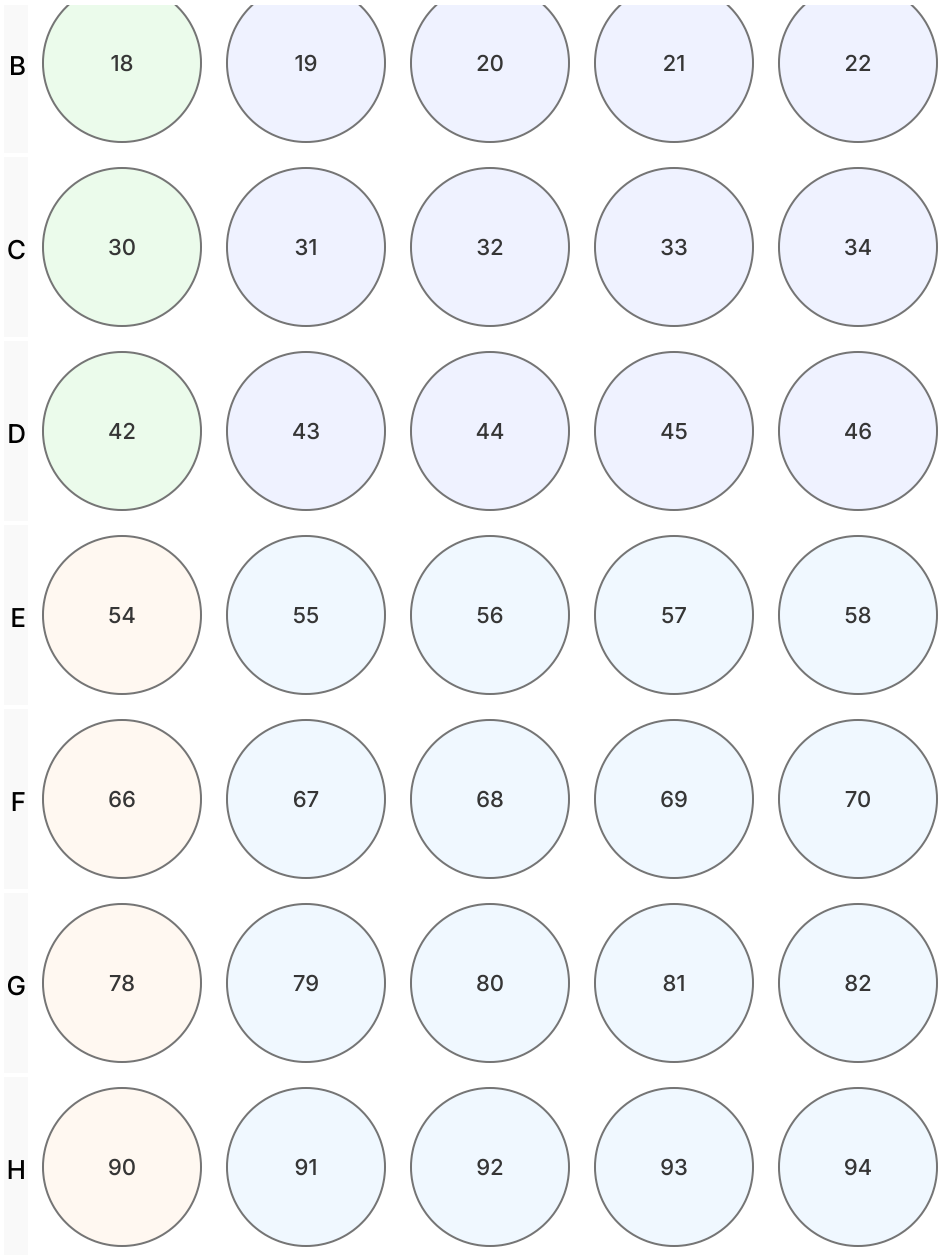
A 1 2 3 4 5
B 13 14 15 16 17
C 25 26 27 28 29
D 37 38 39 40 41
E 49 50 51 52 53
F 61 62 63 64 65
G 73 74 75 76 77
H 85 86 87 88 89
6 7 8 9 10
A 6 7 8 9 10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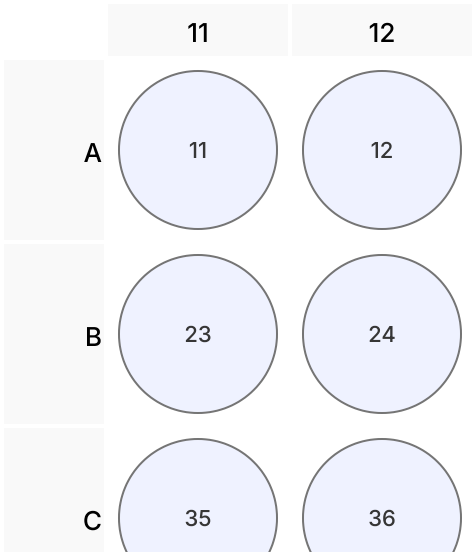
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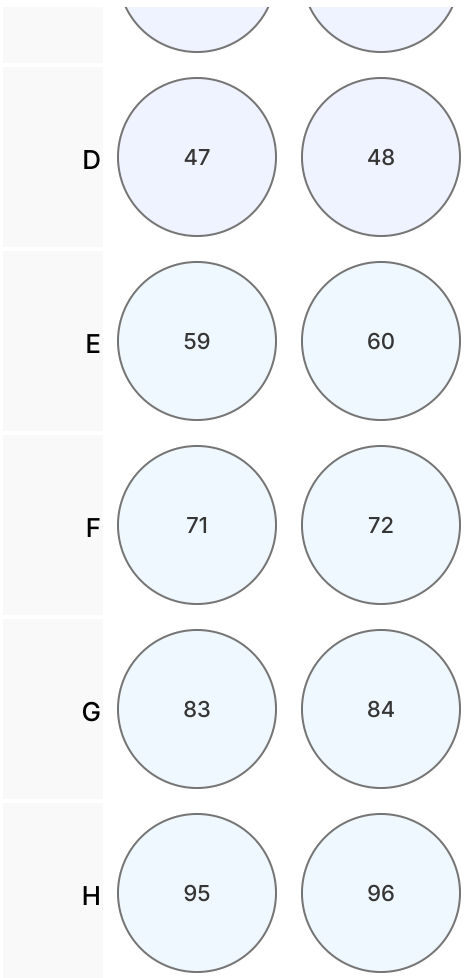
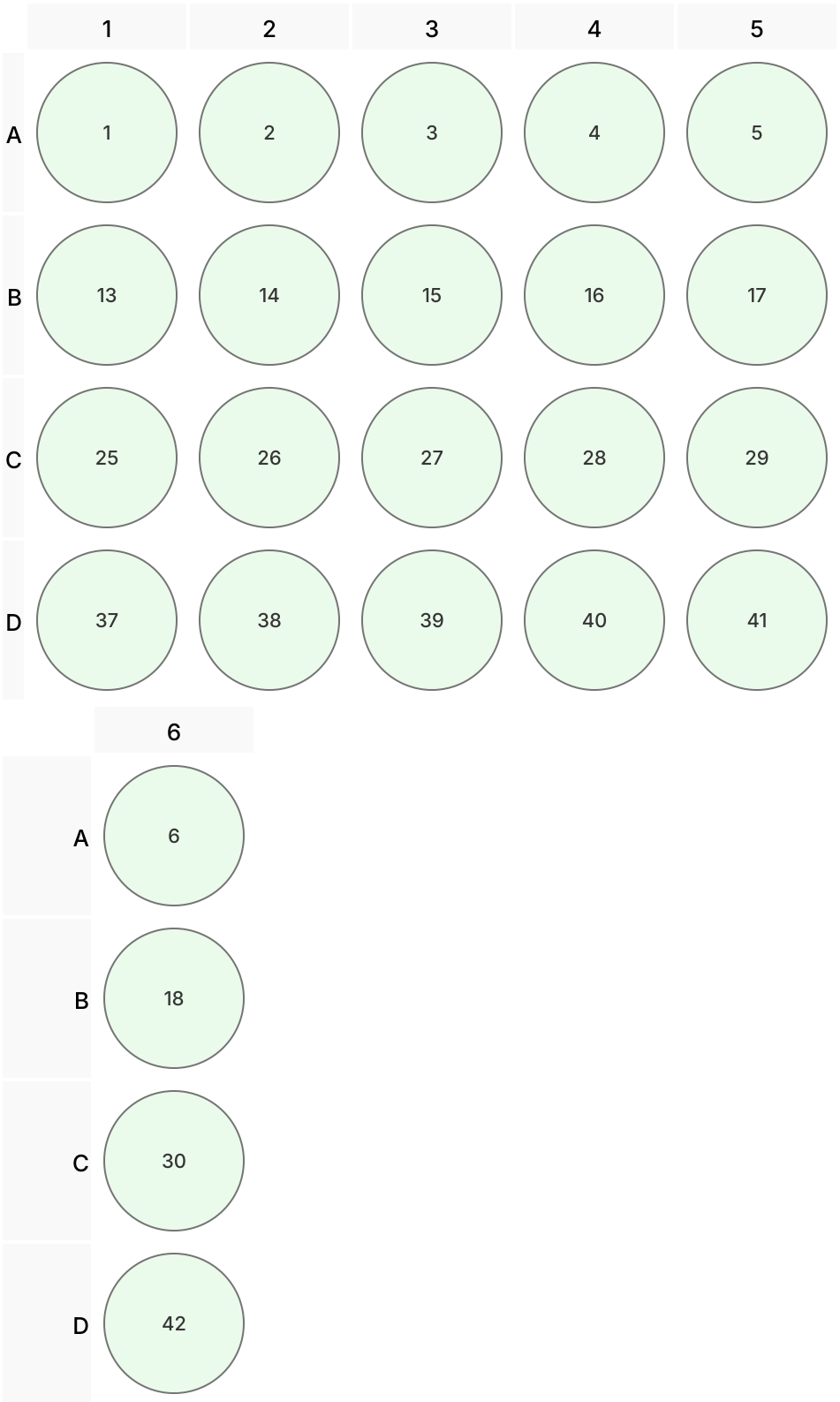


24-well plate 1: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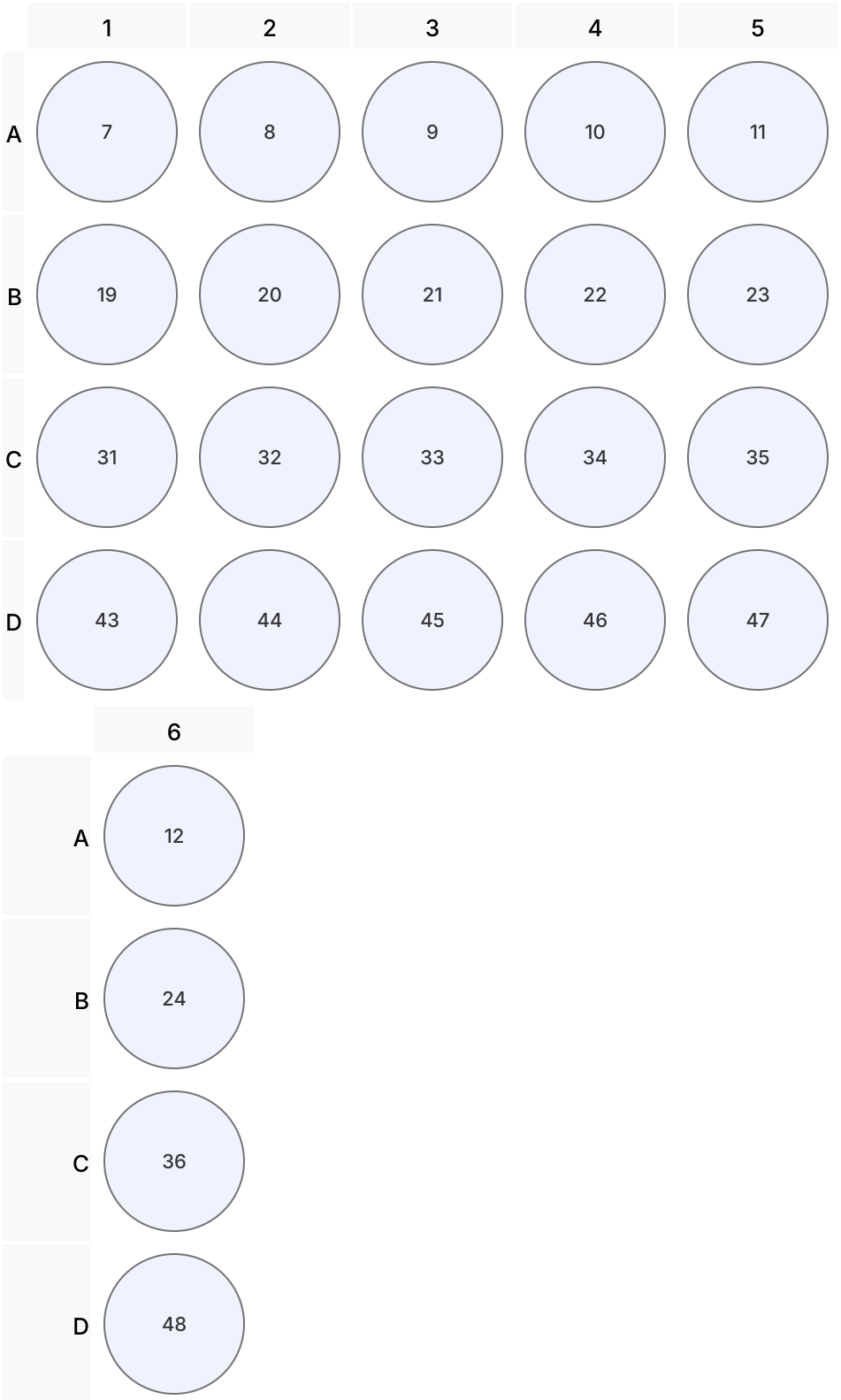
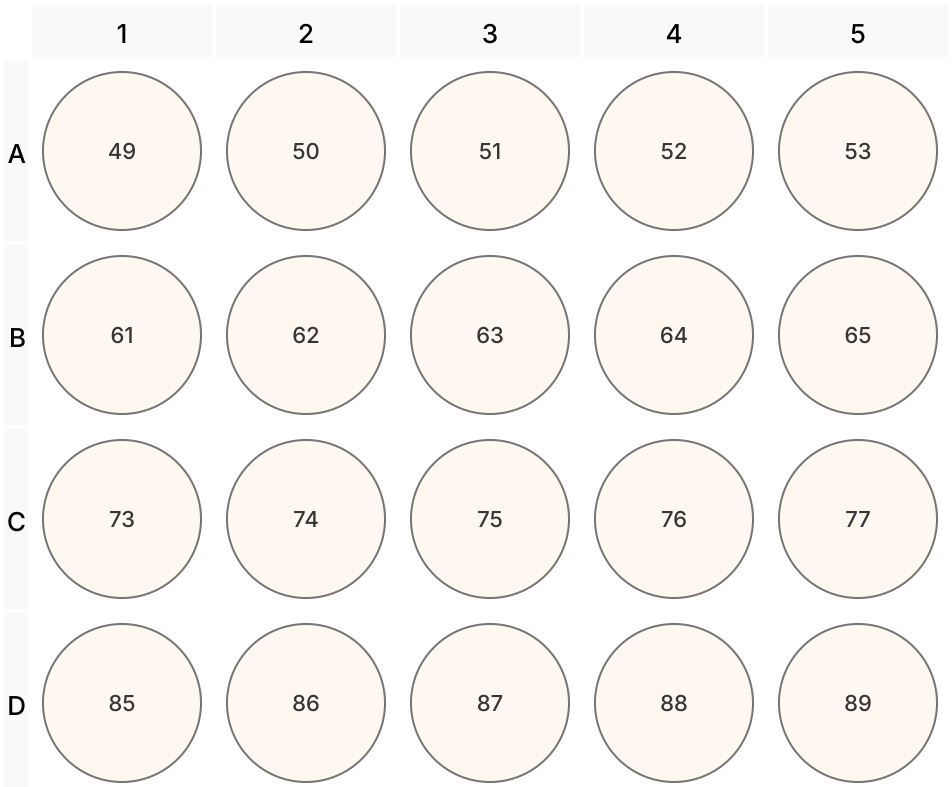
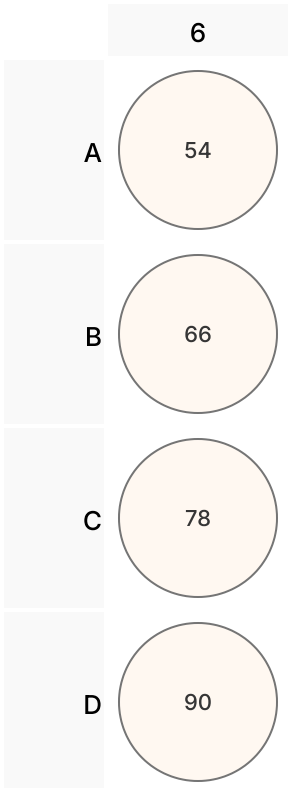
24-well plate 2:

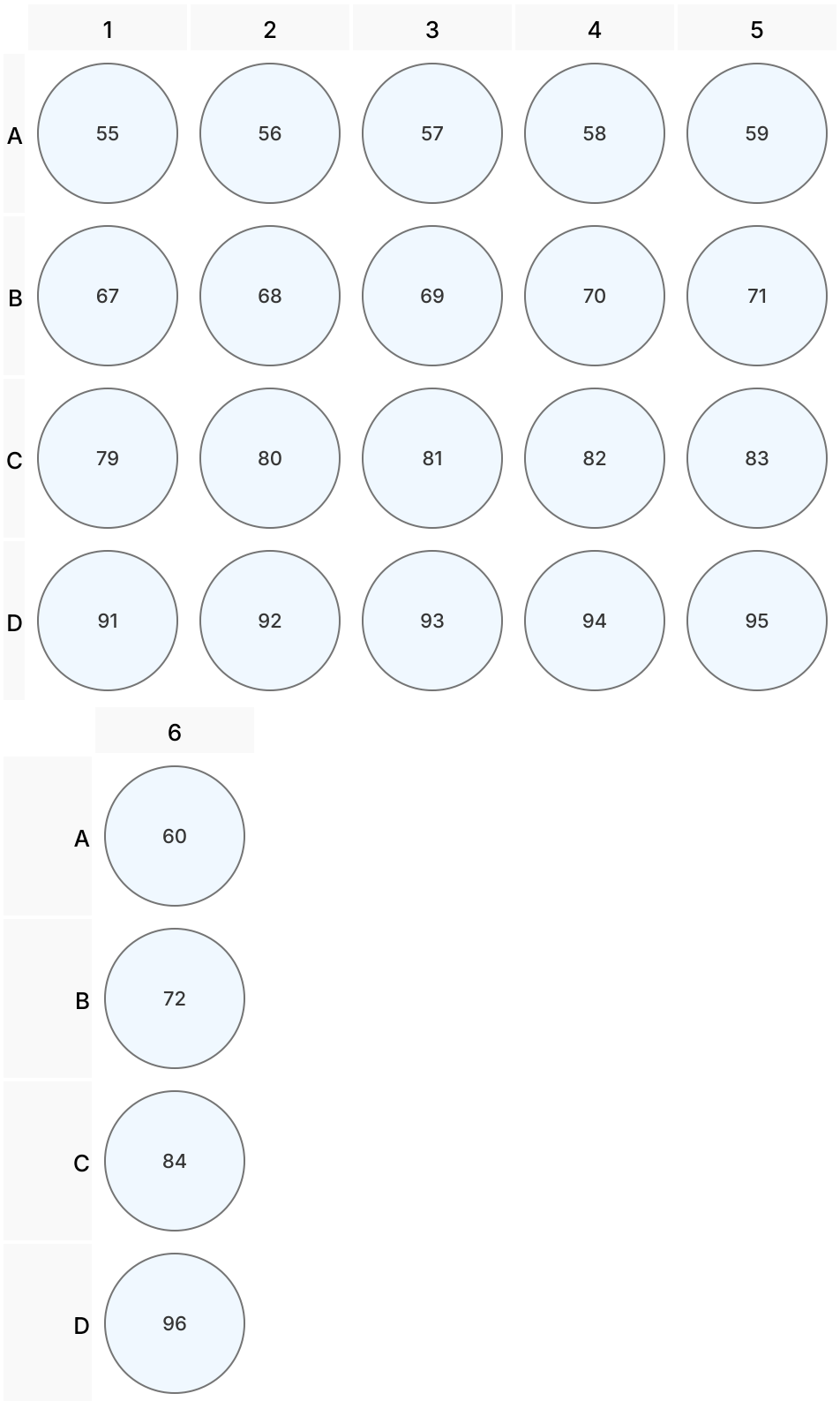


24-well plate 3:





24-well plate 4:



11 Cover plates with breathable film.

12 Incubate cultures 400 rpm, 37°C Overnight . 12h

Harvest (day 2) 47m

13 Add 90 µL LB per well in a 96-well plate. 2m

Note

Should be plate reader compatible.

14 Add 10 µL of culture per well. 5m

Note

Make sure to resuspend if pelleted.

15 Measure OD600 in the plate reader. Remember to include a couple of wells with just LB as blank controls. 10m

Note

OD of the culture will then be 10x this number. This step is qualitative and meant as a quick check of the growth. The values vary between experiments.

16 Take a 15 µl sample of each production from diluted 96-well plate samples and save for SDS-PAGE analysis. 10m

17 Pellet bacteria in the 24-well plate 2500 x g, Room temperature, 00:15:00 15m

18 Remove the supernatant. Freeze 24-well plate or proceed to lysis. 5m

19 Decide on your lysis protocol. This will depend on the stability of your proteins of interest.

STEP CASE

If you have heat stable proteins 16 steps

This protocol will remove many of the endogenous *E. coli* protein in your samples[6].

20 Preheat Thermomixer 95 °C 15m

21 Resuspend the pellets in 500 µL lysis buffer and move to a heat-stable 96-well plate. 10m

Note

If the lysate is very viscous you can increase the volume, but this will decrease the final protein concentration.

22 Heat-lyse the bacteria 95 °C 00:15:00 . 15m

23 Optional to ease filtration: Centrifuge 3000 x g, Room temperature, 00:10:00 and take only the supernatant to the next step. 10m *

24 Transfer the lysate to a 96-well filter plate stacked on a collection plate (96 deep-well plate). 5m

25 Centrifuge 1500 x g, Room temperature, 00:05:00 . If the liquid in some wells has not gone fully through, resuspend with pipette and centrifuge again. 5m

26 Take 15 µl samples from cleared lysates for SDS-PAGE. 15m

27 Estimate protein concentration in the lysates using the Bicinchoninic Acid (BCA) Method. *

Affinity chromatography purification (day 2, optional)

1h 10m

28 Prepare 200 mL binding buffer (20 mM imidazole) and 60 mL elution buffer (500 mM imidazole). Open the plate according to instructions and place it on top of a collection plate (96 deep-well). 15m

29 Centrifuge the plate to remove the storage solution from the resin
500 x g, 4°C, 00:02:00 5m

30 Add 500 µL deionized water/well 500 x g, 4°C, 00:02:00 . 5m

31 Add 500 µL binding buffer/well and mix briefly to equilibrate the resin, centrifuge 500 rpm, 4°C, 00:02:00 . Repeat once. 10m

32 Apply filtered lysate to the wells, mix briefly, and incubate 00:03:00 5m

33 Remove the flow-through by centrifuging
100 x g, 4°C, 00:04:00 , or until the wells are empty 5m

34 Add 500 µL binding buffer/well and mix briefly to wash out unbound sample, centrifuge
500 x g, 4°C, 00:02:00
Repeat once. 10m

35 Add 200 µL of elution buffer/well and mix for 00:01:00 . Change collection plate and centrifuge the plates 500 x g, 4°C, 00:02:00 and collect the fractions.
Repeat twice. 15m

Note

If required, change collection plate between each elution (to prevent unnecessary dilution of the
target protein).

Protocol references

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Citations

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