Expression, purification, and assay of beta-glucosidase B

Day 1: Transform expression cells

MATERIALS

Material	Concentration	Amount per reaction
Chemical competent BLR cells		20 μL
Sequence-verified plasmid	> 20 ng/μL	1 μL
Terrific broth		200 μL
LB agar plates with kanamycin		1
Sterile beads		8–10
PCR tubes		1

METHOD

1. In an ice cold tube, mix 1 μL plasmid and 20 μL competent cells
2. Incubate at 42 C for 60 seconds
3. Incubate on ice for 60 seconds
4. Add 200 μL media and incubate at 37 C for 1 hour

Day 2: Growth cultures

MATERIALS

Material	Concentration	Amount per reaction
Falcon tube		1
Pre-cut tube seal		1
Terrific broth with kanamycin		5 mL

METHOD

1. Aliquot 5 mL terrific broth with kanamycin into Falcon tubes
2. Inoculate tubes with single colonies from plate
3. Seal tubes with tube seals
4. Incubate with shaking at 37 C for 24 hours

Day 3: Expression cultures

MATERIALS

Material	Concentration	Amount per reaction
Terrific broth		5 mL
Pre-cut tube seal		1
IPTG	1000X	25 μL
Kanamycin	1000X	25 μL

METHOD

1. Centrifuge growth cultures at 4,700 RPM for 10 minutes
2. Add IPTG and kanamycin to TB to make enough induction medium for all reactions
3. Add 5 mL induction medium to tubes
4. Vortex to resuspend pellets
5. Seal with tube seals
6. Incubate with shaking at 18 C for 24 hours

Day 4: Protein purification

MATERIALS

Material	Concentration	Amount per reaction
Wash buffer		450 μL
Bugbuster	10X	50 μL
Lysis mix		1 mg
2 mL Goog tubes		1

METHOD

1. Centrifuge expression cultures at 4,700 RPM for 10 minutes
2. Make lysis buffer and aliquot 500 uL into 2 mL Goog tubes
3. Pour off supernatant from cultures
4. Resuspend cell pellet in 500 μL wash buffer
5. Transfer 1 mL of resuspension into Goog tubes with lysis buffer aliquots
6. Rock tubes for 20 minutes
7. Centrifuge at 14,700 RPM for 10 minutes
8. Set microcolumns in rack over waste collector
9. Add 100uL of nickel resin (blue) from 50% slurry to each microcolumn. Make sure resin is thoroughly mixed before pipetting out. Use 1000 μL tips otherwise the beads get stuck!
10. Add 500 μL of wash buffer and allow to drip through
11. Add 500 μL of supernatant and allow to drip through
12. Add another 500 μL of supernatant and allow to drip through
13. Wash six times with 500 μL of wash buffer, allow to drip through each time
14. Transfer column to fresh tube
15. Add 100 μL protein buffer directly to beads and wait five minutes
16. Add second 100 μL protein buffer and spin briefly to ensure you get it all (this contains your purified protein)

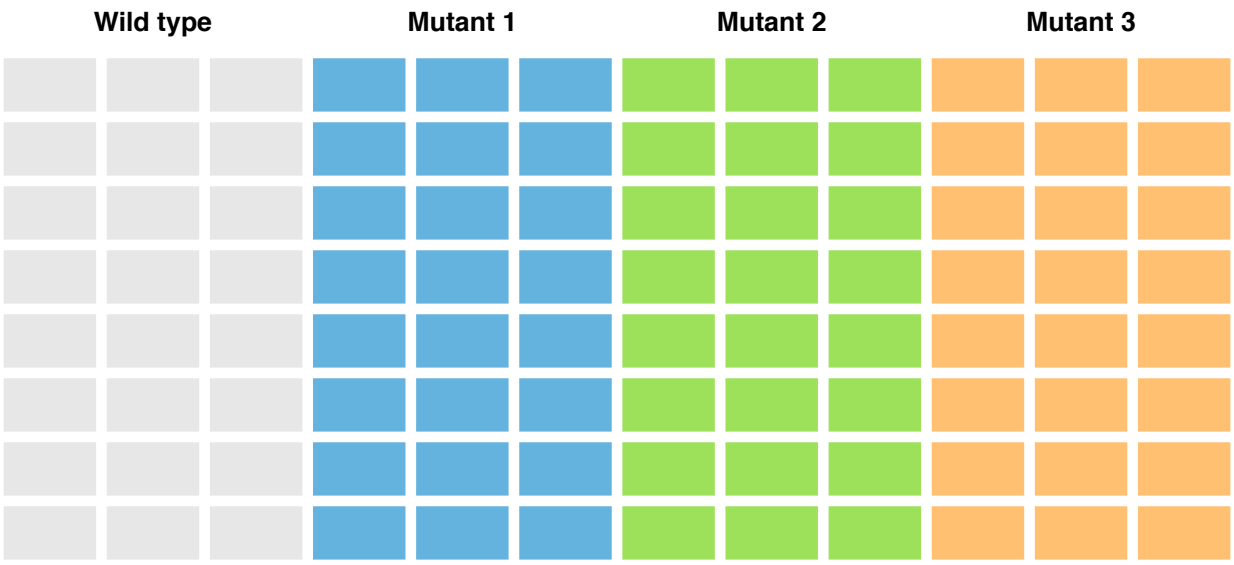
Day 5: Assay

MATERIALS

Material	Concentration	Amount per assay
Protein solution	about 0.01 mg/mL	600 μL each protein
Substrate solution	100 mM	1200 μL
96-well plate (cheap)		1
Costar 96-well half area plate		1

METHOD

1. Aliquot 25 μL of protein solution into wells of black plate as diagrammed below



2. In cheap plate, aliquot 100 μL of substrate stock into the first row and 75 μL of protein buffer into the rest of the rows
3. With the multichannel, remove 25 μL from the first row, add to second row, and mix
4. Repeat, leaving the last row alone
5. Set up plate reader (use program “Model enzyme kinetics”)
6. Transfer substrate from cheap plate to assay plate
7. Place assay plate in reader and press play

Recipes

Plates

LB plates with kanamycin (25 plates)

Autoclave 250 mL water with 2 pellets of LB agar in a 500 mL bottle. After autoclaving, hold in 50 C dry bath until 50 C, then add 250 μL 1000X kanamycin solution. Use a serological pipet to pour 10 mL per plate

Stocks

Lysis mix (100 reactions)

Combine 8 g lysozyme, 1 g DNAse, and 1 g PMSF.

Terrific broth (TB) (250 mL)

Dissolve 12.5 g granulated TB in 250 mL water and autoclave.

1 M isopropyl-beta-D-thiogalactopyranoside (1000X IPTG) (1 mL)

Dissolve 240 mg IPTG in 1 mL wash buffer. Store at –20 C.

Buffers

Wash buffer (1 L)

Dissolve 11.9 g HEPES, 8.7 g sodium chloride, and 0.68 g imidazole in water and adjust pH to 7.4

Protein buffer (1 L)

Dissolve 11.9 g HEPES, 8.7 g sodium chloride, and 7.31 g EDTA in water and adjust pH to 7.4