Blue/White Screening of Bacterial Colonies
X-Gal/IPTG Plates

- Prepare 20 mg/ml X-Gal solution in DMF (See X-Gal Stock Solution Procedure). For reduced DMF toxicity in media, you can alternatively make a 100 mg/ml X-Gal solution in DMF (this concentration is only stable at -20°C for ~1 week).

- Prepare 100mM IPTG solution in dH_2O (or dilute from 1M IPTG Stock Solution).

Agar Media (Recommended)

1. Cool autoclaved growth media agar to 50°C.

2. Add 10 µl X-Gal Solution** (20 mg/ml) per 1 mL of Media (or 2 µl X-Gal Solution (100 mg/ml) per 1 mL of Media).

3. Add 10 µl IPTG (100mM) per 1 mL of Media for a final concentration of 1mM.

4. Add screening antibiotic of choice (Ampicillin, Kanamycin, Carbenicillin, etc).

5. Pour plates and allow to cool to room temperature (usually at least 30 minutes) before use.

6. Spread transformed competent cells as desired.

Note: Blue/White Selection plates are generally stable for only 1 week if stored at 4°C in clear sleeves, but may be stored in the dark (or a dark sleeve) at 4°C for up to 1 month.

** We recommend using a higher concentration of X-Gal than most protocols. In our experiments at Gold Bio, we see a deeper blue color with the increased X-Gal concentration which reduces the amount of ambiguous colonies and the need for rescreening. The higher concentration also helps the blue color to develop faster with good, interpretable results after only an overnight incubation, reducing your wait time and the need to put plates in a 4°C refrigerator for additional time in order for color to develop.

Plate Surface

1. Dry media plates in a laminar flow hood.

2. Add 40 µl 100mM IPTG and 120 µl X-Gal (20 mg/ml) to the surface of each plate and spread over entire surface.

   Note: The edges of the plate are difficult to spread adequately and may give false positives. We advise picking colonies in the middle of the plate, if possible, for best results.

3. Dry X-Gal/IPTG-coated media in a laminar flow hood for approximately 30 minutes before use.

4. Spread transformed competent cells as desired.

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