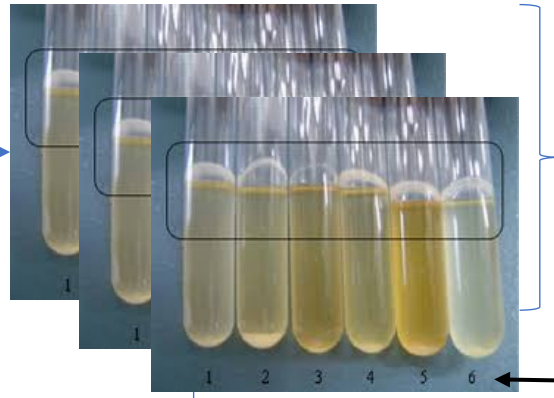
GFP TIsigner variants

- Streak glycerol stocks of each TIsigner variant onto plates for single colonies

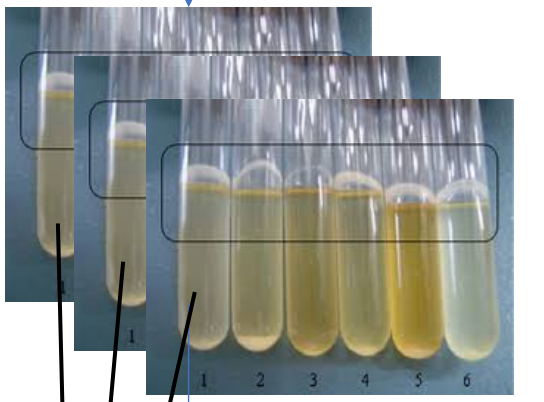
- Pick 3 colonies for each TIsigner variant (i.e., 3× biological replicates)
- Inoculate each colony into 3 mL LB in a 15 mL tube
- Grow O/N at 37 °C



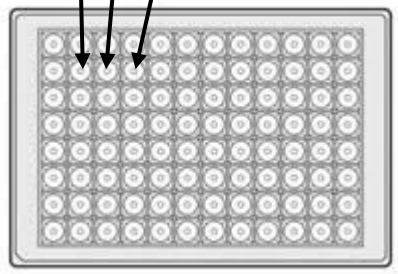
3× biological replicates of each TIsigner variant

TIsigner variant

- Transfer 30 µL of each overnight (i.e. stationary) culture into a new tube containing 3 mL of LB.
- Grow at 37 °C until OD_{600} ≈ 0.6 (i.e., mid-log phase)



- For each biological replicate, transfer 20 µL of the mid-log phase culture into 180 µL of LB + IPTG in a 96-well plate (final OD_{600} = 0.05) (no technical replicates).



- Shake at 37 °C
- Monitor cell density (OD_{600}) and GFP fluorescence every 10 minutes

Work flow for Figure A

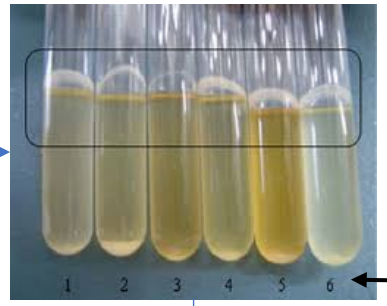
3 biological replicates per plate.

No technical replicates.

GFP TIsigner variants

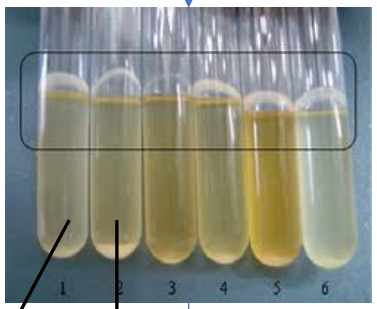
- Streak glycerol stocks of each TIsigner variant onto plates for single colonies

- Pick a single colony for each TIsigner variant (i.e., 1× biological replicate)
- Inoculate the colony into 3 mL LB in a 15 mL tube
- Grow O/N at 37 °C

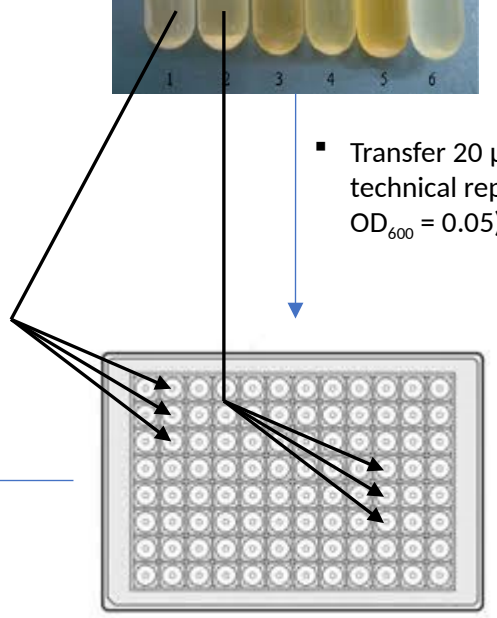


TIsigner variant

- Transfer 50 µL of the overnight (i.e. stationary) culture into a new tube containing 5 mL of LB.
- Grow at 37 °C until OD_{600} ≈ 0.6 (i.e., mid-log phase)



- Transfer 20 µL of the mid-log phase culture in triplicate (i.e., 3× technical repeats) into 180 µL of LB + IPTG in a 96-well plate (final OD_{600} = 0.05)



- Shake at 37 °C
- Monitor cell density (OD_{600}) and GFP fluorescence every 10 minutes

Work flow for Figure B

1 biological replicate per plate.

This work flow was performed on three separate days to generate three biological repeats.