



## DNA Plasmid Stability Test

### e.coli testing of transformed cells



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Immediately before induction, it is advisable to test the culture to determine the fraction of cells that still carry the target plasmid. This involves plating of cells on four different plates.

Plate	cells that grow on these plate
LB plate	all viable cells
LB plate + antibiotic	cells that still carry the plasmid
LB plate + IPTG (1 mM)	cells that have lost the plasmid or mutants that have lost the ability to express the target gene
LB plate + antibiotic + IPTG (1 mM)	only mutants that retain the plasmid but have lost the ability to express the target gene

#### Remarks

- In the presence of IPTG, cells carrying a protein production plasmid do not grow because have dedicated all their resources to the production of the recombinant protein instead of cell maintenance.
- In the presence of the **pLysS** vector, IPTG also prevents colony formation except with certain vector (such as pET-3 and some vectors carrying the T7lac promoter). In the presence of **pLysE**, IPTG usually does not prevent colony formation unless the target protein is toxic.

In a typical culture useful for producing target protein, almost **all** cells will form colonies both on the LB plate and on the LB plate + antibiotic; **less than 2%** of the cells will form a colony on the LB plate + IPTG; and **less than 0.01%** will form a colony on the LB plate + antibiotic + IPTG.

With unstable target plasmids, the fraction of cells that have lost the plasmid will be reflected by an increase in colonies on the LB plate + IPTG and a decrease on the LB plate + antibiotic.

#### Protocol

1. Immediately before induction with IPTG (at OD<sub>600</sub> is approx. 0.6), take a 100-ml aliquot of the cell culture.
2. Make a serial dilution of the cell suspension, including a 10<sup>5</sup> and 10<sup>6</sup> dilution.
3. Plate cells at a dilution of 10<sup>5</sup> on the **LB plate + IPTG** and on the **LB plate + IPTG + antibiotic**.

Plate cells at a dilution of 10<sup>6</sup> on the **LB plate** and on the **LB plate + antibiotic**.

4. Incubate the plates overnight at 37°C.
5. Count the number of colonies on each plate.

Reference: pET System Manual, 8th ed., 1999 (Novagen).