

## Bio 181: Blue/White screening (pBLU)

A central problem of cloning is the identification of a desired clone among countless bacteria, transformed & untransformed.

In the simple cloning system we have used so far, we used **antibiotic resistance** to select the desired clones. This was possible because our desired clones all carried antibiotic resistance genes that untransformed bacteria did not.

But what if you need to select for MORE than just antibiotic resistance? In Labs 18-20, we are cloning a PCR-amplified gene into a plasmid vector (pBLU). After ligation and transformation, we are faced with two variables:

- Transformed vs untransformed bacteria: Bacteria transformed by pBLU are selected from untransformed cells by resistance to ampicillin (pBLU carries the amp<sup>R</sup> gene).
- Bacteria carrying empty pBLU vs bacteria carrying pBLU+PCRproduct (the desired clones): All of these cells will be able to grow on ampicillin. To identify desired clones, use Blue/White screening.

### Blue/White screening: How does it work?

pBLU, and other specially designed cloning vectors, make use of the *lac* operon. pBLU carries the gene for **β-galactosidase** (also known as **lacZ**). This enzyme catalyzes the breakdown of lactose as a food source. It can also degrade an artificial substrate called **x-gal**, which turns BLUE when it is broken down by β-galactosidase.

- Colonies that produce β-galactosidase and are fed x-gal will turn **BLUE**.
- Colonies that do NOT produce β-galactosidase remain white in color, even in the presence of x-gal.

Plasmid vectors designed for blue/white screening have a multicloning site carefully placed early *within the coding region of lacZ*. Successful ligation of a foreign DNA into this multicloning site interrupts *lacZ* and abolishes production of functional β-galactosidase.

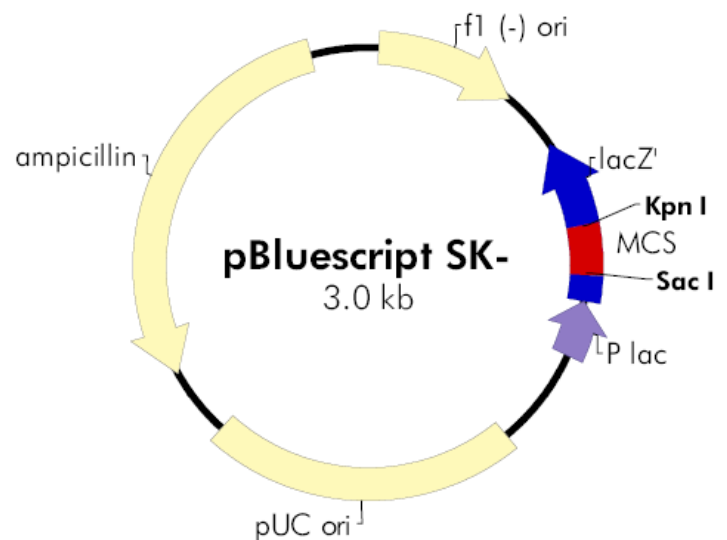
Therefore:

Untransformed bacteria:	No growth on amp
Bacteria transformed with original pBLU:	Blue colonies on amp + x-gal
Bacteria transformed with pBLU + insert DNA:	White colonies on amp + x-gal

Note that blue/white screening is not *selection* (it does not kill the unwanted bacteria); it is *screening*.

In some blue/white screening systems, an additional reagent must be used: **IPTG** (isopropylthiogalactoside). IPTG is an inducer that de-represses *lacZ* expression (it turns the gene on). In some cases, without IPTG, not enough β-galactosidase is produced to turn the colony blue even if the *lacZ* gene is intact.

**f1 (-) origin** 24–330  
 **$\beta$ -galactosidase  $\alpha$ -fragment** 463–816  
**multiple cloning site** 653–760  
**lac promoter** 817–938  
**pUC origin** 1158–1825  
**ampicillin resistance (*bla*) ORF** 1976–2833



**pBluescript SK (+/-) Multiple Cloning Site Region**  
 (sequence shown 601–826)

