

## Keystone Expression System™

- *E. coli* platform designed by Cytovance personnel based on extensive lessons learned from 10 years of contract biomanufacturing experience
- Quality by Design approach from gene construction to GMP manufacturing.
- Proprietary vectors, hosts, and platform fermentation processes are designed to fit each other for efficient and robust scale-up.
- Efficient work-flow enables MCB initiation with growth and titer data from a fed-batch process that is scalable and manufacturable.

### Cytovance's Toolbox Approach

#### HOSTS

Each Keystone™ host was built to be robust and circumvent common scale-up problems (such as genetic instability and nutrient deficiencies).

#### Keystone™ hosts include:

1. BL21 derivatives enhanced for periplasmic expression
2. BLR(DE3) with repaired nutrient deficiency
3. The only available non-DE3 K-12 compatible with the T7 promoter.
4. Platform K-12 host with multiple protease knockouts.

#### DNA

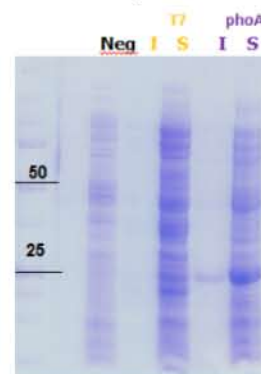
- Keystone™ Vectors were custom-built by Cytovance
- Two Promoters can be tuned to accommodate a variety of products

#### SCREENING

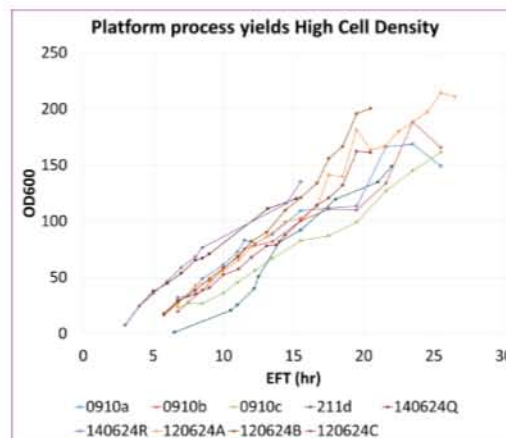
- Efficient primary screen is performed in a 24-well dish (3 mL culture volume)
- Platform fermentation screening processes fit each promoter and yield high cell density

### Sample *E. coli* Strain Development Project

1. Cytovance experts design 12 plasmids: 2 promoters, 2 compartments, 2 periplasmic leaders, 2 copy number
2. DNA2.0 synthesizes codon-optimized genes with GeneGPS™ and performs Electra™ cloning using Keystone™ vectors
3. Transform 2 *E. coli* hosts per plasmid; B and K-12 lineages
4. Evaluate 24 strains in 24-well dish by SDS-PAGE (soluble, insoluble, media fractions)
5. Advance 8 best strains to shake flask screening for confirmation
6. Advance 4 best strains to platform fed-batch fermentation processes
7. Based on fed-batch data, advance best strain to process development and cGMP MCB production



Cytovance hosts, promoters, and screening processes yield high expression levels, and are designed to fit into platform fed-batch processes.



Platform fed-batch processes yield high cell density with a variety of products and enable choice of a strain for cGMP MCB production and initiation of process development with fed-batch titer and growth data.