

# Cell Line Development

**Producing high-expressing,  
stable cell banks from DNA**

ILLUSTRATION:  
CHO cell at 10,500x

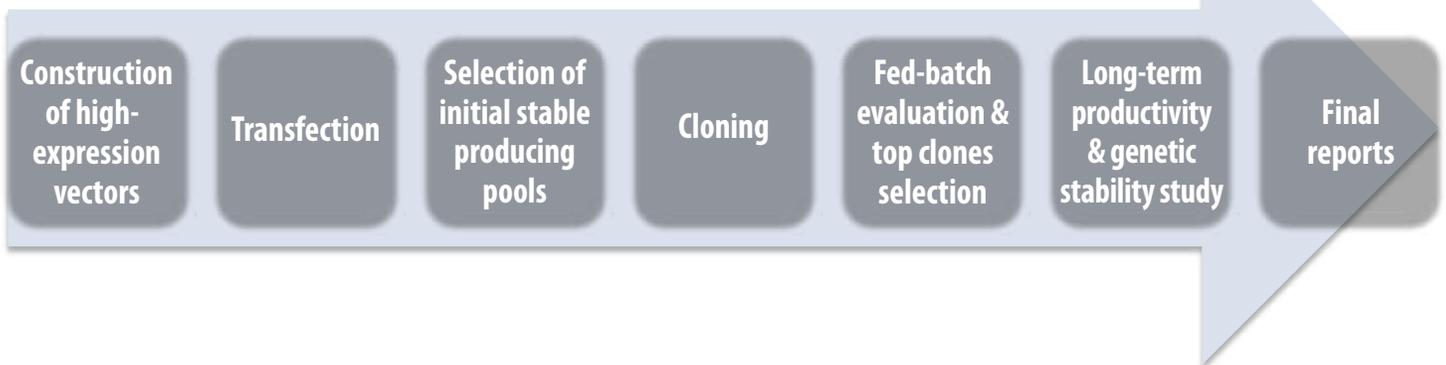
 药明康德  
WuXi AppTec

# Cell Line Development

*Proven, high-yielding development platforms accepted by regulatory agencies worldwide*

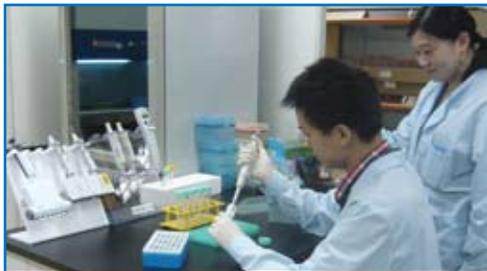
WuXi AppTec, a global R&D and GMP/GLP contract manufacturing and testing company, offers comprehensive cell line development capabilities that start at DNA sequences provided by clients and finish with the delivery of high-yielding stable single clones. We work with you to stay within your timeline and to select top clones according to their productivity and quality.

## The WuXi AppTec Program



### Construction of high-expression vectors

WuXi AppTec offers gene-specific cloning into commercial vectors (e.g., Invitrogen), or client-specified vector systems. The integrity of the target gene cloned into the vector will be confirmed by sequencing prior to transfection into a host cell line.



### Selection of initial stable producing pools

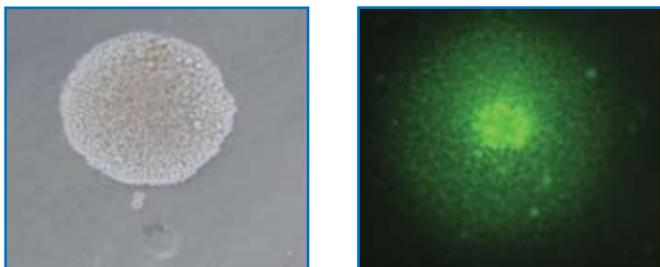
Transfected cells are grown in selective media to generate stable pools. For CHO-DG44 cells, the pools can also be subject to additional MTX selection pressure to increase expression levels. Once the pools are fully recovered, the productivity and quality of the pools will be evaluated to select the top three pools for cloning.

### Cloning

Three pools are plated into semi-solid medium in 6-well plates for clone-picking. High-expressing clones can be captured and visualized by a fluorescently labeled antibody. Approximately 300 clones in 96-well plates are screened utilizing the ClonePix™ system. An HTRF assay is performed to evaluate the expression of these clones, and 20 to 30 clones from each pool are selected and expanded into shake flasks.

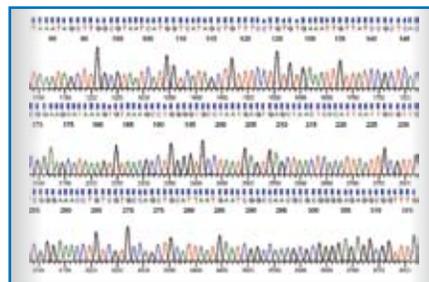
### Transfection

Transfection into one of WuXi AppTec's platform cell lines (e.g., CHO-DG44) or a client-specified host cell line is typically conducted via lipid-based transfection method. Other transfection methodologies are available upon request, such as electroporation, polyethylenimine (PEI) mediated transfection, etc. Parallel transfections will be performed to increase the probability of success and to generate the necessary number of clones for screening.



## Long-term productivity & genetic stability study

PCB protein expression and product quality stability is evaluated for a minimum of 60 generations. (Duration can be extended upon request.) The genetic stability of the target gene(s) for PCB, MCB and WCB is performed by sequencing the full length cDNA. Additional genetic stability studies (e.g., copy number determination, detection of insertions/deletions) can also be conducted. The banks are subjected to adventitious agent detection (e.g., bacteria, fungi, mycoplasma, and viruses such as MVM). Full cell line characterization can be conducted on the MCB and WCB.



## Fed-batch evaluation & top clones selection

The top 20 to 30 clones from each pool are further evaluated based on growth rates. A few vials of a research cell bank (RCB) are made from these clones. The productivity of the selected clones is evaluated in fed-batch culture with a platform process. The top 4-6 clones are determined according to the titer and product quality data and are transferred to the cell culture process development group for analysis in bioreactors. The final clone and the back-up clone are selected based on several rounds of bioreactor screening data. The primary cell bank (PCB) is made for the final clone and the back-up clone. The master cell bank (MCB) and working cell bank (WCB) can be produced for the final clone under GMP conditions.

## Final reports

Reports of cell line development and stability studies are provided to clients for review. After review and acceptance of the reports, cell banks and process will be transferred to clients.

## CELL LINE DEVELOPMENT TEAM

WuXi AppTec's cell line development team has over 30 years' combined experience in developing high-yielding cell lines and comprises experts who are familiar with regulatory requirements of the US FDA, EMA, ICH, and SFDA. The team has developed numerous mammalian cell lines expressing monoclonal antibodies (MAbs) and recombinant proteins yielding multiple grams of target protein per liter in standard fed-batch bioreactors as well as continuous perfusion bioreactors.

The depth and breadth of experience includes:

- Construction of high-expression vectors
- High-throughput clone screening
- High-throughput clone selection and evaluation
- Cell banking
- Cell line stability analysis
- Cell culture process development

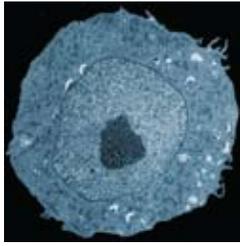


## EQUIPMENT / INSTRUMENTATION

Our well-equipped molecular biology and cell biology labs offer a full range of services required for successful cell line development processes.

Instruments include:

- Beckman Coulter Vi-CELLs: cell counting
- INFORS HT incubators: shake flasks
- Kuhner incubators: high-throughput cell culture in spin tubes and 24 well plates
- Beckman Coulter Biomek® NX: automated high-throughput clone screening
- Molecular Devices ClonePix™: automated clone picking
- SpectraMax M5e plate reader: high-throughput HTRF and for glucose/Lac assay
- Guava: high-throughput cell counting



# Cell Line Development

*WuXi AppTec, a leading global contract services organization, provides services of exceptional quality, leveraging more than 20 years of experience in the discovery, development, manufacturing and testing of mammalian-derived protein therapeutics. This single-source strategy can reduce the time-to-clinic and decrease significantly the cost of our customers' drug development efforts.*

## PROGRAM FEATURES

- Proven high-yielding cell line development platforms accepted by global regulatory agencies.
- Quick turnaround time and competitive costs.
- Well-equipped molecular biology and cell line development labs.
- Capability to develop MAb clones at 2-3 g/L prior to cell culture process optimization.
- Robust cell lines with high productivity and exceptional product quality and stability.
- LIMS system for tracking cell line history and seamless linkage between cell line development, bioprocess development and PAS groups.
- Cell line characterization, including biosafety, genetic and expression stability studies

## ADDITIONAL SERVICES

### Discovery Services

WuXi AppTec offers a full spectrum of discovery services for the generation of high potency novel monoclonal antibody therapeutics. Discovery technologies include hybridoma generation, phage display libraries, antibody humanization and affinity maturation. Through partnership with OMT, WuXi AppTec became the first CRO in China with access to transgenic animal models capable of generating fully human monoclonal antibodies.

### Cell Banking & Cell Line Characterization

Independent cell banking, cell line biosafety testing and cell line characterization services are also offered. Cell banks are manufactured to client specifications, under exacting standards and QA oversight, in secure, monitored cell banking suites. Long-term storage is available at WuXi AppTec sites in highly controlled and regulated GMP facilities. Complete biosafety, identification and characterization assessment of cell banks and cells at the limit of in-vitro cell age is conducted under FDA and ICH guidelines in our in-house laboratories.

### Process Development

Top selected clone(s) can be transferred to our cell culture process development team for further process development and optimization. WuXi AppTec's upstream cell culture process technology platform provides for optimization of media, seeding densities, feeding and bioreactor process control strategies to maximize expression, enhance process reproducibility and facilitate scale-up.

**Contact WuXi AppTec for your cell line development:**

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