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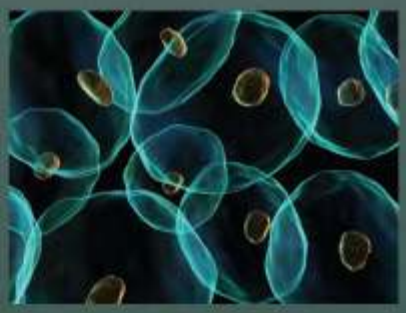
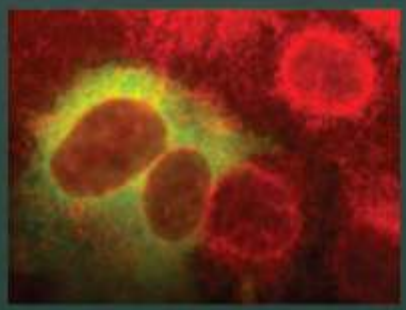
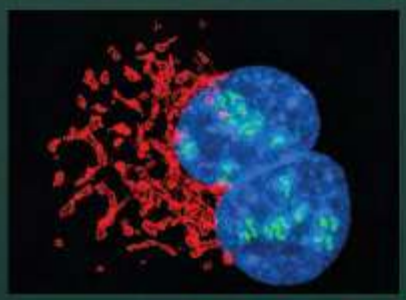
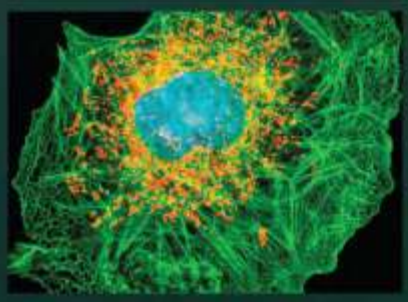


Biopharmaceutical Expression Systems and Genetic Engineering Technologies

Current and Future Manufacturing Platforms







by Ronald A. Rader

FIRST EDITION

Biopharmaceutical Expression Systems and Genetic Engineering Technologies

Current and Future Manufacturing Platforms

by Ronald A. Rader

Title:	***Biopharmaceutical Expression Systems and Genetic Engineering Technologies: Current and Future Manufacturing Platforms***
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Introduction and User Guide

This is the 1st edition of *Biopharmaceutical Expression Systems and Genetic Engineering Technologies: Current and Future Manufacturing Platforms*. Expression systems encompass the technologies - biological materials and associated know-how - needed to genetically modify organisms for the manufacture of recombinant proteins (including glycoproteins and antibodies). This book is designed to be the single most informative source concerning commercial biopharmaceutical product manufacturing-related expression systems and basic engineering technologies, with emphasis on those currently used for biopharmaceutical manufacture and those available for commercial licensing for this purpose; providing basic information for the knowledgeable user to determine relevance for their applications; conduct further research and/or contact technology licensing sources.

The primary goal is to inform the user of the many technologies in commercial use and those claimed to be useful for commercial-scale manufacture of biopharmaceutical products, rather than provide detailed or comparative information about each. This directory is the result of multiple man-months of cumulative effort in information acquisition, organization and analysis. As such, it is a high value-added product that should save you considerable time and effort in finding technologies relevant to your interests. It should reliably cover relevant technologies currently being used commercially, those being actively offered for licensing, those discussed in industry news sources and review articles, and those offered by leading genetic engineering and bioprocessing technology licensors. However, it does not cover every relevant published or patented technology.

Coverage - Simply stated, coverage concentrates on host cells/organisms, basic genetic engineering methods, recombinant constructs and the many technologies available to enable or improve expression of desired proteins, including glycoproteins and antibodies. This directory concentrates on the core genetic materials (e.g., host cell lines and organisms) and related methods and materials, e.g., vectors, promoters, selection and amplification methods, chaperones, etc., used or claimed useful for commercial-scale manufacture of biopharmaceutical products, primarily recombinant proteins and monoclonal antibodies. Thus, this directory concentrates only on what is used or needed for upstream manufacture (and nothing else).

This directory includes broad platform technologies, generally defined by the living host cells/organisms being used, which may be natural or genetically modified to begin with; and the basic genetic engineering technologies needed to get the desired gene sequence(s) into these hosts and get these genes efficiently expressed (transcribed and translated) for commercial-scale manufacture. Thus, this directory includes a number of specific genetic engineering technologies, e.g., vectors, promoters, chaperones, affinity fusion protein purification schemes, etc., useful with all, some or specific platform technologies/host systems.

NOTE!

This reference is based on published and unpublished information. It is recommended that readers confirm information, and obtain updates from license holders.

Technologies involve or can be defined or viewed in many ways or on many different levels. For example, one may broadly refer to yeast or baculovirus expression vector technologies, actually a grouping or classification of multiple technologies. And very often, what is referred to as a specific technology actually involves multiple components, each of which may be considered a technology, e.g, be separately available for licensing. In most or nearly all cases, technologies have been described in or exemplified by patents. Technologies involve know-how or enabling knowledge and related information. With biopharmaceutical manufacturing and genetic engineering technologies, this invariably involves information, e.g., methods and gene/protein sequences, often embodied in genetic constructs and culture collection deposits. In the biopharmaceutical area, just about every technology of interest has been or is in the process of being patented; and most technology acquisition or other technology transfer involves patent licensing. In many cases, all one needs to effectively acquire rights and implement a desired technology is to license related patents. In many other cases technology acquisition/licensing should involve or requires initial or even continuing technical assistance from the inventors or the organization handling licensing.

Coverage includes both technologies currently in predominant use for biopharmaceutical product manufacture, with these primarily based on use of *E. coli*, Chinese hamster ovary (CHO) cells and yeasts, primarily *Saccharomyces cerevisiae*, and new and upcoming alternative platforms/hosts, most of which have not yet been adopted/adapted for commercial-scale manufacture. Much of the older technologies, particularly those in use since the 1980s (including most *E. coli*, CHO and yeast technologies), have in recent years either lost or will soon lose patent protection. Many users of this directory will likely be interested in these proven, regulatory agency-familiar, cheap (now or soon no licensing expenses involved) but, in many respects, inefficient technologies. Most, if not most, directory users are presumed to be interested in new alternatives and/or significantly improving current in-house platform technologies, e.g., by adopting newer technologies offering higher yields.

What is not included - If a technology does not involve genetic materials and their manipulation, generally host cells/organisms and genetic constructs or methods, it has not been included, no matter how relevant to biopharmaceutical manufacture. Thus, this directory does not include;

a) technologies relevant to specific products, e.g., product-specific gene/protein sequences; only technologies relevant to manufacture of all or broad classes of proteins, including glycoproteins and antibodies.

b) protein engineering or other molecule design technologies, unless substantially involving commercial-scale protein expression. Thus, nearly all methods for designing and predicting protein structures are not included.

c) protein screening technologies, including selecting for desired active agent/product characteristics. An incredible number and diversity of screening technologies are available, but are not included.

d) some rather generic genetic engineering, molecular biologic laboratory technologies, with these sometimes discussed in brief generic entries. For example, there are hundreds of different chemical and physical agents and related methods used for transfection or modifying cells so that vectors or other genetic constructs get to and act upon genetic material within cells. Most of the related reagents and materials are readily available from multiple commercial vendors/reagent sellers, and most of the methods are readily available in standard references for molecular biology procedures.

Various basic, broad genetic engineering and molecular biology methods have been included where these have been patented and/or require taking a license for commercial use. Many directory users will be

unpleasantly surprised to learn that many of the most basic genetic engineering and molecular biologic method and reagents are patented, and require taking royalty-bearing licenses for commercial use.

e) generic, non-genetic engineering-based methods for host cell/organism modification, e.g., non-targeted or random mutation-based methods; e.g., exposure to mutagens and selection for desired characteristics or adaptation to specific; e.g., protein-free, culture media by growth, repeated passages and selection of adapting cells/organisms.

d) microorganism and cell culture, culture media, fermentation, and related bioreactor and fermenter technologies. This directory does include various microbes, other organisms and plant, insect and animal cell lines, many adapted to specific types of culture or bioreactors, e.g., adherent or suspension culture, and/or adapted to specific types of culture media, e.g., serum- or protein-free media; but does not include technologies related to bioreactors, fermentors, bioreactor/fermentor control, etc.

e) downstream technologies, including purification. This directory does not include separation, purification, formulation, viral inactivation or other downstream technologies. This directory does include genetic and protein expression-based methods for downstream processing, particularly purification using fusion proteins for affinity-based purification, but does not include chromatography and other technologies for protein purification.

In many cases, technologies, particularly those being offered for licensing, were described as such by their owners/licensors; and the author generally followed how inventors/licensors described their technologies. In many other cases in which a technology description is not clear, this author had to identify and define technologies. Thus, the author often defined and developed his own technology descriptions, presuming that essentially every/any technology, particularly those fully publicly disclosed (e.g,. in patents), is available for licensing (for the right price).

Luckily, the biotechnology industry is a relatively open market for technology licensing, i.e., most every non-product-specific technology is available for licensing, if one bothers to ask. However, there are some exceptions in certain areas, notably with higher plants, e.g., field crops, and transgenic mammals, where some companies simply hoard (do not license) their technologies or are very selective in their licensing., e.g. not licensing them to companies that might be worthy competitors.

Monographs Content - Descriptive entries are provided for ~340 technologies. Data fields are:

1) Title - The various names of associated with technologies and major components are included, optionally followed by a hyphen and the author's annotation of the host/organism system(s) used and/or special capabilities of the technology (e.g., glycosylation; antibodies manufacture).

2) Organizations involved - The major organizations involved are listed along with characterization of their role or involvement in the technology, e.g,. licensor, patent assignee, research, etc.

3) Description - A summary of available information about the technology concentrating on functionality, improvements provided, etc.

4) Use with - Brief characterization of the main host cells/organisms used with the technology.

5) Use to make - A brief characterization of the types of products the technology is designed or claimed to be useful for.

6) Background - An optional field presenting claimed benefits or desirable characteristics of the technology.

7) Patents - Information about relevant patents.

8) Licensing information - Information about licensing contact(s), optionally with information about related commercial actitivies, e.g., know licensees.

9) Products made with this tech. - An optional field presenting information about biopharmaceutical products made using/incorporating the technology.

10) Further info. - An optional field usually presenting citations to related publications.

Organization of Monographs - The monographs are divided into two main sections:

1) The first section presents broadly-enabling, platform-type technologies, particularly novel host cells and organisms.

2) The second section presents more specific, supporting and component technologies. These may be broadly generic, applying to diverse hosts/platforms, or applying to multiple or just one major host/ platform.

Note, these divisions represent purely subjective decisions on the part of the author! It is often very difficult to determine the relevance and utility of these technologies. What may be presented as a more specific, supporting or component technology, e.g., vectors or promoters useful with a specific organism or class of organisms, may along with other technologies be the single critical components enabling or defining a new manufacturing platform technology.

Within each of the two main technology sections, monographs are loosely classified or grouped by broad platform technologies, generally host cell/organism classes, e.g., E. coli, yeasts, mammalian cells, etc. However, keep in mind that most technologies are or can be presumed to either be relevant to multiple broad platforms, as is often presented in monographs, or may actually be relevant to just one specific platform, e.g., vectors claimed useful with yeasts may actually be only or primarily useful with *S. cerevisiae* or another yeast (but available information does not make this clear). The author generally followed the lead of available information from the licensor, including patents, in terms of describing the utility of specific technologies.

Indexes - The following indexes are provided:

1) Company/Organization

2) Subject

3) Primary Host Systems

See the text at the beginning of each of these indexes for further information about their coverage, conventions and limitations.

Information Sources Used - The primary source for the information in this directory was documents collected by the author specifically for this purpose over an approximate 3-year period. The author of this directory is also the author of *Biopharmaceutical Products in the U.S. and European Markets*, the only reference book/database concerning biopharmaceuticals (now in its 6th edition, 2 vol., 1602 pages. Besides deriving information about biopharmaceutical products manufacture from this source, as part of developing/maintaining this publication the author has long engaged in a continuous intensive competitive intelligence gathering and analysis program (i.e., he intensively reviews the world's press releases, industry newsletters, meeting abstracts and every other relevant publicly available information source). Even before starting recent work on this directory, the author had over 2,500 documents collected for use

in developing this directory. Thus, the author is confident that relevant technologies discussed in industry publications and at industry-oriented conferences in recent years have been included.

The monographs were largely assembled by modifying and piecing together text retrieved from diverse sources, mostly those available on the Internet (and within the bounds of fair use). Thus, those using this directory and doing their own research will likely be able to recognize text adapted from or extracted from Web sites, patents, articles, etc. However, in all cases, the author made sure to provide additional, value-added information and analysis. This includes providing what should be useful contact information, including use of the membership directory of the Licensing Executive Society (LES), the database of registered patent attorneys/agents at the U.S. patent office Web site, and otherwise finding E-mail addresses for relevant corporate contacts.

Other information sources and methods used in developing this directory include:

1) meeting announcements and abstacts - The world's major biotechnology-related conferences, particularly those with a commercial orientation or involving relevant sessions, have been monitored for several years.

2) literature searching - Some basic searching of the peer-reviewed biomedical literature, e.g. PUBMED, was performed, including searching for overview and review-type articles concerning broad platform technologies. In many cases, the biomedical literature was also searched concerning specific technologies.

3) patent searching - Much searching of U.S. patents and applications (primarily using U.S. patent office full-text databases) and international patents/ applications (primarily using EspaceNet) was performed. Besides patents often being required to explain or obtain basic descriptive information concerning technologies, patents very often provide analyses of related prior art (previous or competing technologies).

4) Web sites - The Web sites of essentially every company/organization included in this reference, and many others, were examined for technology-related information and to determine optimal contacts for licensing-related inquiries. This included checking the online technologies available for licensing listings of those organizations well known as sources of bioprocessing and genetic engineering technologies/ patents. For example, the University of California and NIH have consistently been among the leaders in obtaining U.S. genetic engineering patents; RCT is the licensor for several basic platform technologies, and the Boyce Thomson Institute and Texas A&M University are sources for various insect cell/ baculovirus expression technologies.

5) federal research funding and contracts - CRISP and other databases covering NIH and other federal agency research funding and contracts were searched. Thus, the various expression systems being developed largely with federal funding, mostly related to biodefense, are included, e.g., the DARPA, DOD, and NIAID, NIH, grants and contracts seeking to develop systems for rapid manufacture of large amounts of recombinant proteins, e.g., millions of doses of vaccines in just several months.

6) licensors/technology sources - The licensing contacts of hundreds of organizations, including the majority of those mentioned, were contacted by the author by E-mail, requesting public/publishable information about relevant technologies, particularly those available for licensing.

As further discussed, there are various reasons why many companies (vs. universities) are hesitant to provide information for directories. Many are unprepared for anyone requesting nonproprietary

information about their technologies available for licensing (making this directory all the more valuable). And despite it being counter-productive, technology transfer/licensing professionals, and many scientists/ inventors involved in licensing and invention marketing simply prefer to avoid disseminating information about licensing opportunities. Many licensing professionals feel that technology transfer/licensing is best practiced, with public information dissemination viewed as a less sophisticated approach.

Information Sources Not Used; Limitations/Caveats- Bound by limitations of time and expenses, the author did not use a number of relevant information resources and acquisition methods (that directory users may want to follow-up with). For example, with over 300 technologies, if an information resource or acquisition method was not free, i.e., involved spending money, it almost certainly was not used. Thus, the author did not use high-end, fee-based online databases, e.g., DERWENT patent databases, online versions of *Chemical Abstracts*, etc. Some fee-based databases were searched using the online databases at a local university library, along with document delivery services, but primarily to retrieve review articles, not to retrieve information about specific technologies. Otherwise, the author concentrated on finding and summarizing information to provide useful, but not all, information about technologies' functions/characteristics, advantages and ownership. Thus, information retrieval was not exhaustive - the author stopped looking when seemingly adequate descriptive and ownership information was retrieved.

Users should exercise caution in interpreting what technologies are actually relevant or useful for! The author generally describes technologies much as described by their licensors and/or inventors. Many times, licensors/inventors tend to restrict their claims about functionality and utility only to what they have studied or documented, while other times they may be too expansive in their claims. The author's descriptions reflect the content of inventions-available-for-licensing descriptions, patent descriptions and claims, i.e., available information. For example, some descriptions (reflecting their source) are probably too broad in their claims, e.g., may be primarily or actually relevant to one or a few members of a class of organisms, while licensors/inventors claim utility for an entire class of organisms (e.g., an invention actually relevant to only human cells may be claimed as relevant to all mammalian cells or eukaryotes). Conversely, inventions may be described as relevant to xyz specific organisms or uses, but may actually be relevant to many others (e.g., an invention claiming relevance to E. coli may actually be useful with all bacteria or all organisms).

Also, be aware that many major sources of biopharmaceutical processing technology simply make it hard for anyone to find and approach them or figure out what licensable technologies they have. And, many technology sources are seemingly only interested in dealing with major players. For example, essentially all of the long-surviving biotechnology/biopharmaceutical companies, i.e., those around for several decades, have amassed considerable portfolios of patented and also unpatented proprietary manufacturing-related technologies. However, few technologies from these major companies, e.g., Genentech, Amgen, Biogen Idec, Wyeth/Genetics Inst., J&J/Centocor, etc., are included in this directory. Adequate information is simply unavailable, with essentially none of these companies publicly disclosing their manufacturing- or basic genetic engineering-related technologies available for licensing, their licenses granted or responding to the author's inquiries. Similarly, most every contract manufacturing organization (CMO) has likely developed in-house proprietary technology and/or licensed-in and is able to offer sublicenses or access to technologies from others. These technologies have been included where information was available, but following the general pattern, seemingly few CMOs bother to disclose their proprietary technologies in their public information or only do it in vague generalities [yet another paradox in the marketing (or lack of it) of biopharmaceutical manufacturing and related genetic engineering technologies].

For many users, examining all entries will be the most effective way to use this directory, in addition to using its organization into topical sections and its indexing. Knowledgeable persons will likely be able to see and make their own connections and conclusions about the relevance of technologies. Many technologies that may seem irrelevant, e.g, from their titles, placement and/or indexing, may actually provide new ways of approaching problems or provide improvements that you had not been looking for.

Finding Further Information - So, with this directory designed to get you started, what can or should you do after finding technologies of interest. Obviously, much depends on your particular interests. Your options include:

a) Search the world's publications, Web sites, patents, etc. Use Google and one or more complimentary Web search engines. Search the biological and chemical literature, e.g., PUBMED, use the online versions of *Chemical Abstracts*, *Biological Abstracts*, and do not ignore the chemical engineering literature, which may also have relevant information. Whether from going through their Web site and/or searching the Web, read up about the licensor organization and any related licensing track record. Use patent databases, including better fee-based ones, to retrieve further information about patents, e.g., what is their status, which countries are patents being sought in, etc. For old(er) technologies, e.g., those invented in the 1980s or with patents granted about 17 or more years ago, check to whether patents have expired in countries of interest. If so, you may not need to take a formal license. If a technology involves a biological material, e.g., cell line, and even if it is in the public domain, it cannot hurt to license this from the original source, vs. getting a derivative from a culture collection or commercial vendor. This may save considerable testing and avoid documentation problems with FDA and other regulatory agencies.

b) Network with and/or delegate or pass-upwards your inquiries to others in your organization, particularly your own technology transfer office or professionals. Licensor contacts, particularly licensing professionals, are more likely to respond to inquiries from other technology transfer professionals, patent attorneys, corporate executives, etc., vs. inquiries from scientists or mid-level managers.

c) Make contact with the licensor - The Company/Organization Index includes a contact point to initiate licensing-related inquiries. The response you get from these or other contacts may depend on your organizational affiliation, e.g., whether you are perceived as a potential client or competitor, and whether you are perceived as having licensing negotiation authority. Of course, it is always best to be prepared and as knowledgeable as one can be when interacting with licensor contacts, many of whom are already overworked with dealing with obtaining patents on inventions, negotiating licenses. It is probably best to volunteer up-front to sign non-disclosure agreements, even if you are only seeking public information, with this showing that yours is more a genuine licensing vs. simply an information or competitive intelligence gathering request. If you don't get a prompt response, make personal contact because many technology transfer professionals prefer personalized information dissemination and they prefer personal contacts before they respond.

Most every licensor will or should be able to offer serious inquirers various options, ranging from sending out information (which may require a non-disclosure agreement), material transfer agreements (MTAs) or other standard agreements allowing access/release of materials, e.g, cell lines or vectors, for further study, usually with many explicit limitations on use; licenses allowing limited in-house technology evaluation; and other options short of a full licensing with big up-front licensing payments and royalties on sales.

d) Contact the inventor(s). Besides being the most knowledgeable, they are more likely to be scientists, and will likely be more responsive at least in terms of providing you with public/published information

and even discussing your potential interest/application. And, many inventors make themselves available for you to hire as consultants or contractors.

e) If you want an outside expert(s)/consultant(s) to do further research and make initial contact, which could include not disclosing your identity contact the publisher, BioPlan Associates, Inc.; info@bioplanassociates.com; 301-921-9074.

Monographs Table of Contents

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Yeasts

Entry Number Monograph Titles

Animals, misc.

Mammalian

Chinese hamster ovary (CHO) cells

Entry Number Monograph Titles

Insects

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More Speci□c and Component Technologies

Bacteria/Prokaryotes

Indexes

Expression Systems and Genetic Engineering Technologies: Opportunities for Innovators, CMOs and Product Developers

by Ronald A. Rader

Introduction:

New expression systems and recent improvements available for current systems have the potential to revolutionize the biopharmaceutical industry! As reflected by currently marketed products, since the advent of genetic engineering in the 1970s, there has been little basic change in the technologies used for commercial-scale manufacture of biopharmaceutical products. Nearly all current products are manufactured using much the same old, familiar technologies – primarily using *Esherichia coli* (*E. coli* bacterium), Chinese hamster ovary (CHO) cells and the yeast *Saccharomyces cerevisiae (S. cerevisiae*) as hosts – technologies invented in the 1970s and commercialized in the 1980s.

Biopharmaceutical Expression Systems and Genetic Engineering Technology

Current and Future Manufacturing Platforms

New expression systems have the potential to revolutionize the biopharmaceutical industry! Until recently, there has been little basic change in the technologies used for commercial-scale manufacture of biopharmaceutical products. Nearly all current products are manufactured using much the same old, familiar technologies – primarily using Esherichia coli (E. coli bacterium), Chinese hamster ovary (CHO) cells and the yeast Saccharomyces cerevisiae (S. cerevisiae) as hosts – technologies invented in the 1970s. Today, a number of factors are rapidly changing the biopharmaceutical manufacturing environment. Scientific and technological advances offer significant advantages. Recombinant protein manufacture that typically involved multi-1000 liter bioreactors and dedicated facilities can now be accomplished using bioreactors an order of magnitude smaller.

Expression systems – These systems encompass the technologies needed to genetically modify organisms for the manufacture of recombinant proteins (including glycoproteins and antibodies). This book is perhaps the single most informative source concerning commercial biopharmaceutical product manufacturing-related expression systems and basic engineering technologies. The primary goal is to inform the user of the many technologies in commercial use and those claimed to be useful for commercial-scale manufacture of biopharmaceutical products. This directory should save the reader considerable time and effort in finding technologies relevant to his or her interests. It should reliably cover relevant technologies currently being used commercially, those being actively offered for licensing, those discussed in industry news sources and review articles, and those offered by leading genetic engineering and bioprocessing technology licensors.

Coverage - This directory concentrates on what is used or needed for upstream manufacture. Coverage concentrates on host cells/organisms, basic genetic engineering methods, recombinant constructs and the many technologies available to enable or improve expression of desired proteins, including glycoproteins and antibodies. This directory concentrates on the core genetic materials (e.g., host cell lines and organisms) and related methods and materials, e.g., vectors, promoters, selection and amplification methods, chaperones, etc., used or claimed useful for commercial-scale manufacture of biopharmaceutical products, primarily recombinant proteins and monoclonal antibodies.

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