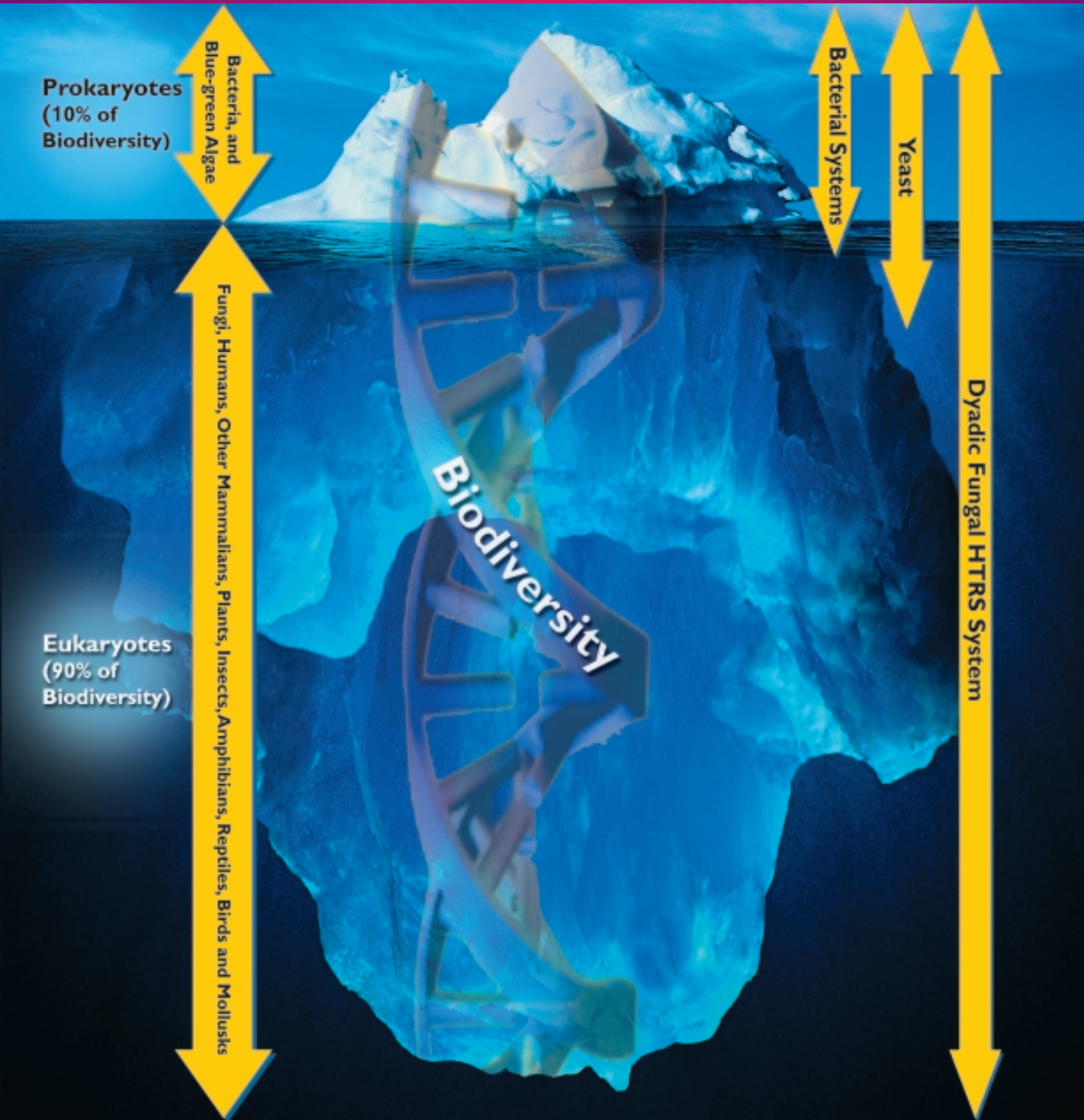


Freedom of Expression

Why stop at the tip of the genomic iceberg?



Rapidly discover **ALL** of biodiversity with
the Dyadic® Fungal HTRS System

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Dyadic®: The “One-Stop Shop” From Eukaryotic Gene Discovery to Commercial Manufacturing

The C1 Fungal High-Throughput Robotic Screening (HTRS) System and C1 Fungal Hyper-Producing Protein Expression System

The Challenge: Developing Products from DNA

Within the *eukaryotic* kingdom exists millions of organisms. These organisms represent a largely untapped source of potential genes that can be used to make new products of commercial value. But to date, only a small number of eukaryotic genomes have been sequenced and only a small fraction of these genes have been isolated and functionally characterized. Among those, even fewer have been translated into commercially viable products. The reasons for this are varied, but in general, most of today's *genomic* and *proteomic* R&D programs face **three major bottlenecks**:

Bottleneck 1

Ability to Rapidly Identify Viable Product Leads

Up to 90% of the *biodiversity* found on the earth, such as yeast, fungi, plants, insects, fish, and other animals including humans, are eukaryotic organisms¹, that is, they are multi-celled with a nucleus. The genes within these eukaryotic organisms generally contain introns. However, current methods for rapidly screening for new product leads – called High-Throughput Robotic Screening (HTRS) – often fail to recognize proteins from eukaryotic organisms. This is because currently used discovery organisms, primarily bacteria but also yeast on a limited basis, are unable to adequately process introns found in native eukaryotic genes. This failure leads to an inability to convert genomic sequences of eukaryotic genes into functional proteins, **which means that up to 90% of the world's potential product leads are still waiting to be discovered.**

Bottleneck 2

Producing Sufficient Protein for Laboratory and Application/Clinical Testing

Even when HTRS assays identify a protein with commercial potential, developers still face the challenge of making enough protein with which to work. If researchers are unable to make enough functional protein, it is impossible to continue research, development and applications testing work, including clinical trials for pharmaceuticals. **Many pharmaceutical and biomaterial research programs have been cancelled due to the inability to produce adequate quantities of these newly discovered biomolecules.**

Bottleneck 3

Difficulty in Bringing Biological Product Leads to Market

Due to the fact that different organisms express proteins differently, genes that produce active proteins in one living organism are often unable to make economically viable quantities of functional proteins when placed in another organism. Mass production technologies of today often require that genes discovered in a bacterial or yeast HTRS system be expressed in a completely different commercial production system, such as animal cell culture like Chinese Hamster Ovary (CHO), yeast or filamentous fungal systems. Consequently, there is no guarantee that a protein, once discovered, can ever be manufactured. **Bottlenecks in commercial scale production—a late stage in product development—are extremely costly and may lead to delays or cancellation of otherwise promising projects.**

¹ E.O. Wilson, F. Baird – The Current State of Biological Diversity “Biodiversity” National Academy of Sciences, National Academy Press, Washington D.C. 1988.

The Dyadic Solution – C1, the “One-Stop Shop”

Dyadic® International, Inc. has developed the foundation to overcome each of these monumental hurdles. Dyadic has developed ***Chrysosporium lucknowense* (C1)** to go from gene discovery to commercial manufacturing using the same host organism (see Figure 1). The C1 Fungal High-Throughput Robotic Screening (HTRS) System (Patent Application #WO 01/25468) and the C1 Fungal Hyper-Producing Protein Expression System (Patent Application #WO 00/20555) utilize the C1 organism to rapidly convert genomic libraries from the world's vast pool of eukaryotic DNA into functional biomolecules, leading to rapid gene discovery and commercial manufacture of valuable products.

Unique Features of the C1 Eukaryotic Technology Platform

The unique Dyadic C1 Hyper-Producing Fungal System was isolated from an alkaline-soil lake and then genetically modified to serve as an optimized eukaryotic host organism for the Dyadic HTRS and C1 Protein Expression Systems. It is being developed to:

- Take DNA from virtually any eukaryotic or prokaryotic organism and translate it into protein.
- Produce enough protein to enable gene discovery and assay screening, utilizing existing HTRS equipment.
- Manufacture the protein in commercial quantities.

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C1: Breaking Through the Bottlenecks

Dyadic scientists are developing the C1 organism to overcome many of the obstacles encountered with other fungal, yeast and bacterial systems that scientists traditionally utilize to translate DNA into protein from biological samples. The primary hurdle is that while most organisms used to screen DNA are *prokaryotic*, or single-cell organisms lacking a nucleus, roughly 90% of the world's biodiversity are *eukaryotic*.¹

Making Sense and Substance from Genetic Diversity

The problem with traditional prokaryotic organisms, such as *E. coli* bacteria, is that they are unable to take DNA from eukaryotes and express it as a functional protein. This is primarily because native eukaryotic genes contain *introns* (genetic sequences not involved in coding for protein), which bacteria, and for all practical purposes, yeast cannot properly splice out. C1, however, is a eukaryotic organism, which can properly process introns similar to those found in humans, other animals and plants²; so it is well suited as a host organism for expressing native genes from such sources. Eukaryotic genes also express proteins that must have glucose or sugar molecules attached in the correct arrangement. This process, called *glycosylation*, is a function bacteria cannot perform. C1 efficiently provides access to a largely untapped eukaryotic world for genetic exploration. By doing so, C1 will be superior to other organisms used to express genes, including yeast, other fungi and cell culture, which often use CHO cells.

cDNA Techniques and the Dyadic C1 Fungal HTRS System

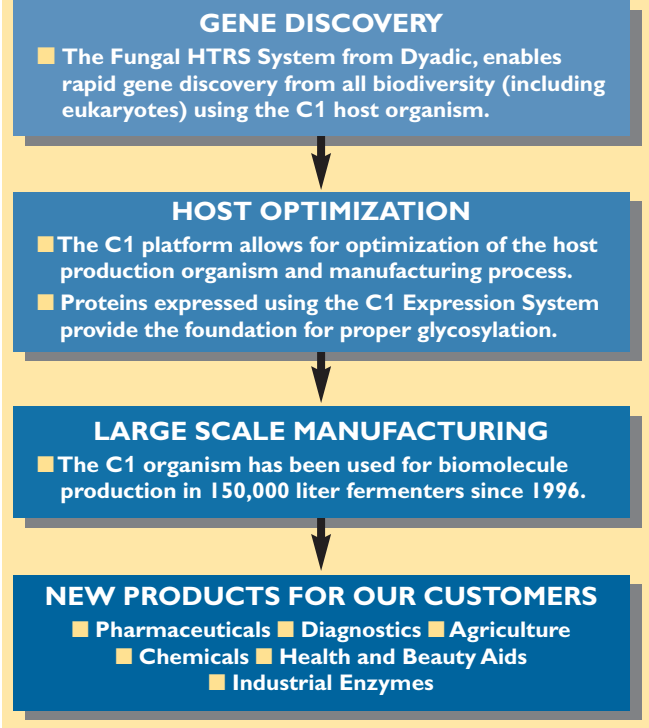
In order to get around the inherent problem encountered in expressing genomic DNA from higher eukaryotes in bacteria and yeast, due primarily to these host organisms' inability to splice introns commonly found in these eukaryotic genes, many scientists have had to resort to the use of cDNA techniques.

The generation of cDNA libraries requires that researchers obtain high quality mRNA after culturing the organism. Since mRNA is highly unstable, it is virtually impossible to obtain mRNA of the desired quality from environmental samples, and not all organisms in an environmental sample are amenable to culturing under standard laboratory conditions.

cDNA libraries inherently represent only a subset of the total genome of an organism because they are influenced by the growth conditions and growth stage of the cultured organism. A further limitation is that these cDNA libraries are biased towards those genes that are most highly expressed. Many genes are over or underrepresented depending on the culture conditions used to obtain the mRNA. The only way to get a complete and unbiased gene library is to generate it from genomic DNA.

In view of the above, cDNA strategies result in many missed opportunities in the discovery of novel genes of commercial value. The proprietary Dyadic C1 fungal HTRS System has the capability of splicing many of the introns found in genomic sequences of eukaryotic genes. Therefore, the proprietary genomic libraries at Dyadic can be rapidly screened utilizing existing High-Throughput Robotic Screening equipment to discover and analyze new functional proteins and other biomolecules from the world's vast pool of eukaryotic DNA.

FIGURE 1: THE C1 ADVANTAGE



The Dyadic C1 host organism does not require cDNA libraries, although this fungal organism is quite proficient in expressing genes from cDNA libraries.

The Advantages of C1

Filamentous fungi are eukaryotic organisms known to have the capability to splice out the introns found in native eukaryotic gene sequences and, therefore, possess the innate ability to convert genomic DNA into functional proteins. However, filamentous fungi are highly matted and viscous (thick) when grown in liquid cultures, making them unsuitable for use in existing HTRS equipment (see Table 1).

Dyadic scientists spent several years engineering and optimizing strains of the C1 organism that can express proteins at many times the level of currently used organisms. And unlike other filamentous fungi, C1 grows in *propagules*, or non-filamentous fragments that behave similarly to single cell organisms, like bacteria, and have very low viscosity when cultured. The C1 propagules allow analysis of individual clones in the culture wells used in HTRS gene discovery systems, which companies use to screen hundreds of thousands of genetic samples for proteins or enzymes of interest. The propagules can also be readily transferred from one microwell to another, which allows for greater ease of use in screening systems.

C1 grows optimally and expresses proteins under low viscosity and neutral pH conditions (shown in Table 2). Furthermore, many low *protease* strains of C1 with strong promoters are available, which make C1 a very powerful expression system for eukaryotic genes from diverse sources.

² M. Deusch, M. Long - Intron-exon Structures of Eukaryotic Organisms. *Nucleic Acid Res* 1999, Aug 1:27(15):3219-28

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Table 1 compares the most commonly used host organisms for expressing native eukaryotic genes.

TABLE 1: CAPABILITIES OF CURRENT GENE EXPRESSION HOST ORGANISMS

	Bacterial Systems	Yeast	Chinese Hamster Ovary (CHO)	Other Fungi	C1
Intron Processing	None	Limited	✓	✓	✓
Expresses Eukaryotic Proteins	Very Limited	Limited	✓	✓	✓
HTRS Compatible	✓	Limited	No	No	✓
Glycosylation	None	Over-glycosylation	✓	Over-glycosylation (less than yeast)	✓
Optimizable Output for Large-Scale Manufacturing	✓	✓	Limited	✓	✓
Single Organism from HTRS Discovery to Manufacturing	Limited	Limited	No	No	✓

Not only can the C1 expression system be used to express protein for HTRS, but the very same host organism, C1, also can be used to mass produce proteins on a commercial scale. In terms of mass producing proteins and enzymes, the C1 organism also holds several key advantages over existing systems. On a commercial scale, **the C1 organism can produce biomaterials in a variety of conditions — including near human physiological conditions (37° C & neutral pH),** which is generally not the case in other fungal expression systems (see Table 2). This is a major advantage for producing functional biomolecules targeting human diseases as these proteins are often sensitive to acidic pH and protease activities. Also, the time it takes to produce a given quantity of biomolecules on a commercial scale, whether enzymes or proteins, is much shorter with C1 than CHO cells, yeast and other commercially used fungal systems. In addition, it is also possible to optimize output and improve expression levels in C1 by engineering and selecting for an improved strain of C1 organism, a process called *mutagenesis*. This is not possible in higher eukaryotes such as CHO cells.

C1, From Gene Discovery to Manufacturing in One Organism

The unique C1 Dyadic systems can be used in every stage in the research and development process (see Figure 1). The process starts by extracting DNA from an organism of interest, be it a fungus, plant or animal in nature, and creating a DNA “library” (see Figure 2). To fill the library, the organism’s DNA is separated and inserted, piece by piece, into thousands of C1 organisms, each one residing in a tiny microwell. The C1 organism takes up the DNA and translates each fragment of unknown sequence data into a unique protein. The proprietary expressed eukaryotic genome libraries at Dyadic can be screened using the Dyadic Fungal C1 HTRS System to assist companies seeking a particular protein or enzyme. This system allows researchers to rapidly search through the hundreds of thousands of samples present. If the target biomolecule is there, the Fungal C1 HTRS System will express the protein of interest inside the tiny microwells used in existing high-throughput screening equipment.

TABLE 2: FERMENTATION CONDITIONS — C1 VS. OTHER FUNGAL SYSTEMS

	Aspergillus	Trichoderma	Dyadic C1
pH	3.0-6.0	3.0-6.0	4.5-9.0
Temperature (°Celsius)	30-37	25-32	25-43
Viscosity (Centipoise)	1,500-2,000	200-1,000	<10
Fermentation (Days)	8-9	6-7	5

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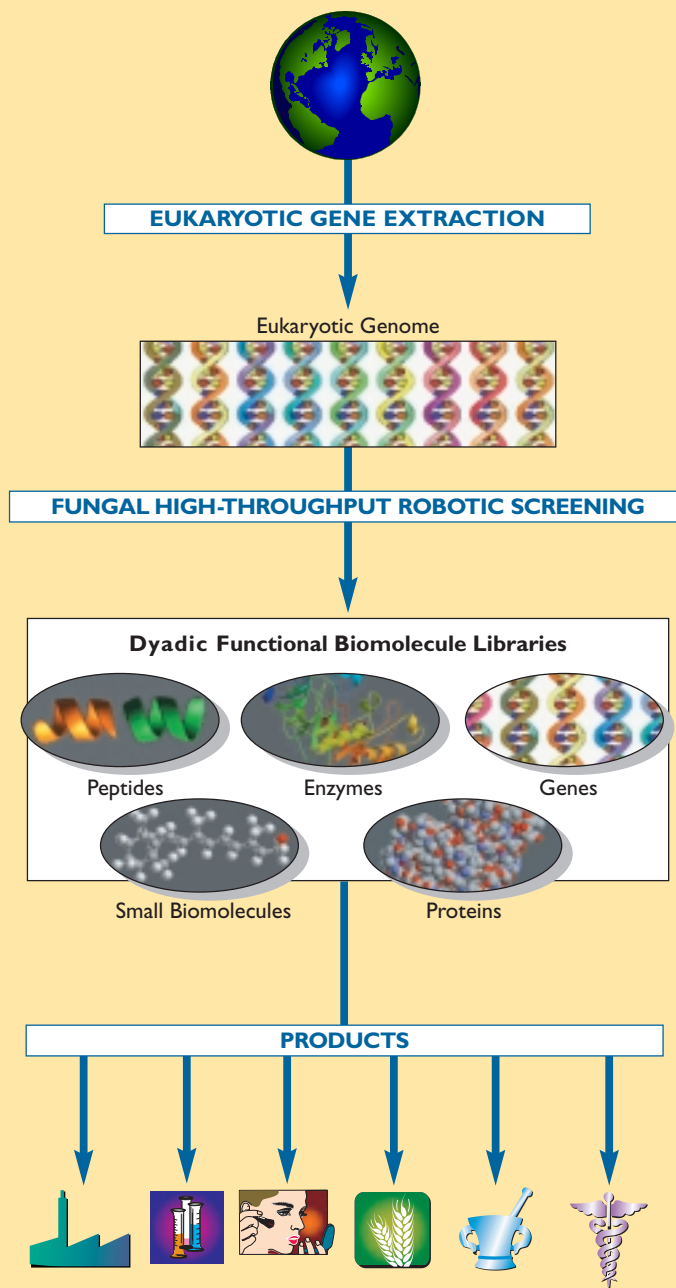
Once the desired protein is discovered, researchers then take the gene that codes for the production of that protein and insert it into another C1 host production organism specially engineered for over-expression. Scientists will then begin studying the protein and characterizing its properties. C1 can efficiently produce large quantities of protein by virtue of its proprietary “expression cassette,” a set of genetic promoter and terminator DNA sequences designed to drive over-expression. Once it has been sufficiently studied, multiple copies of the specific gene can then be taken up by the host production organism using proprietary expression cassettes. This facilitates the development of an optimized production organism and large-scale manufacturing process for the biomolecule of interest.

Commercial Applications of the C1 Fungal Expression System from Dyadic

Dyadic combines C1 Fungal HTRS System (Patent Application # WO 01/25468) with its unique Hyper-Producing C1 Fungal Expression System (Patent Application # WO 00/20555) in an integrated technology platform. Dyadic applies this technology platform in discovering, developing and manufacturing therapeutic, industrial, chemical, agriculture, diagnostic, and other protein products.

The core of the Dyadic C1 Technology Platform is the C1 host organism, which can be used in any industry interested in the manufacture of biomolecules. Dyadic is using its C1 organism to discover and produce various enzymes in 150,000 liter fermenters for use in industrial processes. However, the potential areas for gene discovery and commercial application are many and diverse. One area is the pharmaceutical arena, as companies continue to search for new therapeutic proteins and peptides for use in medicine. There are also agricultural applications, as firms seek to make better, less harsh insecticides and produce hardier plants, as well as enriched food sources that can serve as potential nutraceuticals and vaccines. The chemicals industry can also use the C1 technology to enable cost-effective manufacturing of many fine and specialty chemicals, fibers, plastics, resins and organic solvents for which economically viable chemical manufacturing processes are currently unavailable. As the C1 system uses renewable agricultural feedstock, it can help reduce the environmental burden of many petroleum-based chemical processes. Other important markets that can benefit from enzymes discovered using Dyadic technology include the animal feed, detergent, food processing, starch, textile, pulp and paper, and health and beauty aids industries.

FIGURE 2: BUILDING PRODUCTS FROM OUR FUNCTIONAL EUKARYOTIC LIBRARIES



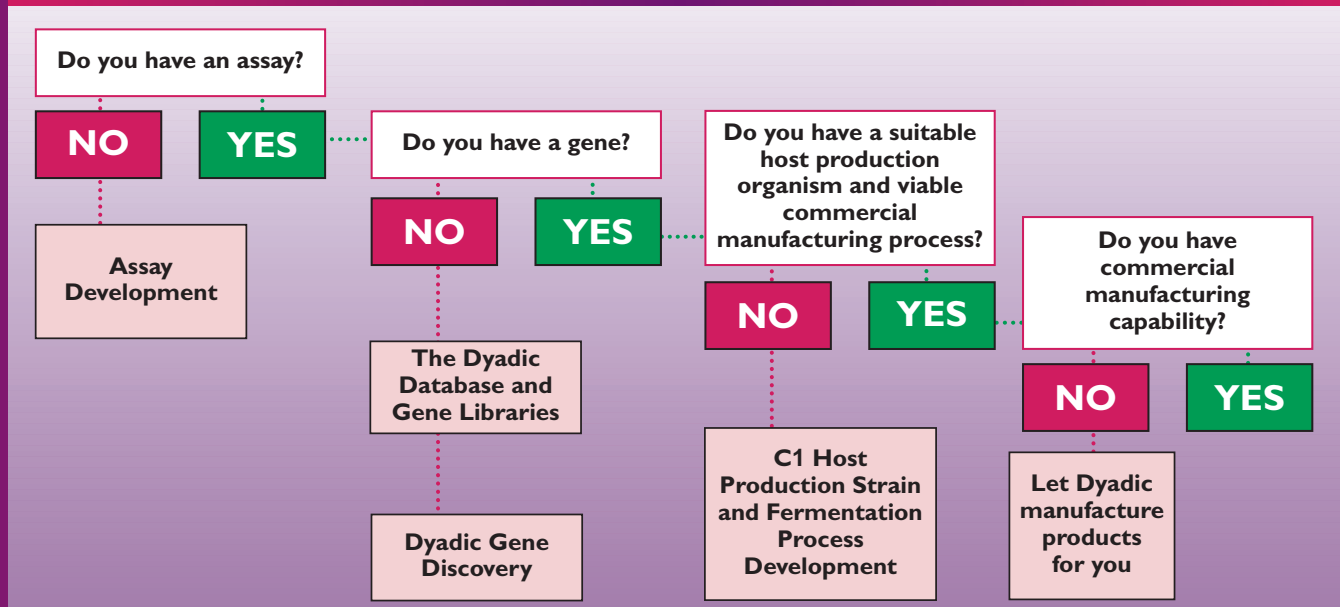
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ABOUT DYADIC INTERNATIONAL

Founded in 1979 by Mark Emalfarb, Dyadic International, Inc. has evolved into a leading biotechnology company with unmatched capabilities in the area of functional proteomics. With a core business in industrial enzymes, Dyadic embarked on a series of international collaborations leading to the development of its proprietary *Chrysosporium lucknowense* (C1) High Throughput Robotic Screening (HTRS) and Protein Expression Systems. Dyadic plans to develop functional biomolecule libraries and license its proprietary C1 technologies for biomolecular research and development projects across a broad cross-section of industries including pharmaceuticals, diagnostics, agriculture, chemicals, health and beauty aids, and industrial enzymes.

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