

Eclonals

An Integrated Platform for Full Length IgG Antibody Discovery, Optimization and Production in Bacteria

The Eclonal technology is an integrated platform for the isolation, engineering and expression of IgG antibodies in bacteria.

- Proprietary system for efficient and reliable expression and screening of IgG antibody libraries in bacteria
- APEx method for rapid screening of IgG combinatorial libraries and the isolation of antibodies with desired specificity
- Antibodies are aglycosylated and therefore have greater stability, but can also be engineered to mediate specific binding to Fc receptors.
- Bacterial fermentation process for reliable and high yield production of homogenous batches of antibodies

As a fully integrated platform, the Eclonal technology allows discovery, optimization and testing of antibodies in one system.

Compared to the existing antibody discovery technologies, the Eclonal technology provides the following advantages:

- Novel technology that is not dependent on existing methods for antibody production
- Production of homogeneous IgGs at industrial titres of 1-1.5g/L
- Possibility to engineer the Fc region that displays specific binding
- Rapid, inexpensive and highly integrated route to the discovery of therapeutic antibodies

Intellectual Property

Clayton Biotechnologies has world-wide intellectual property protection for this technology filed under Research Development Foundation. The following are examples of our US patents and patent applications:

- US60/915183 AGLYCOSYLATED IMMUNOGLOBULIN FC POLYPEPTIDES
- US60/982652 METHODS FOR GENERATING ANTIBODY LIBRARIES
- U.S. Patent 7,094,571, U.S. Patent 5,866,344, U.S. Patent 5,348,867,
- US2007/0099267, US2006/0029947, US2005/0260736, US2004/0058403, US2007/0258954, US2004/0072740, US2003/0036092

Available for licensing or partnering

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The Eclonal Platform

The Eclonal platform technology has been developed by Professor George Georgiou who heads the scientific team at the University of Texas, Austin in a research program conducted by the Clayton Foundation for Research. The Eclonal technology uses *E.coli* bacteria as the host cell for the discovery, optimization and production of full length IgG antibodies. Large libraries of human IgG antibody variants have been constructed and expressed in bacteria. Cells producing antibodies that bind to the desired target molecule with high affinity are isolated by high throughput screening using magnetic and flow cytometric cell sorting and other techniques. The scientific team has demonstrated the isolation of very high affinity IgG antibodies using the Eclonal platform, both from immune and from synthetic libraries. Eclonal antibodies are selected to exhibit high stability and expression characteristics.

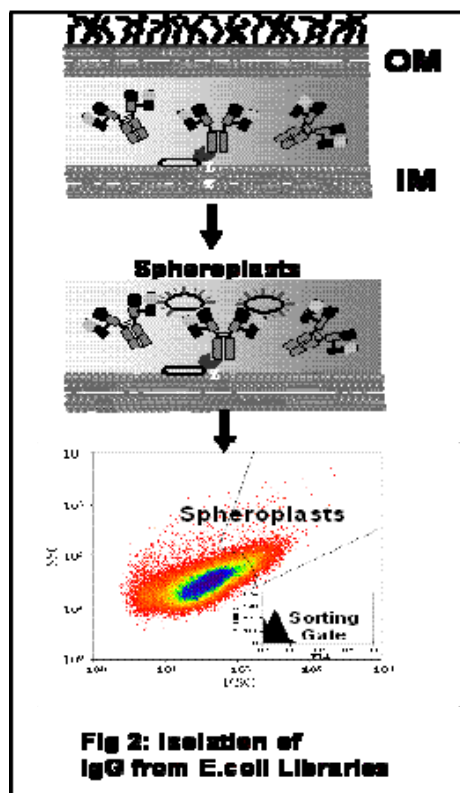
Bacterially produced IgG antibodies are not glycosylated. Normally, without glycosylation IgG antibodies cannot interact and recruit the innate immune system. Dr. Georgiou's team has isolated mutations which bypass the need for glycosylation. These engineered IgGs (containing mutations in the Fc domain of the antibody) interact with innate immune proteins with high specificity and affinity similar to or higher than that displayed by human glycosylated antibodies. Evaluation of the therapeutic efficacy of the engineered aglycosylated antibodies in cancer models is in progress.

Eclonal antibodies are produced by bacterial fermentation. Earlier studies have demonstrated that bacterial fermentations can yield IgG titers of 1 g/L or more. Bacterial fermentation is technically much simpler, faster and cost effective than the current production methods that involve mammalian cells.

Competitive Advantages of the Eclonal Platform

Homogeneous product	⇒ More predictable pharmacodynamics, high therapeutic efficacy.
Unique therapeutic optimization strategy leading to fine-tuned interactions with effector innate immune cells	⇒ Potential for increased therapeutic potency
Greatly accelerated therapeutic discovery and development timeline	⇒ EClonal platform will be able to reduce the time from target to preclinical from 16-20 months to 60 days.
Low cost manufacturing	⇒ Lower costs
Minimized third party royalties	⇒ Lower costs
Seamless integration of discovery, optimization and production using one host cell system and a single antibody format IgG.	

Technical Description of the Eclonal Technology



Eclonal Discovery platform IgG libraries are constructed and expressed in *E.coli* where they accumulate in the periplasmic space, between the inner (IM) and outer (OM) membranes of the bacterium. The IgG antibodies are expressed as a dicistronic operon that circumvents existing IP positions for antibody expression in *E.coli*.

Cells are grown under conditions that ensure the optimal assembly of heavy and light chains into IgG. Within the *E.coli* periplasm, the IgG is captured on the surface of the inner membrane (IM). One preferred way for the capture of IgG molecules on the surface of the inner membrane is via co-expression of Protein A fused to a lipoprotein anchor sequence that tethers it stably to the inner membrane¹. This strategy is called Anchored Periplasmic Expression or APE_x and has been described in several high profile scientific papers and is protected by several issued and pending patents.

Subsequently, the outer membrane (OM) is stripped from the cells by treatment with Tris-EDTA-lysozyme and thus the cells are converted to spheroplasts. Following incubation with fluorescently labeled antigen, spheroplasts displaying IgG that recognize the antigen become labeled and are isolated by high throughput flow cytometry or by fluorescently activated cell

sorting (FACS). We have thus obtained antibodies with sub-nanomolar affinities from IgG libraries derived from immunized animals or from synthetic libraries where sequence diversity had been introduced by complete randomization of the hypervariable loops.

The screening of large libraries of antibody producing cells by FACS instrumentation is time consuming. To accelerate this process we have introduced prescreening steps aimed at eliminating clones producing unrelated, non-specifically binding antibodies. Library prescreening has been accomplished by a novel, proprietary method that utilizes non-covalent display of IgG on bacteriophage. We have also implemented the pre-screening of spheroplasts (outer membrane stripped cells) by magnetic sorting. The combination of pre-screening by phage/magnetic sorting followed by FACS allows the isolation of the best possible antibodies in terms of affinity, stability and suitability for expression.

¹ Mazor, Y., Van Blarcom, T., Mabry, R., Iverson, B.L. and G. Georgiou, "E-clonal Antibodies: Isolation of Engineered Full-Length IgG from Libraries Expressed in *E. coli*" *Nature Biotechnology* **25**:563 (2007).

Additionally, several special features of the Eclonal screening platforms critical for the discovery of antibodies for specialized applications have been demonstrated:

- Seamless conversion of IgGs to FAB antibody fragments and back without subcloning allowing switching from bi- to monovalent formats to facilitate the isolation of ultra-high affinity antibodies.
- Selection of antibodies that exhibit optimal characteristics for high level expression in bacteria and can be produced in high yields.

Therapeutic Optimization; Fc Engineering of Eclonal antibodies for ADCC and CDC. Variations of the Eclonal screening platform have been developed for the optimization of additional pharmacological properties of IgG antibodies including:

- Endowing the aglycosylated Eclonal antibodies with specific binding to Fc γ receptors and to complement proteins for ADCC and CDC.
- Generation of sets of Fc domains exhibiting specific binding to only a single Fc γ receptor or to a select panel of Fc γ receptors for fine tuned ADCC and enhanced killing of cancer or pathogen target cells.
- Long serum half-life via the engineering the interaction with the Fc γ Rn protein
- Increased stability to serum proteases

In one notable example, we have engineered a mutant Fc region having two amino acid substitutions in the CH3 domain that exhibits very specific binding to the Fc γ RI receptor. Eclonal IgG containing these mutations binds to the Fc γ RI receptor with an affinity 1,000 higher than that exhibited by the parental aglycosylated antibody. In fact, even though the engineered IgG is non-glycosylated it binds to Fc γ RI with an affinity indistinguishable from that of glycosylated antibodies produced by mammalian cells. This engineered Eclonal IgG binds specifically to the Fc γ RI receptor (which is expressed in macrophages, neutrophils and dendritic cells) but does not interact at all with any of the other human receptors. Similarly aglycosylated Eclonal Fc domains that bind selectively to Fc γ RIIa, Fc γ RIIIa and Fc γ RIIb are being developed. In this manner any variable domain having a desired specificity can be fused into various engineered Fc to give rise to a panel of antibodies each of which recruits a specific subset of effector cells for optimal ADCC. In parallel, the Georgiou group is engineering Eclonal antibodies with varying abilities to activate complement, from none at all to much higher than that of mammalian antibodies.

Bioprocessing: Eclonal IgG antibodies are expressed in bacteria. The Heavy and Light chain polypeptide chains are secreted into the periplasmic space of *E.coli* where cysteine residues are oxidized to form disulfide bonds essential for folding. As mentioned above Eclonal antibodies are aglycosylated and therefore do not exhibit the glycan heterogeneity that is a major issue in the current methods of antibody production which rely on mammalian tissue culture. Currently, we obtain yields in the 100-200 mg/L range by bacterial fermentation. However, we expect that much higher yields, exceeding 1 g/L, can be attained by optimizing the production conditions and bacterial strains. Efficient downstream processes for the purification of Eclonal IgG antibodies with very high purity will also need to be established.

Advantages of the Eclonal Technology

The Eclonal technology is a highly integrated and comprehensive platform for antibody discovery, optimization and production. The Eclonal platform provides a series of unique advantages compared to the existing antibody technologies:

1. Unique advantages in therapeutic optimization: The Fc domain of Eclonal antibodies has been engineered to specifically bind to a single Fc γ R receptor. This property allows Eclonal antibodies to specifically recruit a desired subpopulation of effector cells in turn affording precise and more efficient elimination of target disease cells in cancer and autoimmunity. By contrast, normal glycosylated antibodies exhibit binding to multiple Fc γ R receptors and thus do not provide a high degree of targeting.

2. Production of homogenous IgG: Whereas mammalian antibodies are heterogeneous because of the diversity of their attached carbohydrate component, Eclonal antibodies are non-glycosylated and are produced as a single molecular species. The high homogeneity of Eclonal antibodies can result in a homogeneous therapeutic product with predictable PK/PD and potentially higher efficacy.

3. Accelerated discovery of full length IgG antibodies: The Eclonal platform is the only technology that delivers the isolation of full length IgG antibodies. In contrast, existing library screening technologies result in the isolation of antibody fragments which then must be converted to full length antibodies for therapeutic purposes. The ability to isolate full length IgG antibodies offers the following benefits:

- Demonstrated greater diversity of different target specific antibodies
- No need for time consuming reformatting of antibody fragments to full length IgG

4. Reliable generation of antibodies with high antigen affinity/stability/expression properties.

5. Discovery optimization and production are all carried out in bacteria: The use of a single host organism throughout the entire drug discovery process provides an unprecedented degree of integration to the drug development process which translates in more rapid generation of molecules exhibiting the desired pharmacological properties as well as biomanufacturing characteristics.

6. Rapid bioprocess development: Eclonal antibodies are produced by bacterial fermentation processes which are more than five fold faster than the conventional mammalian expression process resulting in dramatically lower capital costs for manufacturing. Also the development of scalable bioprocesses for bacterial expression is orders of magnitude simpler and faster than tissue culture expression.