AvantGen Inc.
Your Partner for Antibody Discovery and Optimization

AvantGen Technology Platform Overview

AvantGen is a San Diego-based biotechnology company with Therapeutics and Diagnostics divisions. To date the company has successfully fulfilled 100s of projects for government, universities, well-known pharmaceutical and biotechnology companies, facilitated and accelerated their antibody-based therapeutic development through services, partnership collaborations, and technology licensing.

- **Proprietary Yeast Display System**
  - Robust and high copy number display of human antibodies in various formats, including Fab, scFab, scFv, etc.
  - Enabled by proprietary peptide display motif fused to the constant region of heavy chain
  - Stable system can be amplified without loss of diversity
  - FAC based techniques allow multiple parameter selections
  - The system also allows antibodies to be secreted to culture media in 96 well plates for ELISA, FACS and functional analysis of individual clones prior to subcloning, sequencing, and purification

- **Large Human Antibody Library and Sequence Database**
  - Over **200 billion clones** (20 sub-libraries in various formats)
  - Rationally designed to mimic diversity in our human antibody database with superior developability
  - Human antibody database compiled from deep sequencing of human antibody repertoires >500 different individuals to inform the library design

- **Antibody Humanization and Optimization**
  - Simultaneous humanization and affinity maturation using epitope guided approach to retain epitope specificity and rationally designed focused library for affinity improvement

    or
  - CDR grafting with minimum back-mutation to retain affinity

- **Rapid Affinity Maturation and Optimization**
  - Mimics natural sequences observed for antibodies derived from their relevant variable region genes and by length for CDR3 for the heavy chain
  - **10 to 1000-fold affinity improvement** readily achieved

- **Novel Rabbit Monoclonal Antibody and VHH nanobody Discovery**
  - Optimized rabbit and alpaca immunization protocol
  - Generates antibodies with affinities in the low pM range

- Particularly useful for differentiation between subtle structural variations, such as single point mutation, different cleavage sites, different isoforms, and blocking and non-blocking anti-idiotype antibodies.
- Well suited for immunodiagnostic, research tool (IHC, FC *etc*.) and PK purposes.
- Can be adapted for therapeutic purposes by following discovery with humanization

1. Novel Yeast Display System for Antibody Discovery and Optimization

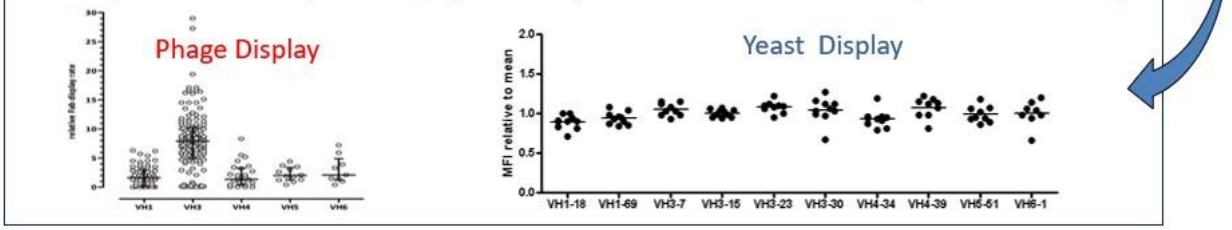
AvantGen's proprietary yeast display system is a highly robust system based on a novel synthetic anchoring peptide. Although phage display has been widely used for protein engineering, including antibody discovery and optimization, it has been well documented that phage display is highly biased in its ability to display antibodies derived from different human antibody germline variable region genes, or even antibody clones that differ with only a single point mutation.

AvantGen's display system, in contrast, shows no such bias. The figure below is the comparison of phage display and AvantGen yeast display of human antibodies. AvantGen's innovative display system is used for the discovery of both novel human and rabbit monoclonal antibodies, as well as for antibody humanization and optimization. It is well suited for displaying rabbit antibodies, where the light chains normally have an intra-light chain disulfide bond that is required for maintaining the high affinity and specificity of rabbit antibodies.

Comparison of in vitro Antibody Discovery and Optimization Platforms

Features	Phage Display	Yeast Display
Antibody Format	Limited	Extensive
Display Diversified Libraries [1]	Limited/Biased	Excellent
Developability	Low	High

[1] Yeast System Provides Improved Display of Diversity Across Diverse Human Antibody V Families vs. Phage



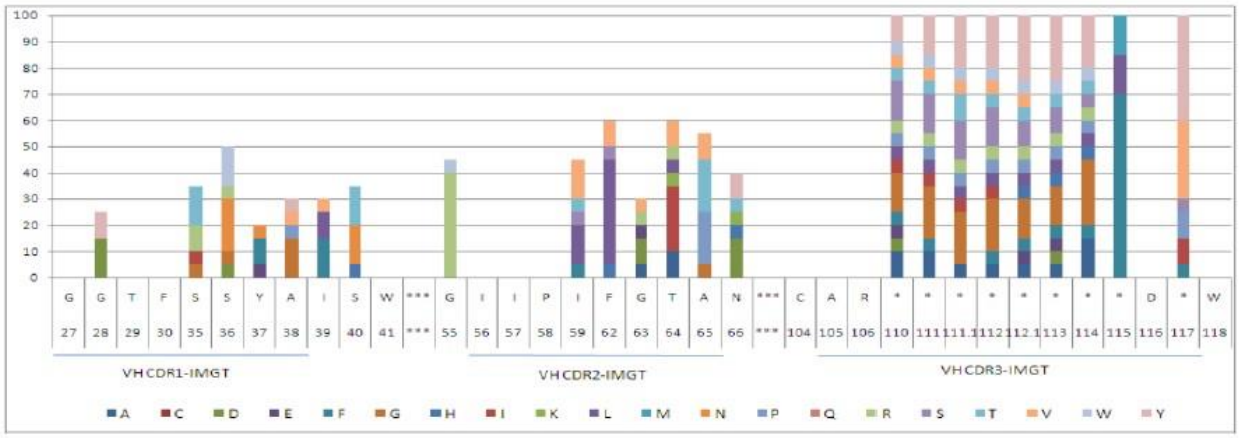
As shown in the figure, AvantGen's yeast display is highly uniform: < 2-fold difference in antibody display levels of all different human VH/VL pairs tested (chart on the right); while with phage display (chart on the left), showed prokaryotic expression coupled with constraints of assembly into the phage coat results in > 30-fold difference in antibody display between different variable region families (Morphosys; mAbs 5:3, 445-70; 2013)

2. Large Human Antibody Library and Sequence Database

Therapeutic antibodies are the fastest growing segment in the pharmaceutical industry. Fully human antibodies are ideal for therapeutic development since they are not normally immunogenic and can carry all the necessary effector functions. Several different approaches have been pursued by others to obtain full human antibodies, but to date, each has its limitations such as affinity, specificity, degree of “humanness”, developability, and time to obtain ideal antibody candidates.

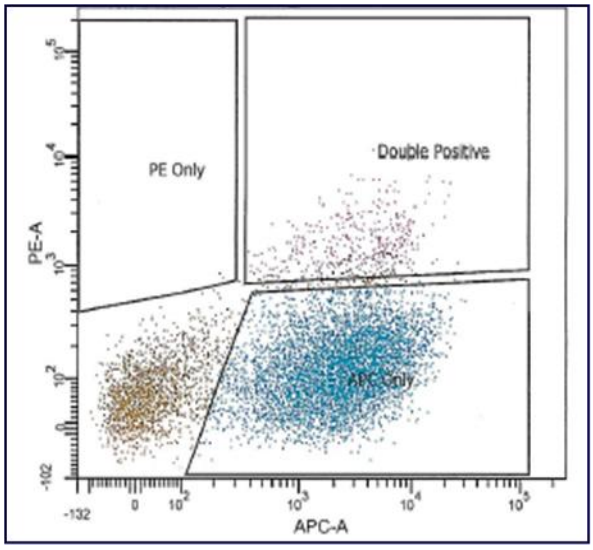
To address these issues, AvantGen has evaluated sequences from deep sequencing of human antibody repertoires derived from >500 different individuals and generated a database where the frequencies of each amino acid at each position of all 6 CDRs in the light and heavy chains were determined for each of the human antibody germline variable regions, stratified by length for CDR3-H.

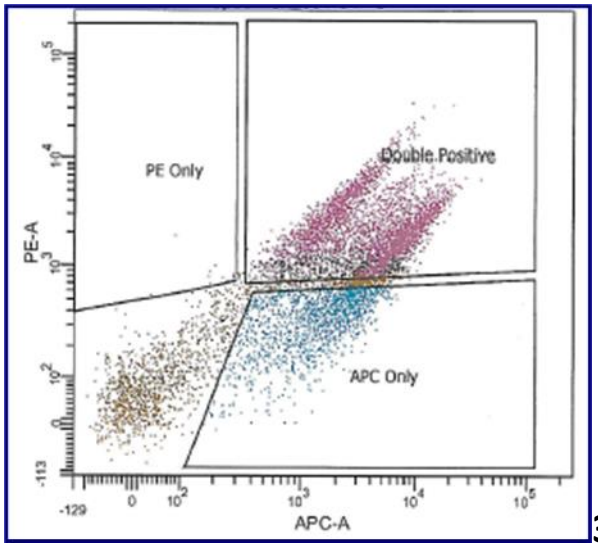
AvantGen has used the data to design and construct human antibody libraries for novel antibody discovery and antibody optimization that closely match natural human antibodies in vivo more than other synthetic human antibody libraries.



The Figure above represents one of the amino acids frequencies used in 3 CDRs of human heavy chain germline VH1-69. Highly biased usage of amino acids at the CDR positions is evident.

Using the type of screening illustrated below, our libraries has yielded sub nM to single digit nM binders for more than 200 antigens to date.





3.

1st round FACS (with **100 nM** biotin-Ag) Labeled with streptavidin-PE Goat-anti-huFab and anti-goat-APC	4th round FACS (with **1 nM** biotin Ag) Labeled with streptavidin-PE Goat-anti-huFab and anti-goat-APC

3. Rapid Antibody Library Screening

AvantGen's human antibody libraries can be rapidly screened by magnetic bead based sorting (MACS) followed by fine-tuned fluorescence-activated cell sorting (FACS) for antibody clones with the desired characteristics, including the following features:

- Specificity for isoform or conformation/epitope of interest
- Species-cross reactivity, such as recognizing human, Cynomolgus, Rhesus and mouse antigen
- Blocking activity

These features can be designed and incorporated into the library screening that can result in generating human antibody clones with the desired characteristics in **a few weeks**.

The Figures below showed a few examples of *de novo* library screening for isoform and active form-specific antibodies.

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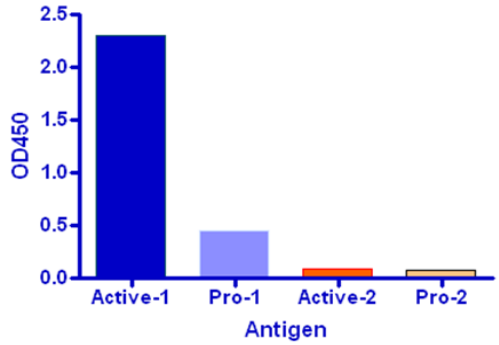
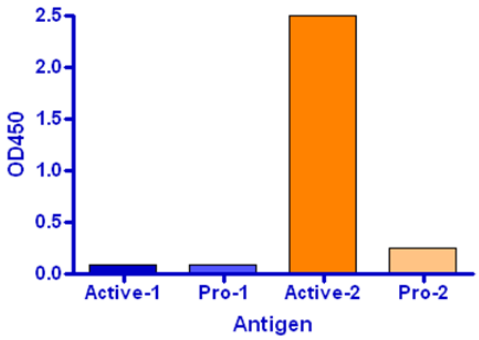
A.



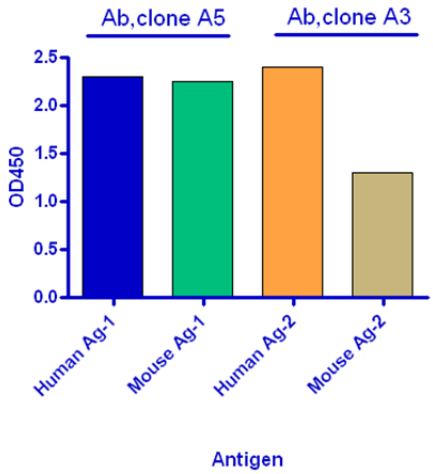
B.



A. clone A5 is specific for the active form of enzyme 1, but not the pro-enzyme or enzyme 1.

B. clone A3 is specific for the active form of enzyme 2, but not the pro-enzyme or enzyme 1.

C.



C. both clone A5 and A3 recognize human and mouse protein; D) both Ab#1 and Ab#2 show high affinity receptor blocking activity.

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4. Antibody Affinity Maturation and Optimization

Therapeutic antibodies need to meet stringent criteria for development and testing. Antibodies from many discovery methods may have interesting properties but may not meet the required profile.

Affinity maturation and optimization of a pre-existing antibody can preserve the epitope specificity and its functional activity. Optimization can also boost the antibody's potency to the desired level. In addition, it can be useful to remove potential modification sites for improved developability.

Using our antibody database and yeast display system, we are able to quickly design focused libraries based on the antibody to be optimized, and simultaneously screen for antibodies with higher affinity and higher levels of expression, while in the meantime, removing undesirable post-translational modification sites. Our system can improve the antibody affinity 10-1,000 times or greater compared to the parental antibodies.

Sample ID	K_D (M)	K_{on} (1/Ms)	K_{off} (1/s)	Full R^2	Fold Improvement
WT	5.24E-07	2.75E+05	1.44E-01	0.99	1
Clone A	2.60E-10	6.08E+04	1.58E-05	0.99	2000
Clone B	<1.0E-12	8.28E+04	<1.0E-07	0.99	>1000

5. Generation of High-Specificity and High-Affinity Antibodies

The rabbit immune system generates and affinity-matures antibodies by mechanisms that differ from those of mice and other rodents. Rabbit monoclonal antibodies normally have 10 to 100-fold higher affinity for antigen than mouse monoclonal antibodies. In addition, the rabbit immune system can generate antibodies that are able to distinguish between very similar molecules with subtle structural variations.

We combine an optimized rabbit immunization protocol and our robust yeast display system to generate a large library of antibodies, a sufficient size that allows us to isolate remarkably high affinity monoclonal antibodies. The yeast display system is uniquely suited for rabbit antibodies, which have an additional disulfide bond in their light chain. This disulfide bond formation is believed to be important for the high affinity and specificity observed for rabbit antibodies. In contrast, phage display normally does not support such modifications.

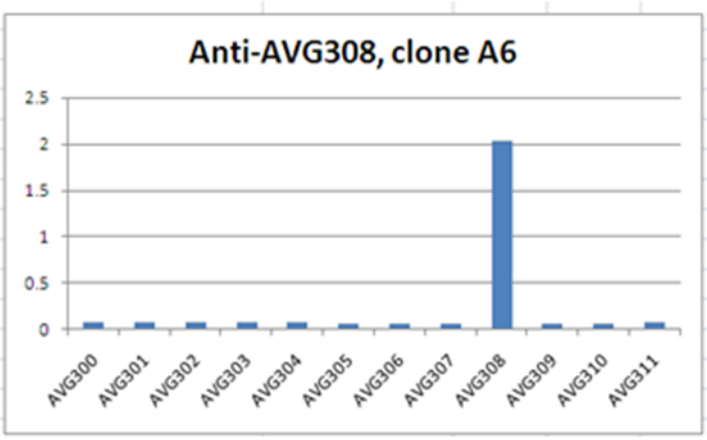
Our approach overcomes issues associated with rabbit hybridoma-based technology, such as low fusion efficiency, unstable cell lines and low antibody yield. It also greatly shortens the time required to generate monoclonal antibody producing stable cells. Finally, antibodies generated

with our technology often exhibit affinities in the range of 0.001-1.0 nM as demonstrated by a recently completed contract project for the National Cancer Institute (NCI) [HHSN261201300023C].

a) Rabbit Monoclonal Antibodies with High Affinity and Specificity

Examples of monoclonal antibody clones generated for this project with AvantGen's technology are shown below including one example that can specifically distinguish a peptide in its phosphorylated and non-phosphorylated form and two examples of pairs of high affinity clones that can form quantitative ELISAs for NT-ProBNP and polysaccharides from group A Streptococcus (Strep A).

Antigen	Capture Antibody	Affinity (nM)	Detection Antibody	Affinity (nM)
NT-proBNP	A10	0.20	F10	0.03
Strep A	H2	0.05	E10	0.05
AVG300	D4	0.01	LALQAQPVPDELVTK	
AVG301	E11	0.01	DITSDTSGDFR	
AVG308	G11	0.04	**Phospho-peptide specific** VADPDHDHTGFLTEyVATR	
AVG309	H4	0.04	**Phospho-peptide specific** RPHFPQFsYSASGTA	



K_D = 40 pM
AVG308: VADPDHDHTGFLTEyVATR
AVG310: VADPDHDHTGFLTEYVATR
y indicates phosphorylated residue

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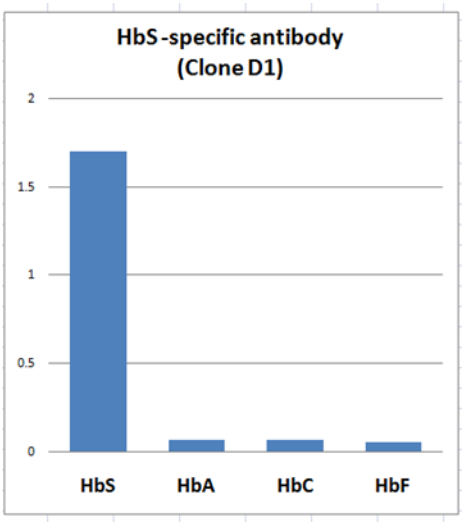
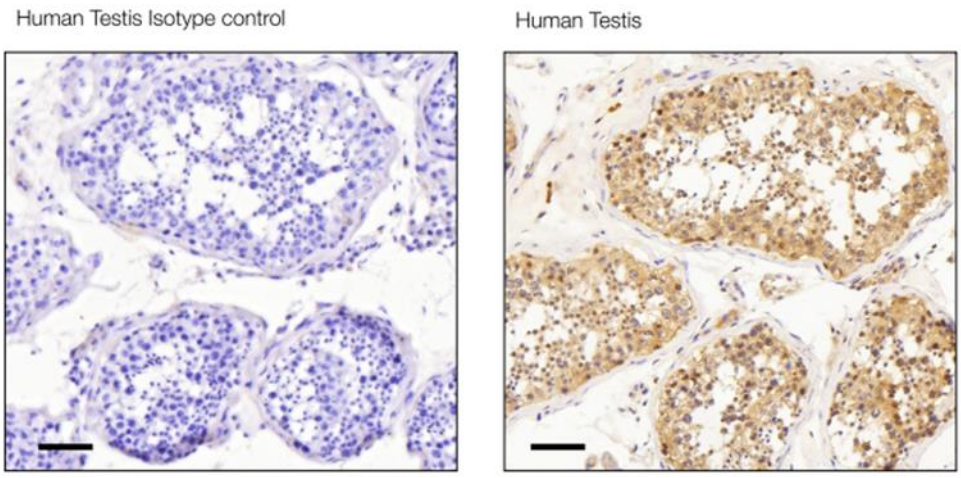
b) Rabbit Monoclonal Antibodies that Distinguish Point Mutations

The Figure below shows an example of a high affinity rabbit clone, D1, that can specifically recognize hemoglobin S, but not adult hemoglobin or hemoglobin C, which differ by only one point mutation.



HbS: VHLTP**V**EKSAV
HbA: VHLTP**E**EKSAV
HbC: VHLTP**K**EKSAV
HbF: GHFTEEDKATITS

c) Rabbit Monoclonal Antibodies for IHC and PK Studies (Anti-Idiotype)



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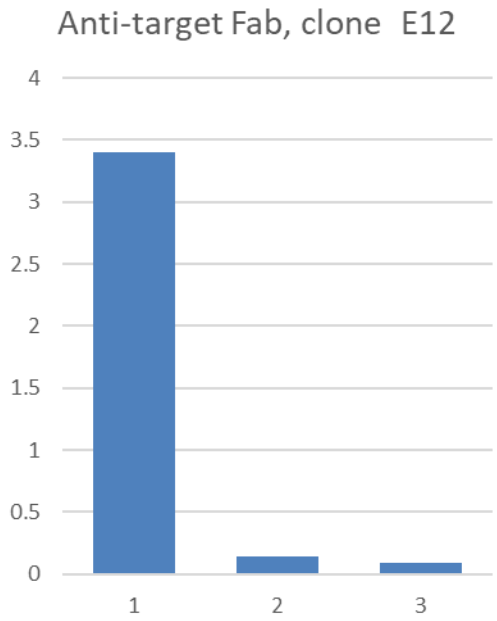

1. Target: Human Fab
2. Irrelevant human Fab A
3. Irrelevant human Fab B

6. Comparison of Rabbit mAb Discovery Approaches

	AvantGen Yeast Display	Phage Display	Hybridoma Based
Display System	**novel and uniform**	commonly used, but high biased antibody display	N/A
Rabbit Strains	no constraint	need to use basilar B9 rabbit strain to avoid intra-light chain disulfide bond	no constraint
Library Construction/ Fusion	captures **all** of the antibody repertoire	captures only some of the antibody repertoire	inefficient fusion ($1:10^6$), may lose good clones
Library Screening	MACS & FACS for clones with the desired properties from **>100 billion clones**	panning to capture all positive clones, time consuming to identify clones with the desired properties	FACS for clones with desired binding properties but from a very small pool of fused cells
Deliverables	antibody genes, purified antibody, and antibody-expressing mammalian cells	antibody genes, purified antibody, and antibody-expressing mammalian cells	Hybridoma cells, low yield and not very stable
Timeline	~ 5-6 months	~6 months	~8 months

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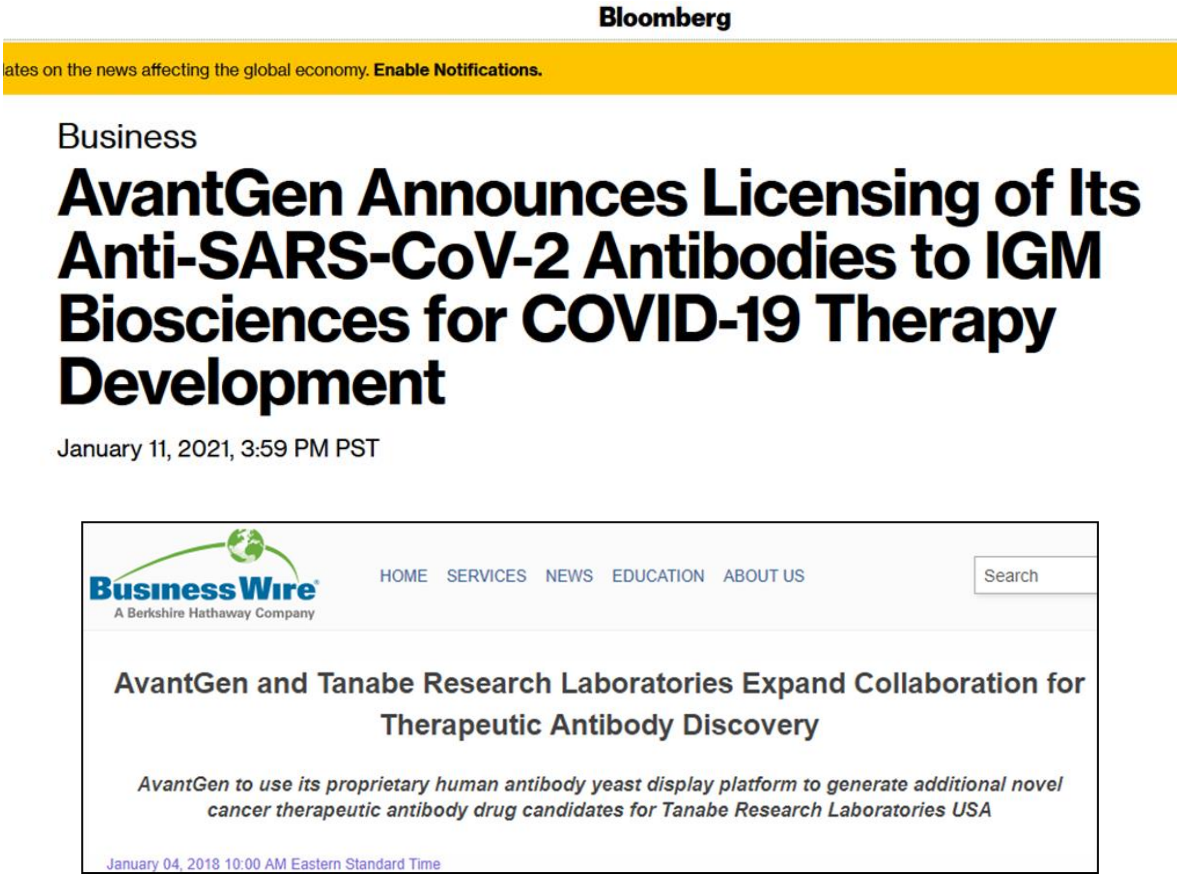
7. Summary of AvantGen Technology Platform

Antibody Attributes	Performance
Affinity	Majority of antibody clones isolated from our libraries exhibits affinity in sub-nM to single digit nM range
Specificity	Specificity can be tailored to be highly specific to the antigen of interest with or without cross reactivity to other homologous proteins or isoforms
Diversity	Typically, a panel of 10s-100s unique antibody clones can be isolated from our libraries against a given antigen
Developability	Antibody is designed to be of high developability
Humanness	Libraries rationally designed to mimic diversity in human antibody database compiled from >500 different individuals
Speed	Ag-specific Ab clones can be isolated in 5-6 weeks and full-length IgG can be produced from mammalian cells in 8-10 weeks

8. Examples of Successful Collaborations



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Business

AvantGen Announces Licensing of Its Anti-SARS-CoV-2 Antibodies to IGM Biosciences for COVID-19 Therapy Development

January 11, 2021, 3:59 PM PST

BusinessWire
A Berkshire Hathaway Company

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AvantGen and Tanabe Research Laboratories Expand Collaboration for Therapeutic Antibody Discovery

AvantGen to use its proprietary human antibody yeast display platform to generate additional novel cancer therapeutic antibody drug candidates for Tanabe Research Laboratories USA

January 04, 2018 10:00 AM Eastern Standard Time

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9. AvantGen Services and Business Collaboration Opportunities

Competitively priced for Antibody discovery, affinity optimization and humanization, AvantGen cordially welcomes the opportunity for the following business collaborations.

- Contracting services using AvantGen's proprietary technologies to rapidly generate antibodies that meet and exceed your design goals.
- Technology transfer package if you are interested in partial or complete internalization of AvantGen's technology platforms.
- Access to AvantGen's proprietary and comprehensive antibody database, which will enable you to design and generate libraries tailored to your unique interests.

AvantGen
Proprietary Yeast Display System
Exceptional Results

CONTACT

WWW.AVANTGEN.COM

ROBIN DONG **DIR. OF BUSINESS DEVELOPMENT**
EMAIL **RDONG@AVANTGEN.COM**