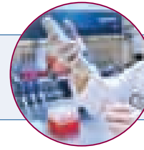
ProCode™

A Rapid Flexible MAb-Like Discovery Platform for Creating Diagnostic Antibodies

Meridian Life Science,® Inc.

www.MeridianLifeScience.com

CUSTOM MONOCLONAL DEVELOPMENT



Meridian Life Science, Inc. (MLS) is an industry leader in the commercial production of critical raw materials for the diagnostic market. Through an exclusive partnership with Vybion, Inc., MLS offers a unique technology (ProCode™) for the development of antibody-like molecules. ProCode is a recombinant monoclonal antibody technology that overcomes the limitations of traditional hybridoma development. It is based on a proprietary synthetic library and a unique selection process natural to *E. coli*. ProCode's innovative features enable the rapid isolation of target-specific antibodies without the labor intensive screening common to other recombinant and non-recombinant methods.

BENEFITS OF PROCODE COMPARED TO HYBRIDOMA TECHNOLOGY

Higher sensitivity and specificity
Affinity (Kd) of 10^{-6} to 10^{-12}
Specificity to a single point mutation
Direct selection of sandwich pairs

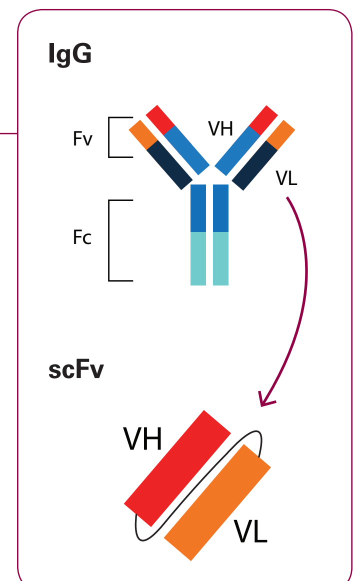
Superior control of downstream production
Cost effective at 3g/L yield in *E. coli*
Enables unlimited, consistent, and fully defined reagent supply
Subcloning never required

HYBRIDOMA	vs.	PROCODE
24 weeks	**Project Lead Time**	4-10 weeks
Unpredictable	**Specificity**	Direct Selection
Unpredictable	**Affinity (Kd)**	10^{-6} to 10^{-12}
Not guaranteed	**Paired Antibodies**	Guaranteed
Limited to what can be injected into an animal	**Types of Antigens**	Peptides, proteins, toxins, tissues, organs, modified compounds
2-4mg	**Amount of Antigen Required**	<0.5mg
20mg/mouse 35mg/L cell culture	**Production Efficiency**	3g/L
Ascites & *In vitro*	**Production Method**	High-throughput, *In vitro*
Unpredictable	**Stability**	Infinite

scFv ANTIBODY LIBRARY

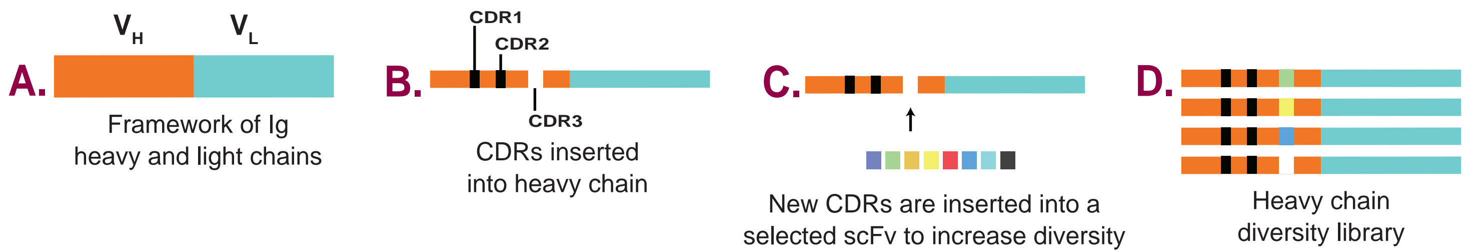
ProCode scFv libraries (size 107kb-109kb) are synthetically created to represent the structural diversity of the human antibody repertoire
scFv represents the variable region (Fv) of the heavy and light antibody chain
ProCode scFvs can be modified into various formats
(e.g., Fab fragments, full length IgG, etc.)
Heavy chain, light chain or both (heavy and light) diversity libraries are available

ProCode Libraries are proprietary and have enhancements/modifications:
In vivo filters to eliminate aggregation and stops
PCR mutagenesis to mimic somatic mutation
The libraries contain genes that represent:
- Constant regions of the heavy and light chains (framework)
- Hypervariability regions (via 3 CDR cassettes)



ProCode™ is a trademark of Vybion.

scFv LIBRARY CONSTRUCTION



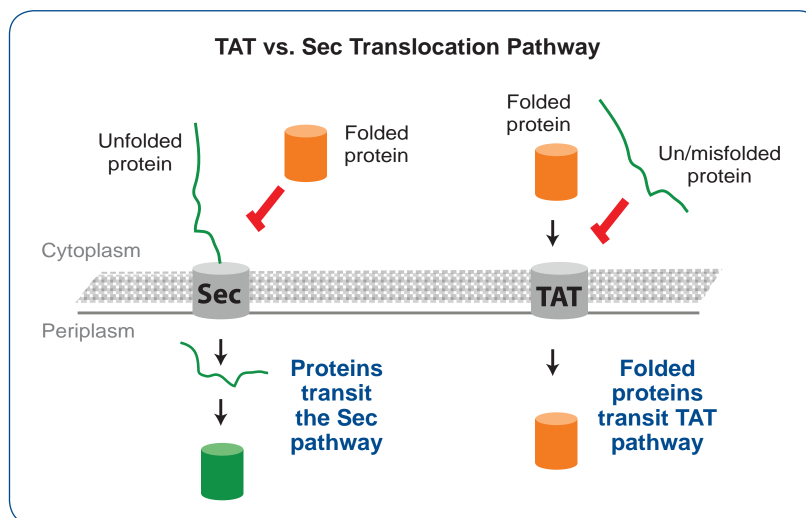
TAT TRANSLOCATION SYSTEM

ProCode scFv libraries are expressed in *E. coli* and use the TAT system as an internal quality control for correct scFv folding

- scFvs are expressed in the cytoplasm of *E. coli* and must be actively transported to the periplasm
- Tat pathway only exports folded proteins that have already attained their native conformation in the cytoplasm
- Transport is mediated by a unique leader sequence

Most conventional phage display systems use the Sec translocation pathway

- Sec transports via an unfolded state
- Proteins that require the cytoplasmic environment and/or cytoplasmic components for folding are not compatible with the Sec pathway



HOW scFv SELECTION WORKS

1. Antigen is screened against the scFv library; 2 methods available

   A. FLI-TRAP: Functional Ligand-binding Identification
   - Antigen is co-expressed with scFv library within *E. coli*
   - Primarily focused on epitope specific scFvs
   - Enables bispecific antibody selection

   B. MADT-RAP: Membrane Anchored Display
   - Library is first expressed in *E. coli* then screened against the antigen by FACs or panning
   - Provides for an unlimited range of targets
   - Novel proven protocols to drive affinity maturation

2. Positive hits are screened by ELISA (end-point dilution)
   - Additional screening (i.e. counter screen) is optional

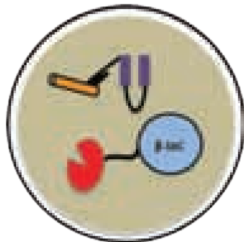
3. Up to 10 best clones are selected for production or for progress to Phase II
   - In Phase II the ten scFVs undergo further rounds of random PCR mutagenesis to create a "new" target specific scFv library
   - This library is further screened for affinity, sensitivity, etc.

4. scFvs can be modified to other formats
   - Modification of scFv to a bivalent scFv, scFv-Fc, full length IgG molecule or other Fab fragment possible
   - Addition of fusion constructs: possible to engineer detection via a one-step rapid readout system (e.g. luciferase)
   - Conversion to expression in yeast or mammalian cell systems

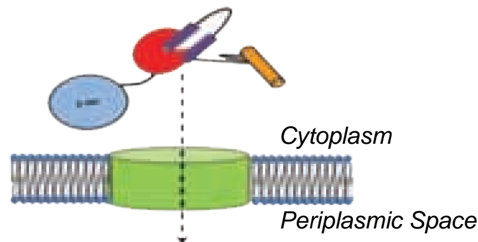
SELECTION METHODS

FLI-TRAP

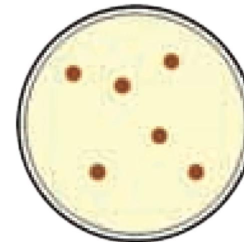
Functional Ligand-binding Identification (FLI-TRAP) selects the handful of scFvs capable of interaction with the antigen that is co-expressed with the library



scFv library is co-expressed with the target antigen in the *E. coli* cytoplasm



scFv binds to antigen and the complex translocates to the periplasm thru the TAT transporter–leader sequence is embedded into membrane and a protease cleaves off the scFv

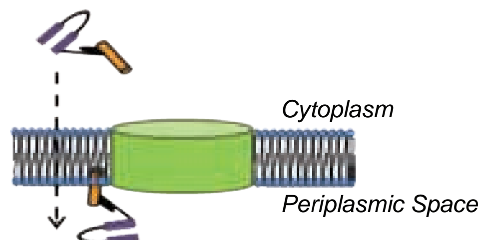


Cells are plated on AMP plates and only scFvs complexed with antigen/ß-lac survive

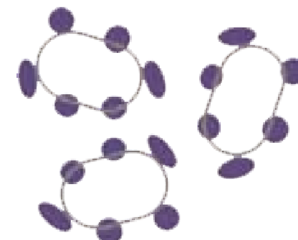
MAD-TRAP

Membrane Anchored Display (MAD-Trap) expresses libraries anchored in the bacterial plasma membrane & binder panels are selected by FACS or panning

scFv library is expressed in the *E. coli* cytoplasm

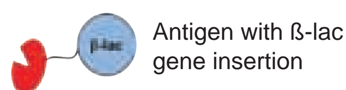


scFv translocates to the periplasm thru the TAT transporter–leader sequence is embedded into membrane but a sequence mutation prevents cleavage at the scFv by the protease

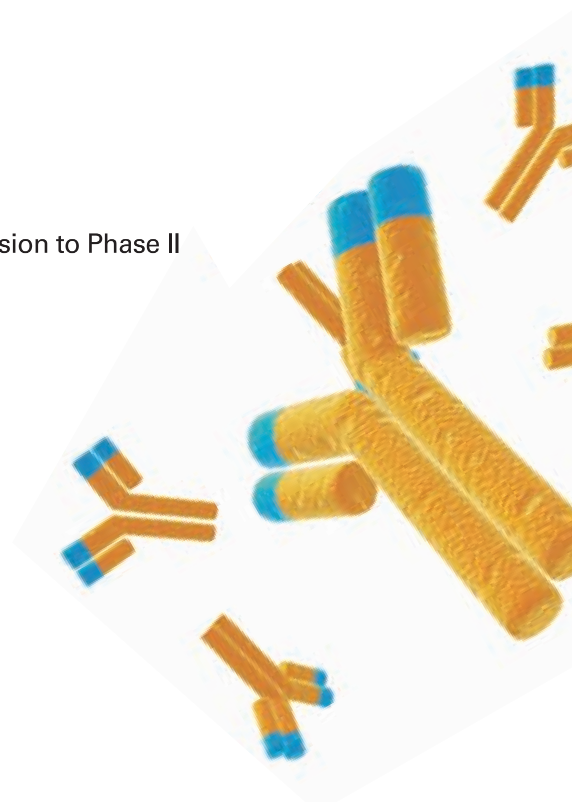


E. coli cell wall is stripped to isolate spheroplasts expressing an scFv library-this is further screened against the antigen by panning or FACs

DIAGRAM KEY



PROCODE PROJECT OUTLINE



PHASE I
Enrichment

Selection against library with one round of enrichment
Screen positive scFvs (up to 200 colonies) for reactivity (ELISA) and affinity
Protein expression (4-5mg) from 10 clones (affinity of ~10^{-6}) provided or progression to Phase II
Success guaranteed – no charge for initial selection if no hits are generated

PHASE II
Affinity Maturation

Two rounds of affinity maturation (PCR mutagenesis)
FACS and/or ELISA screen (up to 100 colonies) by endpoint dilution
Determination of affinity (Biacore™)
Protein expression (4-5mg) from10 clones (affinity typically in pM range)

ADDITIONAL OPTIONS
Further rounds of selection to increase affinity
Conversion to *Pichia pastoris*, CHO, mammalian or fusion constructs
Cloning into full length human antibody IgG1
Stable cell line development

CONTRACT MANUFACTURING SERVICES:

Pilot (ug) to commercial (g) scale contract manufacturing for:
- Monoclonal antibodies (*In vitro* and *In vivo*)
- Recombinant proteins (*E. coli*, yeast, insect and mammalian expression systems)

Upstream and downstream process development
Cell banking and storage
Analytical testing
Validation lots

Meridian Life Science, Inc. has an exclusive partnership with Vybion® for diagnostic applications of ProCode™

Biacore™ is a trademark of GE Healthcare Companies.
ProCode™ is a trademark of Vybion, Inc.

FREQUENTLY ASKED QUESTIONS

1. Does each spheroplast express only one scFv? What is the size of an scFv?

   Yes. 27kD.

2. What is the stability of the scFv?

   Due to the novel nature of these scFvs, long term stability studies have not been performed to date. However, they have a DCS profile in the high 60s and storage (-80°C) has not resulted in development of aggregates.

3. Have all scFvs generated to date had a yield of > 3g/L?

   Yes, in a fermentor scFvs produce at 3g/L which has been confirmed by SDS PAGE.

4. Do you use a human library? What is the library size?

   Yes, we use Human libraries (both an NNK and a protein frequency library) which range from 107kb or over 109kb after removal of stops and aggregation. Aggregation is removed by passing the sequences through our quality control transport system which is part of the core ProCode IP.

5. How many antibodies have been created to-date using ProCode?

   So far, ProCode has been utilized to develop over 50 specific scFvs.

6. Has the *E. coli* been mutated in some way to prevent translocation by the Sec pathway?

   We use standard strains of *E. coli* that have not been modified. Instead, there is a leader sequence on the scFvs that facilitate transport through the TAT pathway exclusively. This sequence is incorporated into our proprietary expression vectors which contain all the necessary machinery to express an scFv.

7. How are the scFvs purified?

   We are currently using a His tag for purification, which is purified under standard laboratory conditions without reducing agents.

8. In the case that only 1-2 hits are generated in the initial screening, would a re-screen be performed to try to generate more hits? Would a cost be associated with this?

   If it is a standard antigen we would perform a re-screen without any additional cost. However, for unusual antigens only a few hits may be generated. These details can be discussed on a case-by-case basis.

9. Is it possible to generate scFvs that recognize a phosphorylated molecule? Will a ProCode antibody distinguish between phosphorylated and non-phosphorylated antigen?

   Yes, scFvs can be generated that recognize and bind to a phosphorylated molecule. By using the proper counter screen with the non-phosphorylated counterpart, we can select for specific single chains that only recognize the phosphorylated target..

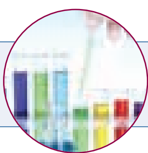
10 Is it possible to generate an scFv that recognizes both an antigen in its non-phosphorylated and phosphorylated state?

   Since a scFv will recognize only one epitope, a bispecific antibody (that recognizes two antigens, such as the phosphorylated and non-phosphorylated form) could be created to recognize both targets.

11. How much scFv antibody is required compared to a standard monoclonal antibody in a diagnostic assay (ELISA, LF, etc.)?

   The amount used will vary depending on both the antigen and affinity of the scFv, but generally it is in the same range as a standard antibody.

RELEVANT PUBLICATIONS



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2. Lim HK, Mansell TJ, Linderman SW, Fisher AC, Dyson MR, DeLisa MP. (2009). Mining mammalian genomes for folding competent proteins using Tat-dependent genetic selection in Escherichia coli. Protein Sci.;18(12):2537-49.

3. Waraho D, DeLisa MP. (2009). Versatile selection technology for intracellular protein-protein interactions mediated by a unique bacterial hitchhiker transport mechanism. Proc Natl Acad Sci U S A. ;106(10):3692-7.

4. Lee LL, Ha H, Chang YT, DeLisa MP. (2009) Discovery of amyloid-beta aggregation inhibitors using an engineered assay for intracellular protein folding and solubility. Protein Sci. ;18(2):277-86.

6. Fisher AC, DeLisa MP. (2009) Efficient isolation of soluble intracellular single-chain antibodies using the twin-arginine translocation machinery. J Mol Biol. ;385(1):299-311.

7. Panahandeh S, Maurer C, Moser M, DeLisa MP, Müller M. (2009). Following the path of a twin-arginine precursor along the TatABC translocase of Escherichia coli. J Biol Chem.;283(48):33267-75.

8. Fisher AC, DeLisa MP. (2008). Laboratory evolution of fast-folding green fluorescent protein using secretory pathway quality control. PLoS One.;3(6):e2351.

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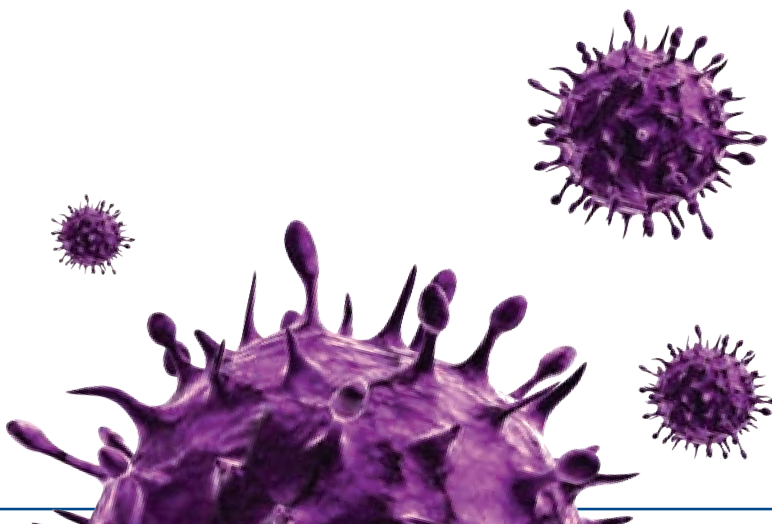
10. Tullman-Ercek D, DeLisa MP, Kawarasaki Y, Iranpour P, Ribnicky B, Palmer T, Georgiou G. (2007). Export pathway selectivity of Escherichia coli twin arginine translocation signal peptides. J Biol Chem. ;282(11):8309-16.



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