Isolation of Alternative Affinity Reagents for *Entamoeba histolytica* Detection Utilizing an Accelerated Pipeline

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The Accelerated Molecular Probe Pipeline

**GOAL:** Making alternative antibody-like molecules a viable and cost-effective substitute to animal monoclonal antibodies

**LIMITATIONS:**

- scFv cloned off display surface lose activity
- Uniquely, we are attempting to employ scFv still in the environment in which they were selected

**ADVANTAGES:**

- Yeast library of $10^9$ diversity
- 2–3 week production, completely in vitro
- 1–2 rounds of magnetic selection
- 1–2 rounds of FACS

Yeast scFv Bioassays

- **Flow Cytometry**
  - ELISA
  - **Whole Yeast Cell** (not to scale)

- **Polyclonal Antibody**
  - Anti-agglutination

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Yeast scFv Bioassays (Label-free)

- Fragmented yeast scFv (nano-scFv)

350-E2 nano-yeast-scFv (Buffer w/ Controls)

350-E2 (500 pg/mL)

No Ag

Grewal et al., Chem. Commun., 2013

Activity of 350-E2 Yeast-scFv Post-Lyophilization

- Kahn et al., unpublished

In 1:5 “negative” stool:

i) no Ag

ii) 500 pg/mL Ag

Our reagents can see target in complex biological samples.

Yeast scFv Lyophilization Studies

- Kahn et al., unpublished

No Ag

Fe2+/3+ Fe3+/2+


Yeast scFv Bioassays (Label-free!)
**Entamoeba histolytica Biomarker Validation**

Attempting to validate a new biomarker for *Entamoeba histolytica*:
- Tricky pathogen to diagnose in settings in which it is endemic
- Focusing on the cyst stage, which is shed and transmitted, yet is not the focus of current tests

Select and express candidate biomarkers:
- Biased in cyst *M*/*N*´
- Transcriptomics
- Sequenced divergence with *E. dispar*
- Solubility and cell system

Whole and nano-yeast-scFv

Select yeast-scFv from library

Characterize and validate yeast-scFv

Validate biomarker in clinical stool

**scFv Inventory**

<table>
<thead>
<tr>
<th>Target</th>
<th>Annotation</th>
<th>Unique scFv</th>
<th>LOD (pM) Whole Yeast Sandwich</th>
<th>LOD (pM) Nano-scFv SPE Electrode</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHI_008180</td>
<td>14-3-3 Protein 3</td>
<td>3</td>
<td>156-600 ND</td>
<td>ND</td>
</tr>
<tr>
<td>EHI_104930</td>
<td>Phosphatase 2</td>
<td>3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>EHI_03290</td>
<td>Pyriophosphate-binding protein</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>EHI_07070</td>
<td>Rho GTPase</td>
<td>14</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>EHI_181050</td>
<td>Rad 11 Pase F2</td>
<td>3</td>
<td>156 ND</td>
<td>ND</td>
</tr>
<tr>
<td>EHI_11350</td>
<td>Chromosomal DNA-binding protein</td>
<td>1</td>
<td>313</td>
<td>0.59</td>
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<tr>
<td>ECI_014540</td>
<td>Cell wall protein Jacob2</td>
<td>3</td>
<td>20-70 ND</td>
<td>ND</td>
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<tr>
<td>EHI_101240</td>
<td>Staphylococcal nuclease-like protein</td>
<td>TBD</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>200-md0090</td>
<td>Calmodulin heavy chain</td>
<td>TBD</td>
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</tbody>
</table>

**scFv Inventory: Spotlight on Jacob2**

Jacob2 is a known outer cyst wall lectin.

It has a very divergent spacer which has been expressed in the lab.

We have 3 scFv that bind recombinant *E. histolytica* Jacob2, but not to the recombinant *E. dispar* Jacob2.

[Graph showing LOD (Avrg) for different pH values and scFv binding]
Jacob2 scFv Epitope Binning

Do the 3 scFvs have unique epitopes? Mouse monoclonals we have developed in parallel aided us in that investigation.

Conclusion: Each scFv has a unique antigen epitope, though the Jacob D9 epitope seems to overlap the other two.

<table>
<thead>
<tr>
<th>Jacob scFv Epitope Binning: Ag-Binding Cells (%) of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacob D9</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>18.78%</td>
</tr>
<tr>
<td>16.26%</td>
</tr>
<tr>
<td>14.73%</td>
</tr>
<tr>
<td>350‐E2</td>
</tr>
</tbody>
</table>

Summary

- We can isolate scFv molecules specific to antigens of interest within 2-3 weeks, completely in vitro.

- Whole or fragmented ("nano-") yeast-scFv are maintained in the environment in which they are selected and have been successfully integrated in a number of assays.

- Freeze-dried yeast-scFv still function after 11-12 months of storage.

- We have 34 yeast-scFv to 7 recombinant E. histolytica cyst biomarkers, 3 of which are specific to the pathogen’s outer wall protein.
  - Future directions will investigate the performance of these scFv in complex samples (purified parasite, spiked stool, clinical stool)

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Yeast scFv Selections

ROUNDS 1&2: Magnetic Separation

ROUNDS 3&4: FACS

Isolate and Pick Colonies

OUTPUT: Antigen-Binding Yeast Clones