



*Aspergillus
fumigatus*

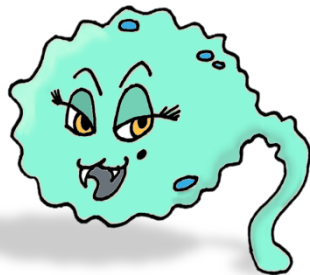


*Candida
albicans*

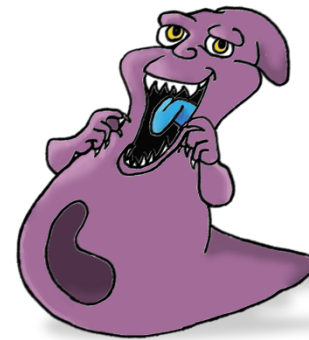


*Aspergillus
nidulans*

iGEM'S
MOST
WANTED
& STUDIED
10th microorganisms
Anniversary
2004 - 2014



*Candida
glabrata*



*Toxoplasma
gondii*

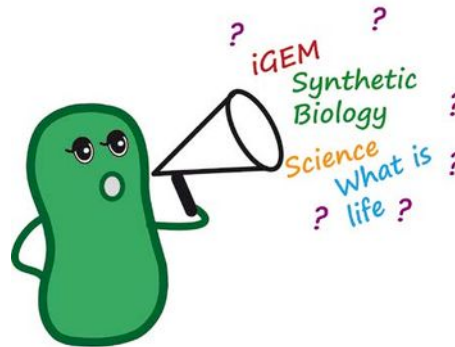


PepTag

A small change for man, a giant pain to germkind!

18.08.2014

What is iGEM?



- The International Genetically Engineered Machine is a Synthetic Biology design competition for undergraduate student teams
- Organized by MIT in Boston
- Aim: introduction to synthetic biology, innovative research ideas and public awareness

About the team 2014



Pathogenic fungi

> 300 million sufferers worldwide

- cause long term illness which lead to death
- the majority remain undiagnosed

➔ *A. fumigatus* cause aspergillosis with a mortality rate up to 90%

➔ **Early diagnosis = increased patient survival rate**

Current detection methods

1.

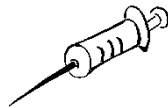
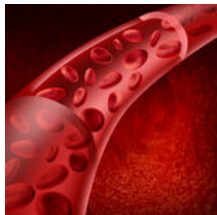


Cultivation of fungi



Inefficient, time consuming, deep tissue removal is necessary

2.



Identification fungal detection marker in body fluids via PCR



Only possible in advanced stages, unspecific

Too Late



Idea:



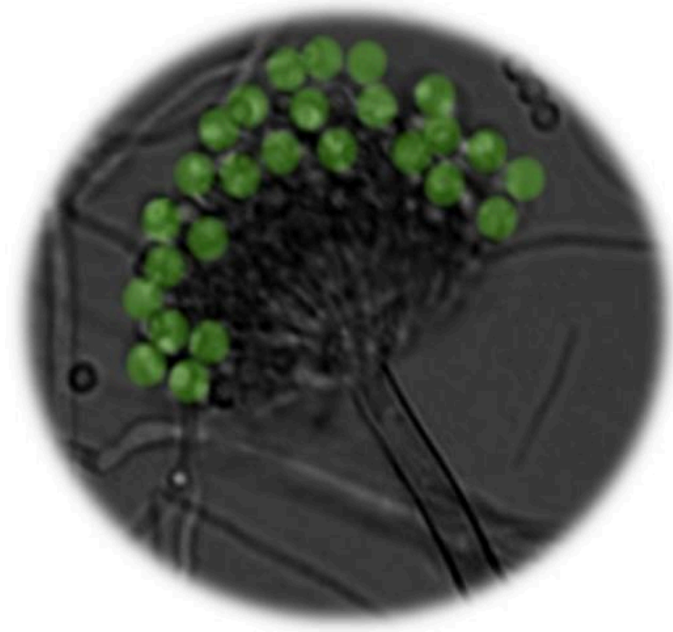
Make fungi cells visible!

Idea: Make fungi cells visible

Before



After



Ask the Experts

Prof. Dr. Uwe Groß



UNIVERSITÄTSMEDIZIN
GÖTTINGEN **UMG**



High specificity



Low detection limits



Robust



Easy to perform



Low cost

Prof. Dr. Sven Krappmann



**Universitätsklinikum
Erlangen**
Institute of Microbiology

Clinical Microbiology, Immunology
and Hygiene

Dr. Med. Johannes Wagener



LMU
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MÜNCHEN
MEDIZINISCHE FAKULTÄT
MAX VON PETTENKOPF-INSTITUT

Criteria for suitable fungi surface proteins



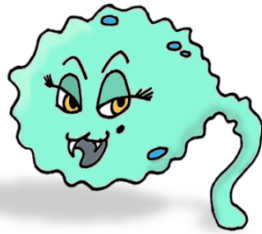
- Really on the outer surface
- Ideally no enzymatic reactions and no glycosylations
- Vaccine candidates

Selection of 35 different fungi surface proteins



Candida albicans

- ✓ *Mpb5*
- ✓ *Tos1*
- ✓ *Als1*
- ✓ *Als3*
- ✓ *Sim1*



Candida glabrata

- ✓ *Ssr1*
- ✓ *Pir4*
- ✓ *Scw4*
- ✓ *Pir3*
- ✓ *Utr2*



Aspergillus fumigatus

- ✓ *RodA*
- ✓ *RodB*
- ✓ *Crf2*
- ✓ *Crf1*
- ✓ *BglE*
- ✓ *Prm1*
- ✓ *Ecm33*
- ✓ *EglC*
- ✓ *Sun1*



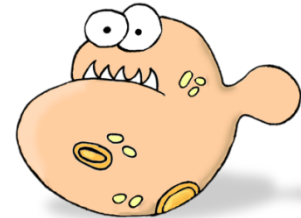
Aspergillus nidulans

- ✓ *RodA*
- ✓ *EglC*
- ✓ *XlnA*
- ✓ *Sho1*
- ✓ *Npc2*



Toxoplasma gondii

- ✓ *Ama1*
- ✓ *Rom5*
- ✓ *Gra4*
- ✓ *Rom4*
- ✓ *Gra7*



Saccharomyces cerevisiae

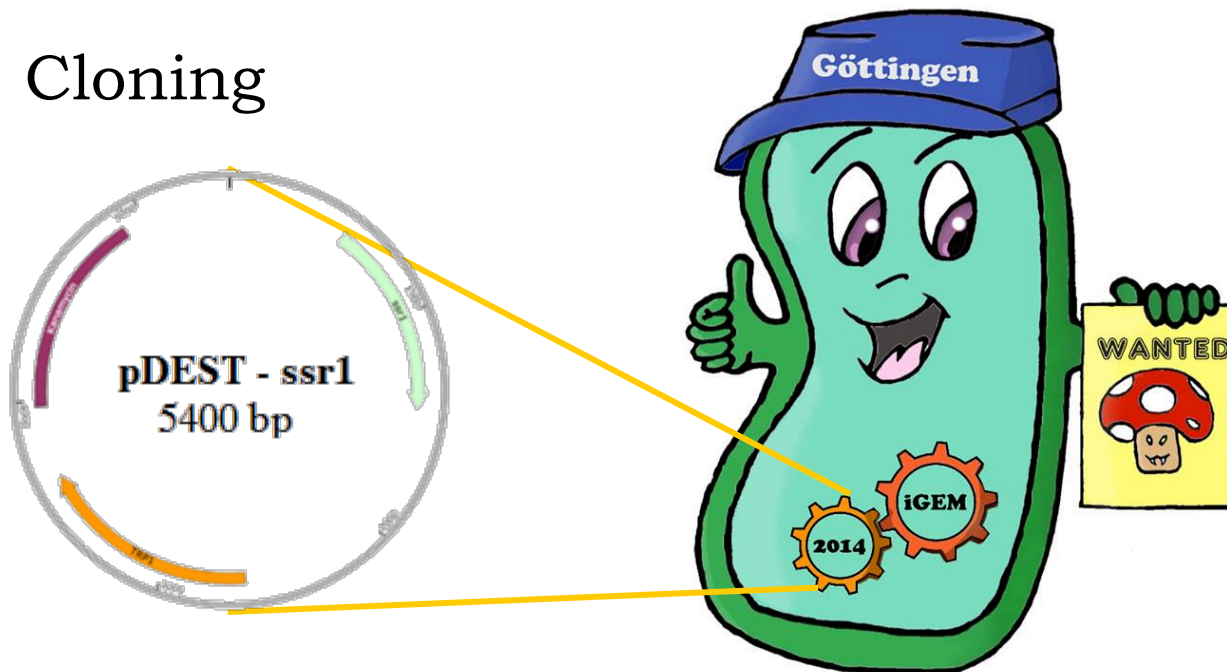
- ✓ *Cwp1*
- ✓ *Cwp2*
- ✓ *Tir1*
- ✓ *Sed1*
- ✓ *Tir4*
- ✓ *Mid2*

PCR and Cloning

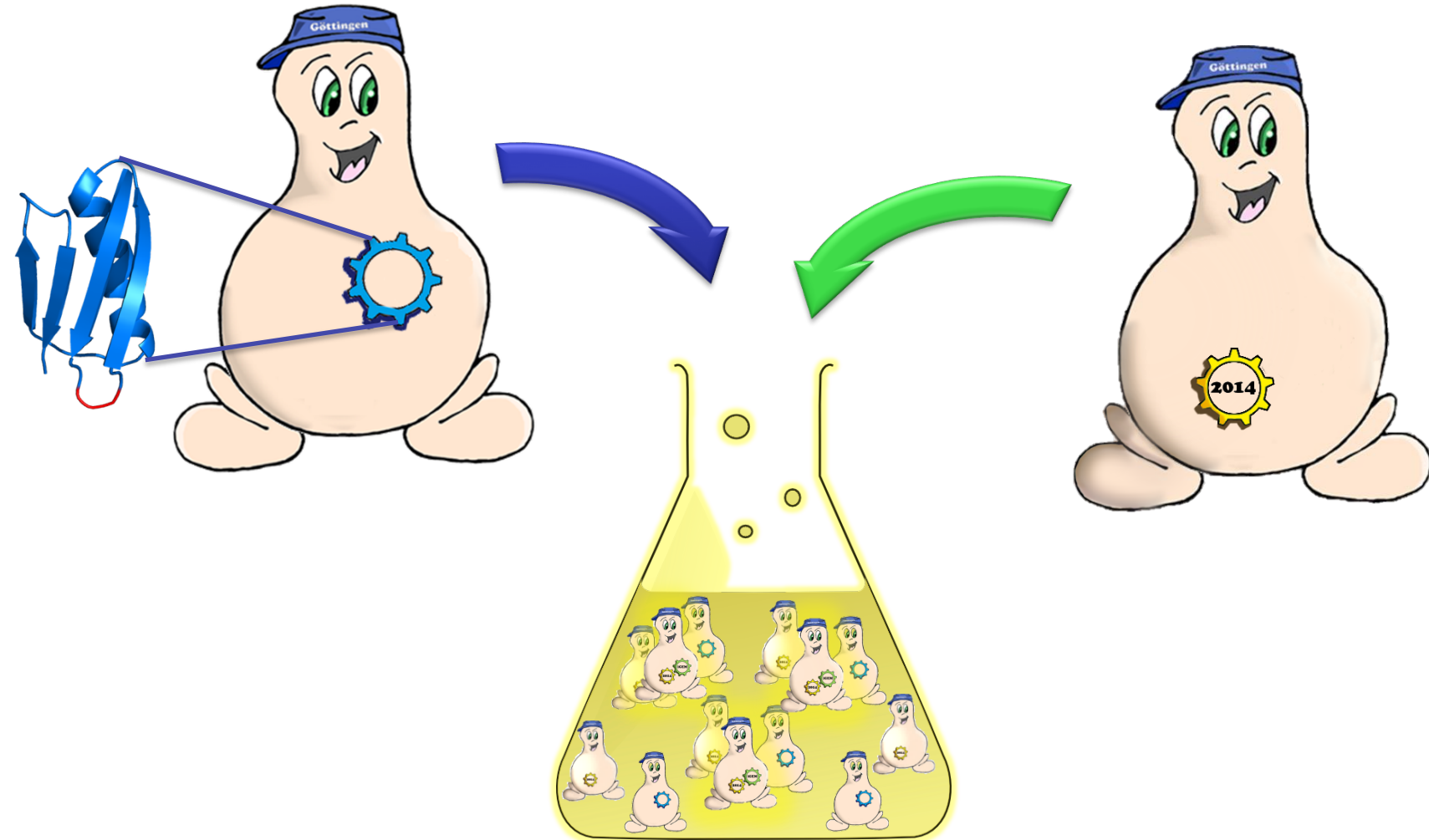
PCR Amplification



Cloning

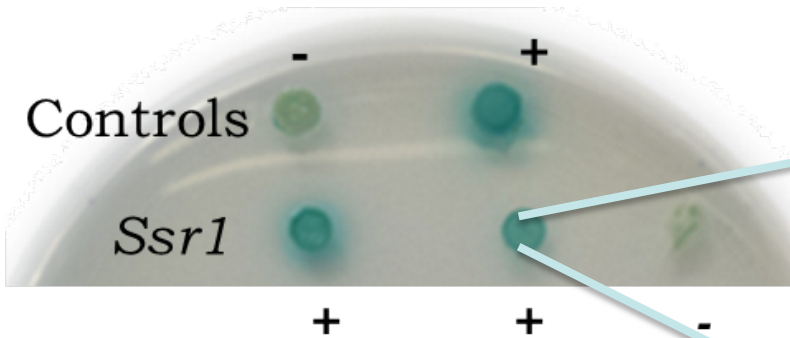


Yeast-two-Hybrid in Liquid Medium

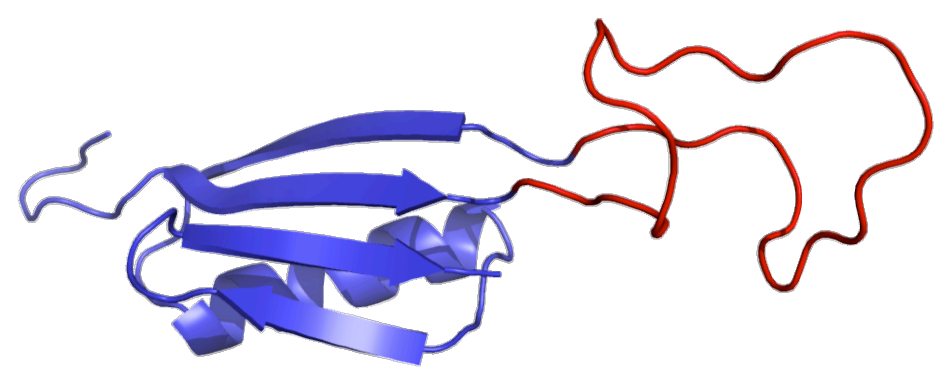
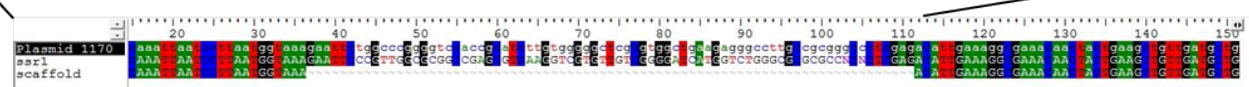
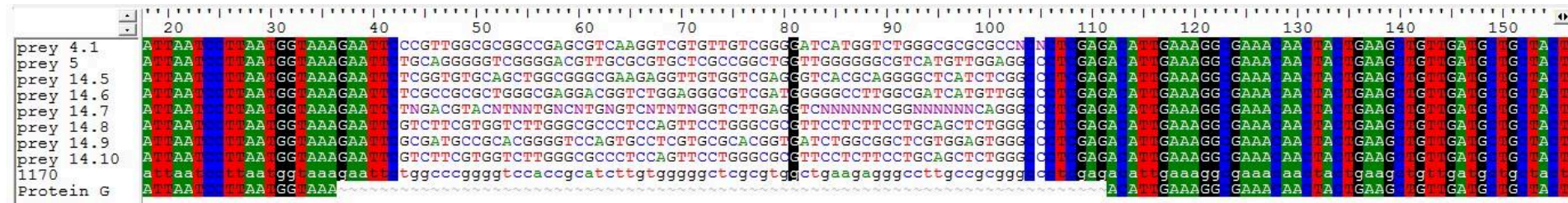


Yeast-two-Hybrid Assay Results

Peptide – Protein Interaction



Sequencing and Homology Modelling



25 Amino Acid Sequence

GB1 Scaffold

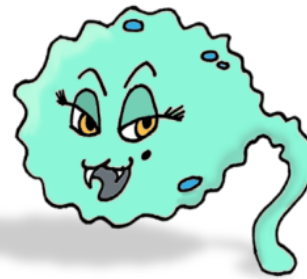
Interacting Peptide

In-vivo Experiments

Strep-tag
+
Peptide

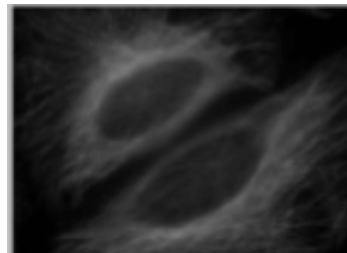


In vivo test

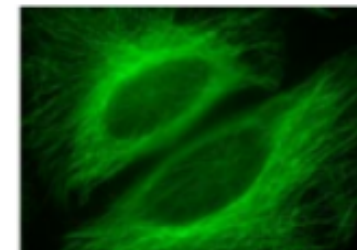


*Candida
glabrata*

ssr1
Knock-out

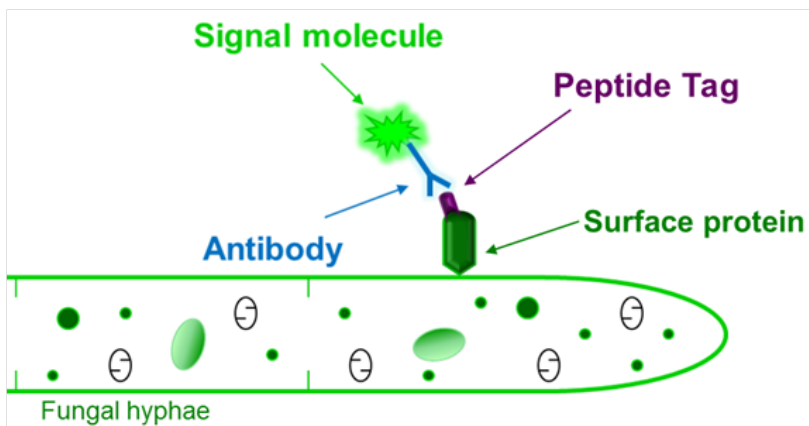


Wild type

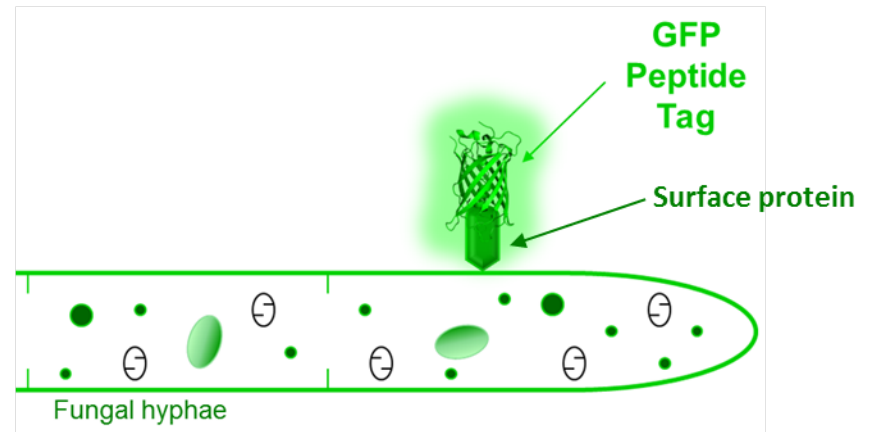


Outlook → Make it even faster!

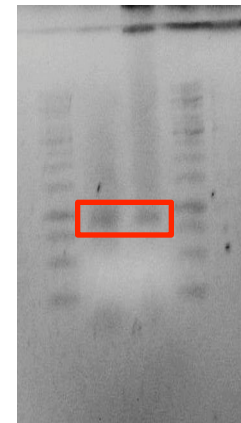
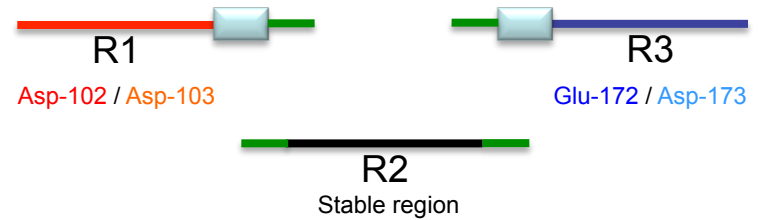
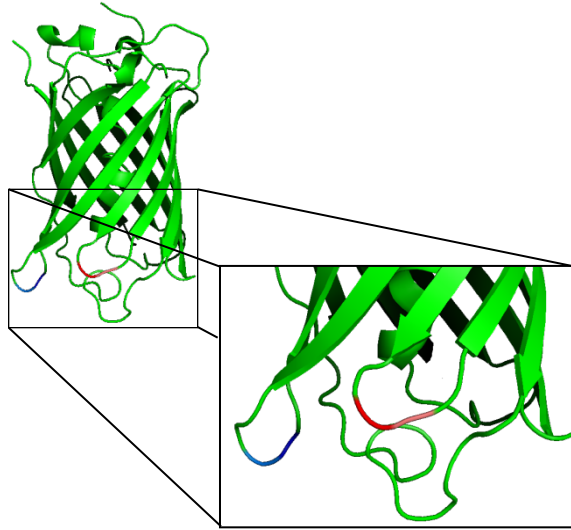
1ST BRICK



2ND BRICK



Our Own Peptide Library



Assemble

**GFP Scaffolds
Fragmented in
3 Regions**

**Plasmid
construction**



Outside the Lab



Our sponsors!



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GÖTTINGEN

Thank you for your attention!

