Functional Genomics in Plasmodium: current status and future prospects
Plasmodium life cycle
Functional Genomics:

- Sequence
- Expression
- Function
# Genome Sequence

## P. falciparum vs. P. berghei

<table>
<thead>
<tr>
<th></th>
<th>P. falciparum</th>
<th>P. berghei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coverage</td>
<td>15X</td>
<td>4X</td>
</tr>
<tr>
<td>Genome Size (Mb)</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>Number of Genes</td>
<td>5300</td>
<td>?</td>
</tr>
<tr>
<td>Variant Genes</td>
<td>~150 (var)</td>
<td>~180 (bir)</td>
</tr>
</tbody>
</table>

Conserved core of 4500 genes in central regions of chromosomes

More than 60% of the genes remain of unknown function
Gene Expression

Microarrays (Pf, Pb)
Mass spectrometry (Pf, Pb)
SSH (Pb)

RBC STAGES

OOOCYST → SPZ
OOKINETE

ZYGOTE → LIVER STAGES

Microarrays (Pf)
SAGE (Pf)
Microarrays of *Pf* RBC stages

**RBC cycle = 48 h**

**Synchronization**

Each gene, every hour

\[ \downarrow \]

80% of the genes have a wave-like expression pattern

\[ \downarrow \]

Gene clusters (co-expressed genes)
Microarrays of *Pf*: Identifying Protein Function

7 RBC-invasion genes (*)
Top 5% with similar exp. prof.

262 ORFs
28 already known
189 hypothetical

likely involved in RBC invasion
<table>
<thead>
<tr>
<th>Year</th>
<th>Method</th>
<th>P. falciparum</th>
<th>P. berghei</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>Episomal</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1996</td>
<td>Integrative *</td>
<td>+ (circular)</td>
<td>+ (linear)</td>
</tr>
<tr>
<td>1997</td>
<td>Gene KO</td>
<td>+ (weeks)</td>
<td>+ (days)</td>
</tr>
</tbody>
</table>

* 100% Homologous Recombination !!

- **RBC stages**
- **other stages**
Reverse Genetics by Gene Targeting

Poorly Efficient! (50 genes/10 years)

Essential Genes  Refractory
Conditional Mutagenesis

Site-specific Recombination

FRT  FRT

Flp
Conditional Mutagenesis

+ Rec

St Sp

target

+ Rec

St Sp
Conditional Mutagenesis

TRAP

FlpL

+ 

target
Conditional Mutagenesis

- CTRP
- TRAP
- UIS4
- LS

FlpL
Forward Genetics?

How Can We Identify Essential Genes?

- Random Mutagenesis
  - Transposition
  - Non Homologous Recombination

- Systematic Mutagenesis
  - RNA Interference
  - Homologous Recombination
Systematic Gene Knock-out

Construction of gene KO fragments inefficient
Frequencies of DCO poor

but
**Step 1: Construction of KO fragments by Transposition in E. coli**

**Recipient**

*TOP10 (dap+, Km*)

**Donor**

*β2155 (dap-, Km*)

**Transconjugant**

*XL10*

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*oriR6K*

*bla*

*oriT*

*mini-Tn5*

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*pir*

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*P. berghei shuttle mini-Tn5*
Step 2: Increasing DCO frequencies in P. berghei

New transfection protocol and AMAXA technology

Diagram showing the process of increasing DCO frequencies in P. berghei with input DNA MW and par. pop. MW.
Identifying new essential genes in parasite RBC stages

<table>
<thead>
<tr>
<th>Gene</th>
<th>Homol. arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAP-1</td>
<td>600-1200</td>
</tr>
<tr>
<td>TRAP-2</td>
<td>700-1100</td>
</tr>
<tr>
<td>AMA1</td>
<td>800-900</td>
</tr>
<tr>
<td>MSP1</td>
<td>700-900</td>
</tr>
<tr>
<td>UIS21</td>
<td>800-200</td>
</tr>
<tr>
<td>S6</td>
<td>900-200</td>
</tr>
<tr>
<td>S15</td>
<td>500-800</td>
</tr>
</tbody>
</table>

UIS21 essential for RBC stages
Identifying new essential genes in other parasite stages

green gene essential for liver stages
Functional Genomics in Plasmodium

- **Sequence**: P falciparum, P berghei, P yoelii, P chabaudi, P vivax
- **Expression**: Genome categorization into gene clusters (microarrays, mass-spectrometry)
- **Function**: Reverse genetics and systematic gene KO