Integration of Phage Genomics with Target Identification and HTS

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With its unique phage genomics platform, PhageTech identifies and exploits the natural mechanisms developed by bacterial viruses (phages) that lead to the death of their bacterial hosts, including human pathogens. PhageTech has assembled a large collection of phages and a unique proprietary database of the genomes of phages that kill *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*. Functional genomics tools are used to identify candidate growth inhibitory proteins (ORFs) from the phages; proteomics tools are then applied to identify the cognate bacterial targets of these phage-derived growth inhibitory proteins. Several growth inhibitory protein families and their bacterial targets have been identified. The targets are then used to develop robust, homogeneous, fluorescence-based HTS assays, including FP and TR-FRET, to screen for small molecule mimics of the growth inhibitory proteins. Different from 'phage therapy', this approach supports the development of potent drug candidates that selectively target essential bacterial functions.

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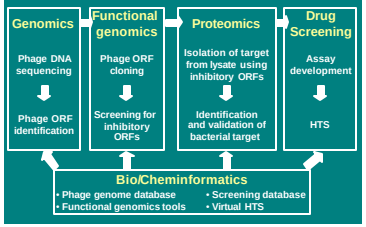
PhageTech Drug Discovery Tools



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The PhageTech Advantage in Antimicrobial Discovery

- Proprietary screening assays developed at PhageTech exploit the interaction between phage-encoded antimicrobial proteins and their cognate targets in bacteria
- PhageTech aims to identify small molecule inhibitors that mimic the effects of the inhibitory ORFs on their targets

Key advantages of the PhageTech approach:

- **speed of analysis** of small phage genomes
- **inherent validation** of the bacterial target by evolution
- rapid access to **diversified targets**
- requisite **positive control** for target screening assays

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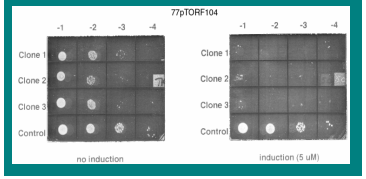
Identification of 77ORF104 as an Antimicrobial Protein

Transform *S. aureus* with cloned phage ORFs → Dot clones onto medium +/- inducer → Identify inhibitory ORFs under induced conditions



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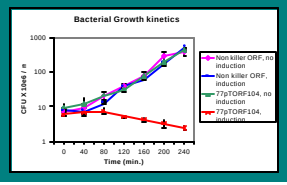
Induction of 77ORF104 is Bacteriostatic for *S. aureus*

Transform *S. aureus* with inhibitory ORF → Grow clones +/- inducer → Determine phenotype of growth inhibition



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Success of PhageTech's Genomics and Functional Genomics Platforms

Bacterial pathogen	Phages collected	Genomes sequenced	ORFs screened	Inhibitor families
S. aureus	150	27	964	31
S. pneumoniae	50	8	264	5
P. aeruginosa	70	10	210	9
Others	59	1	-	-
Total	**329**	**46**	**1438**	**45**

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Proteomics Approach to Target ID

Affinity chromatography of bacterial lysate over immobilized inhibitory ORF

↓

Trypticpeptide mapping, mass spectrometry of eluted proteins

↓

Target identification

↓

Target validation { Confirm ORF-Target interaction; Confirm target essentiality; Determine target function

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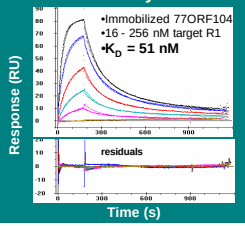
Confirmation of ORF-target Interaction by BIAcore



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Essentiality Analysis of *S. aureus* Target Genes

Gene inactivation/ replacement

Target gene → Selective marker

Growth: target not essential — No growth: suggests target essentiality

Regulated gene expression

P_{ind} Target gene

$P_{inducible}$

Growth *without* inducer: target not essential — Growth only *with* inducer: suggests target essentiality

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Validation of Bacterial Targets from the Proteomics Platform

- Several targets were identified from *S. aureus* lysates using immobilized inhibitory phage ORFs
- Direct interactions between inhibitory phage ORFs and their cognate bacterial targets were confirmed by:
  - Yeast two-hybrid analysis
  - Far western (protein blotting)
  - BIAcore
  - Time-resolved FRET (TR-FRET)
  - Fluorescence polarization (FP)
- These bacterial targets are:
  - essential
  - attractive targets for antibiotic discovery

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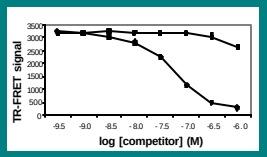
Specific Inhibition of 77ORF104-R1 Interaction in TR-FRET

1. Mix tagged binding proteins + competitor, incubate
2. Add anti-tag donor and acceptor Abs, incubate, read



Conclusion: inhibition reflects BIAcore data

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Pilot Screening with R1

Established: robust TR-FRET assay for R1 screen

Caveat: donor and acceptor antbody conjugates increase complexity and cost of assay

Objective: develop FP assay as alternative for HTS

Comparison: TR-FRET and FP screens were conducted with 640 compounds

Results: 1. Both assays are robust: Z = 0.6-0.7
2. hit rates at 3*S.D. are similar: 1.6% -2.5%

Conclusion: FP assay was selected for HTS; TR-FRET assay was retained for hit confirmation

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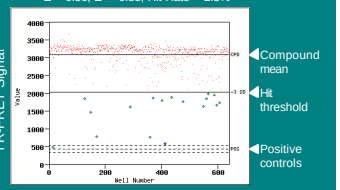
Pilot-Scale TR-FRET Screen with R1

Z = 0.56; Z' = 0.88; Hit Rate = 2.5%



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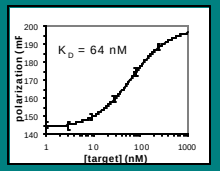
Development of FP Assay for R1

1. Purified 77ORF104 (8 kDa) was labeled with fluorescein
2. Pure *S. aureus* target R1 (~40kDa) was titrated into FL-ORF



Conclusions: FP assay reflects BIAcore data; signal window sufficient for HTS

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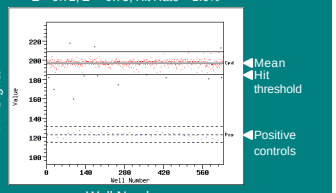
Pilot-Scale FP Screen with R1

Z = 0.72; Z' = 0.73; Hit Rate = 1.6%



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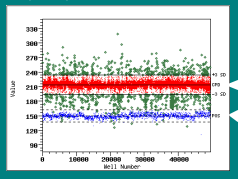
R1 HTS by FP

- FP assay selected based on performance in pilot-scale screen and low cost (<$0.05/well)
- 100 000 compounds were screened (first 50 000 shown)
- Intensity filter was applied to reject autofluorescent compounds
- Z' = 0.6; Z = 0.5; hit rate = 1%



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Confirmation of Hits from R1 HTS

1141 hits from 100 000 compounds (1.1%)
↓ screen cherry-picked hits against R1 by FP
677 reproduced hits
↓ screen against R1 by TR-FRET
366 format-independent hits
↓ counter screen against irrelevant targets by TR-FRET
150 confirmed hits from 100k compounds

- **Confirmed hits are now the focus of SAR / Cheminformatics Programs**

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In-House Bio/Cheminformatics

- Management of world's largest phage genome and phage ORF database
- Comparative mapping of phage genomes
- Automated annotation of phage ORFs
- Database of >5M suppliers' compounds with multiple descriptors for docking and 3D QSAR
- Clustering analyses; scaffold identification
- Generation of focused libraries around PhageTech proprietary targets for SAR

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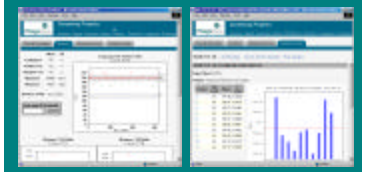
In-House Screening Database

Hit identification and assay validation | Control of pipetting quality with statistical mean tests



- Filtering of autofluorescent compounds
- Automated IC50 analyses

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Summary

- PhageTech has sequenced the genomes of 45 phages of *S. aureus*, *S. pneumoniae*, *and P. aeruginosa*
- 45 families of phage-derived antimicrobial ORFs were identified
- Several novel, essential bacterial targets have been discovered
- Proprietary ORF-target binding assays and enzymatic assays have been developed around these targets
- Diverse libraries of small molecules have been screened for inhibitors
- Key advantages of the PhageTech drug discovery platform include:
  - **speed of analysis** of small phage genomes
  - access to **diversified targets**
  - **inherent validation** of the bacterial target
  - requisite **positive control** for target-based screening assays