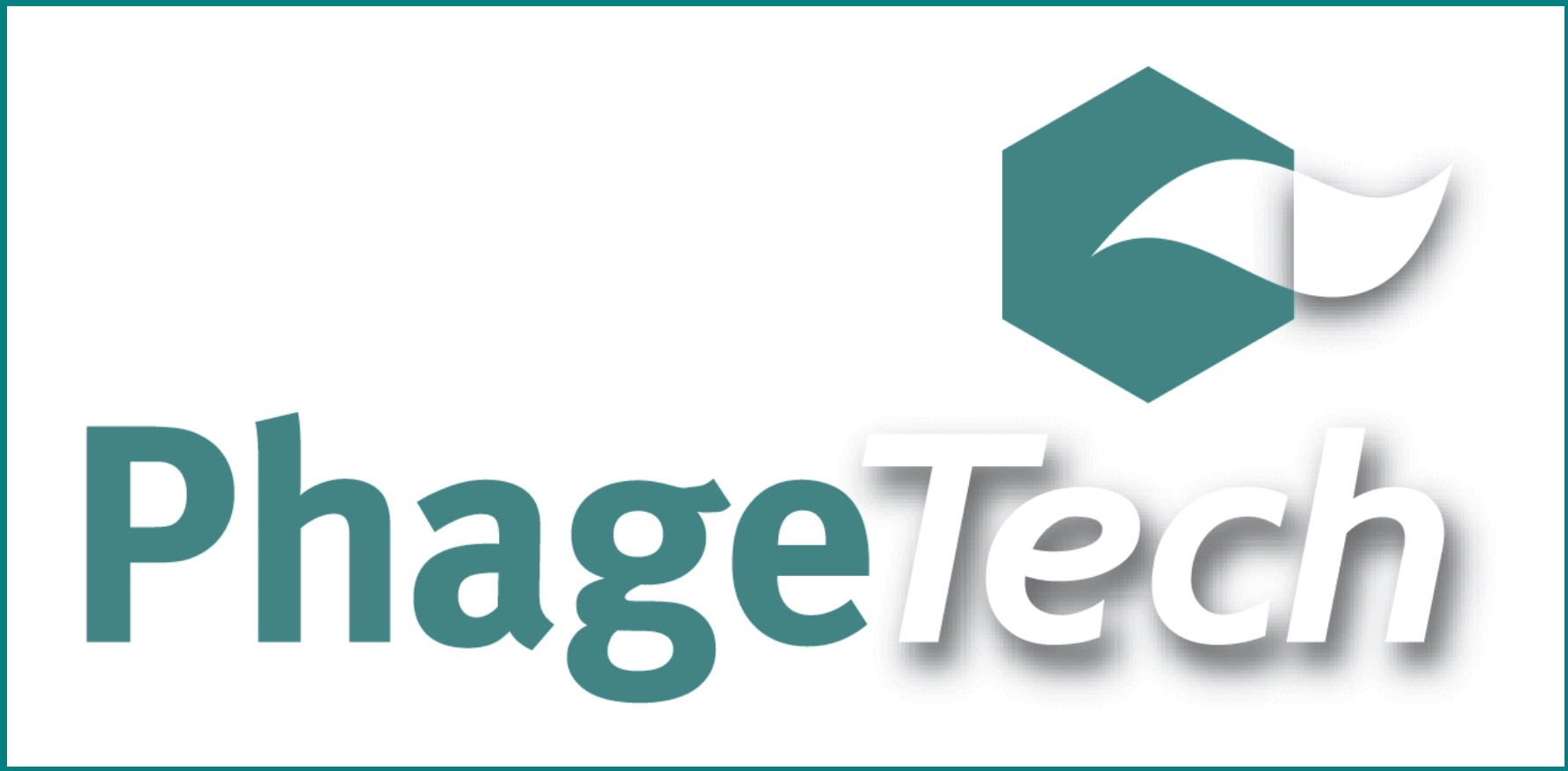
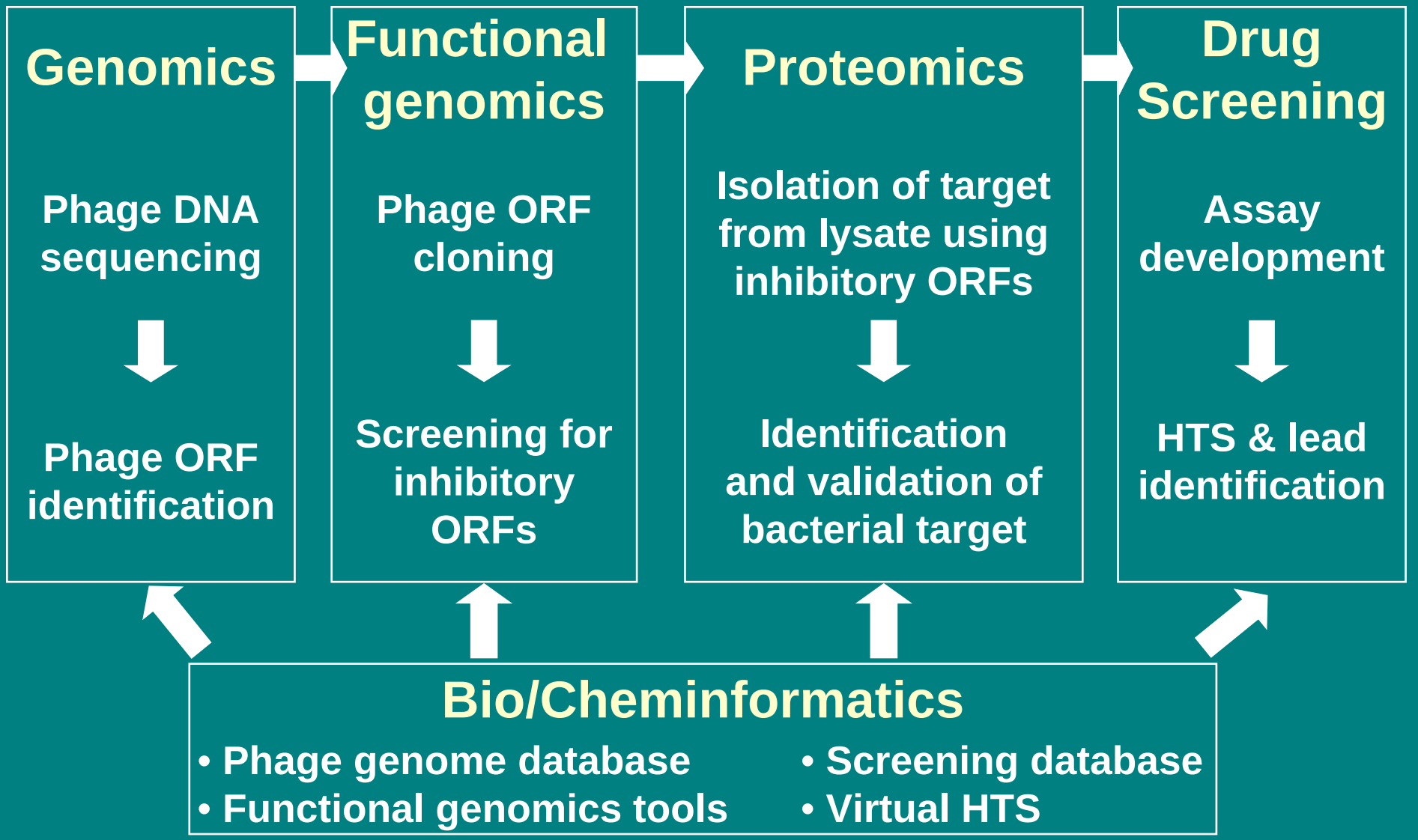


New Classes of Antibiotics From Phage Genomics

From Phage Genomics To Antimicrobial Drug Discovery

PhageTech

PhageTech Drug Discovery Tools



The PhageTech Advantage in Antimicrobial Discovery

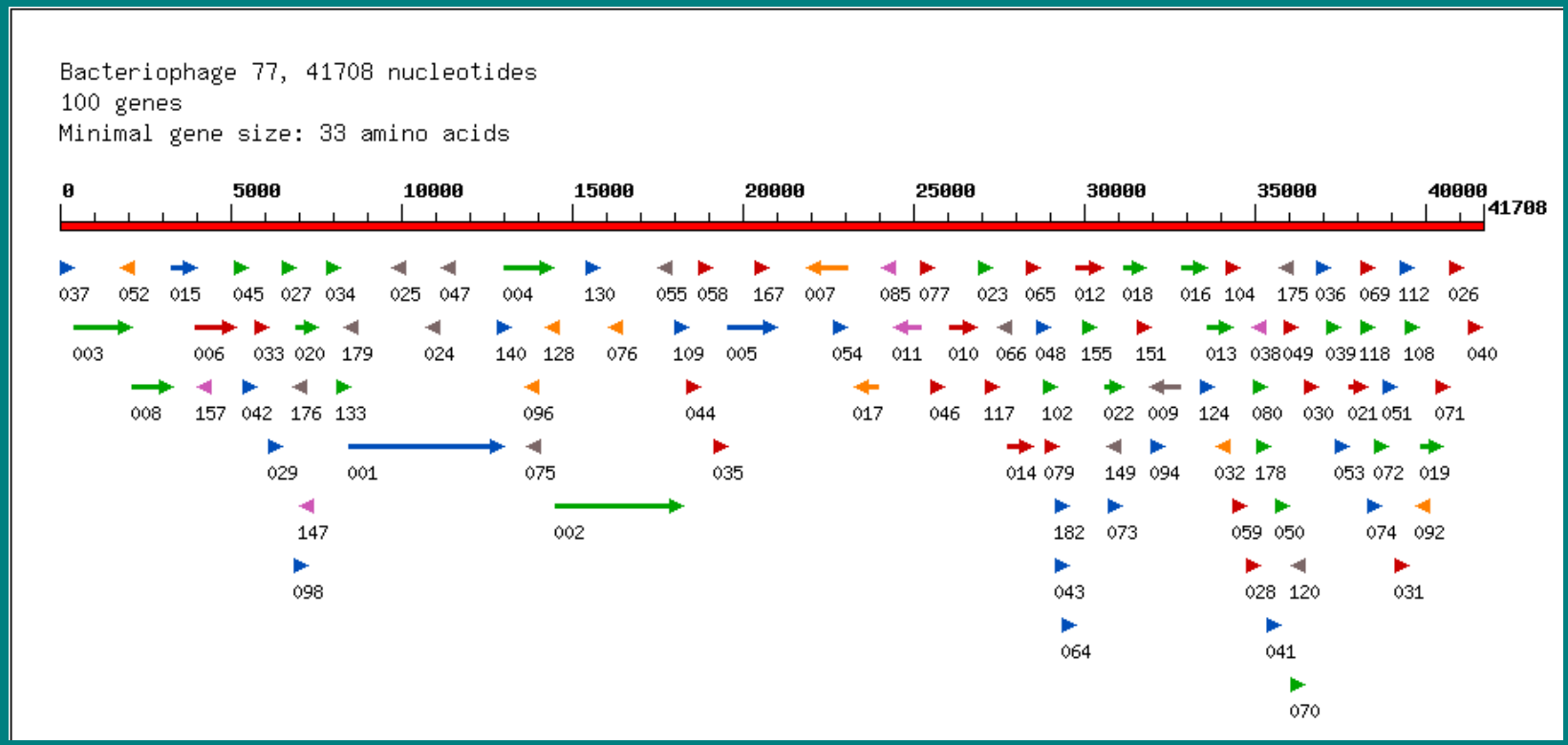
- Proprietary screening assays and tools developed at PhageTech exploit the interaction between phage-encoded antimicrobial proteins and their cognate targets in bacteria

- PhageTech aims to identify small molecule drug candidates that inhibit the same targets as the phage inhibitory ORFs do

Key advantages of the PhageTech approach:

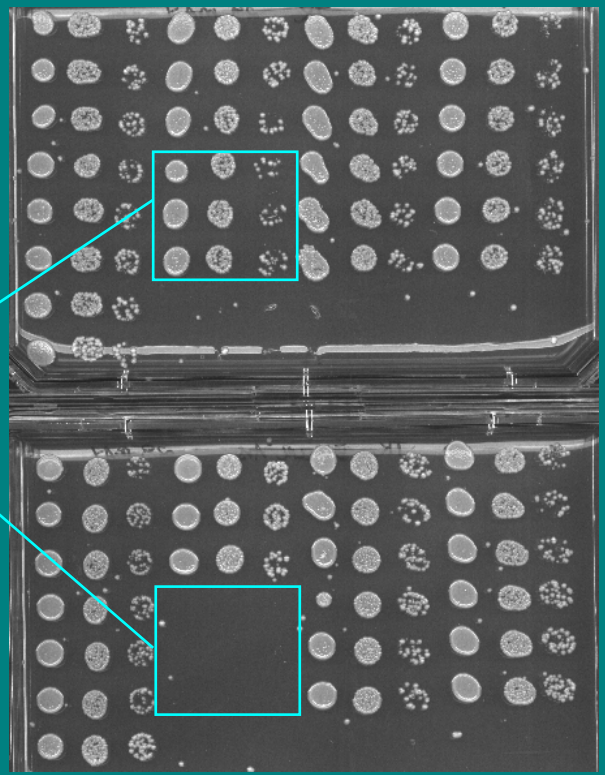
- **speed of analysis** of small phage genomes
- **inherent validation** of the bacterial target by evolution
- rapid access to **diversified targets**
- requisite **positive control** for target screening assays

Genome Map for *S. aureus* Phage 77



High Throughput Screening for Phage Inhibitory ORFs

Systematic clone phage ORFs in inducible shutter vector → Transform *S. aureus* with cloned phage ORFs → Dot clones onto medium +/- inducer → Identify inhibitory ORFs under induced conditions



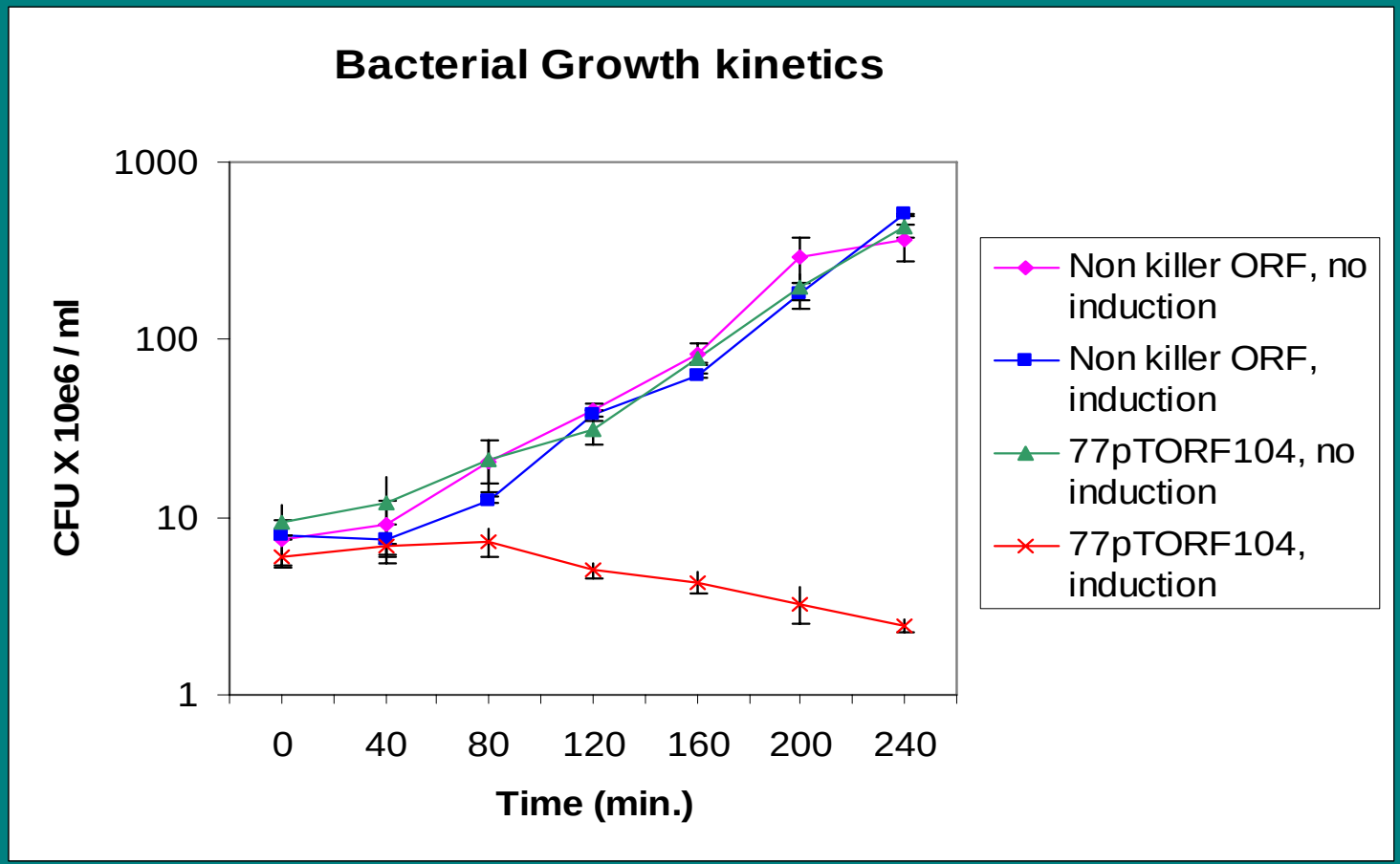
Identification of inhibitory 77ORF04

Without inducer

With inducer

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

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



Summary of PhageTech's Genomics and Functional Genomics Programs

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	11	210?	9?
Total	**329**	**47**	**1438?**	**45?**

Target Identification and Validation

PhageTech

ORF Affinity Approach to Target ID

Affinity chromatography of bacterial lysate over immobilized inhibitory ORF

↓

Tryptic peptide mapping, mass spectrometry of eluted proteins

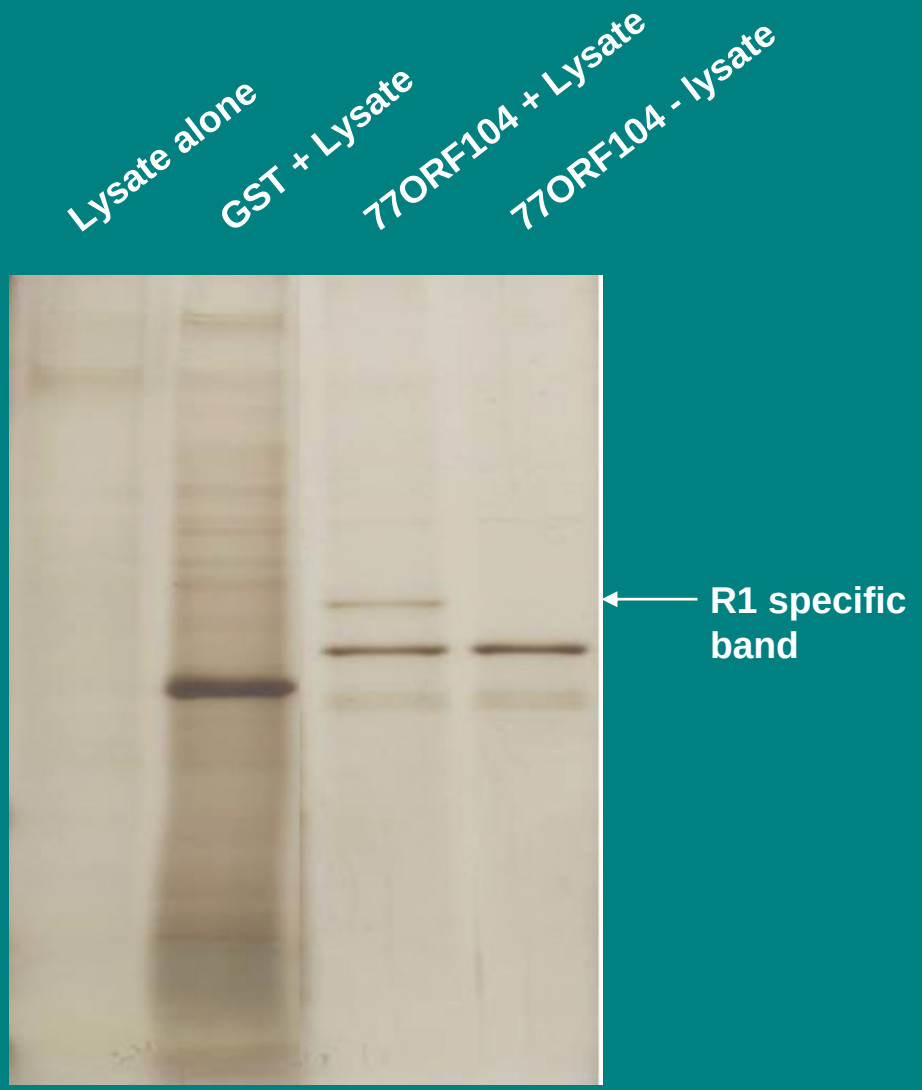
↓

Target identification

↓

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

Identification of DnaI as a *S. aureus* Target of 77ORF104



Identification of the cellular target of 77ORF104 by affinity chromatography:

Purified 77ORF104 and GST proteins were crosslinked to affigel and then incubated with S. aureus lysate.
Specific bands were eluted by 1% SDS and proteins were resolved on a 12% SDS gel. Specific band was excised from the gel and subjected to mass spetrophotometry for identification.

Validation of Bacterial Targets from the Proteomics Platform

- Direct interactions between inhibitory phage ORFs and their cognate bacteral targets were confirmed by:
  - Yeast two-hybrid analysis
  - Far western (protein blotting)
  - BIAcore
  - Time-resolved FRET (TR-FRET)
  - Fluorescence polarization (FP)

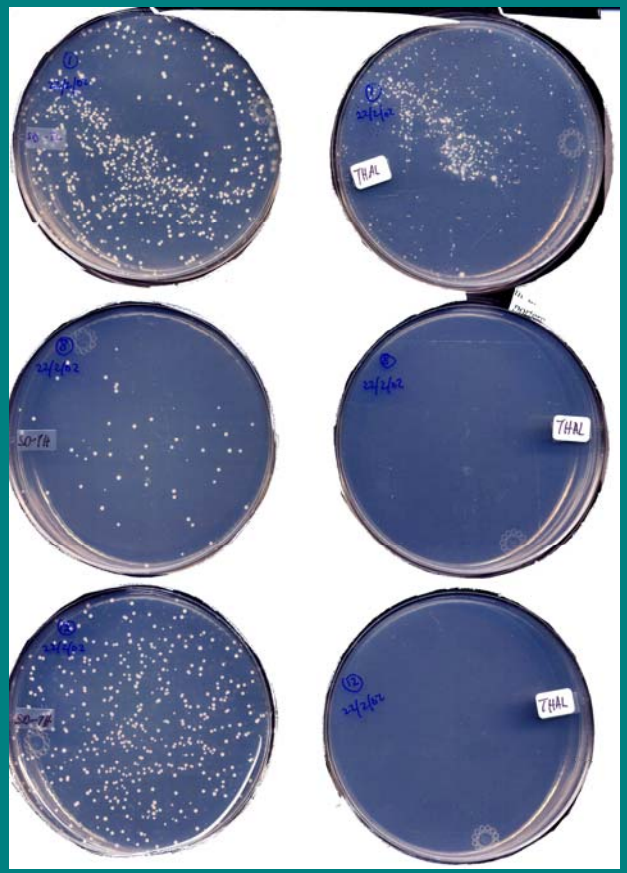
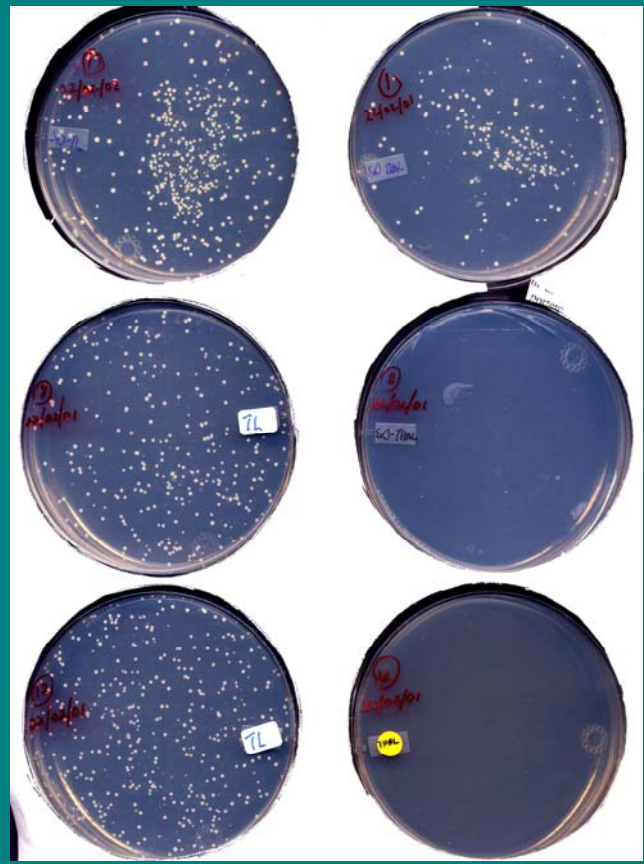
- These bacterial targets are:
  - essential
  - attractive targets for antibiotic discovery

Yeast Two-Hybrid Analysis

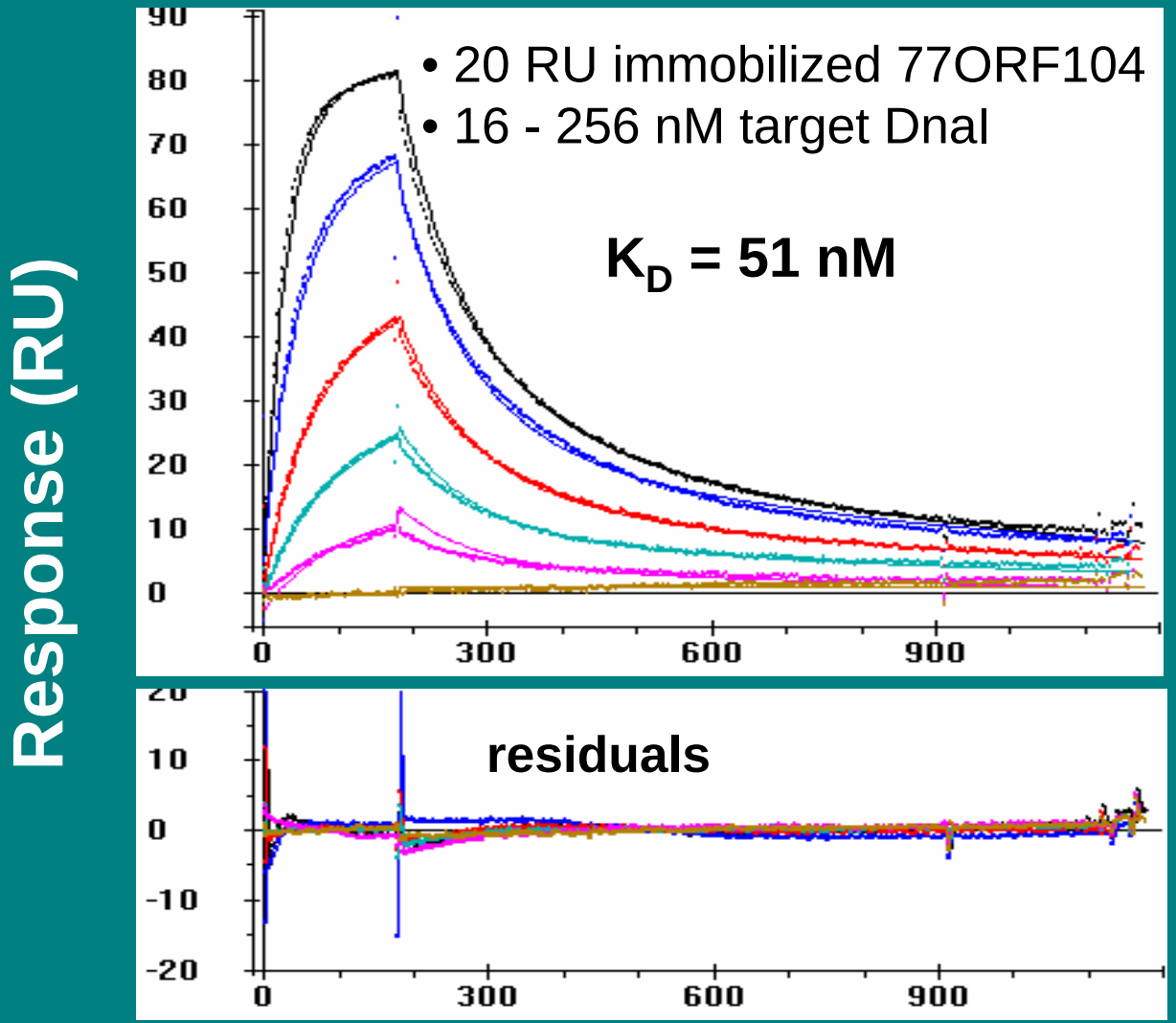
DnaI interacts with 77ORF104

DnaI in activating domain; 77ORF104 in binding domain

DnaI in binding domain; 77ORF104 in activating domain

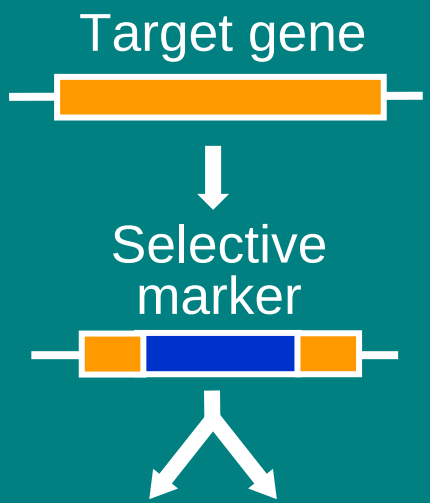
	SD-TL	SD-THAL	SD-TL	SD-THAL	
DnaI and 77ORF104					DnaI and 77ORF104
DnaI and LamC					DnaI and Large T
77ORF104 and LargeT					77ORF104 and LamC

Confirmation of 77ORF104/DnaI Interaction by BIAcore



Essentiality Analysis of *S. aureus* Target Genes

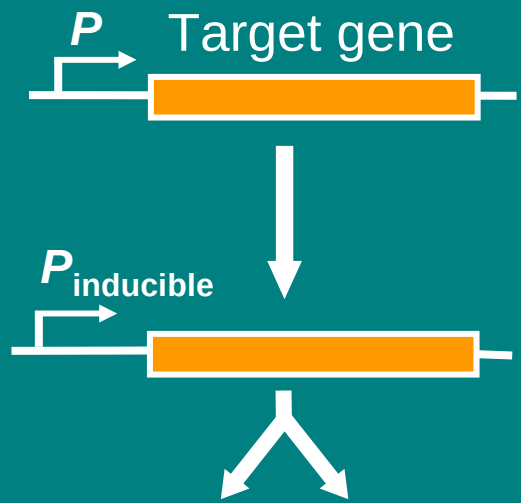
Gene inactivation/ replacement



Growth: target not essential

No growth: suggests target essentiality

Regulated gene expression



Growth *without* inducer: target not essential

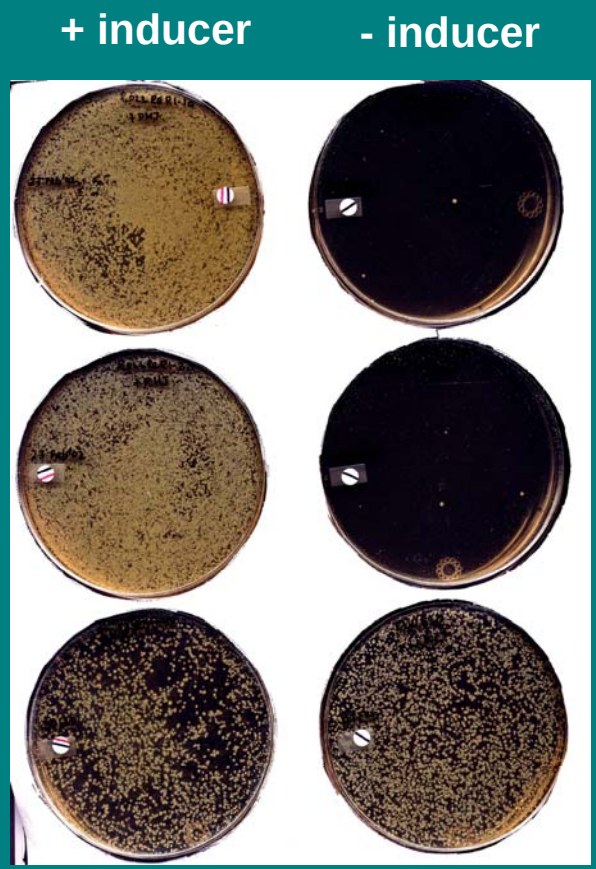
Growth only *with* inducer: suggests target essentiality

DnaI Is an Essential *S. aureus* Gene

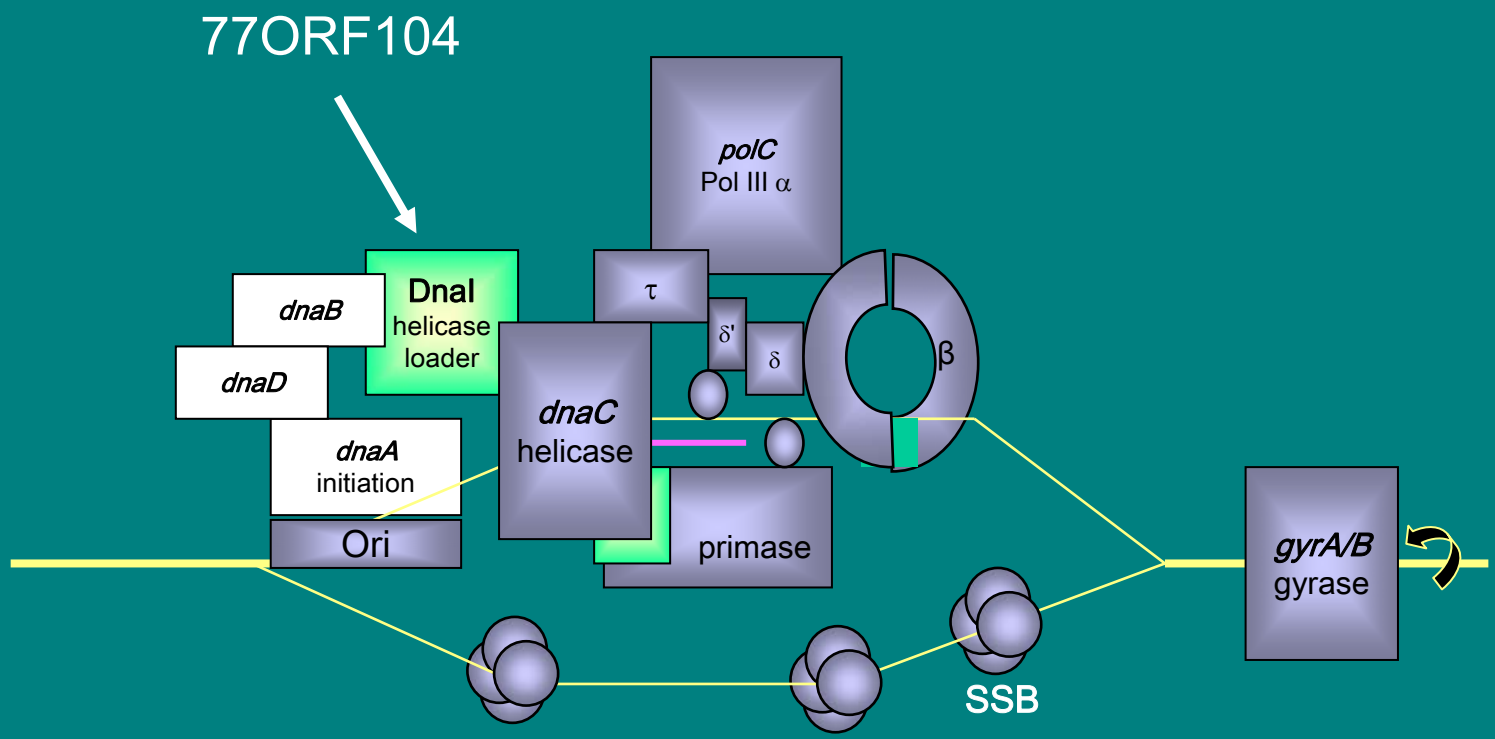
Gene inactivation/ replacement

No viable clones obtained.
Indication of gene essentiality.

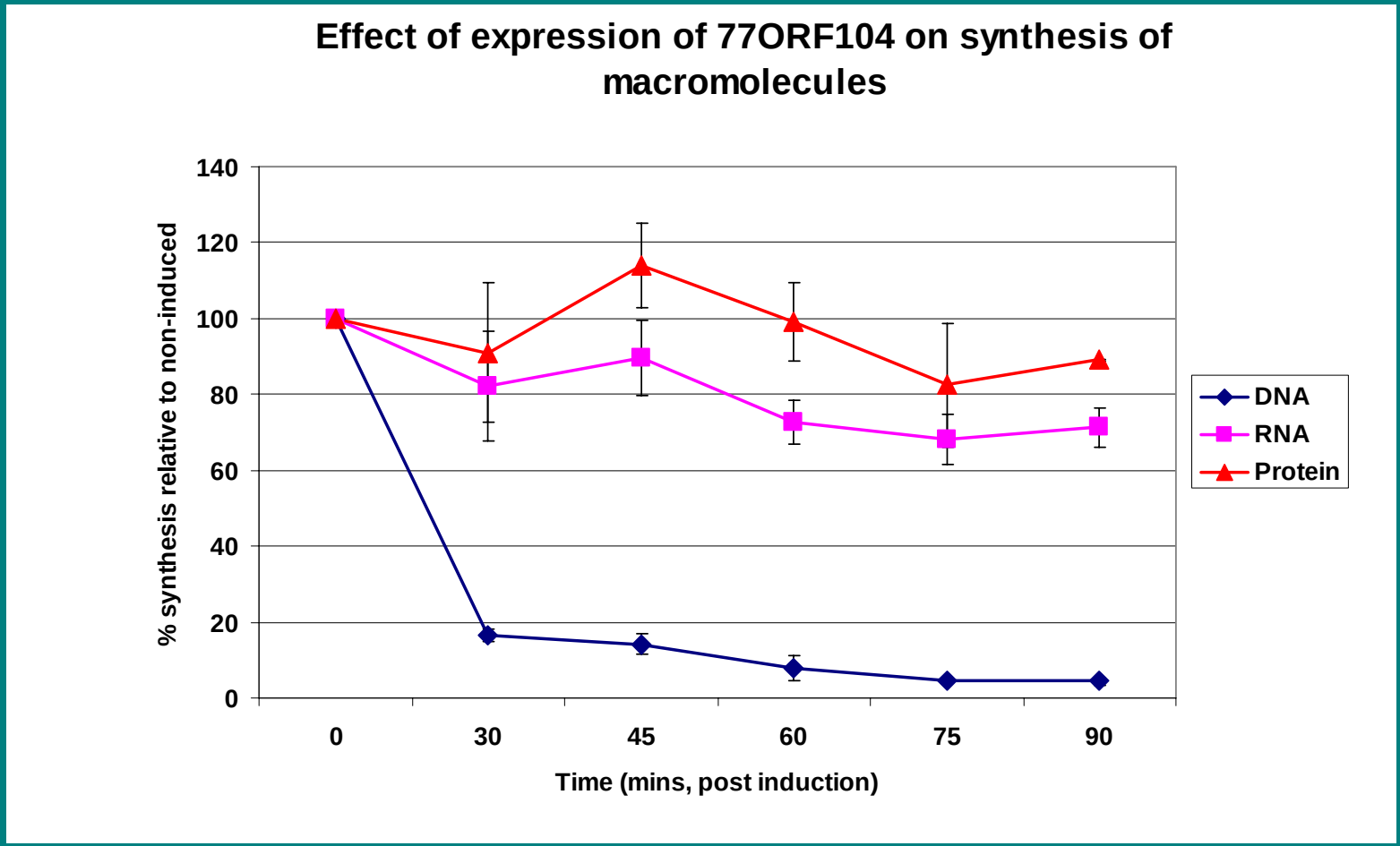
Regulated gene expression



DnaI Involved in DNA Replication Initiation



77ORF104 Inhibits ^{3}H-thymidine Incorporation



Representatives of Small Molecules with Antibacterial Activity against *S. aureus*

Small molecule	77ORF104-DnaI interaction IC_{50} (μM)	MIC (μg/mL)	DNA synthesis IC_{50} (μg/mL)
PT10056053	3	0.5 – 2	1
PT10023719	8	8 – 16	20
PT12008187	0.4	0.125 – 0.25	2

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S. aureus DNA Replication Proteins Identified by Phage Inhibitory ORFs

Representative of inhibitory ORF family[a]	ORF size (aa)	Bacterial target identified	Function of target	Essentiality of target[b]
77ORF104 ORF016	52 297	DnaI	Helicase loader	Essential
ORF025 ORF168 ORF240	58 74 58	DnaN	DNA Pol III β subunit	Essential
ORF078	71	DnaG	DNA Primase	Essential
ORF140	101	PT-R14	Involved in DNA replication	Not determined

Summary

- PhageTech has sequenced the genomes of 46 phages of *S. aureus, S. pneumoniae, and P. aeruginosa*
- ? families of phage-derived antimicrobial ORFs were identified
- Several novel, essential bacterial targets, including *S. aureus* DnaI, have been discovered and patented
- Proprietary ORF-target binding assays and enzymatic assays have been developed around these targets
- Diverse libraries of small molecules are being 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

Acknowledgements

Francis Arhin, Pascale Bauda, Dominique Bergeron, Mario Callejo, Mohammed Dehbi, Vincent Ferretti, Nhuan Ha, Tony Kwan, John McCarty, Greg Moeck, Ramakrishnan Srikumar, Dan Williams, Jinzi Wu and all technicians

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