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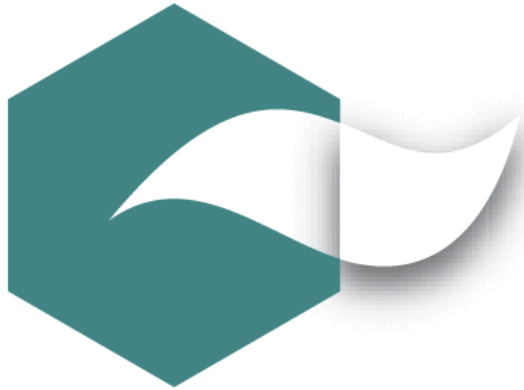
Phage-inspired antibiotics
Transdermal drug delivery
Visualizing receptor dimerization



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Phage-inspired Antibiotics

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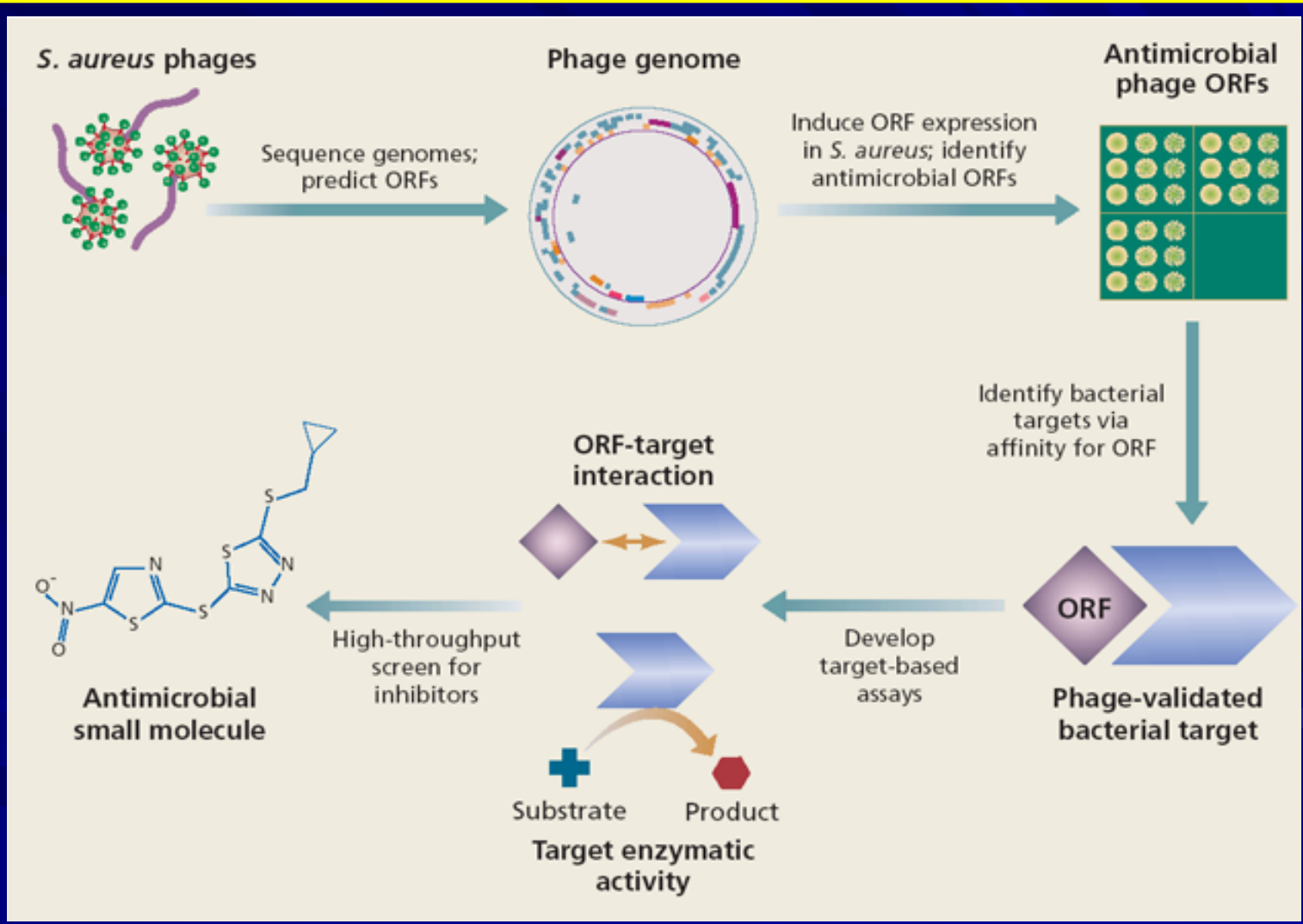
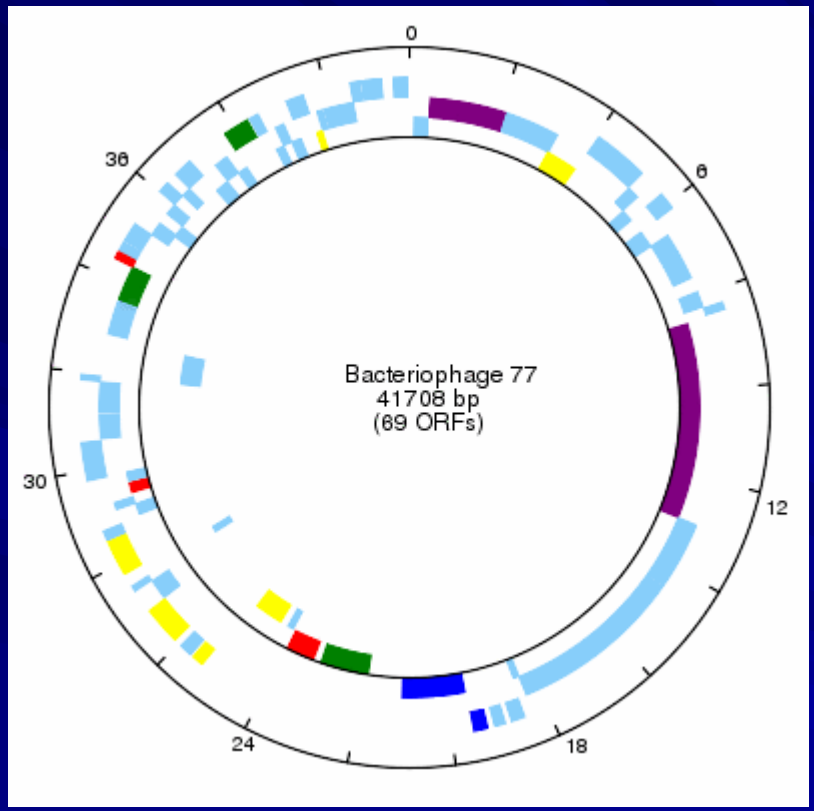
Bacteriophages (Phages)

- Phages are viruses that infect and kill bacteria
- Almost all bacterial species have known phages
- Over billions of years of evolution, phages have evolved highly efficient mechanisms to kill their hosts
- This information is contained in a compact genome

PhageTech Drug Discovery Platform



Genome Map for *S. aureus* Phage 77



- Unknown function
- Morphogenesis
- Bacterial growth inhibitor
- Regulation
- Cell lysis
- DNA/RNA Metabolism
- Intermediary metabolism

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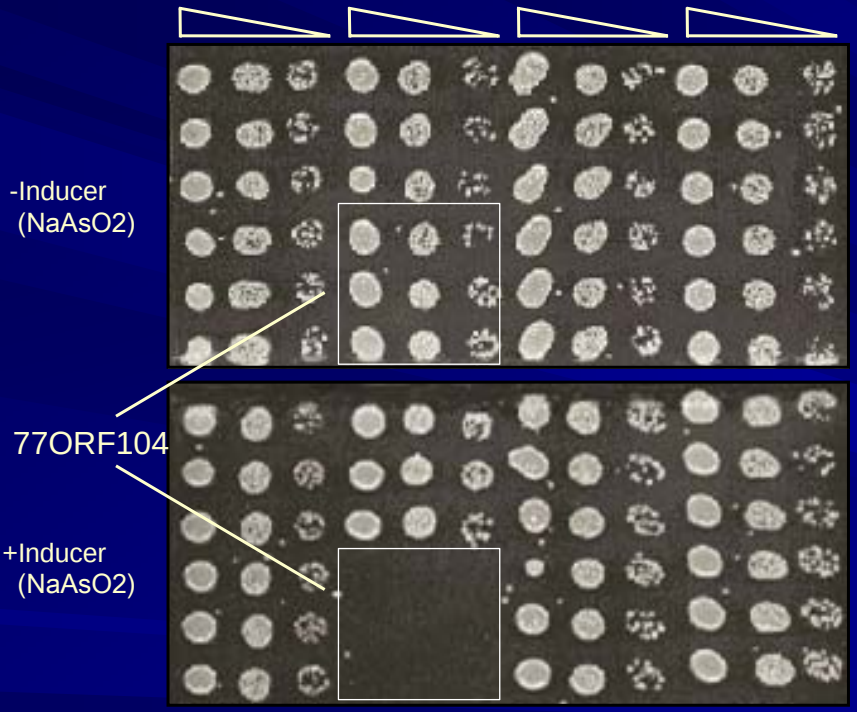
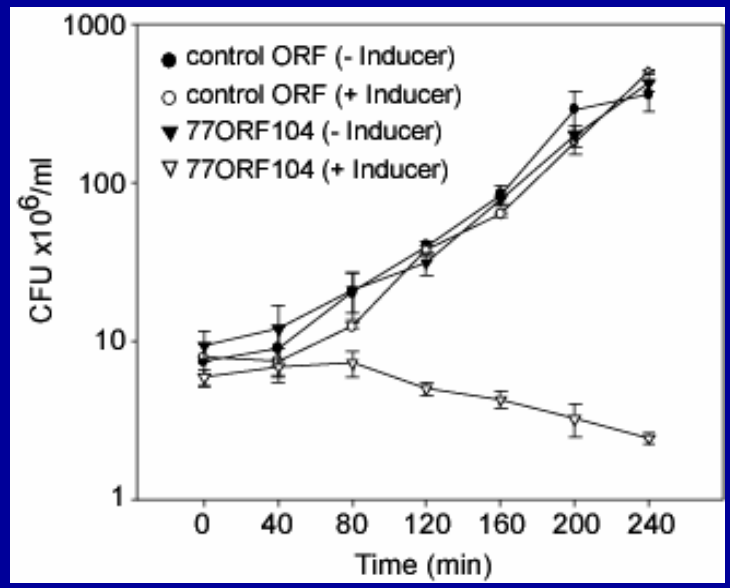
High Throughput Screening for Phage Inhibitory ORFs





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PhageTech's Genomics & 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	749	24
Total	**270**	**46**	**1977**	**60**

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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

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S. aureus DnaI is the Cellular Target of 77ORF104

Validation of Bacterial Targets Identified by Phage Inhibitory ORFs

- Direct interactions between inhibitory phage ORFs and their cognate bacterial targets were confirmed by:
  - yeast two-hybrid analysis
  - far western
  - BIAcore
  - time-resolved FRET
  - fluorescence polarization

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

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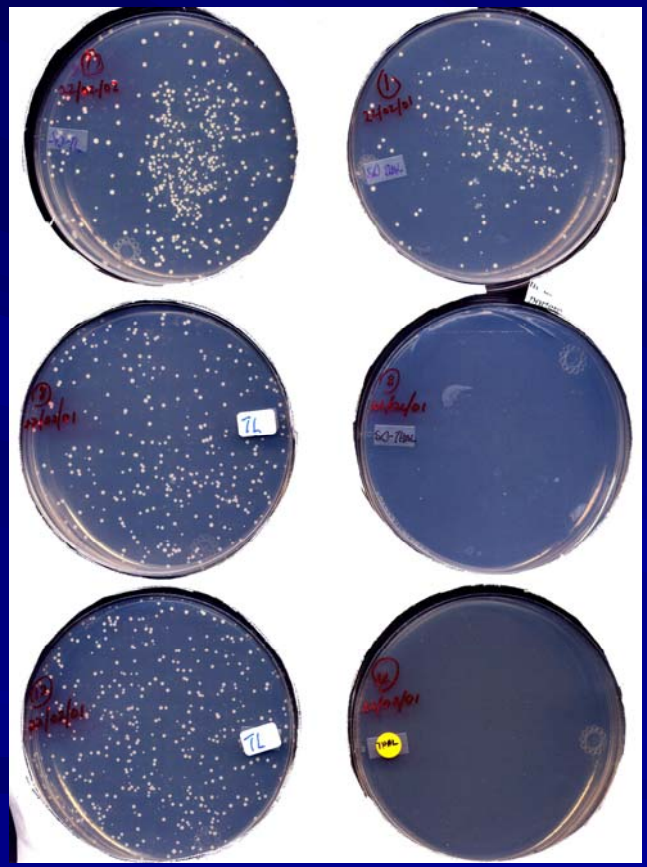
Yeast Two-Hybrid Analysis

SD-TL SD-THAL



DnaI + 77ORF104

DnaI + SV40 Large T-antigen

77ORF104 + LaminC

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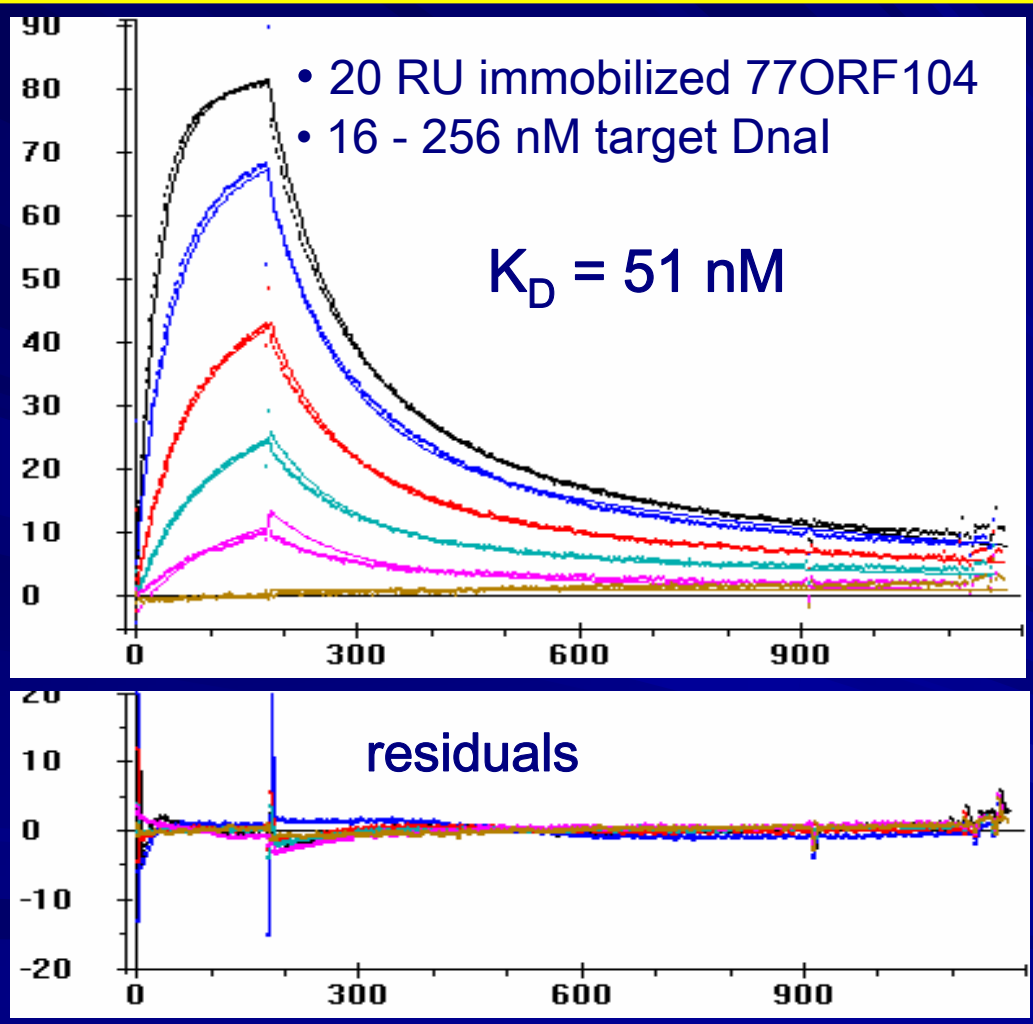
Confirmation of 77ORF104/DnaI Interaction by BIAcore



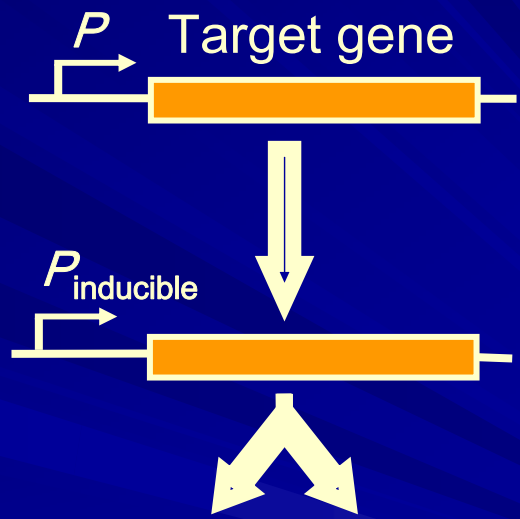
Essentiality Analysis of *S. aureus* Target Genes

Gene inactivation/ replacement

Growth: target not essential

No growth: suggests target is essential

Regulated gene expression



Growth *without* inducer: target not essential

Growth only *with* inducer: suggests target is essential

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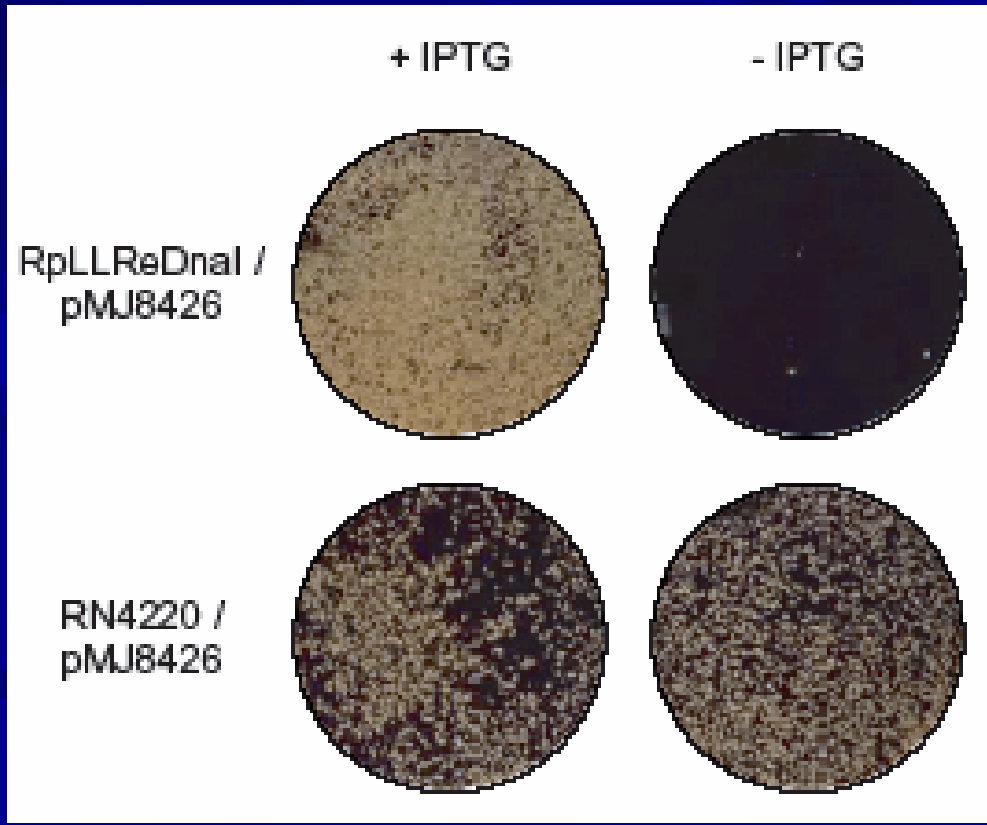
DnaI Is an Essential *S. aureus* Gene

Gene inactivation/ replacement

No viable clones obtained;
Indication of gene essentiality.

Regulated gene expression



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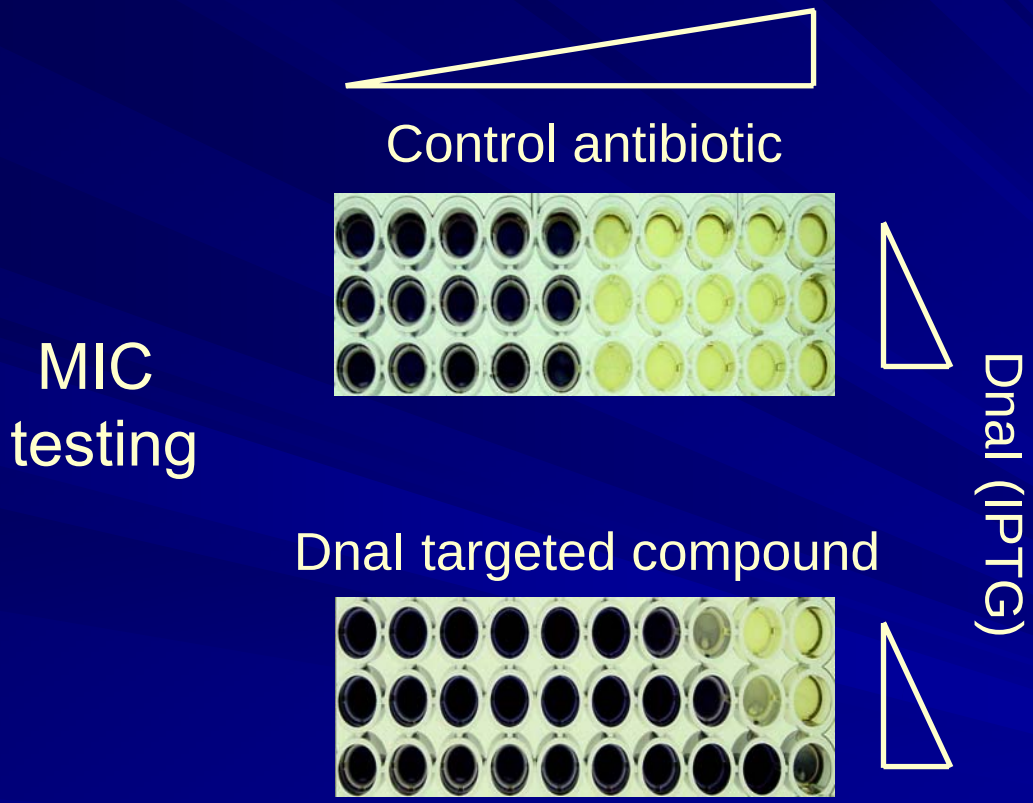
Mechanism of Action Studies Using Target (DnaI) Titrating System



For DnaI targeted compounds, increased DnaI expression correlates with increased MIC

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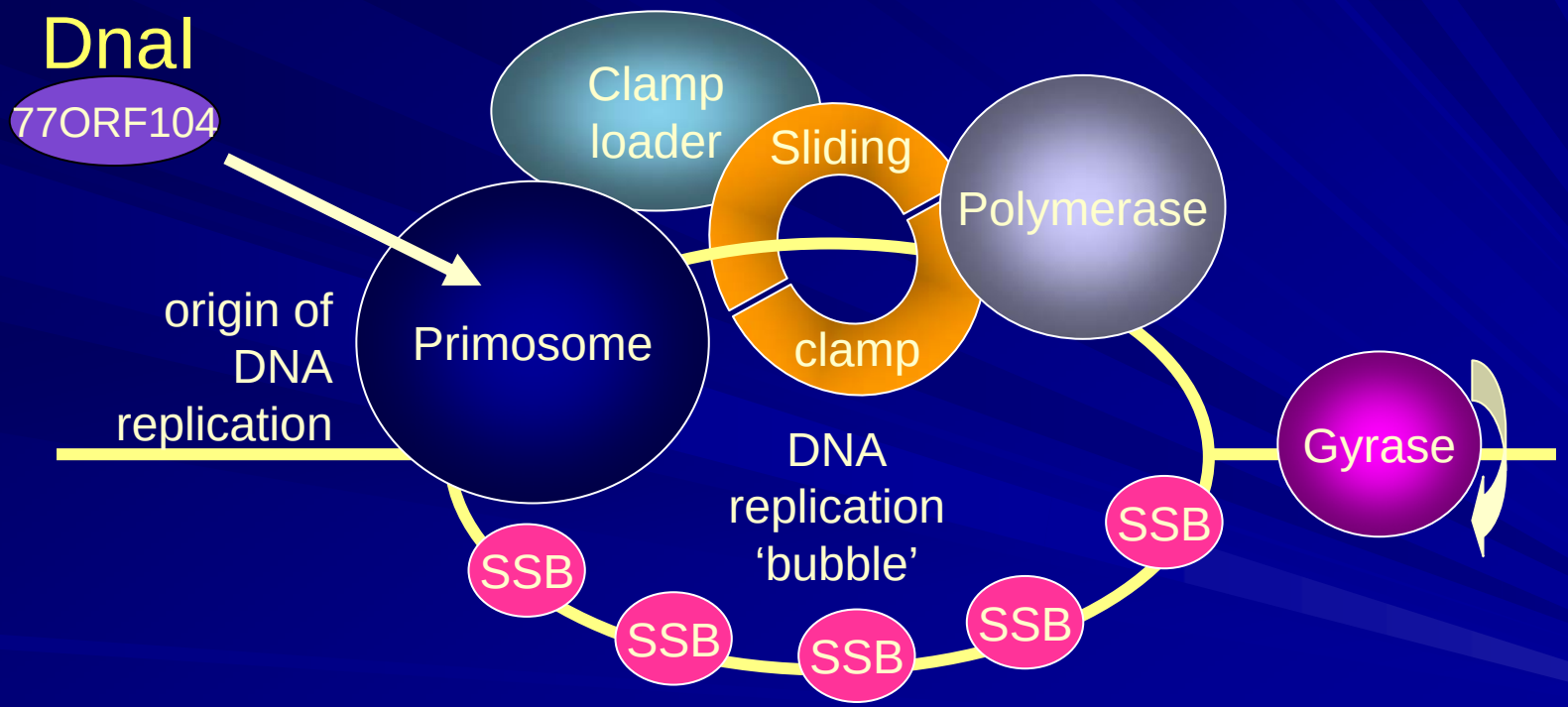
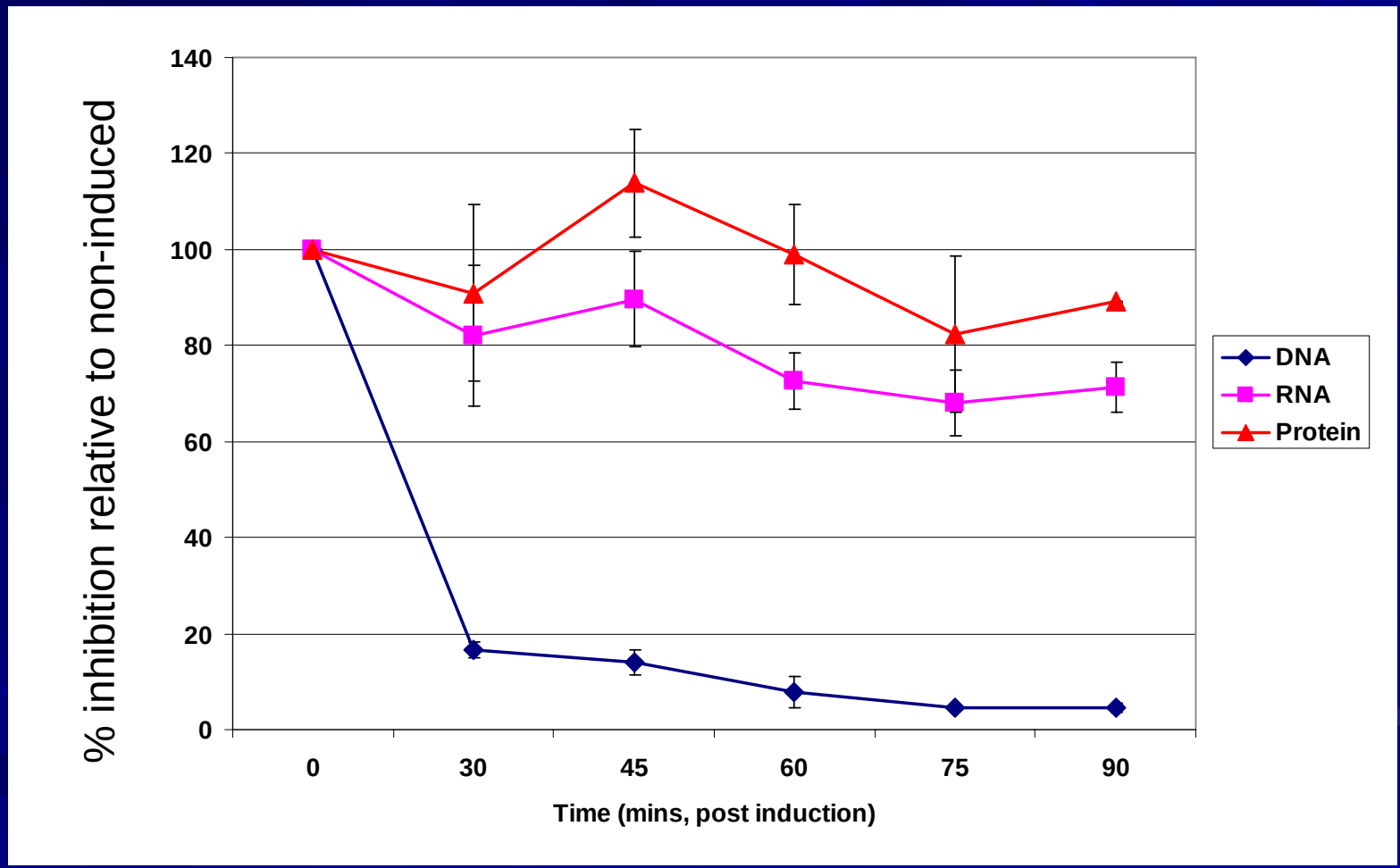
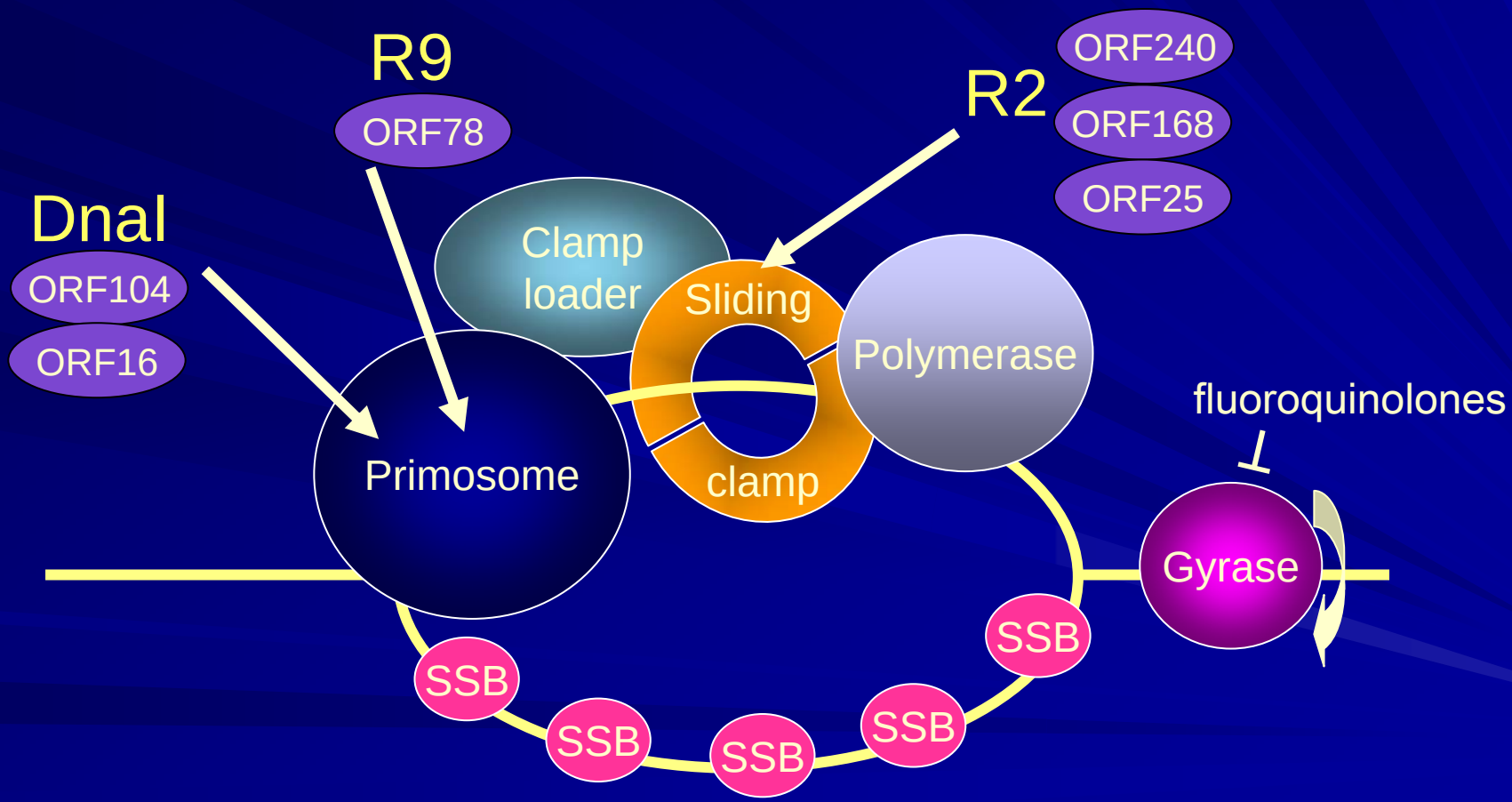
DnaI is Involved in DNA Replication Initiation



77ORF104 Inhibits DNA Synthesis



DNA Replication Machinery is Targeted by Phage Inhibitory ORFs



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PhageTech Targets

Target	Identity	Biochemical Pathway
R1	Helicase loader	DNA Replication
R2	Clamp loader	DNA Replication
R9	Primase	DNA Replication
R12	Sigma factor	Transcription
R4	PLSX-like	Lipid synthesis?
R14, F2, F3	Not revealed	DNA Replication

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Screening Strategy

Small molecule compounds

↓ HTS (ORF-target interaction or enzymatic activity)

Actives

↓ Confirmatory, counter screening

Confirmed actives

↓ Dose response testing

Hits

↓ *In vitro* functional assay

Confirmed hits

↓ Susceptibility testing, compound profiling

Lead

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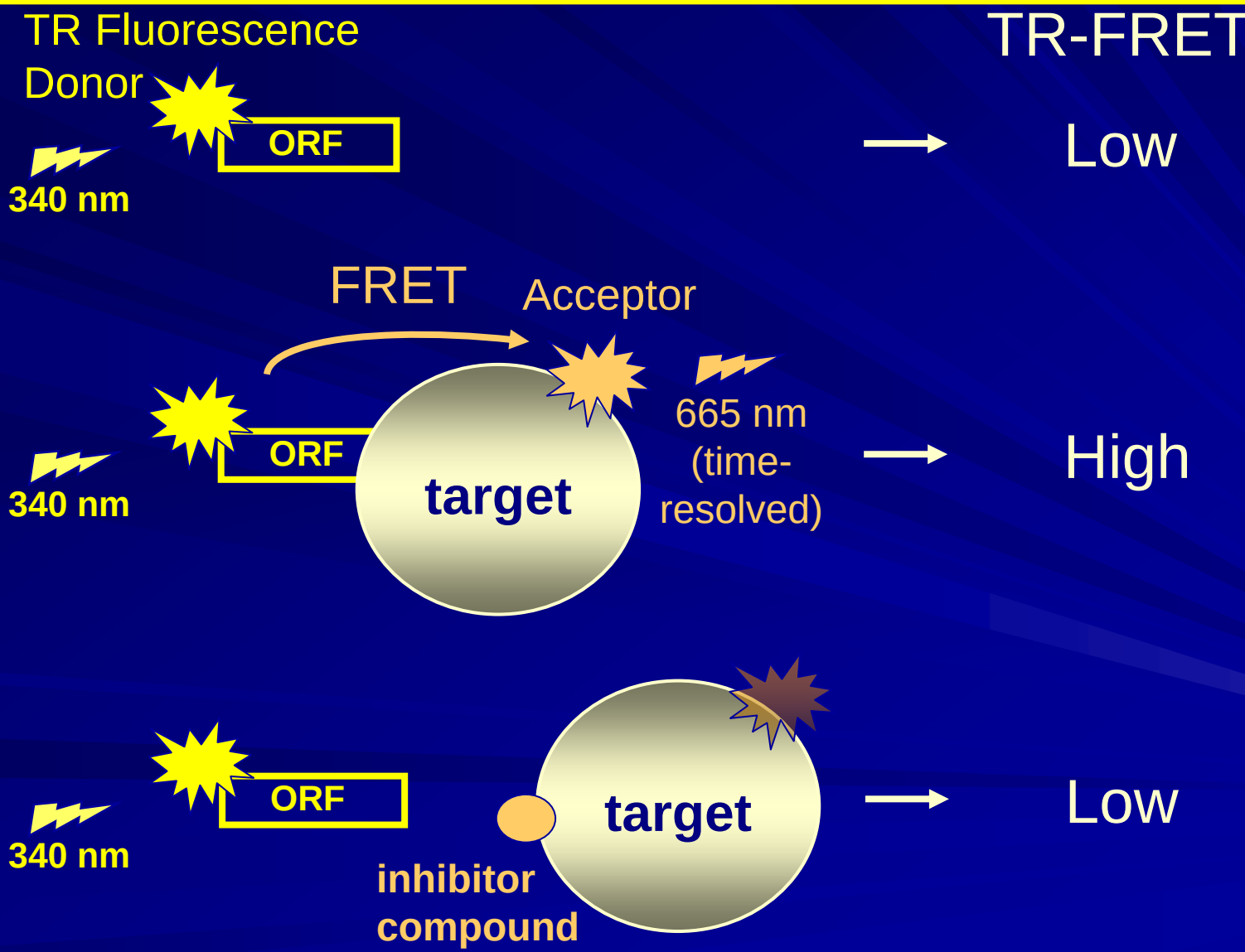
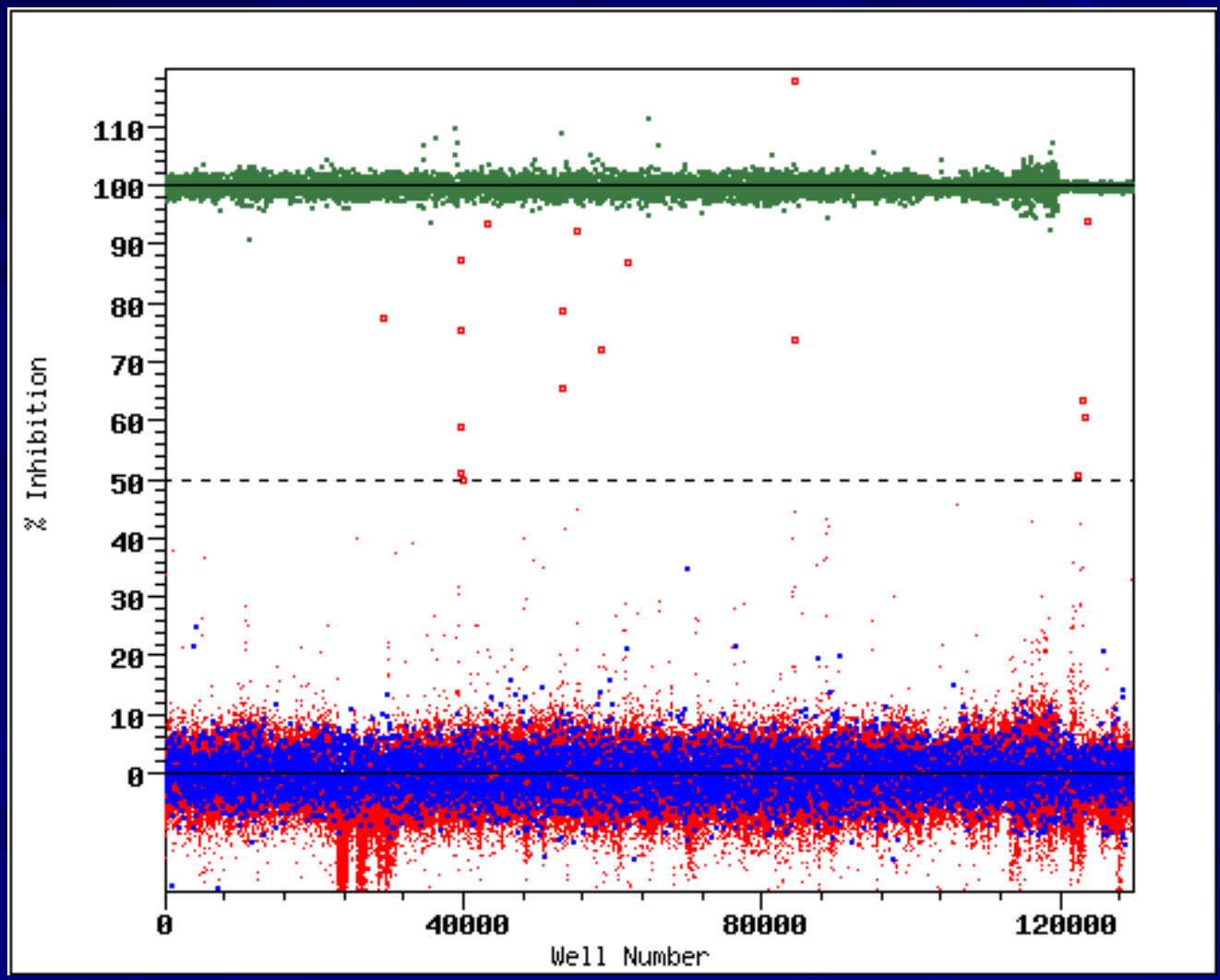
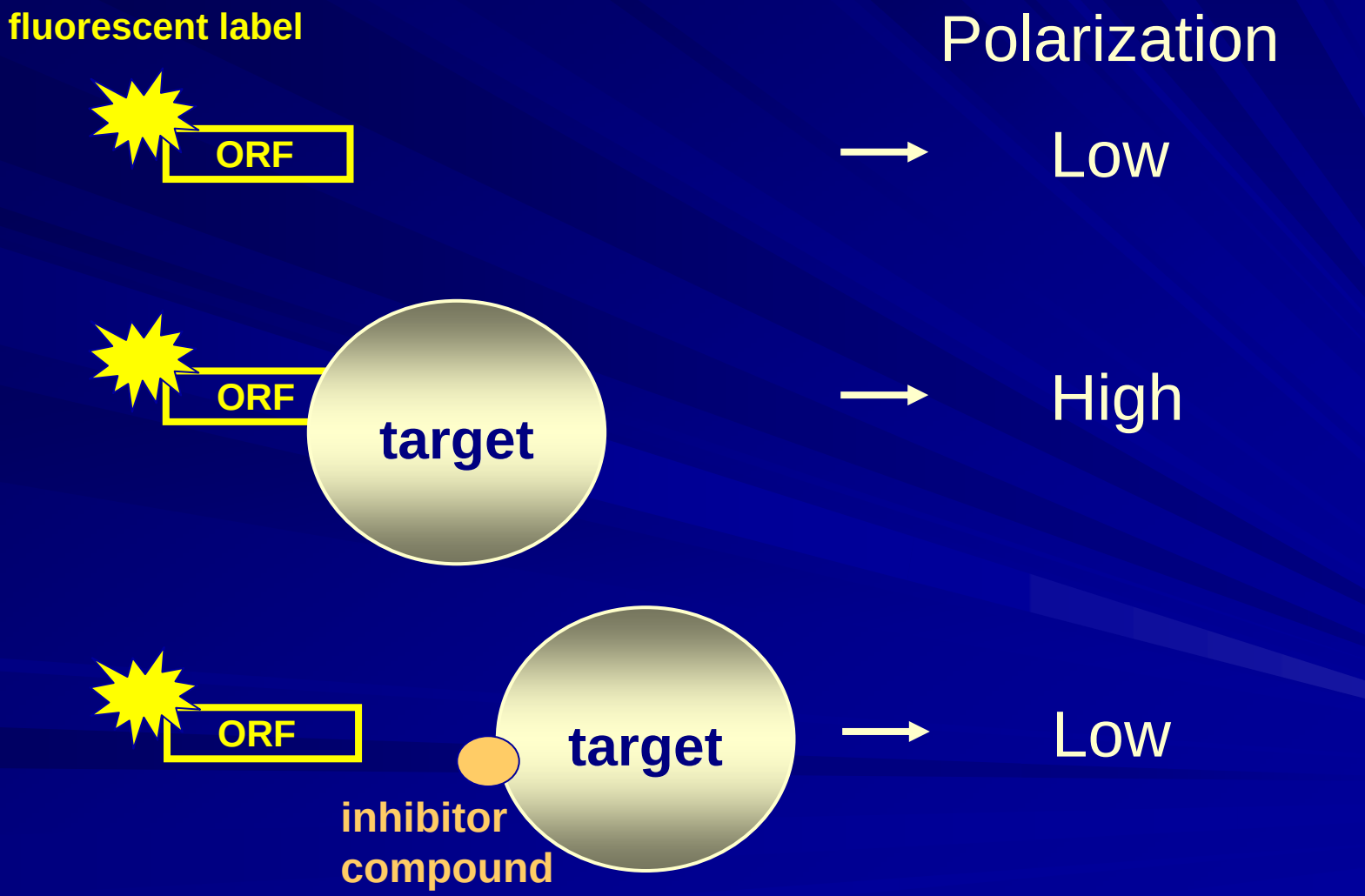
TR-FRET Assay for ORF - Target Interaction



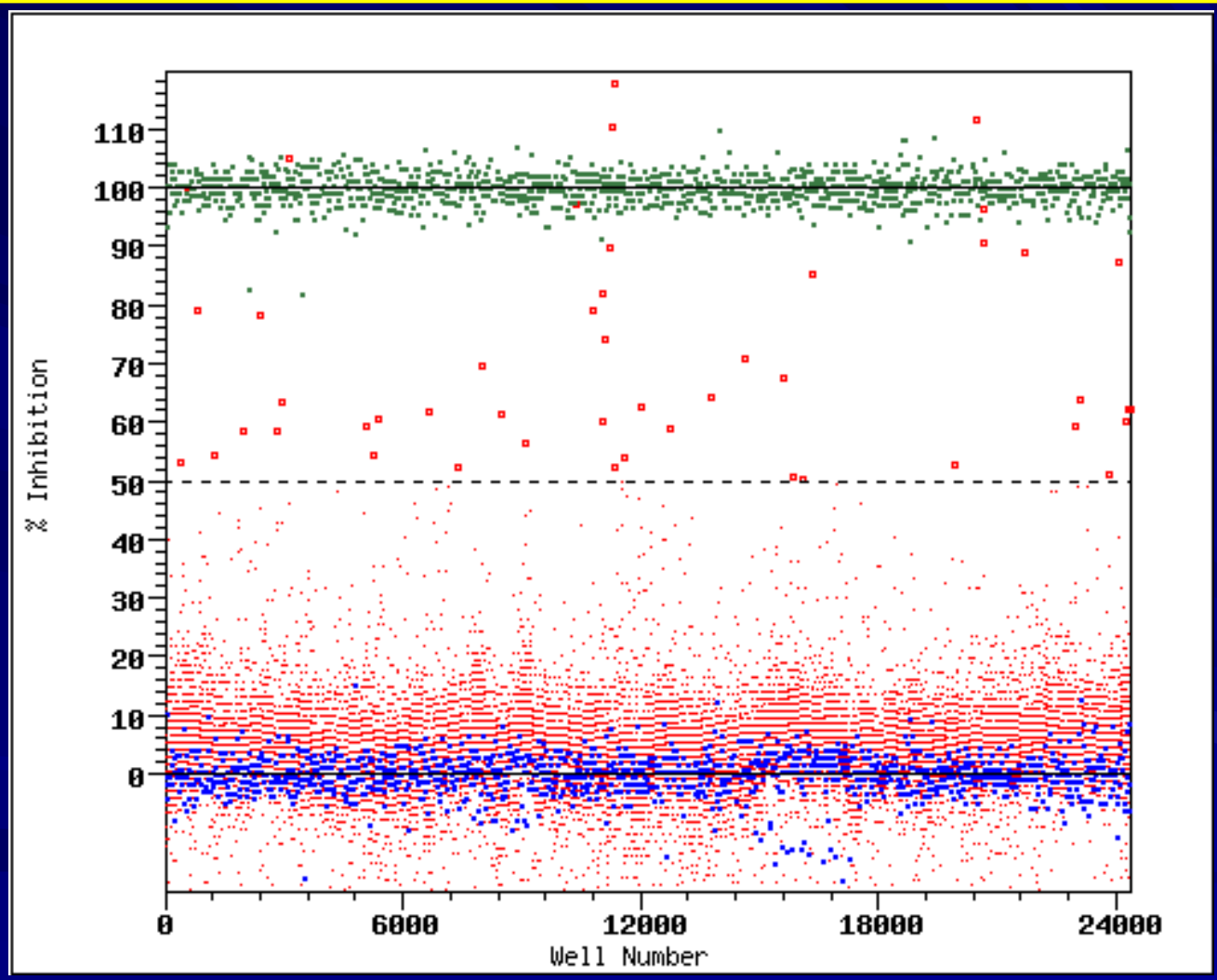
R12-ORF TR-FRET Screen



FP Assay for ORF - Target Interaction



R1-ORF FP Screen



+ CTRL

actives

- CTRL

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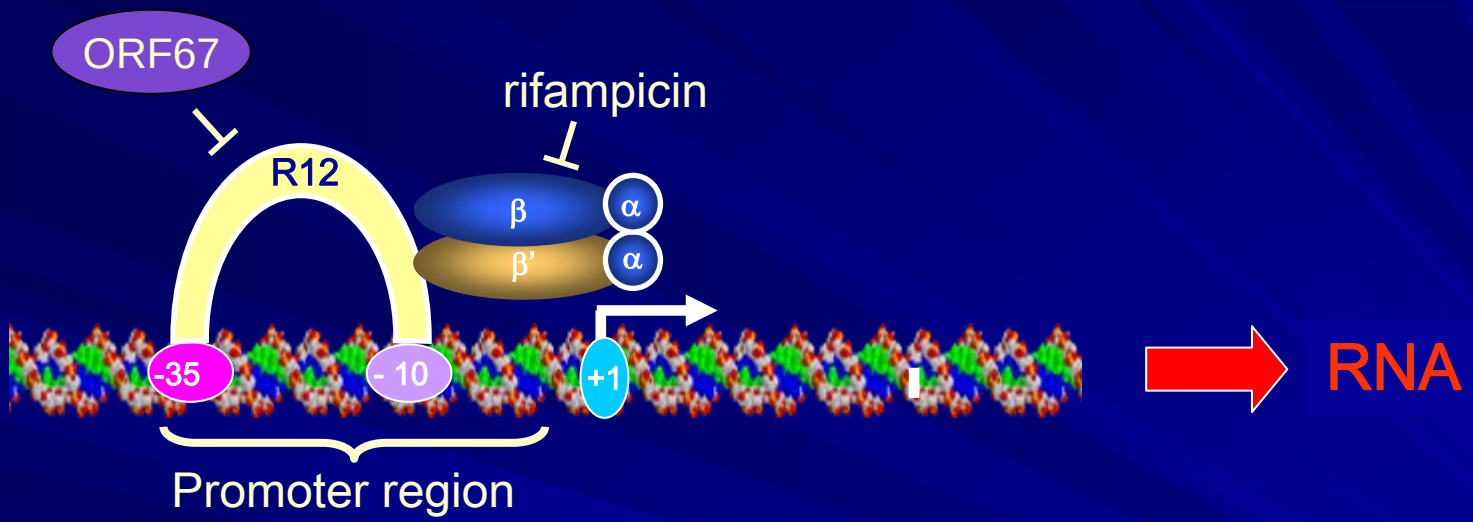
R12-Dependent *in vitro* Transcription Assay



- multi-component enzyme
- DNA template
- NTPs
- radiolabel
- *+/- compound or R12-specific ORF*

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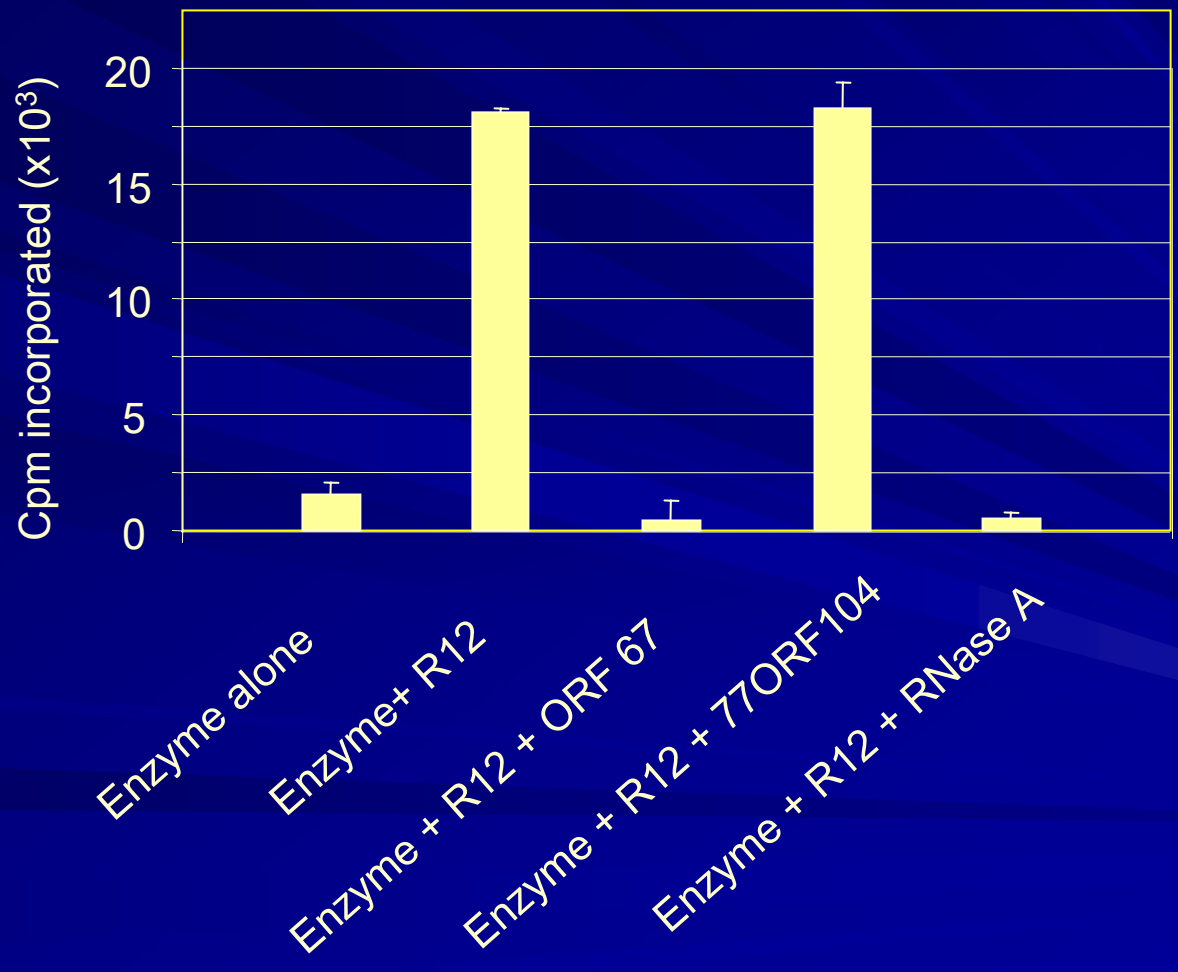
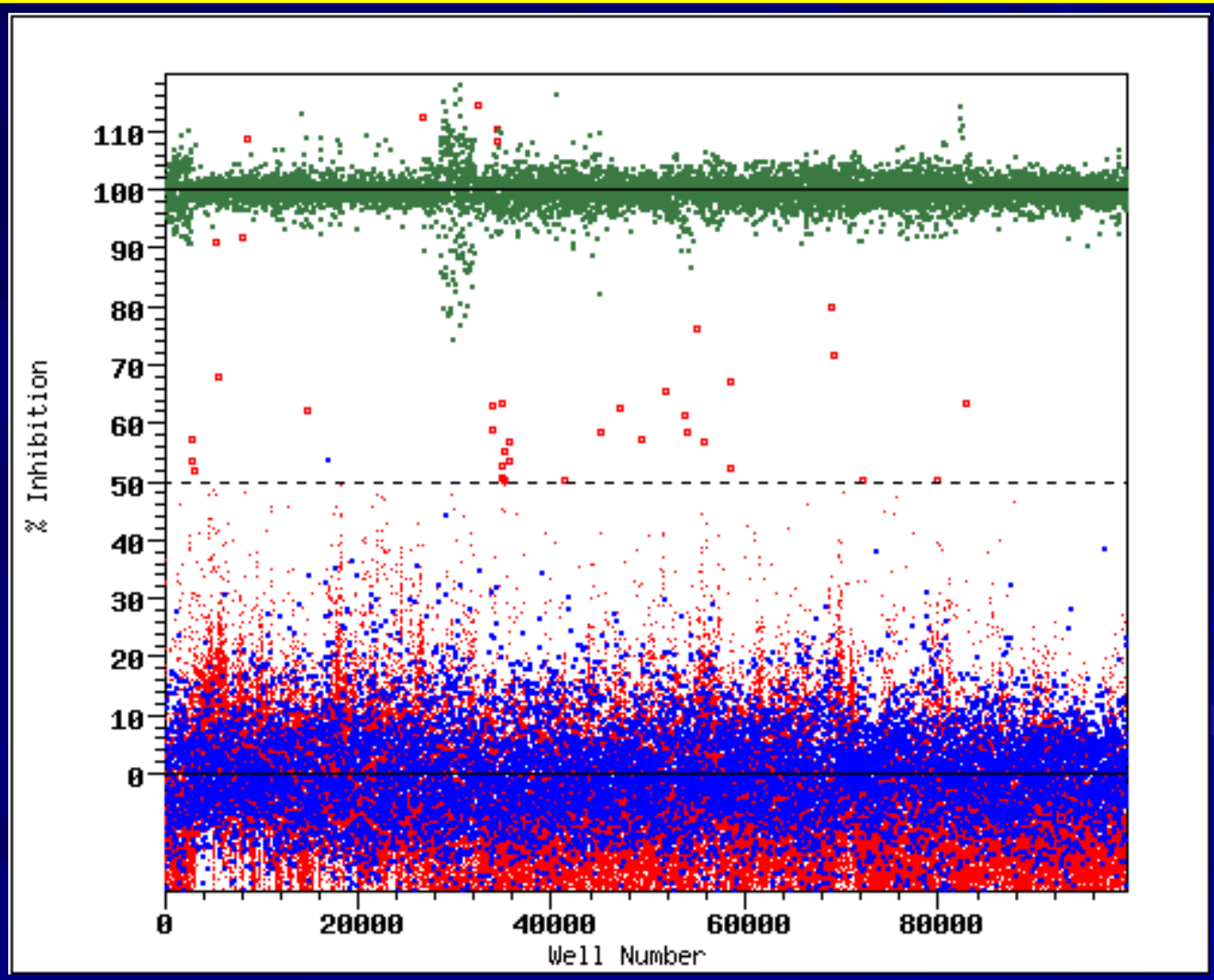
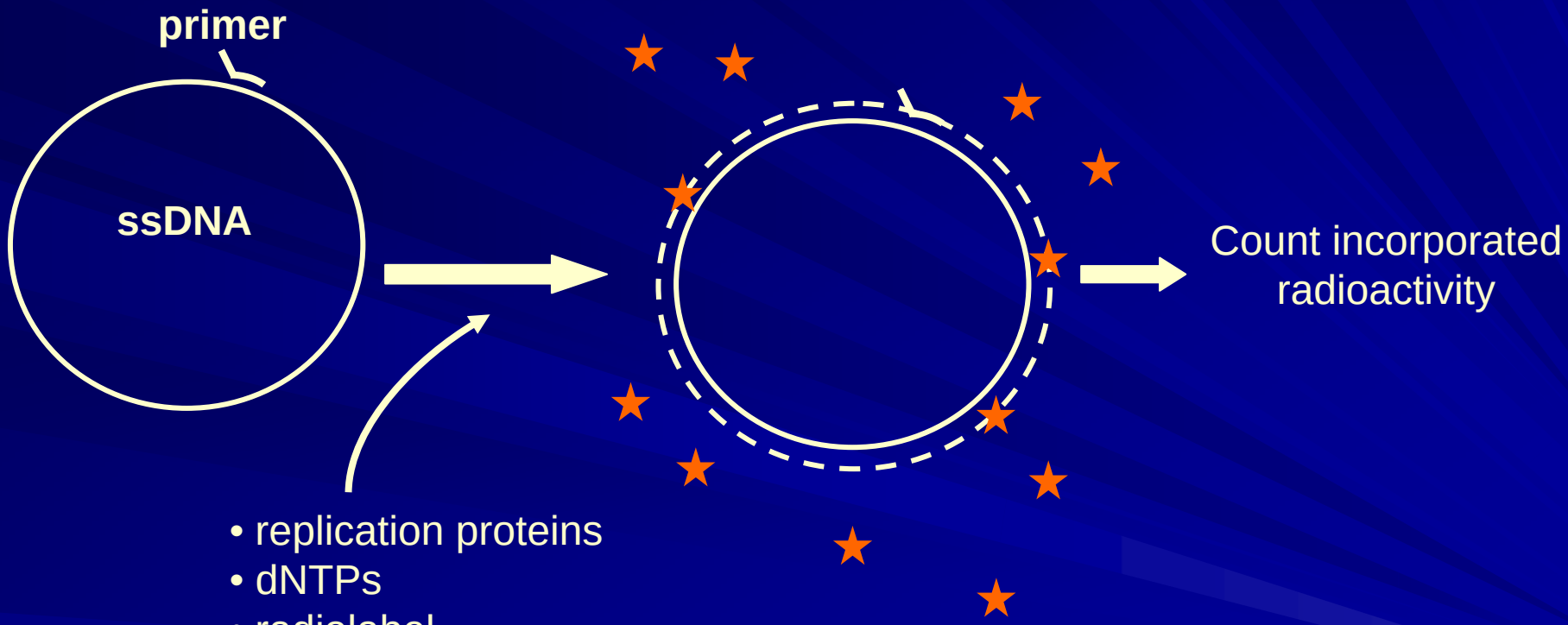
R12-Dependent *in vitro* Transcription



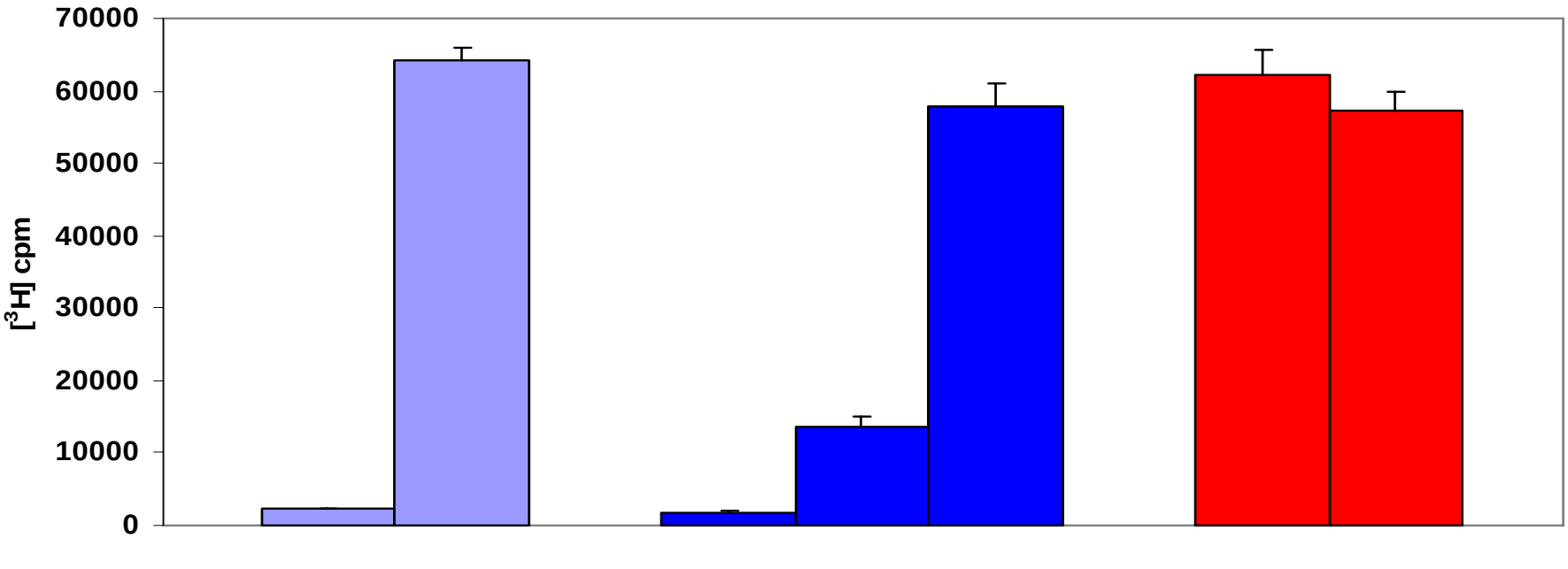
R12-Dependent *in vitro* Transcription Assay



R2-Dependent DNA Replication Assay (MCA)



Inhibition of R2-dependent DNA Replication by ORF



Advantages of the Phage-inspired Drug Discovery Platform

1. Inherent (evolutionary) validation of
   - new antibacterial targets
   - ORF-binding domains of target that can be used to identify small molecule inhibitors

2. Use of proprietary natural inhibitors (phage ORFs) as specific reagents in
   - ORF – target interaction assays
   - multicomponent replication and transcription assays

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Summary

- PhageTech has sequenced over 40 phage genomes of *S. aureus, S. pneumoniae,* and *P. aeruginosa*
- 60 families of phage antimicrobial ORFs were identified
- Biologically validated bacterial targets, including those essential for DNA and RNA synthesis were discovered
- Proprietary ORF-target binding assays and multi-component enzymatic assays that make use of these ORFs as specific reagents have been developed around these targets
- Diverse libraries of small molecules are being screened and selected compounds are being characterized

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Acknowledgements

- PhageTech's scientists and technical support staff

- PhageTech's scientific founders:
  M. DuBow, P. Gros and J. Pelletier