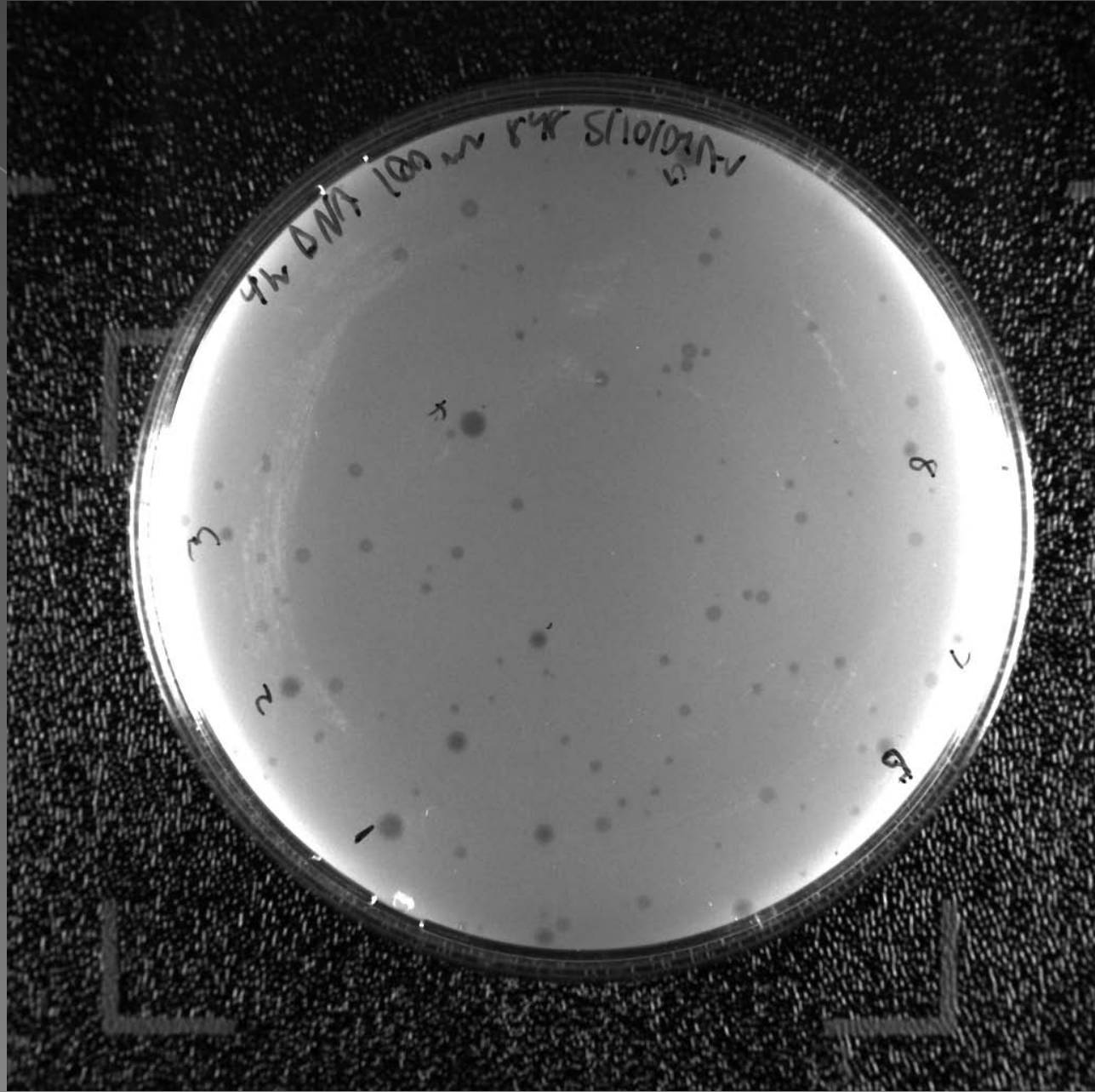


Sequencing and Annotation of the HK239 Genome

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Bacteriophage

- ◉ Viruses that infect bacteria
- ◉ Temperate phage can adopt two lifestyles:
 - > Lytic: the phage will lyse the host bacterium, killing it (visualized by a plaque on a lawn of bacteria)
 - > Lysogenic: the phage will incorporate its genome in the host genome



Lysogens are important!

- ◉ Most prophage genes are not expressed
- ◉ Those that are encode a variety of functions
 - > Virulence: cholera, diphtheria
 - > Exclusion

HK239

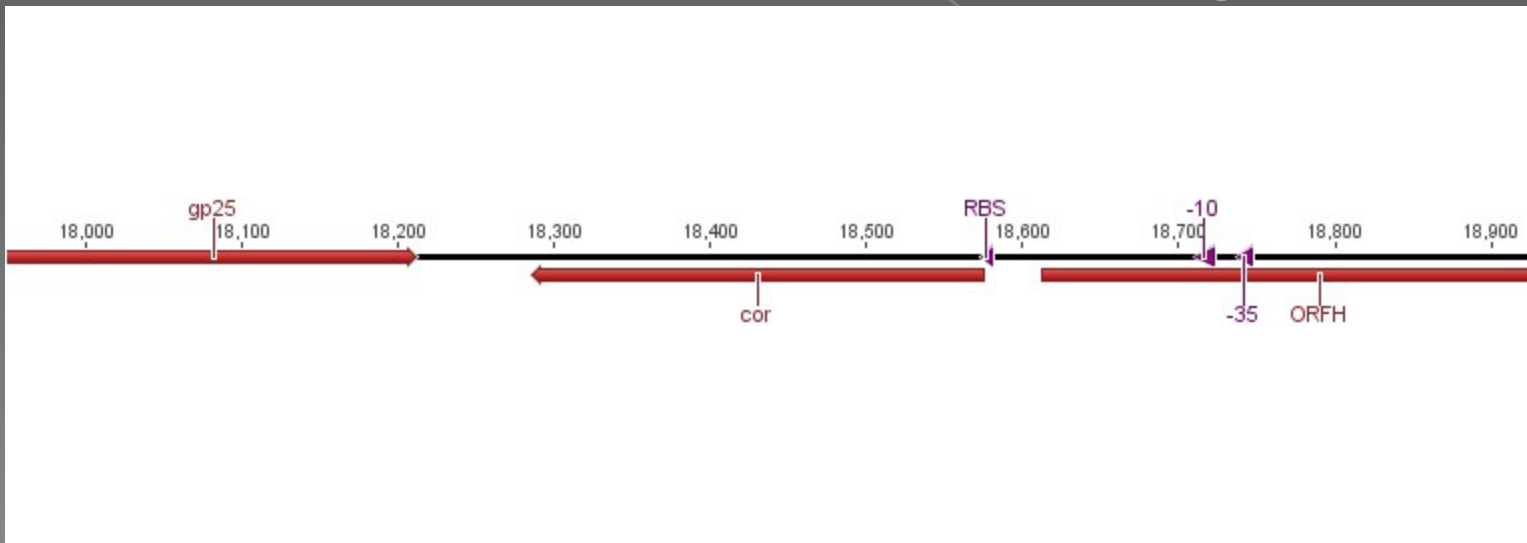
- ◉ Lambdoid phage
- ◉ Wide range of exclusion: λ , P1vir, T4rII, P2 and HK022 (Dhillon and Dhillon, (1973)Virology 55:136-142)
- ◉ Interested in how this exclusion is achieved



Picture by Ramadavi Bonthu
Western Kentucky University

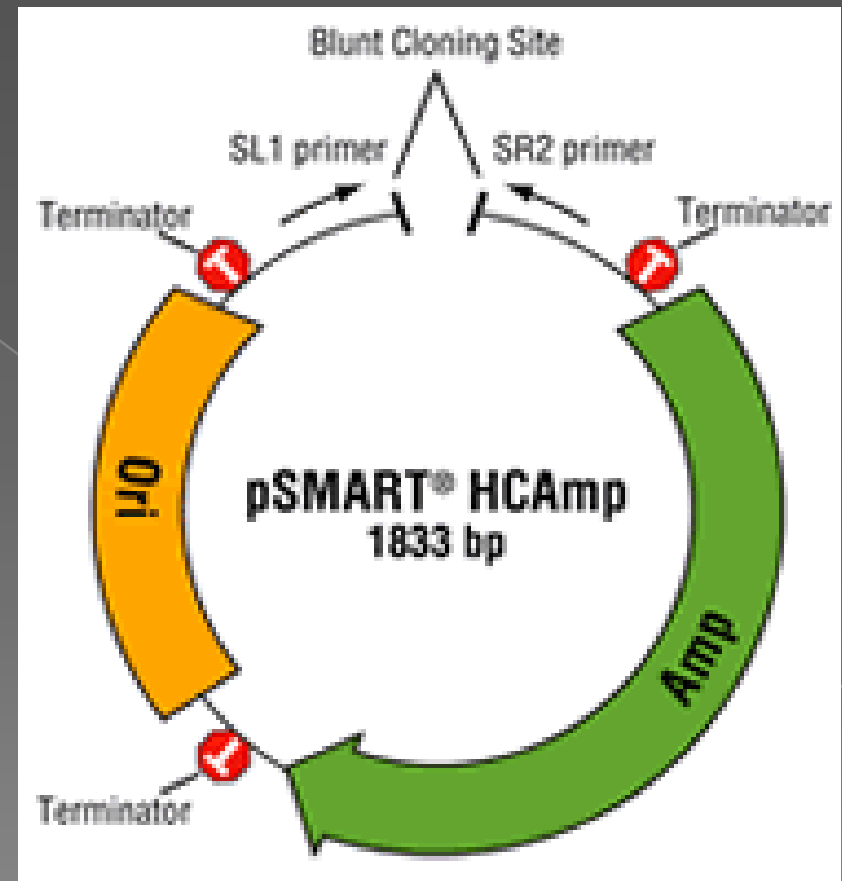
Previous work

- Only a lytic mutant is available
- Used shotgun cloning to isolate genes encoding exclusion functions
- Identified a phi80 cor homolog



Sequencing the Genome

- Sheared into ~1.1kb fragments
- Fragments were ligated into a pSMART HCamp vector
- Transformants were generated
- Inserts from isolated plasmids were sequenced
- Data was analyzed using BLAST



Results and Further Sequencing

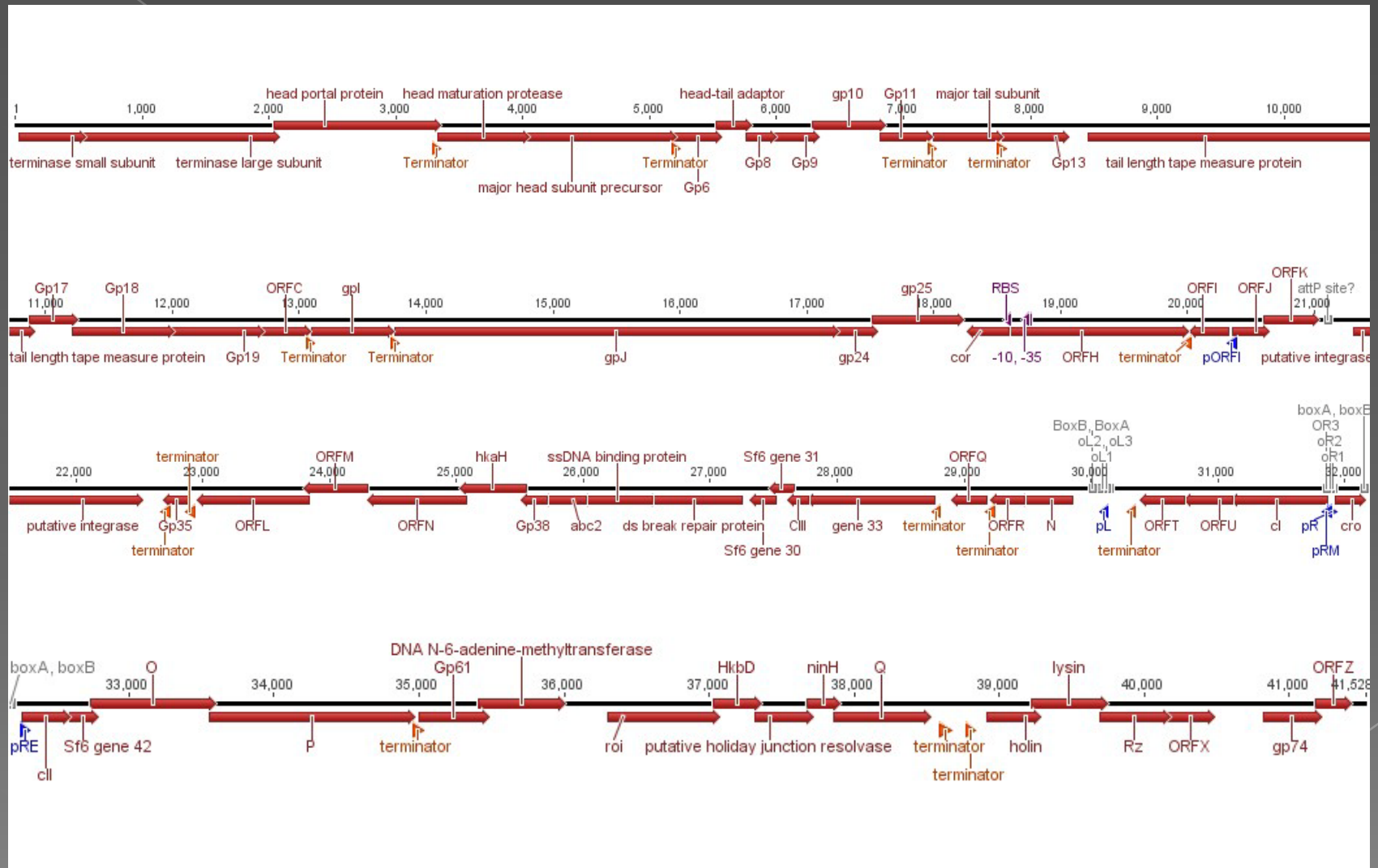
37511-38190	forward	GS132R	
37522-38095	reverse	GS252L	(1-573) part vector, see lambda, HK620 and Sf6
37561-38120	forward	GS252R	see lambda, HK620 and Sf6
39681-40166	forward	GS105L	
40129-40695	reverse	GS132L	
40118-40717	reverse	GS105R	
40577-40751	reverse	GS337L	(278-453) see above, part vector
40637-40751	forward	GS337R	(1-116) see above, part vector

- Sequencing data were assembled into contigs based on overlapping homology
- Initially had 9 contigs
- Sequenced from genomic DNA to close gaps
- Also sequenced areas with low coverage
- Verified assembly by restriction digest

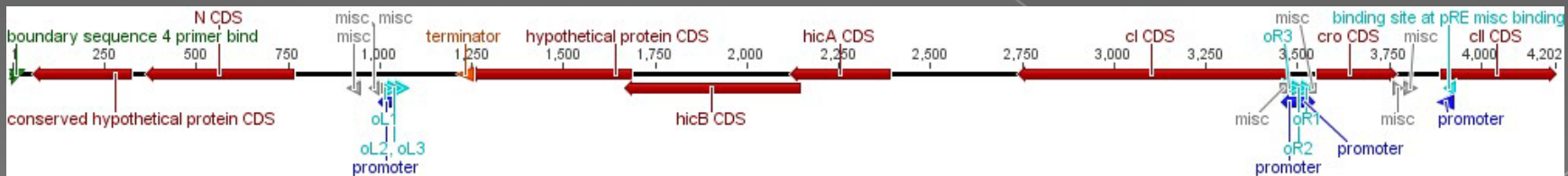
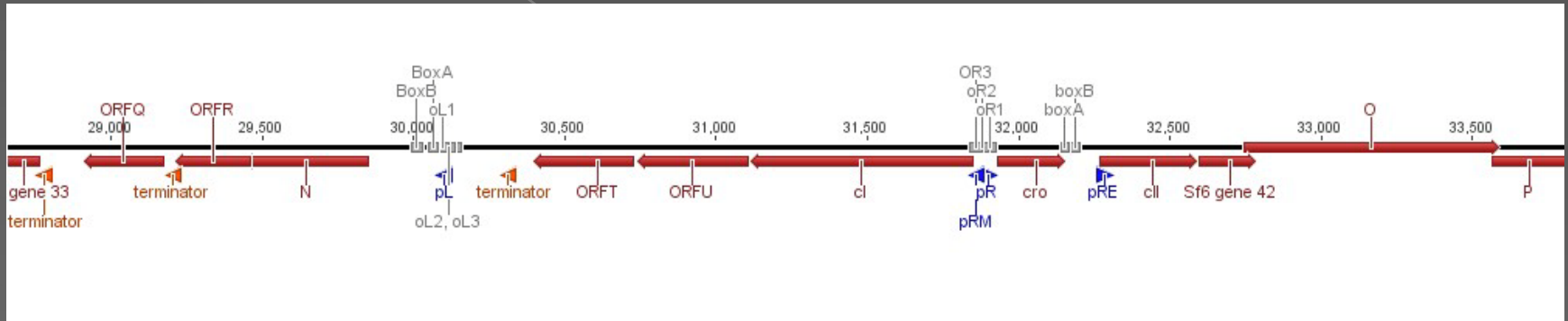
Annotating the HK239 Genome

- ◉ Looked for open reading frames using VGO and Genemark
- ◉ Looked for terminators using TransTerm
- ◉ Also looked at homology in other phage genomes for promoters and other elements

HK239 Genome



The Immunity Region

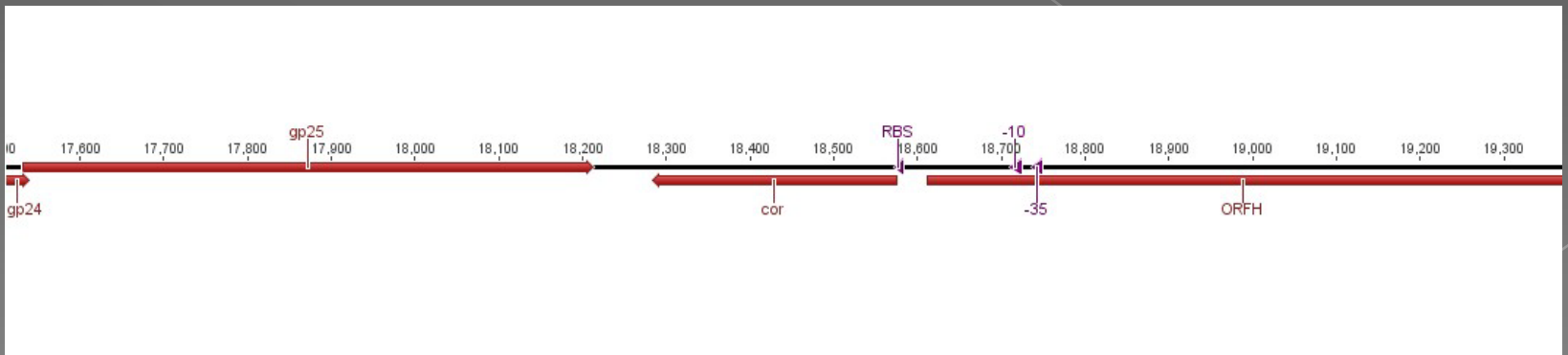
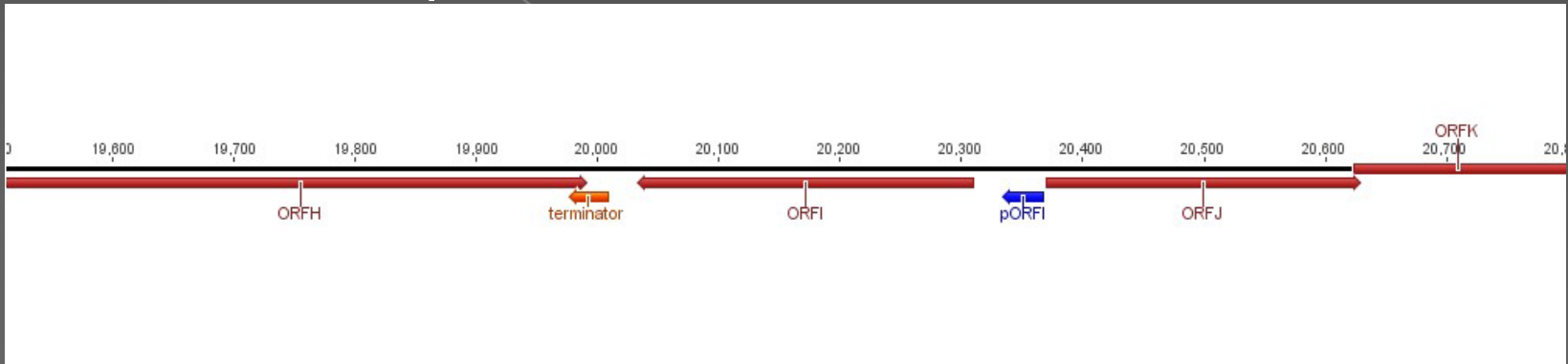


Points of interest

- ◉ No additional exclusion homologs identified
- ◉ Three genes of unknown function are of interest
 - > ORFI
 - > ORFT
 - > ORFU

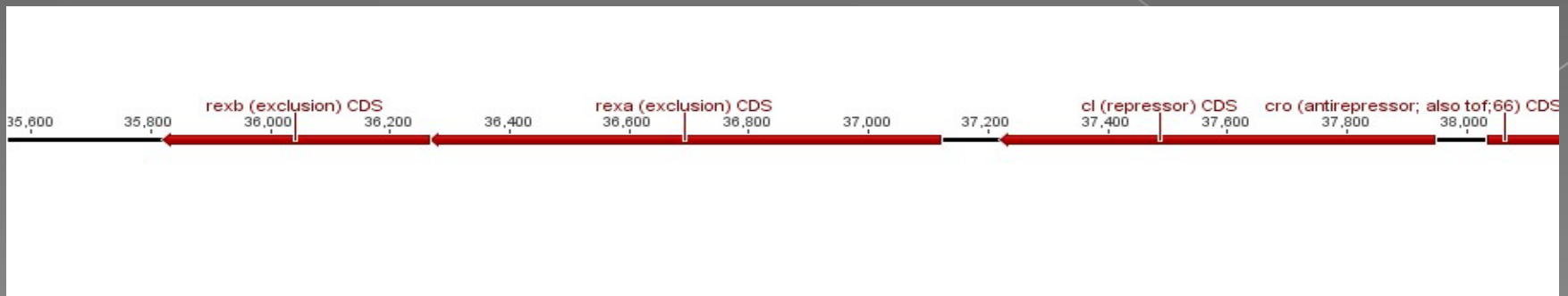
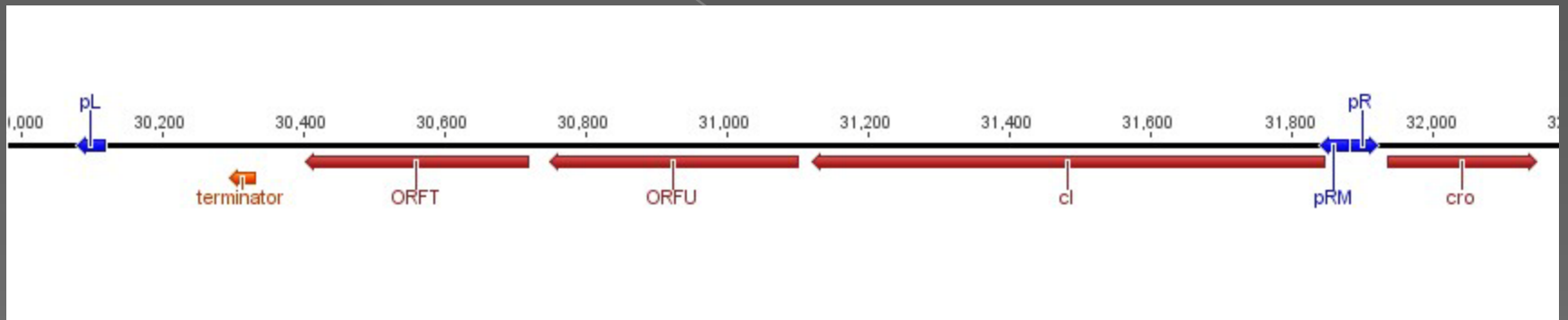
ORFI

- Antisense
- Putative promoter and terminator



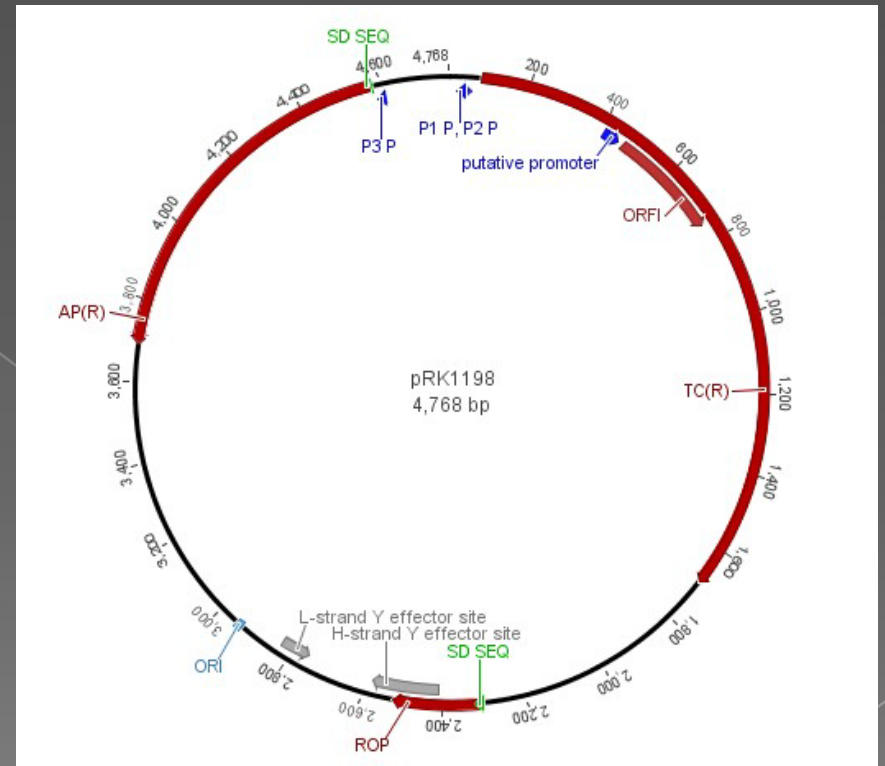
ORFs T and U

- Positioned similar to Rex genes in lambda
- No obvious promoter, may use pRM



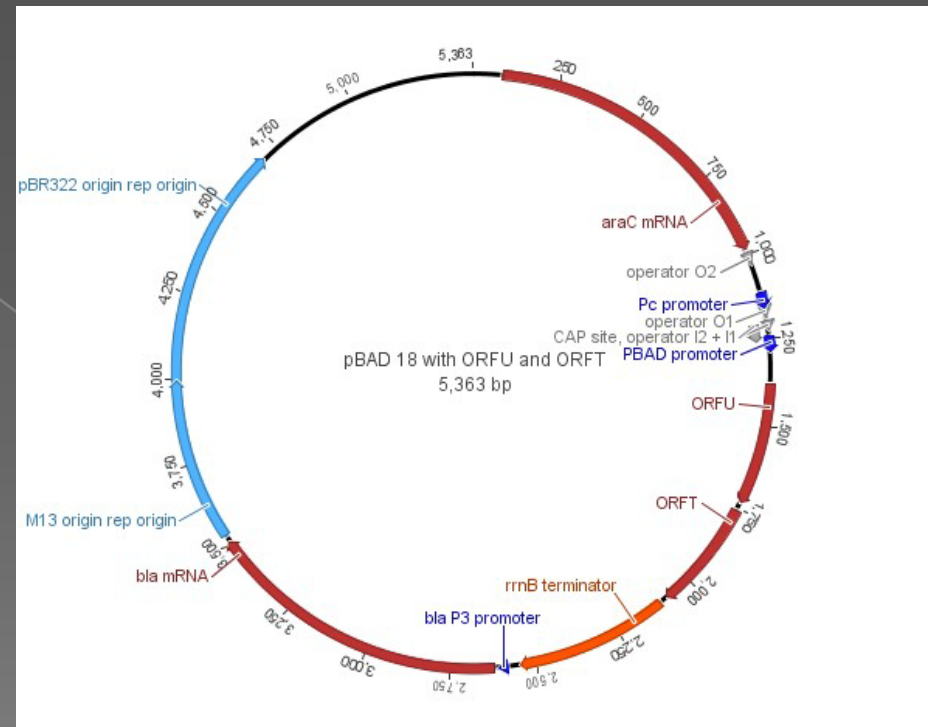
Progress with ORFI

- Cloned ORFI and putative promoter into pBR322
- Will check for exclusion of T4rII, λ , P1 *vir* and P2



Progress with ORFs T and U

- Construction of vector in progress
- Inserting genes into pBAD 18
- Will check for exclusion of T4rII, λ , P1 *vir* and P2



Summary

- ◉ Genome is sequenced and annotated
- ◉ No known exclusion homologs identified
- ◉ Currently investigating genes based on genomic location

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- ◉ Deana Owens
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