Proposed introduction of a tetracycline-resistant (*tetR*) cassette into *C. trachomatis*

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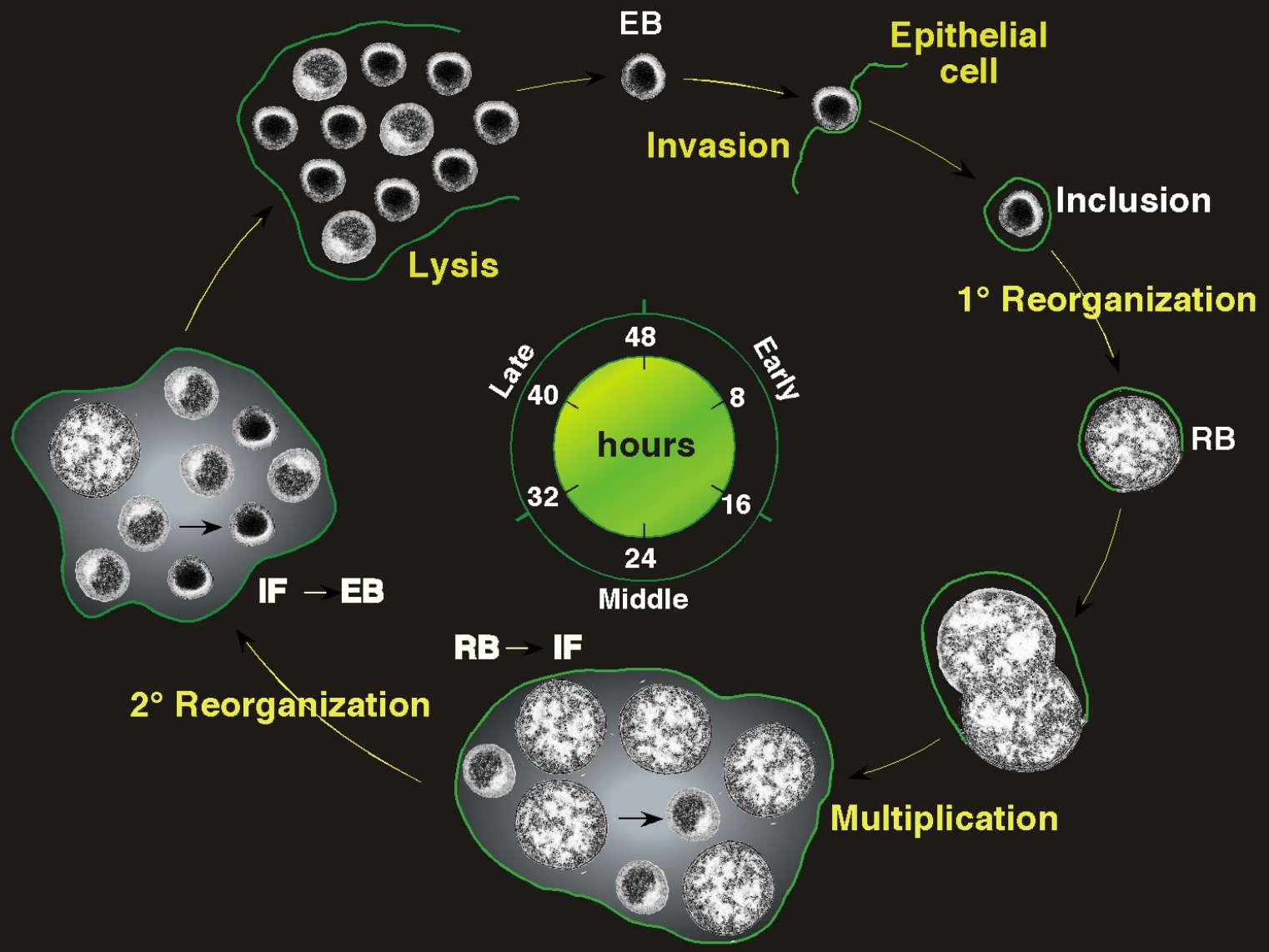
Major Chlamydial Diseases of Humans

Chlamydial species	Disease	Significance
Chlamydia trachomatis		
Serovars A, B, Ba, C	Trachoma	Worlds leading cause of preventable blindness (300-500 M)
Serovars D - K, L1 - L3	Sexually transmitted diseases	5 million cases/yr/US 3-5 billion dollars
C. pneumoniae	Pneumonia, bronchitis	10% community acquired pneumonia

Chlamydial Developmental Cycle



Control of Chlamydial Diseases

- Socioeconomic and behavioral change
  - Most effective but difficult to implement
- Diagnosis and antibiotic intervention
  - Not sustainable, fails to eradicate, and may impact natural immunity
- Microbicides
  - Not efficacious in pre-clinical trials
- Vaccine
  - Most effective but marginal progress

Vaccine Challenges

- Infections of humans are characterized by poor protective immunity
- Virulence factors that function in immune avoidance are largely uncharacterized
- Lack of a tractable genetic system to identify virulence factors

Obstacles in the development of a chlamydial genetic system

- Obligate intracellular parasitism
- Bi-phasic life cycle
  - Neither infectious EB or non-infectious RB highly amenable to transformation strategies
- Unavailability of effective antibiotic selective markers
  - Chloramphenicol and Kanamycin –
    - MBC 50 μg/ml – concentrations that are toxic to eukaryotic cells
    - Generates false positive resistance and prevents plaque cloning
  - Rifampicin
    - High frequency of spontaneous mutation (10^8)
  - Tetracycline is a more attractive selective marker
    - MBC 2-4 mg/ml concentrations that are non-toxic to eukaryotic cells
    - Reduces false positives and allows plaque cloning

Antibiotic treatment options for *C. trachomatis* infections and *in vitro* anti-chlamydial inhibitory properties

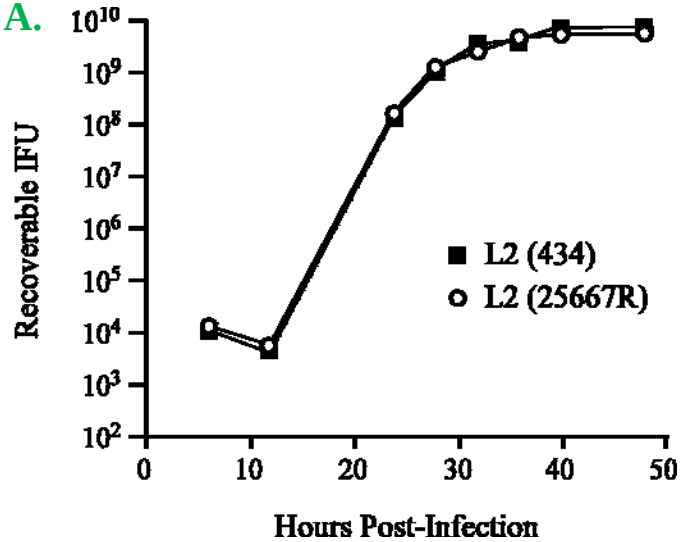
Antibiotic Class	MIC (µg/ml)	MBC (µg/ml)
Macrolides (protein synthesis inhibitor)		
Azithromycin	1	
Erythromycin	1	4
Tetracyclines (protein synthesis inhibitor)		
Doxycycline	0.025	
Tetracycline	0.3	2.4
Quinolones[a] (DNA synthesis inhibitor)		
Ciprofloxacin	2	2
Levofloxacin	0.5	
Ofloxacin	1	1
Trovafloxacin	1	
Penicillins (cell wall synthesis inhibitor)		
Amoxicillin	>4	>50

Why the selection of a LGV strain as a TetR transformation target?

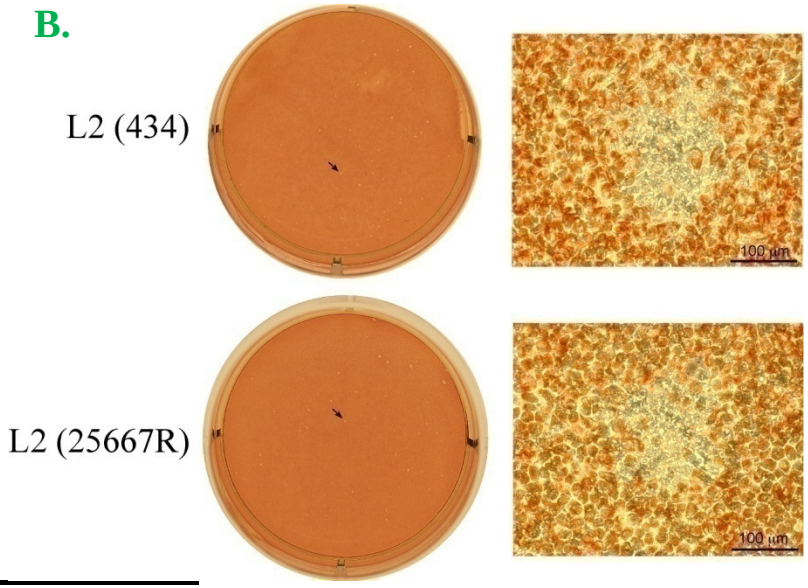
- Low prevalence of natural infection and transmission restricted to sexual contact
- Strain L2(25667R) naturally occurring plasmidless isolate
- L2(25667R) highly attenuated for the female mouse genital tract
  - 400 times greater ID50 than plasmid positive LGV strain
- L2(25667R) fully virulent for cultured eukaryotic cells
  - Similar growth and plaquing characteristics as the plasmid positive LGV strain

Comparative *in vitro* and *in vivo* growth characteristics of L2(434) and L2(25667R)

A.



B.



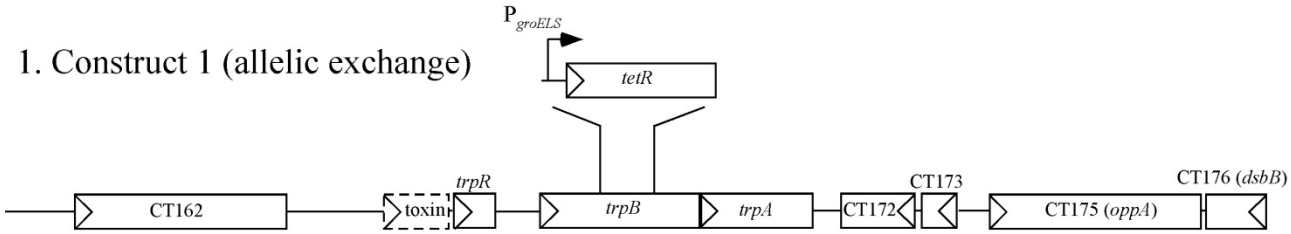
C.

Challenge dose (IFU)	No. of mice culture positive per group at different times post challenge									
	L2 (434)					L2 (25667R)				
	3[a]	7	14	21	28	3	7	14	21	28
10^7	6/6	6/6	2/6	2/6	1/6	5/6	4/6	0/6	0/6	0/6
10^6	5/6	5/6	2/6	3/6	3/6	0/6	0/6	0/6	0/6	0/6
10^5	5/6	2/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6
10^4	4/6	1/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6
10^3	0/6	0/6	nd	nd	nd	0/6	0/6	nd	nd	nd
10^2	0/6	0/6	nd	nd	nd	0/6	0/6	nd	nd	nd
10^1	0/6	0/6	nd	nd	nd	0/6	0/6	nd	nd	nd

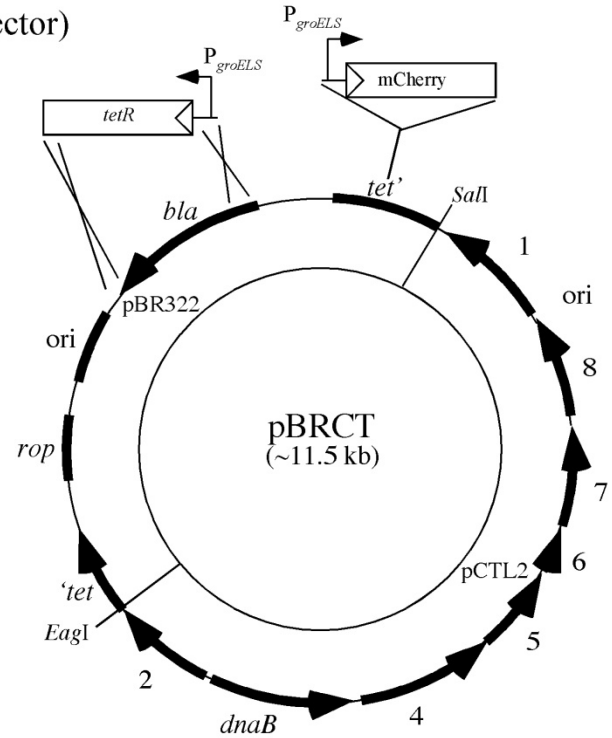
	ID_{50} (IFU)
L2(434)	$1x10^4$
L2(25667R)	$4x10^6$

Proposed *tetR* constructs

1. Construct 1 (allelic exchange)



2. Construct 2 (shuttle vector)



Primary differences between our proposed experiments and the previously approved study

Rockey et al.	Our proposal
Horizontal gene transfer of co-infected cells	Transformation of EB via electroporation
Virulent serovars used [F, J, L2(434)]	Attenuated serovar L2(25667R)
Multi-drug resistance selected [Tet^R, Ofl^R, and Rif^R]	Single drug resistance selected [Tet^R]

Risk Mitigation

- Planned Modifications to SOP
  - Replace probe sonication with cup
  - Modify exposure reporting to be consistent with Medical Services Program for after-hours notification

Risk Mitigation

- Laboratory Facilities
  - BSL-2 **PLUS**
    - ✓ Restricted access via electronic card reader
- Practices and Procedures
  - All workers trained on SOP
  - All work with infectious material performed in a BSC wearing lab coat, gloves, N100 respirator
  - Work with ocular serovars will not be performed at the same time as experiments involving Tet^R
  - All potential and confirmed Tet^R strains will be stored in the restricted-access lab
  - Inter-facility transfers of Tet^R strains coordinated and approved through RML BSO

Chlamydiae

- Obligate intracellular Gram-negative like bacteria.
- Complex biphasic life cycle (infectious and non-infectious organisms).
- Many species with broad host infection tropism.
- Genetically intractable.
- Small genomes (1Mb).
- Numerous genomes sequenced and annotated.
- Good in vivo and in vitro infection models.
- Amenable to functional genomic analysis for studies on immunity and pathogenesis.