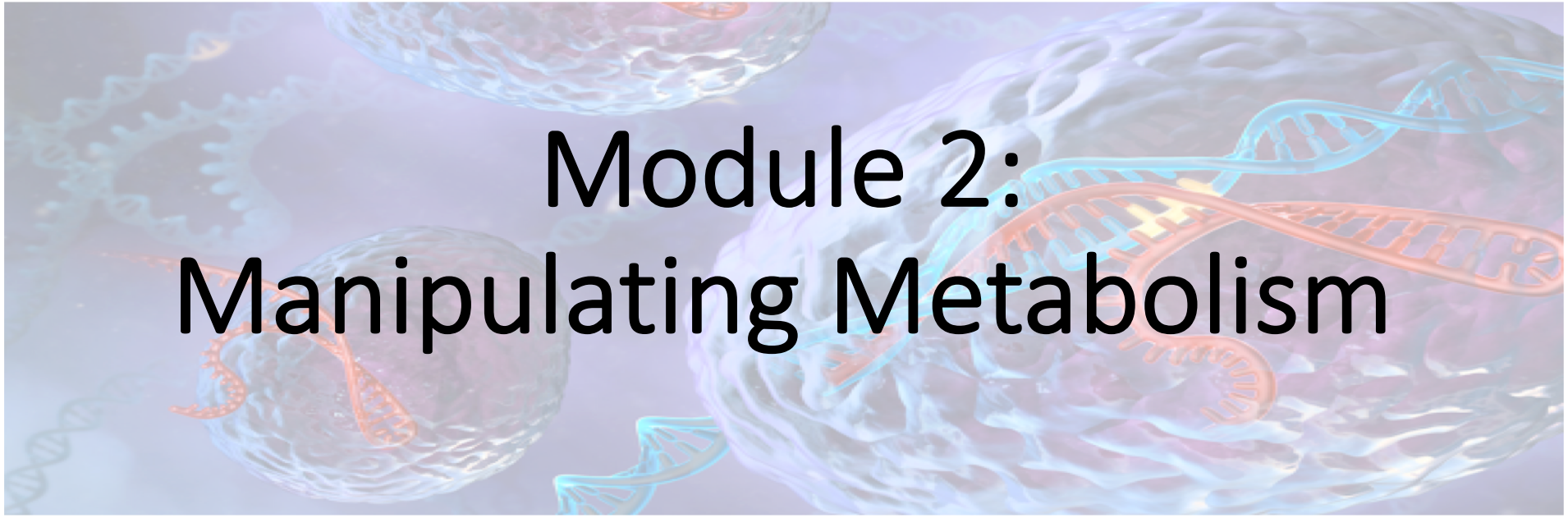


Module 2: Manipulating Metabolism

CRISPR: bacterial adaptive immunity

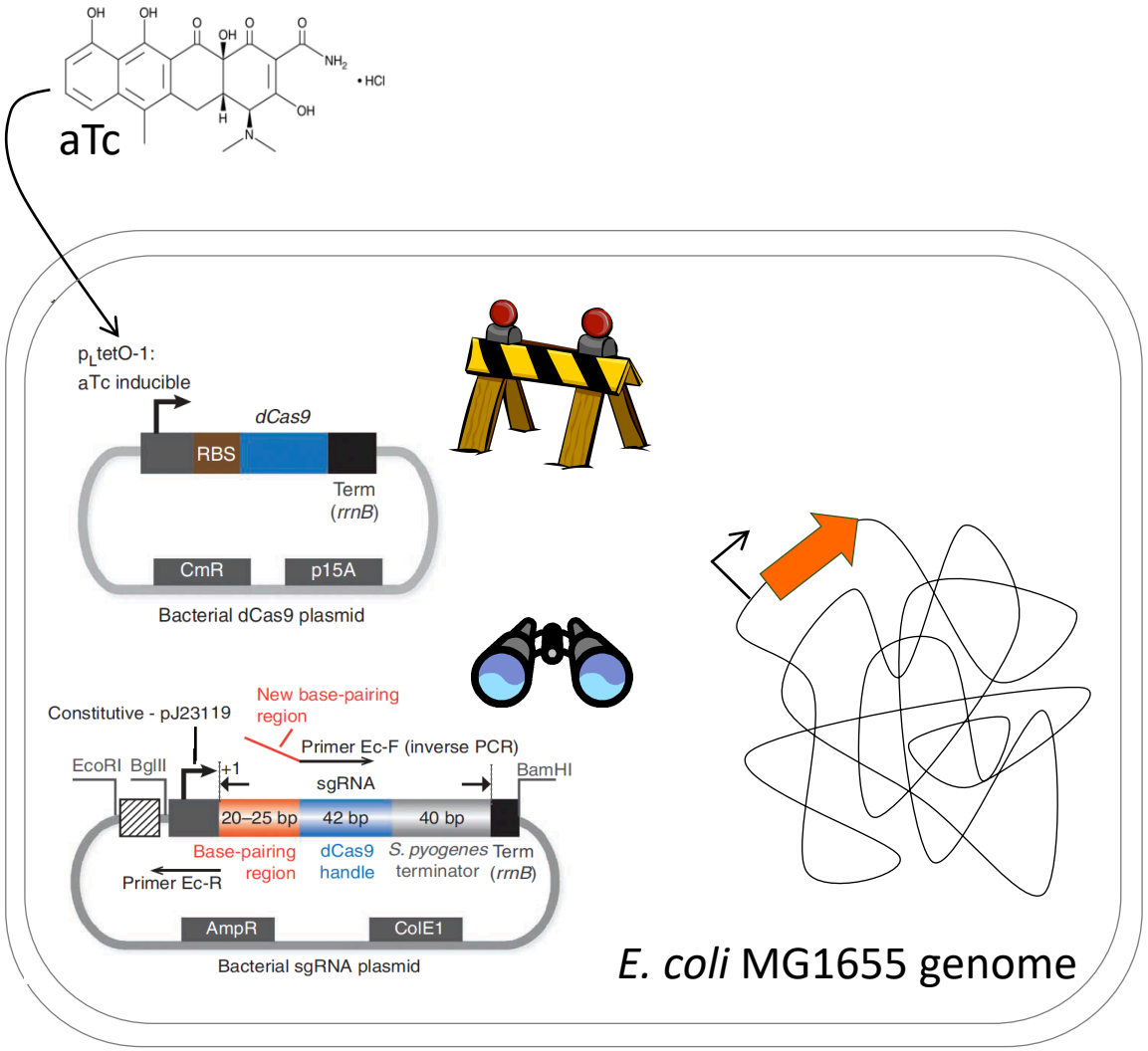
10/22/19

A quick review…

What are the key components?

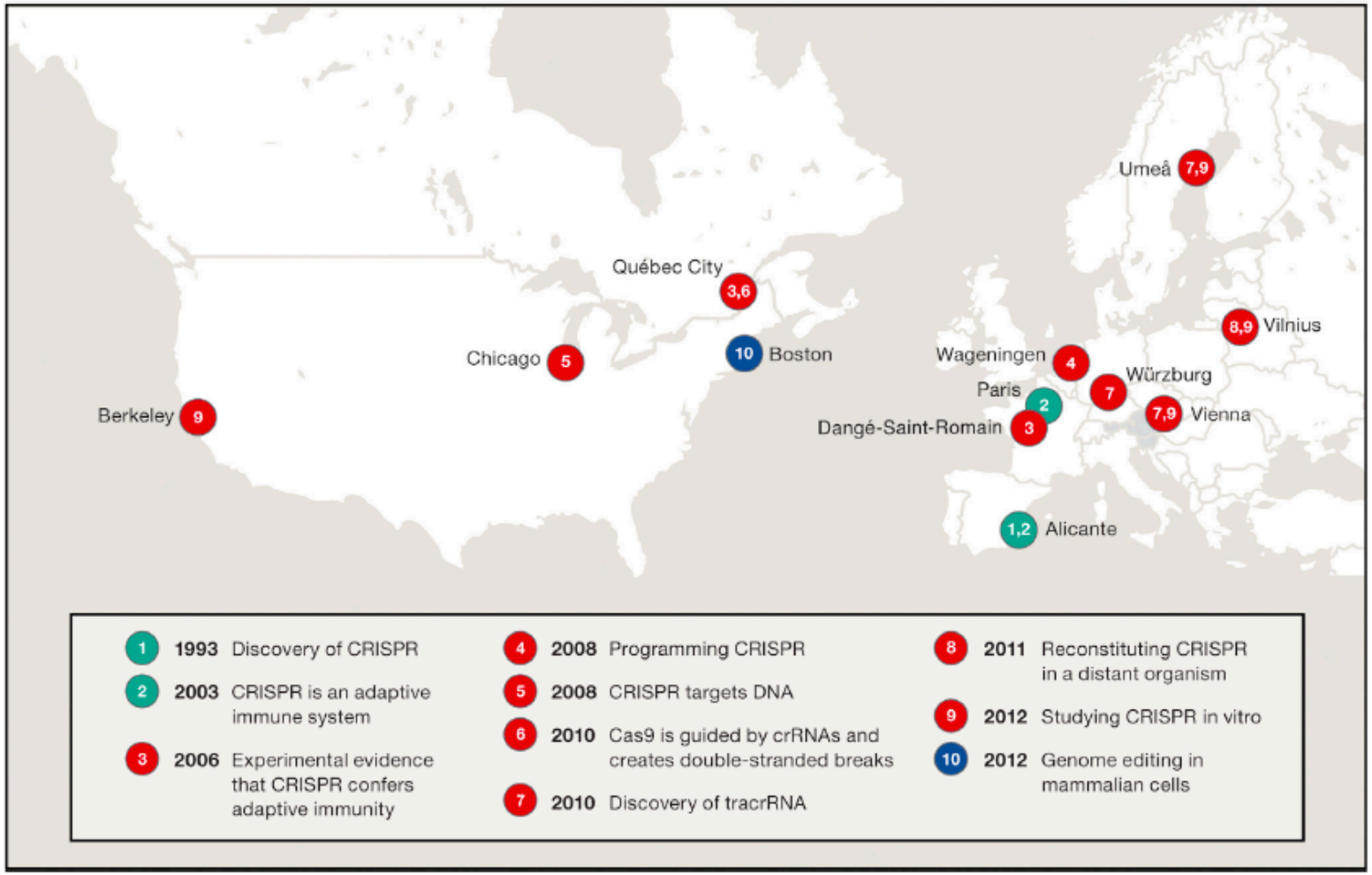
What is the role / function of each component?

How is expression of each component controlled?



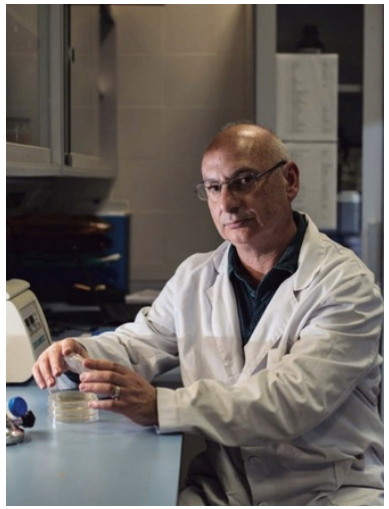
Why communicate your science?

Why communicate your science?



Discovery of repeat sequences in archaea

- 1993 While studying non-related anomaly in DNA fragments, identified multiple copies of 30 base repeats separated by 36 base spacers
- 1995 Found similar repeats in related organisms
  - Other work reported repeat sequences in *E. coli*
- 2000 Repeat loci identified in 20 microbes
- 2003 Spacer sequence from *E. coli* matched to P1 phage



Francis Mojica

Proposed role for repeat sequences

2003 • 88/4500 spacer sequences similar to phage
  - 2/3 matched phage known to infect host microbe

2005 • *Y. pestis* spacer sequences similar to prophage present with genome of strains
  - New spacers present at the ‘front’ end of loci

MICROBIOLOGY
Publishing high-quality research since 1947

C. Pourcel,[1] G. Salvignol[1] and G. Vergnaud[1,2]

2005 • Speculated that transcripts from spacers worked via anti-sense RNA inhibition

MICROBIOLOGY
Publishing high-quality research since 1947

Alexander Bolotin, Benoit Quinquis, Alexei Sorokin and S. Dusko Ehrlich

Evidence of adaptive immunity

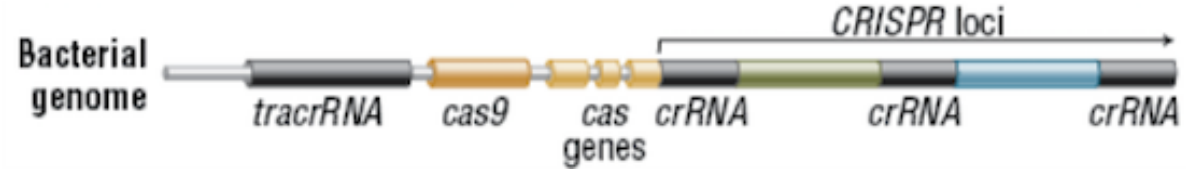
- 2004 • Correlation between spacers and phage resistance in *Streptococcus thermophilus*
- 2007 • Genetic selections used to isolate phage-resistant *S. thermophilus*
  - Strains carried phage sequences at repeat loci
  - Insertion of multiple spacers correlated with increased resistance
- 2007 • Phage with mutations in corresponding spacer sequence able to infect microbial host

Rodolphe Barrangou[1], Christophe Fremaux[2], Hélène Deveau[3], Melissa Richards[1], Patrick Boyaval[2], Sylvain Moineau[3], Dennis A. Romero[1], Philippe Horvath[2,*]

Discovery of genes associated with repeats

- 2000 Genes identified in the immediate vicinity of repeat sequences
  - Assumed to be related to spacer function
  - Hypothesized roles: gene regulation, replicon partitioning, DNA repair, etc.
- 2007 Cas7 required in acquisition of resistance, but not in resisting phage attack
- 2007 Cas9 required for resistance
  - Contains two nuclease motifs: HNH and RuvC

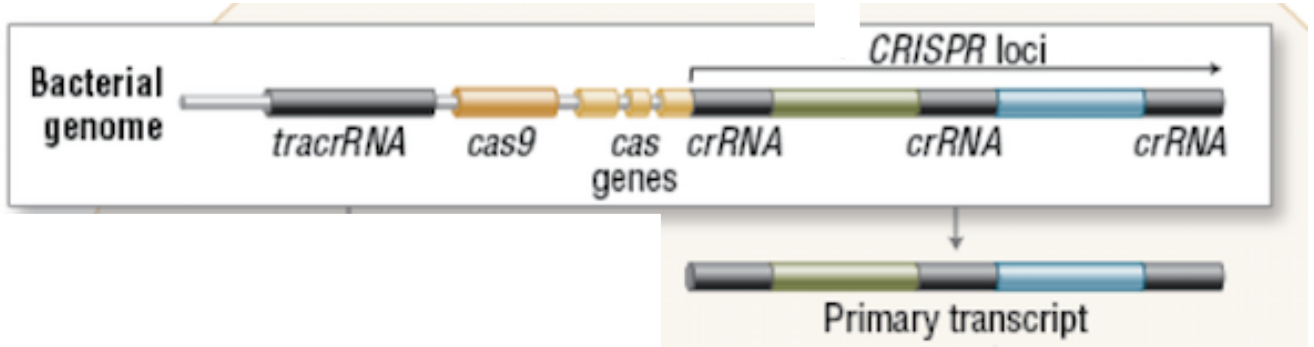
CRISPR loci components



- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
  - Repeats are roughly perfect, palindromic sequences
  - Spacers correspond to phage sequences
- CRISPR-associated (Cas) genes

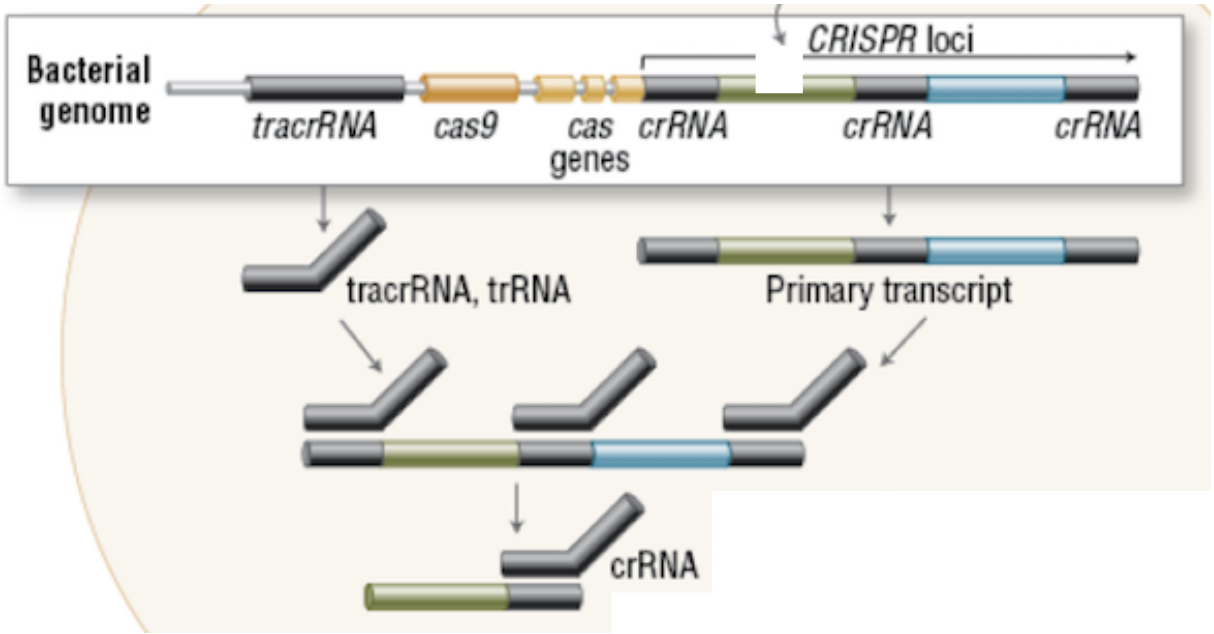
Function of CRISPR RNA (crRNA)

- Precursor RNA transcribed from CRISPR loci is cleaved into crRNAs by RNase III
  - Cleaved sequences start with last 8 bp of repeat (5' handle), followed by complete spacer, end with first bp of repeat (3' handle that forms hairpin)
  - Cas9 required for primary processing
    - Binds / positions molecules



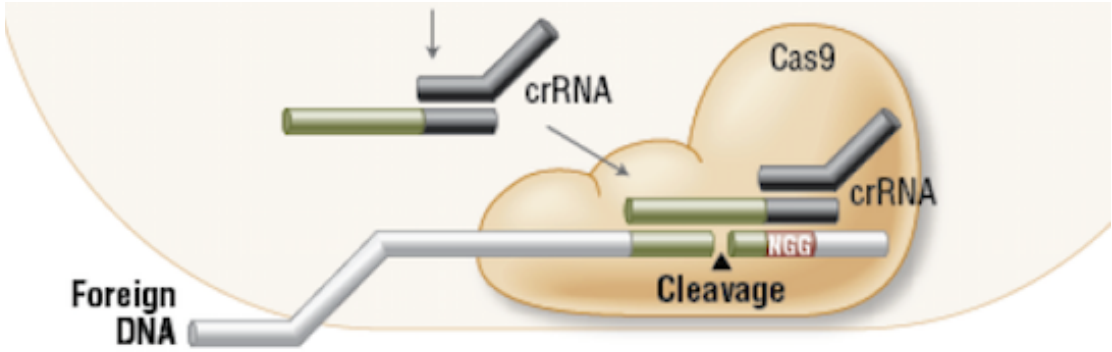
Function of *trans*-activating CRISPR RNA (tracrRNA)

- Third most abundant type of transcript
- Encoded by sequence immediately adjacent to CRISPR loci
  - 25 bp of near-perfect complementarity to repeats



DNA cleavage mediated by Cas9 with crRNA and tracrRNA

- crRNA / tracrRNA complex promotes structural change in Cas9
  - Formation of central channel that binds DNA
- Cas9 / RNA scan DNA for crRNA target (PAM)
  - Bind target sequence to enable strand displacement
- Cas9 cleaves DNA via single blunt cut



DNA is the target of Cas9 cleavage

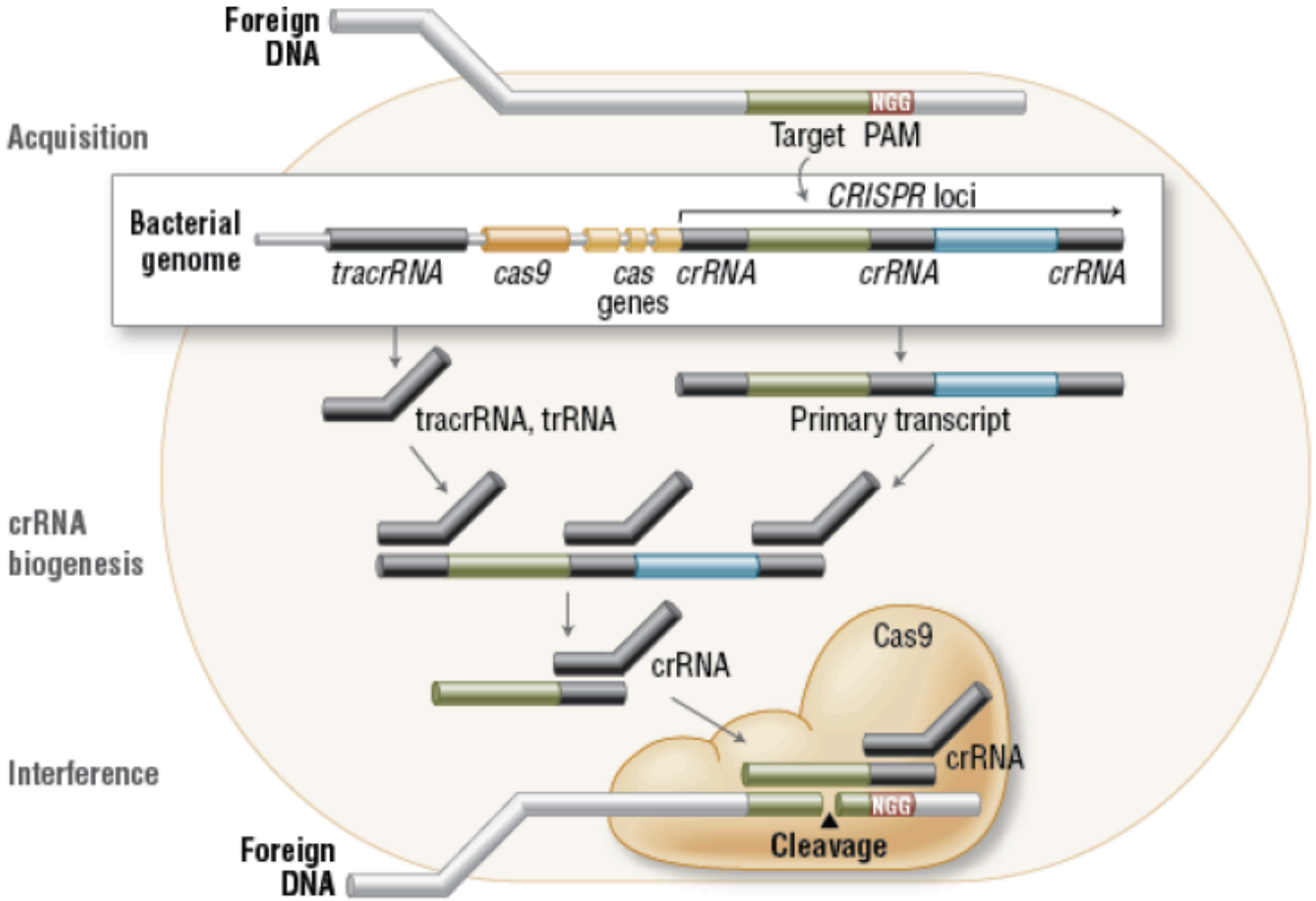
- Plasmid conjugation blocked in *S. epidermidis* strains that carried corresponding spacer
- Modified plasmid such that self-splicing intron disrupted target corresponding to spacer
  - If target is RNA, sequence will ‘splice out’ and CRISPR/Cas9 will recognize and cleave
  - If target is DNA, sequence will not be recognized and CRISPR/Cas9 will not recognize and cleave

https://www.youtube.com/watch?v=MbJ7Hnc2K3Q

Acquisition of immunity

- Phage DNA recognized and fragmented
  - Possible synergy with restriction enzyme system
- Suitable spacers selected by detection of protospacer adjacent motif (PAM)
- Spacer inserted into CRISPR loci by Cas1/Cas2
  - Leader end nicked for insertion
  - PAM-dependent orientation

Taken together, …



Other roles for CRISPR system

- Group behavior in *Myxococcus xanthus*
  - Disruption of *cas7*, *cas5* decreases sporulation
- Virulence in *Campylobacter jejuni*
  - Expression of *cas9* in CRISPR- strain increases virulence
  - Absence of *cas9* in CRISPR+ strain increases swarming, decreases cytotoxicity
- DNA repair in *E. coli*
  - Deletion of *cas1* increases sensitivity to DNA damaging agents

In the *laboratory*…

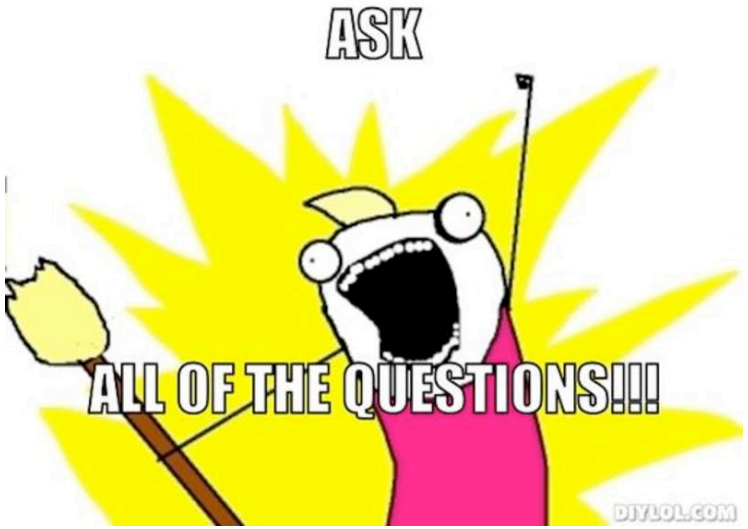
Journal club presentations at 1p in 16-336

"Welcome to Journal Club. The first rule of Journal Club is: you practice. The second rule of Journal Club is: you practice even more."

- Former 109er

Journal Club presentation notes

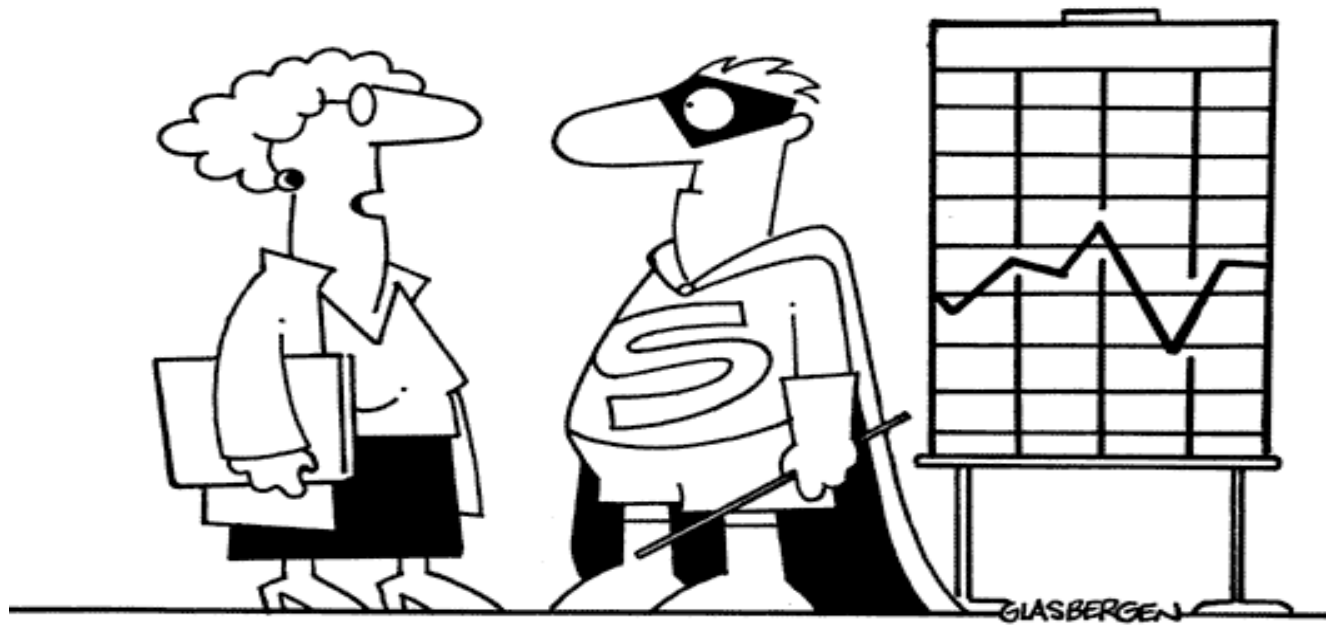
- Speakers
  - Please arrive early, if possible, to check the formatting of your slides
  - Laser pointer, slide changer, timer available for use
- Audience members
  - Please arrive on time
  - Enjoy snacks quietly (no refills during the presentations!)



How can I overcome my fear of public speaking?

1. Know your topic
2. Get organized
3. Practice, practice, practice
4. Visualize success
5. Deep breathing
6. Get support

MAYO CLINIC



"Fear of public speaking is quite common. If dressing up as Speaker Man makes you feel more confident, then so be it."

Put on your capes!