METAGENOMIC RECONSTRUCTION OF BACTERIAL CRISPR LOCI CONSTRAIN POPULATION HISTORIES

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LaTeX

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OVERVIEW

Metagenomic approach to look at *Leptospirillum* CRISPR loci and phage

- ”detect and recover genome sequences from uncultivated phage and link phage to their hosts”
  - reconstruct phage genome from extracted spacer sequences
  - determine who goes with whom
- time series data to look at population history

CRISPR-CAS

Bacterial and archaeal immune system against plasmids and phages

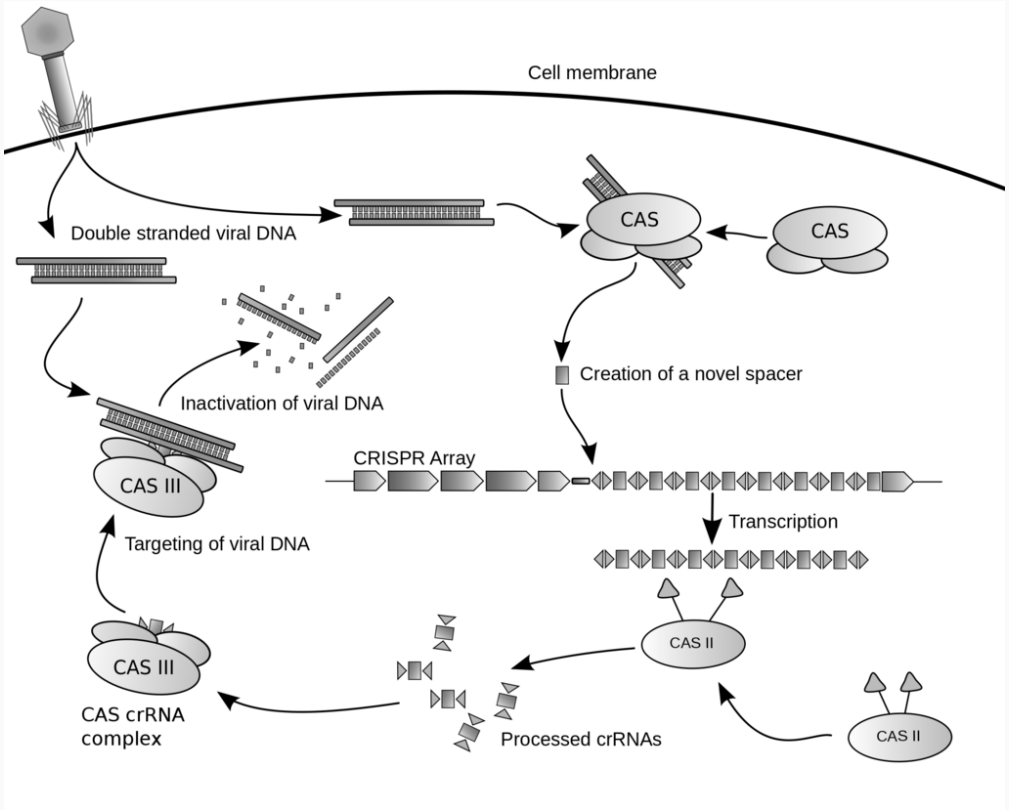

Figure 1: Diagram of the possible mechanism for CRISPR

[1]James Atmos CC BY-SA 3.0 or GFDL, via Wikimedia Commons

CRISPR LOCUS

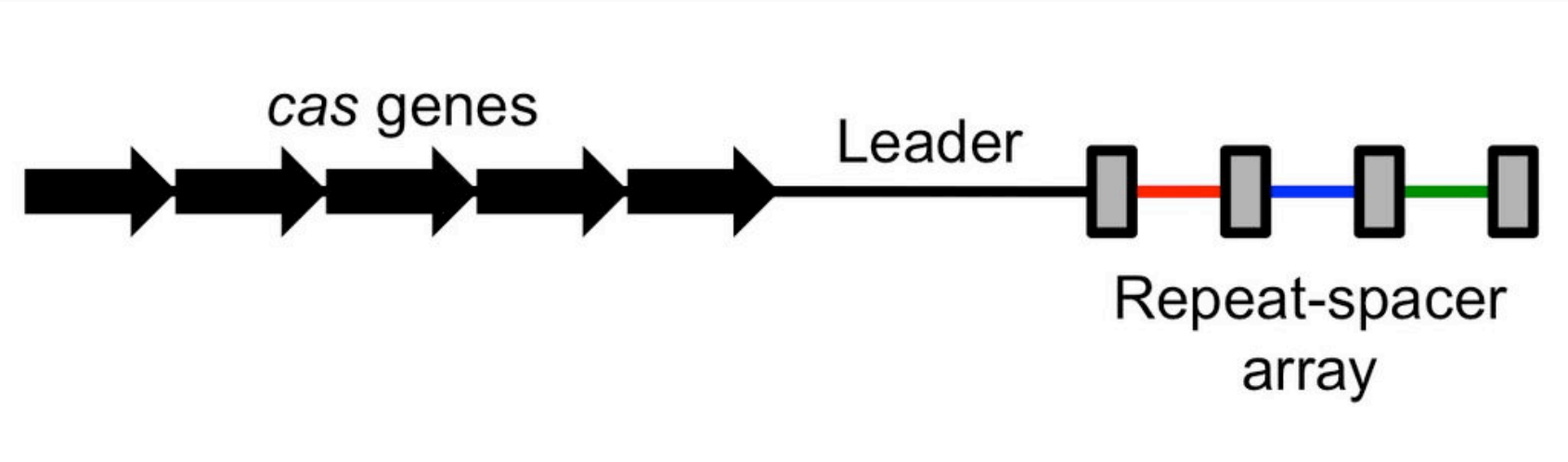

Figure 2: Simplified diagram of a CRISPR locus

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[2] AnnaJune CC BY-SA 3.0 or GFDL, via Wikimedia Commons

METHODS AND RESULTS

SAMPLING AND GENOMIC DATA

- 9 biofilm communities from the Richmond Mine
- Sanger sequencing
- 5way and UBA samples had *Leptospirillum* group II CRISPR locus amplified with CRISPR primers and sequenced with 454

SPACER RICHNESS AND DIVERSITY

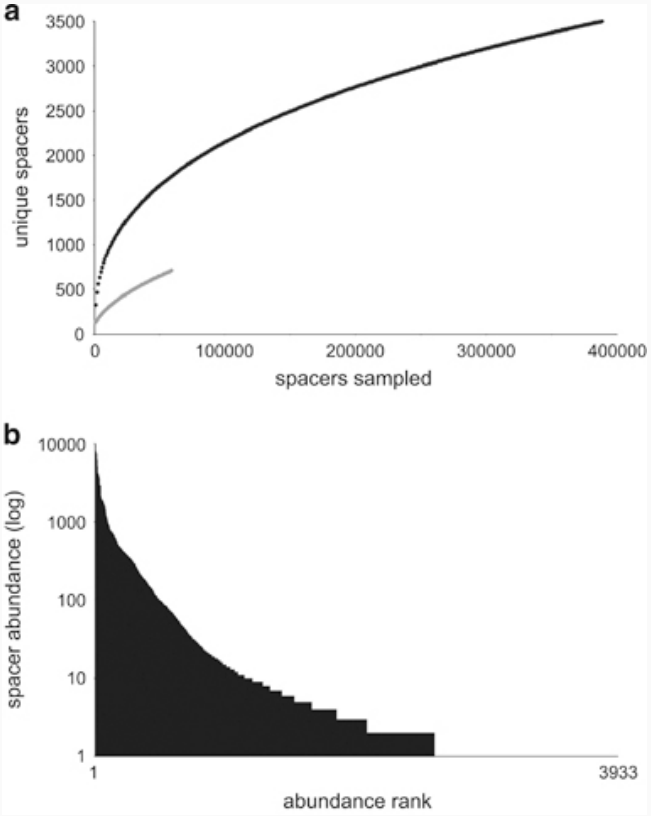
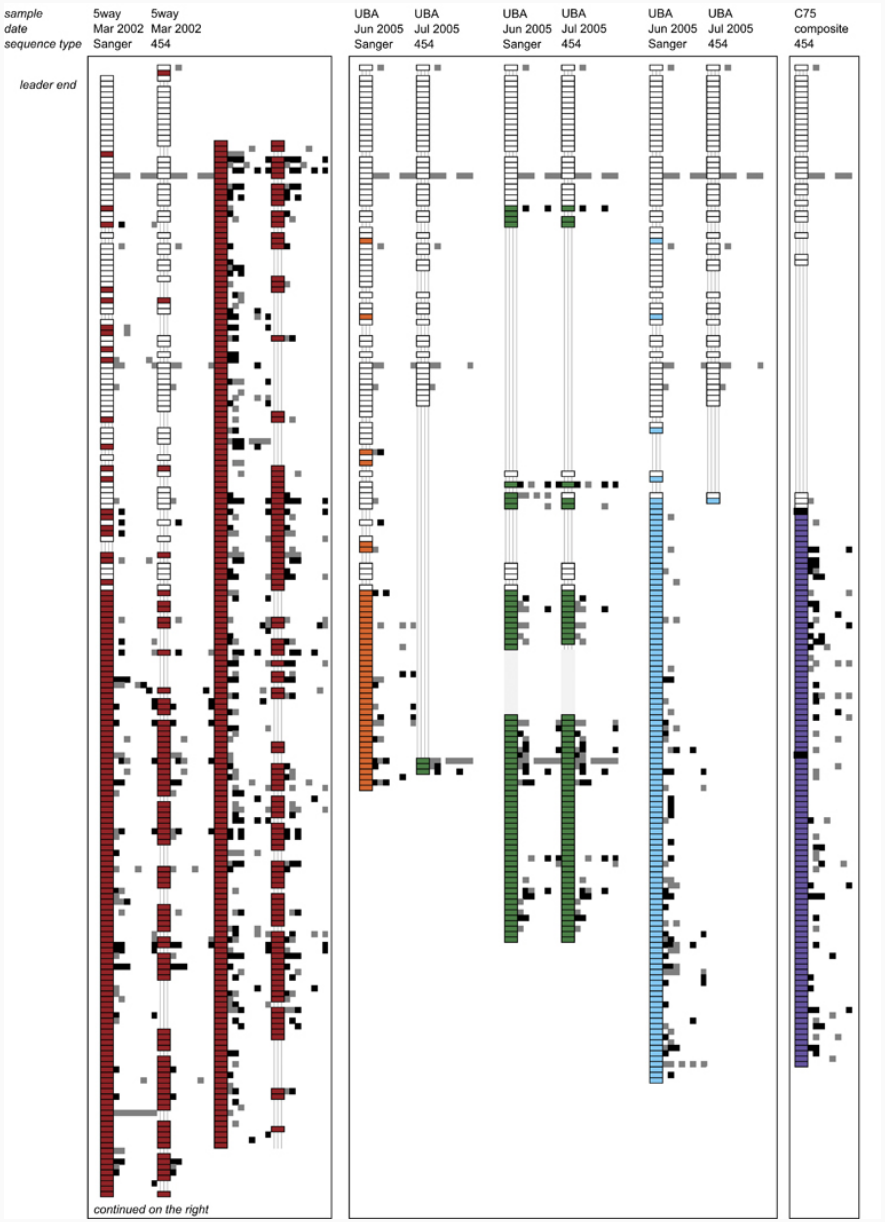


Figure 3: Paper Figure 1

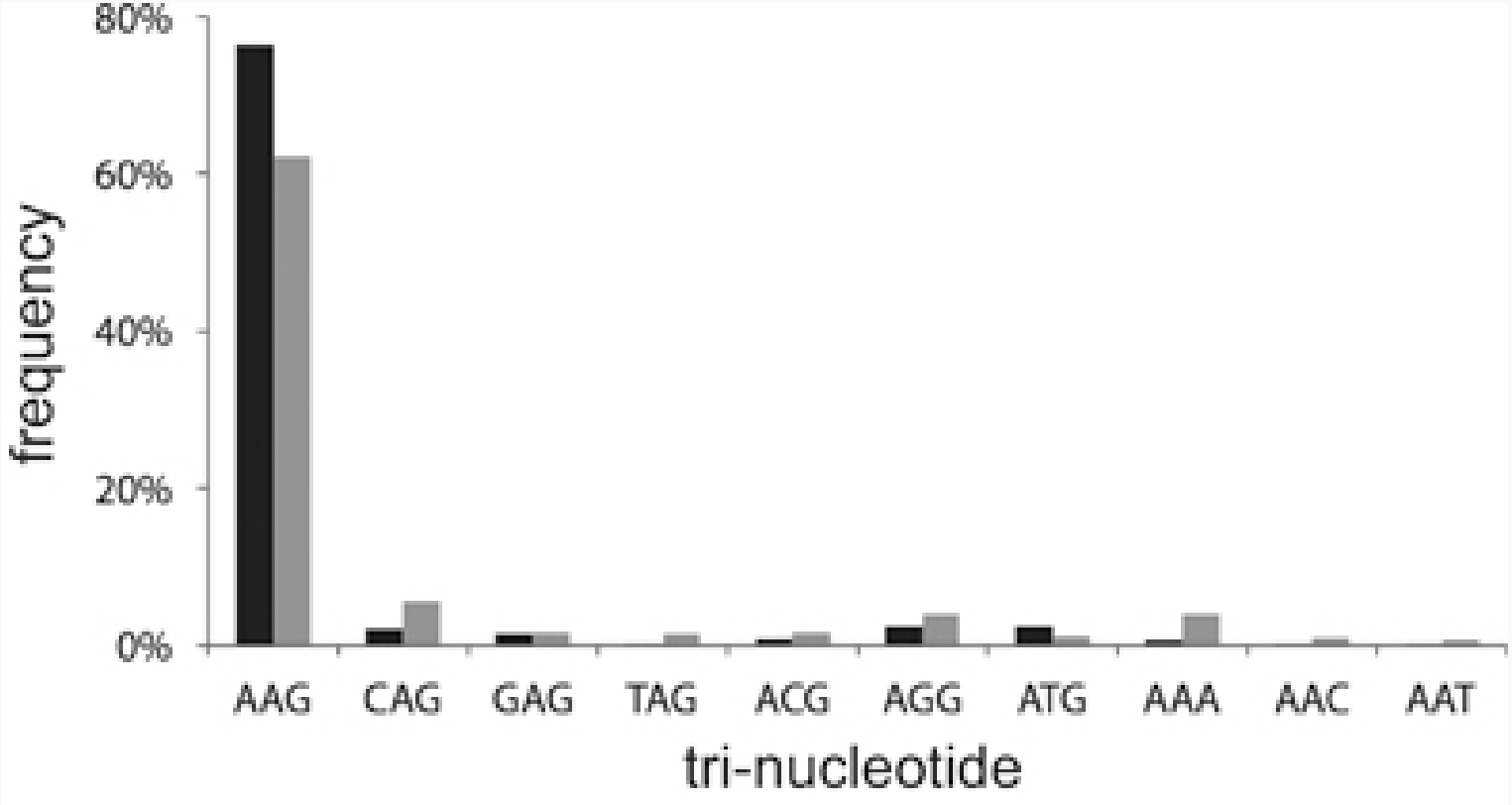
- Rarefaction curves don't approach saturation
- Due to high error rates, spacer were clustered into groups based on length and identity
  - 3933 unique group II groups
  - 296 unique group III groups
- most unique groups only occur a few times across all datasets

LOCUS RECONSTRUCTION



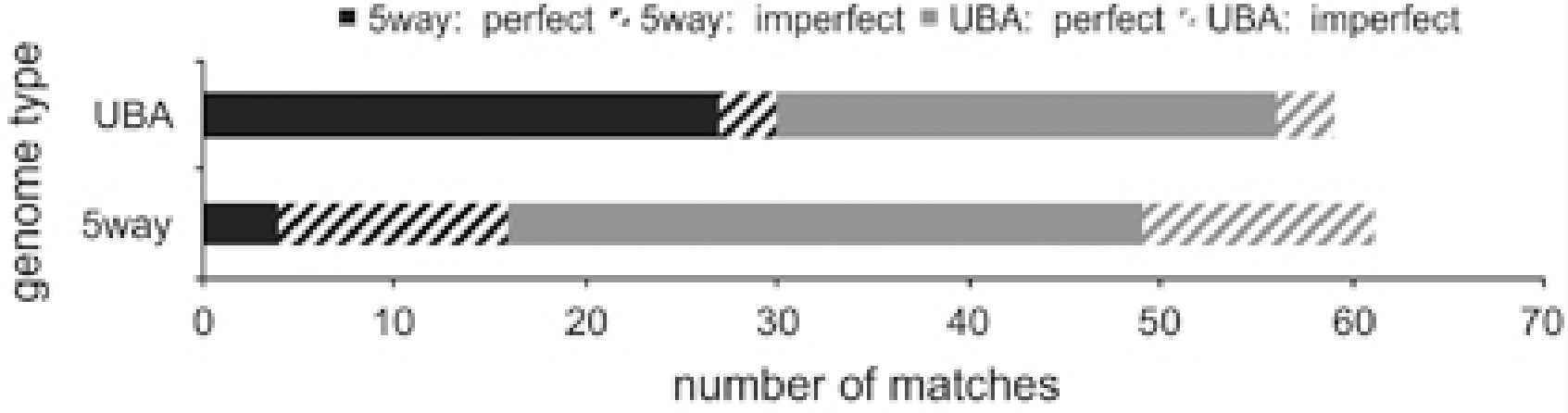
PAM SEQUENCES

- PAM sequence for each *Leptospirillum* group was found by comparing proto-spacer flanking sequences using WebLogo
- Conserved tri-nucleotide flanking sequence AAG for group II and di-nucleotide sequence AA for group III



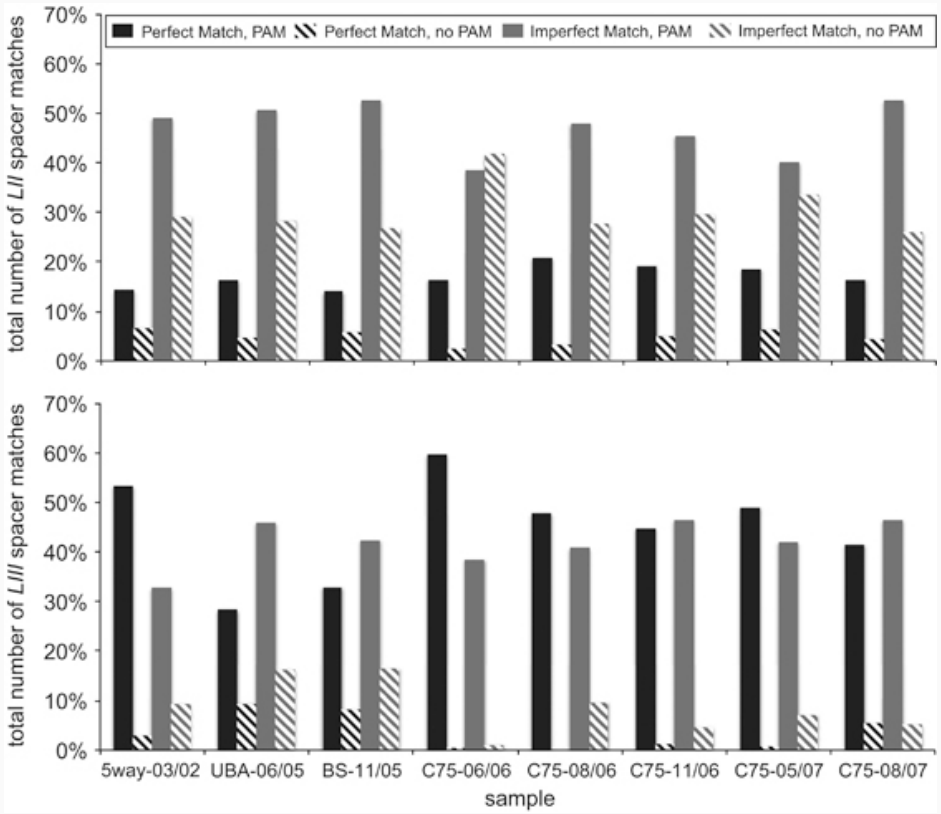
SPACER MATCHES TO GENOME

- Host self targeting
- Most often 5way spacers match genes from UAB groups and vice versa

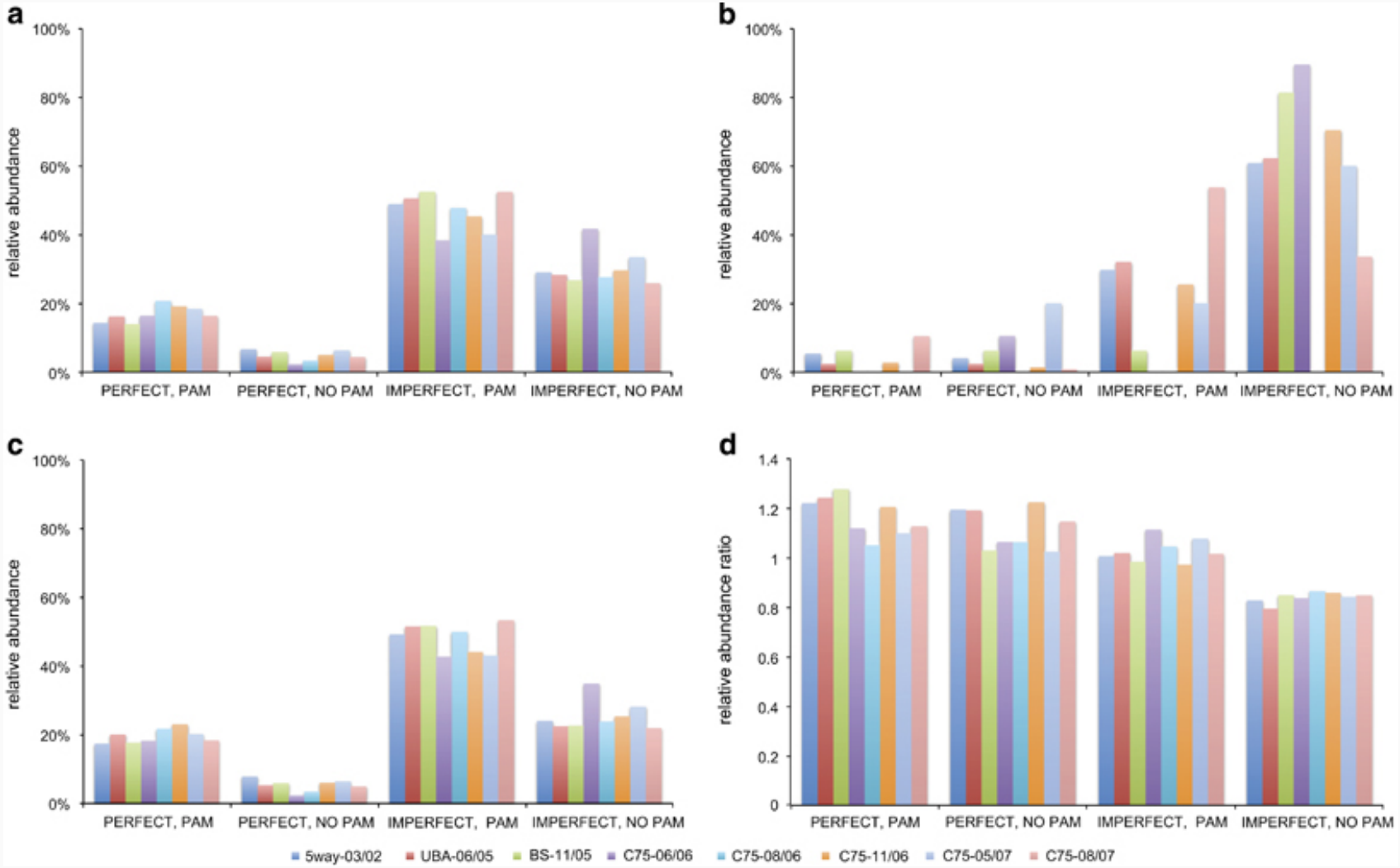


SPACER MATCHES TO PHAGE AND MOBILE ELEMENTS

Reads that don't map to CRISPR or host genome are likely from phage or mobile elements



SPACER MATCHES TO PHAGE AND MOBILE ELEMENTS



SPACER MATCHES TO PHAGE AND MOBILE ELEMENTS

Spacers with mutations (and a perfect PAM) are 9.2 times more common than PAMs with mutations (associated with a perfect spacer)

History of targeting of the known *Leptospirillum* phage AMDV1

- AMDV1 is a *Leptospirillum* group II phage
- 9 spacers that perfectly match longest AMDV1 contig
- Spacer matches occur throughout all time points
- Blocks of spacers from June 2005 that don't contain matches, possible fluctuation in phage exposure

DISCUSSION

- Few self-targeting spacers, mostly to mobile elements in the genome
- Targets may not exist in the same genome!
- The trailer end *Leptospirillum* group II spacers were largely conserved over the 5-year study period
- Most spacer diversity occurs at the leader end. Rarefaction curves show lack of saturation. Cells can contain different CRISPR loci.

CRISPR loci can provide population history

- older and new spacers target essentially the same phage population, a result that points to the persistence of *Leptospirillum* in an environment with the same phage population over the time period represented by the locus (>5 years)
- missing AMDV1 spacers mid locus may suggest period of fluctuation in phage exposure
- Spacer regions mutate before PAM at frequencies expected with random mutation

Questions?