



# Immunity in bacteria and archaea?

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## Prokaryotic silencing (psi)RNAs in *Pyrococcus furiosus*

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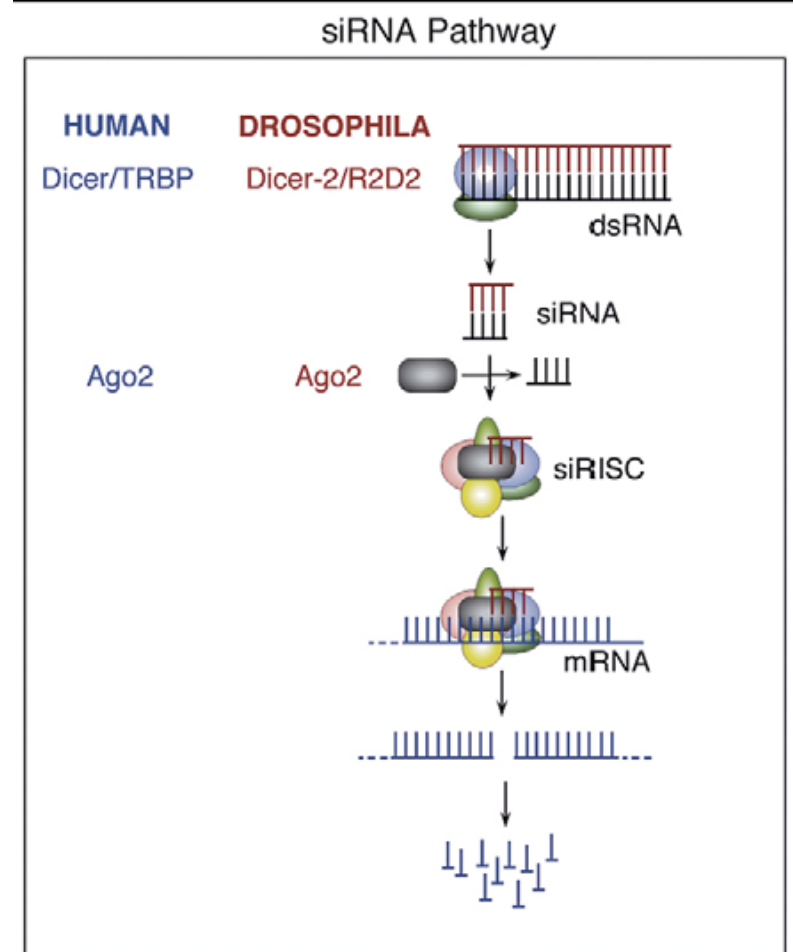
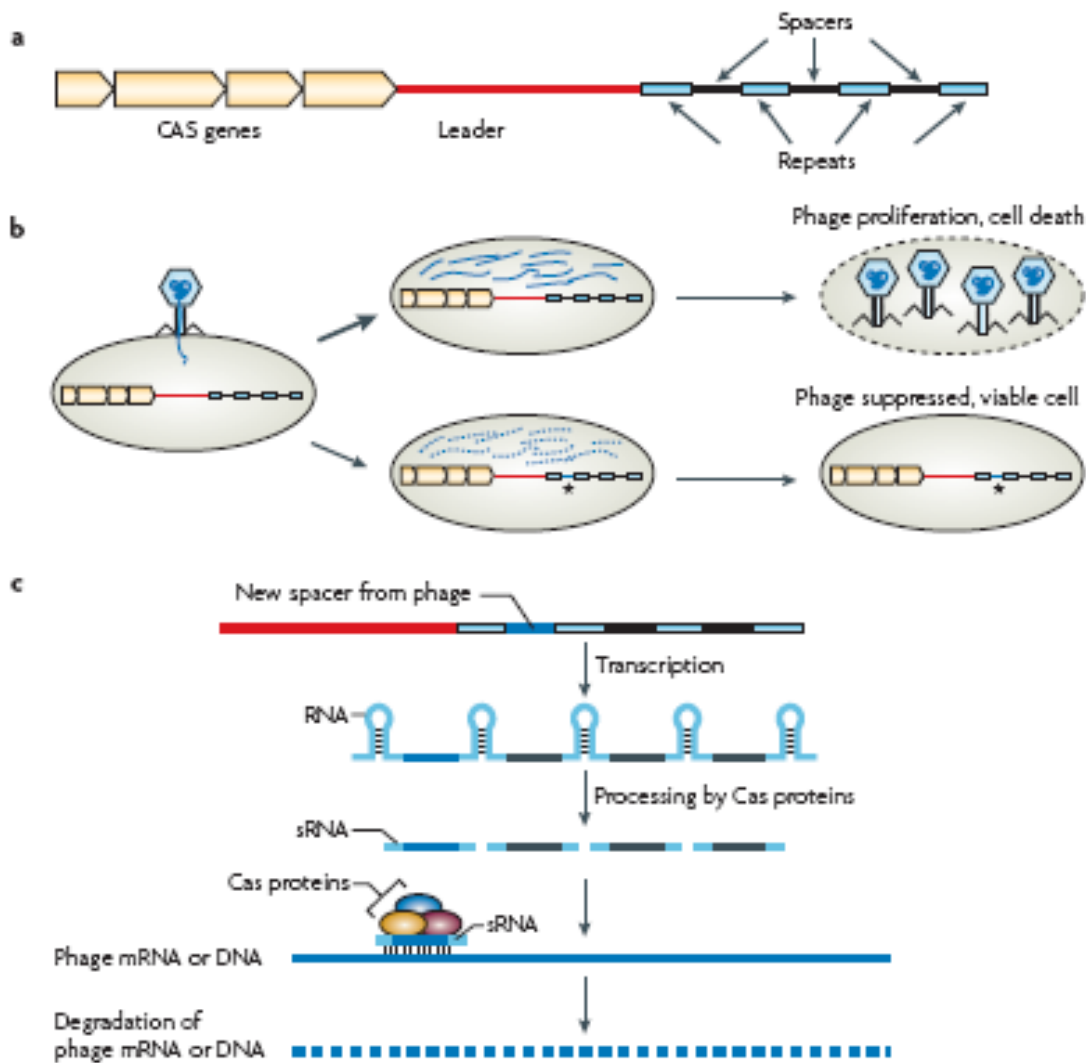
# Phage

- The most abundant forms of life on Earth
  - Seawater contains an estimated 5-10 phage per bacterial cell
  - How do they deal with this?
    - Development of phage resistance mechanisms
      - Restriction enzymes, abortive infection and CRISPR
    - CRISPR- **clustered, regularly interspaced short palindromic repeat system**
      - Confers acquired phage resistance to bacteria and archaea
      - Consists of arrays of short repeats (palindromes) that are separated by non-repetitive spacer sequences
      - 40% of bacteria and 90% archaea contain CRISPR

# CRISPR loci

- CAS – CRISPR associated genes
  - Contain functional domains including:
    - endo and exonuclease, helicase, RNA and DNA binding
- Repeats- Palindromes give a RNA stem-loop secondary structure
- Spacer- Unique sequence complementary to phage
- Leader
  - AT rich, possible promoter

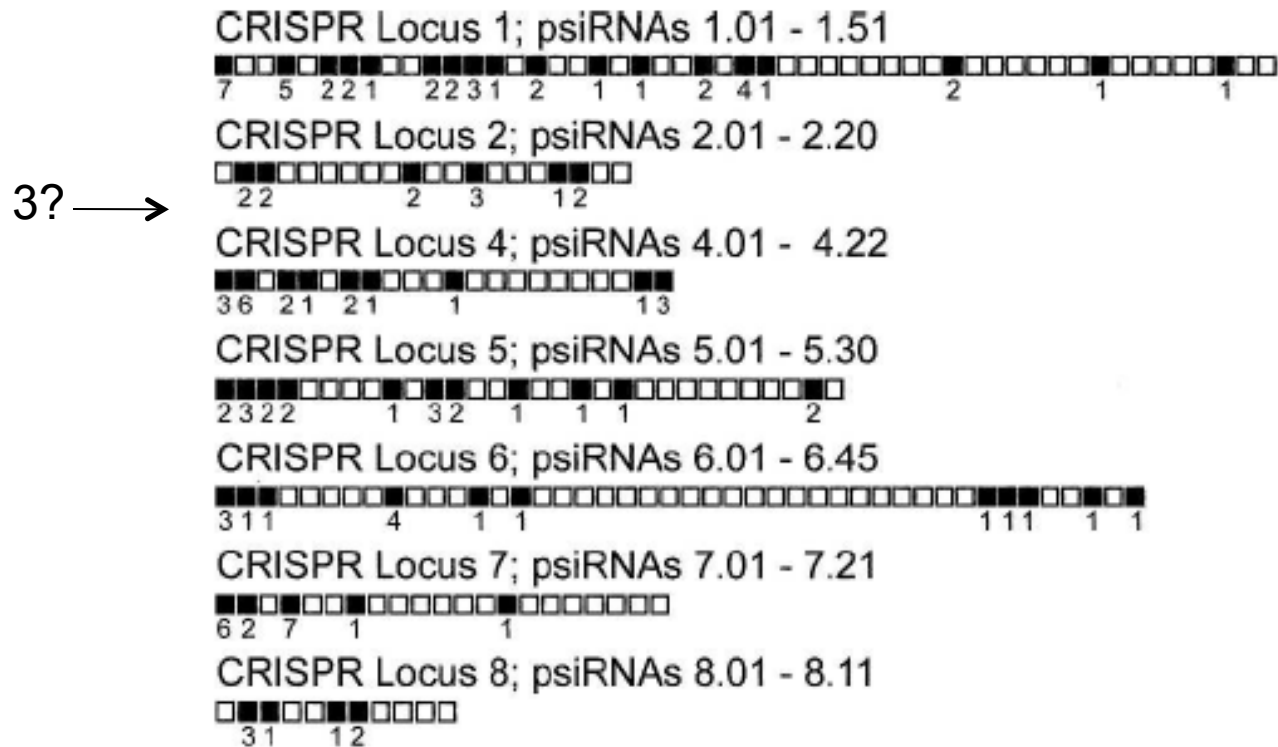
# CRISPR



# Are psiRNA's produced from the CRISPR loci of *P. furiosus*?

- *P. furiosus* contains 7 CRISPR loci encoding 200 potential psiRNA's (11-51 per loci)
- Method:
  - Isolated and cloned small RNA's for sequencing
    - RNA extraction, PAGE resolved
    - Isolated and eluted 20-50 nt RNA's
    - Cloned using standard microRNA cloning procedure
      - pCRII TOPO vector and TOPO10 cells

# CRISPR loci and distribution of psiRNA's identified



\*psiRNA sequences ranged from 12-40nt

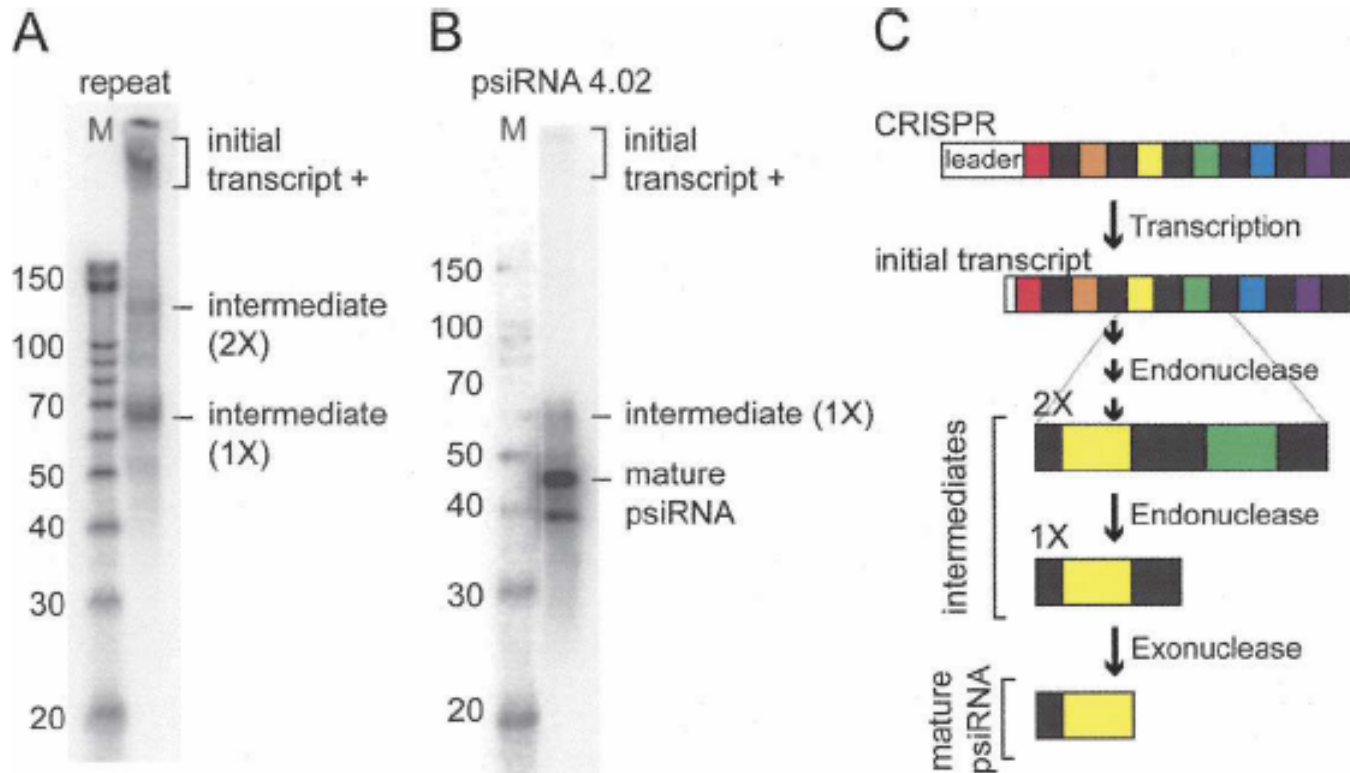
\*repeat sequences were 0-8nt at the 5' and between 0-22nt at the 3'end

\*Of the 872 clones sequenced, 144 represented CRISPR loci

# Are the psiRNA's detectable by northern analysis?

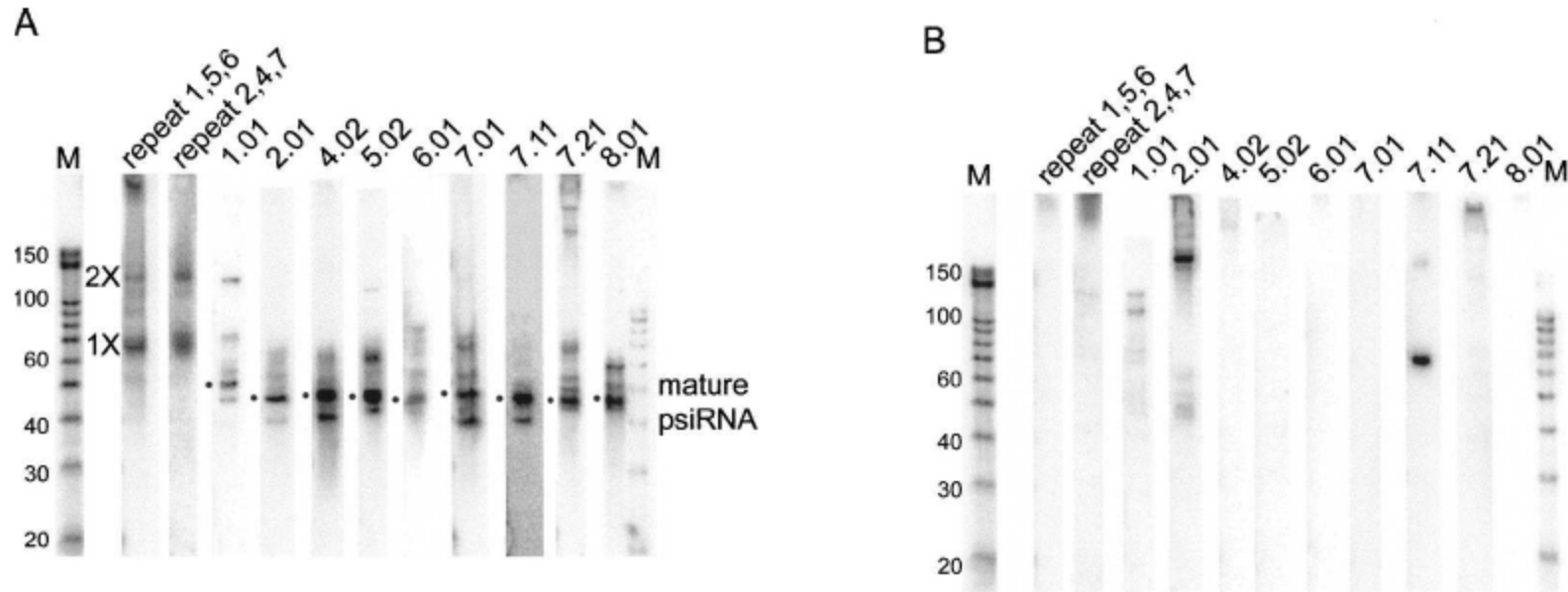
- Sequencing revealed potential small psiRNA's but they were not of uniform size or composition
- Are they really psiRNA's
- Probe construction:
  - Made probes against both repeat and psiRNA sequences and radiolabeled

# CRISPR repeat and psiRNA's are detected



- A) Repeat probe detecting conserved region of CRISPR loci 1, 5 and 6  
B) psiRNA probe for 4.02

# Other CRISPR loci are also detectable



A) Mature psiRNA are detected from each loci w/ sense strand probe

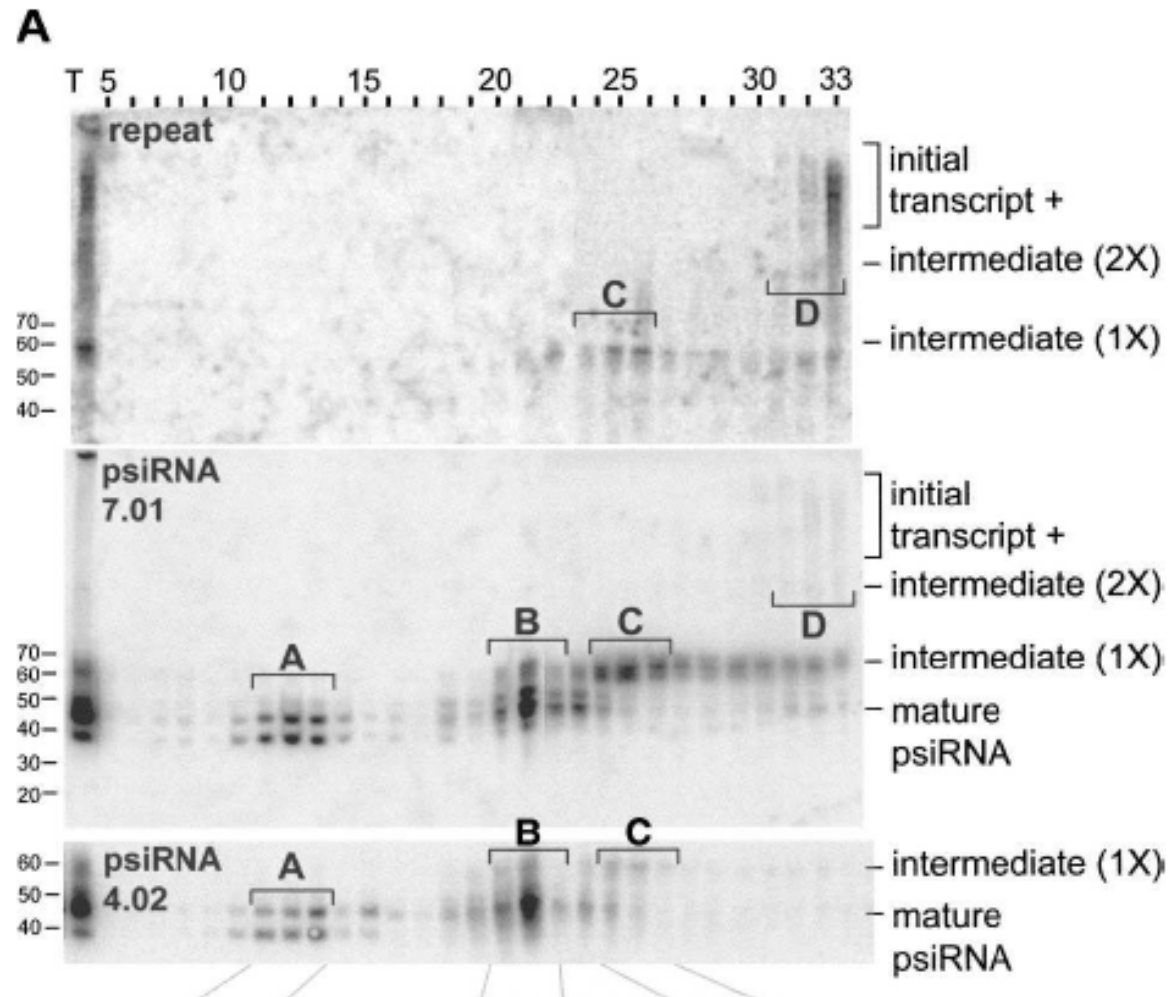
B) No psiRNA detection using antisense probe

\*Primary mature psiRNA's contain between 5-10nt of repeat

# Are the CRISPR RNA's associated with proteins

- Eukaryotic and insect RNAi involves siRNA/  
protein interaction for functionality
- Performed anion exchange chromatography
  - Fractions were evaluated by northern blot analysis  
with probes against repeat and psiRNA sequence

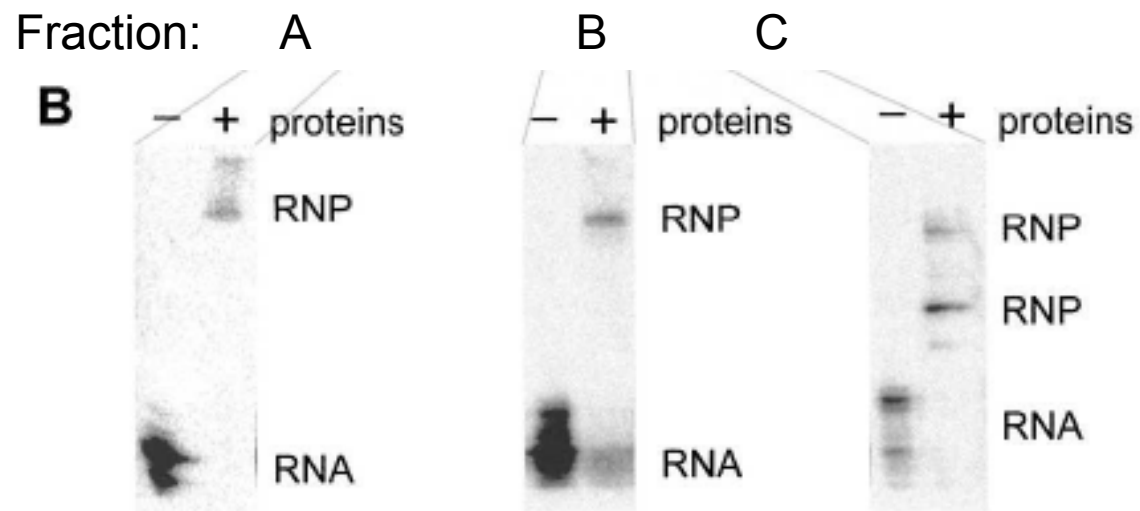
# There are distinct complexes



**A-** mature psiRNA's **B-** mature and 1X **C-** 1x **D-** 2x and initial transcript

There appears to be multiple complexes that contain distinct subsets of CRISPR RNA's

# CRISPR RNA-protein complex confirmation



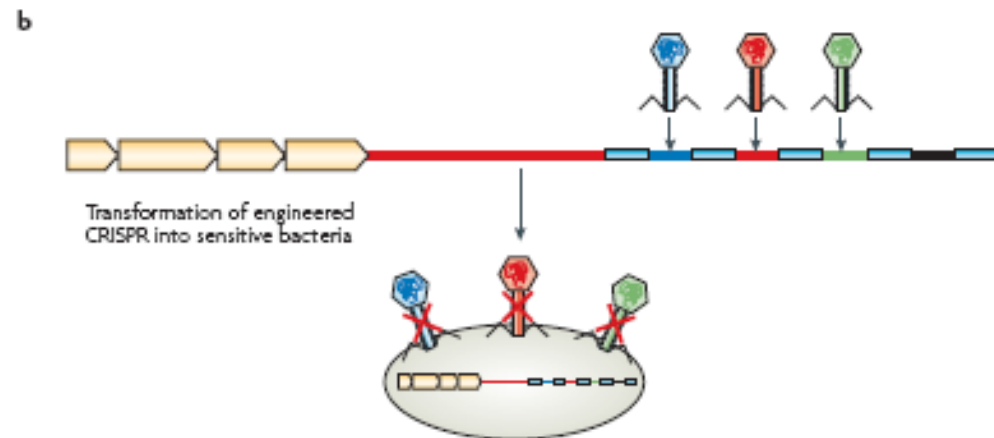
Used non-denaturing gel electrophoresis and Northern analysis against psiRNA 7.01

# Conclusions

- Identified psiRNA's that are gene products of the CRISPR loci
- These psiRNA's are in protein complex's
  - May be the representative effector complex of the pRNAi pathway

# Future Directions

Engineered defence against Phage: Dairy and wine industries



Selective silencing of endogenous Genes: Replace gene KO's

