Transcriptional Recording by CRISPR Spacer Acquisition from RNA

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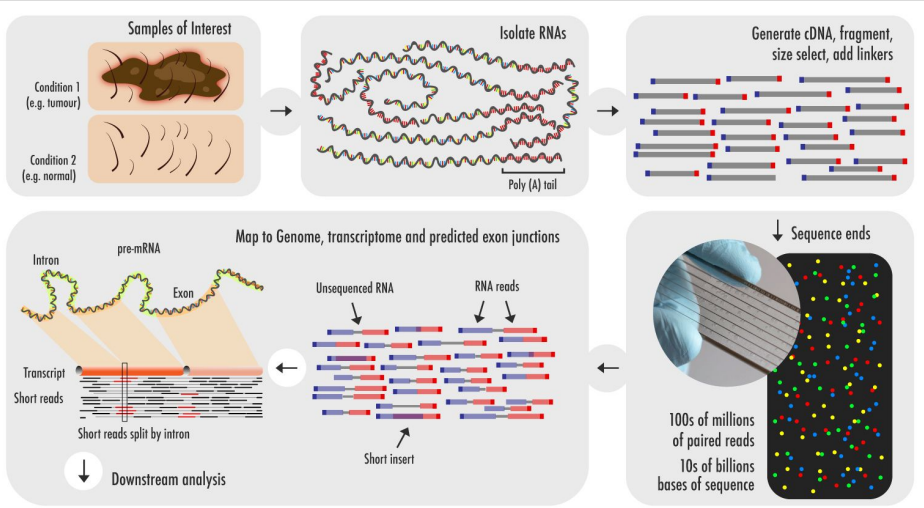
RNA Sequencing

- RNA sequencing can examine the quantity and sequences of RNA in a cell sample using next generation sequencing

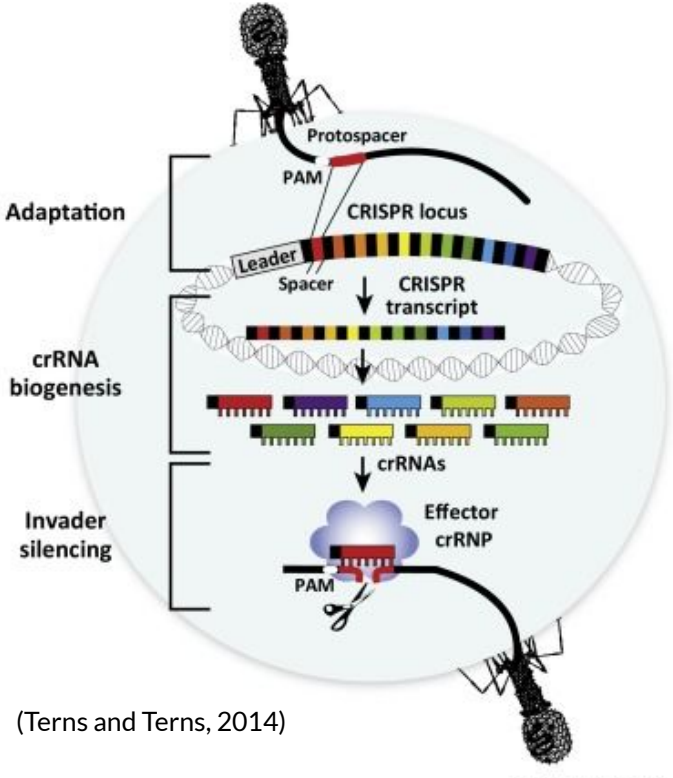
1. Convert isolated RNA into cDNA fragments
2. Addition of adapters to ends of fragments
3. NGS analysis
4. Aligned to a reference genome
5. Assembly

- Limitations:
  - Destructive methods
  - One snapshot at a time

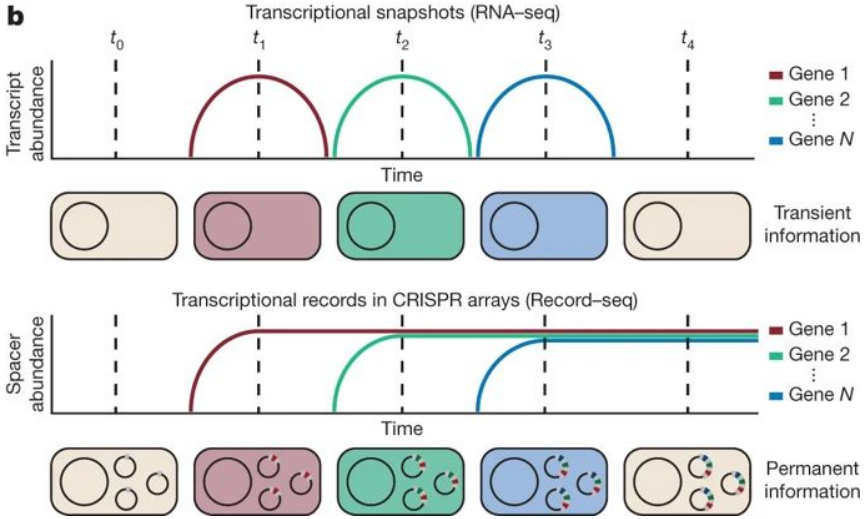


(Mackenzie, 2018)

CRISPR-Cas Immune System



(Terns and Terns, 2014)



(Schmidt *et al*., 2018)

Significance

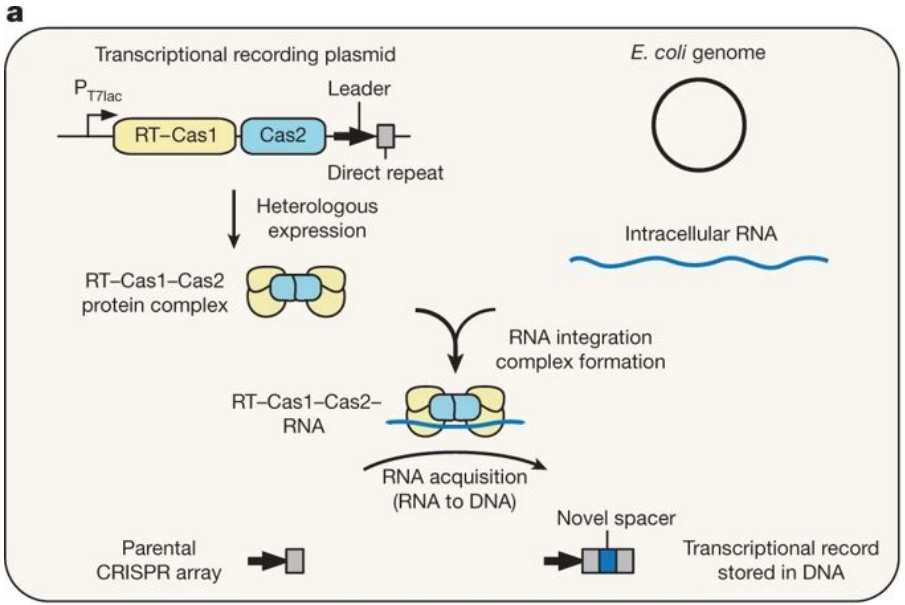
- Allows understanding of our transcriptome, including:
    - mRNA, rRNA, and tRNA
- Form connections between genome information and functional protein expression
- Cell biology and associations with disease

Hypothesis

- CRISPR spacer acquisition from RNA could be leveraged to store transcriptional records in CRISPR arrays to provide a temporal perspective of cellular activity

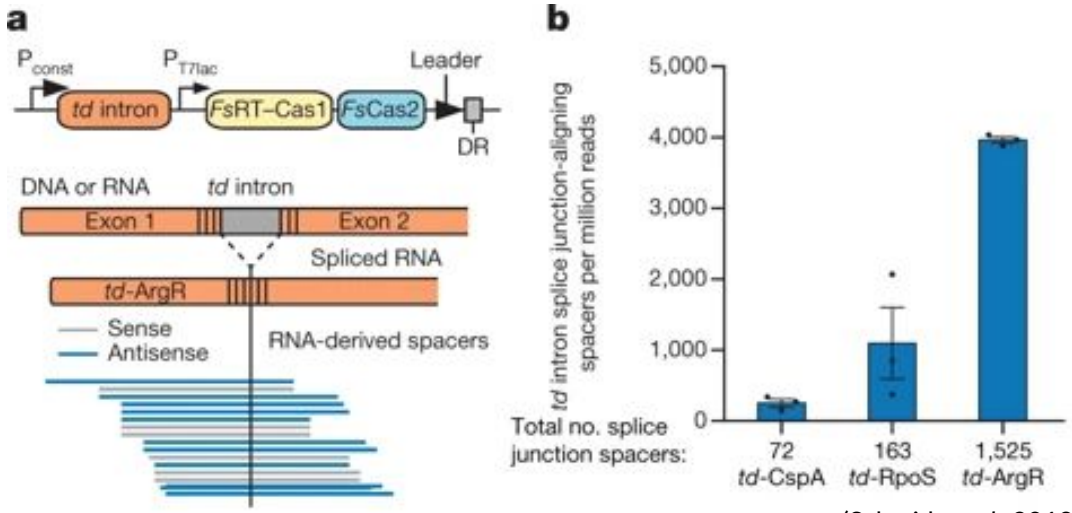
*Fs*RT–Cas1–Cas2

- Overexpressing reverse-transcriptase (RT) Cas1 containing CRISPR-Cas system orthologues in E. coli cells
- Identified an ortholog from *Fusicatenibacter saccharivorans*
- *Fs*RT-Cas1-Cas2 = FCC



RNA Acquisition

Self-Splicing td Group I Intron



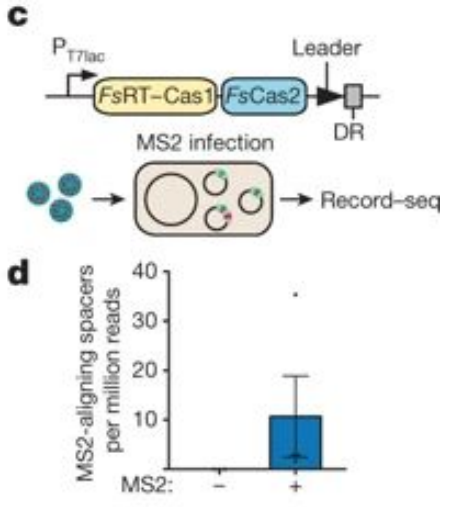
(Schmidt *et al.*, 2018)

- This intron is a functional ribozyme that catalyzes its own excision from the pre-mRNA

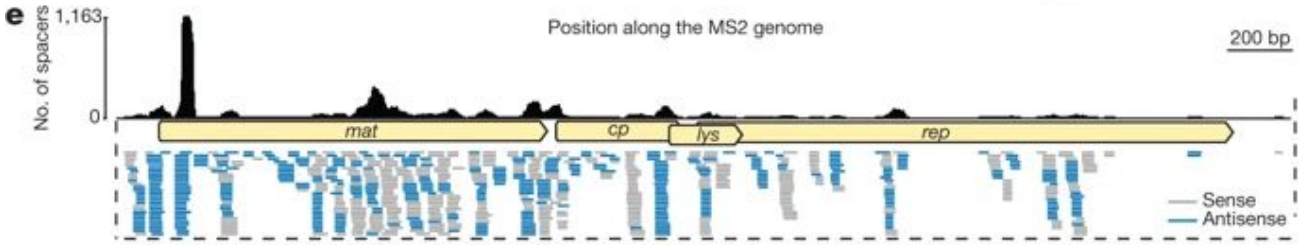
RNA Acquisition

Enterobacteria phage MS2

- These phage can exist with both sense and antisense single stranded RNA during life cycle
- No DNA intermediate



(Schmidt *et al.*, 2018)

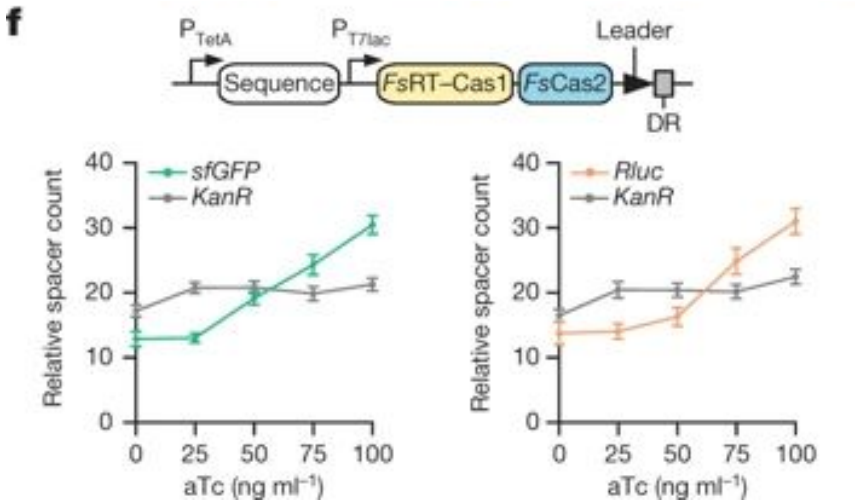


(Schmidt *et al.*, 2018)

Quantitative Recording

sfGFP & Rluc

- Inducible expression system to determine if spacer acquisition is based on RNA abundance
- Under control of anhydrotetracycline (aTc)-inducible P_{tetA} promoter
- Dose-dependant increase in amount of spacers
- KanR control

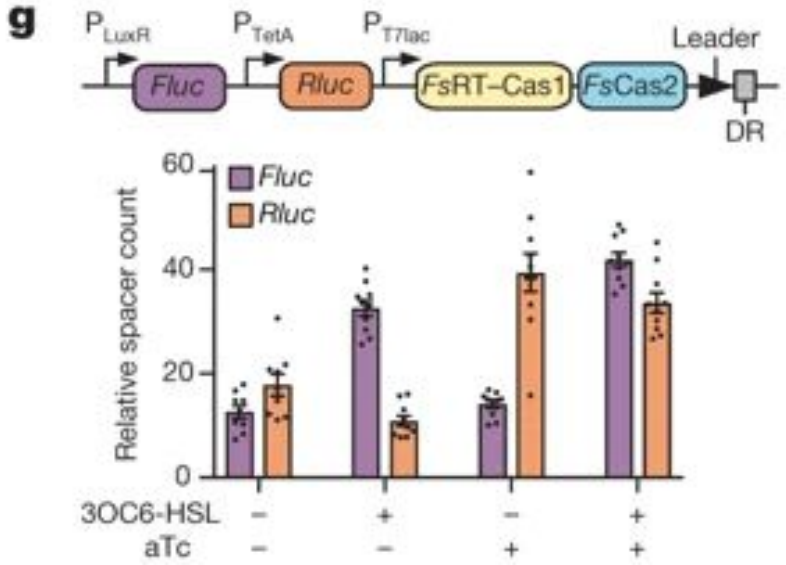


(Schmidt *et al.*, 2018)

Quantitative Recording

Fluc

- Second inducible expression system
- Under control of 3-oxohexanoyl-homoserine lactone inducible P_{luxR} promoter
- Combined both expression systems
- Transcriptional records are quantifiable



(Schmidt *et al.*, 2018)

Proof of Principle

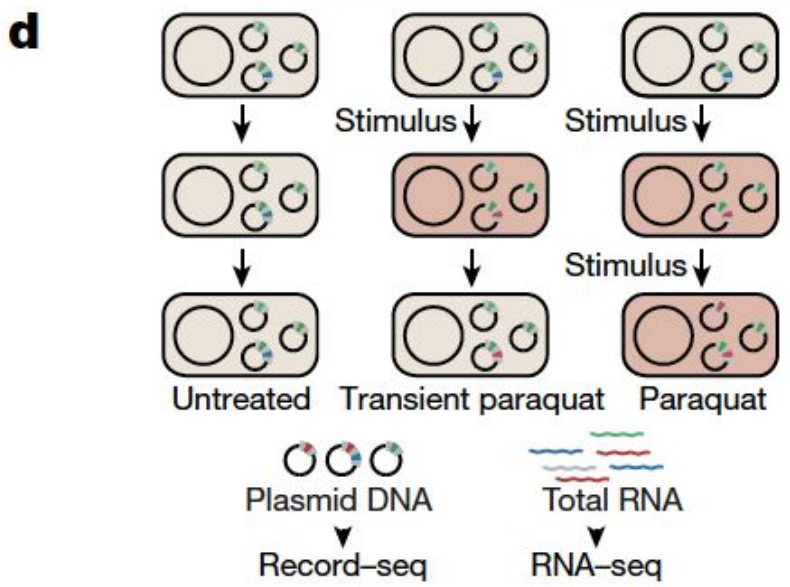
Oxidative & Acid Stress

- Used Record-seq to record and describe complex cellular behaviours
- Analyzed cumulative gene expression counts
- Detected RNA upregulation within bacterial populations due to stress by treatment with:
    - Hydrogen peroxide
    - Acid

Proof of Principle

Paraquat

- Bacteriostatic herbicide
- Three cell conditions:
  - Untreated
  - Transient paraquat
  - Paraquat



(Schmidt *et al.*, 2018)

- Record-seq showed bacterial record of genes transiently upregulated by paraquat while RNA-seq was unable to detect these genes

Critical Assessment

- Systematic approach
- Low efficiency of spacer acquisition
    - One spacer acquisition event per 20, 000 *E. coli* cells
- Further extrapolated with future studies with mammalian cells
- Discovery and use of alternate RT-Cas1 orthologues
- Alternate Cas proteins (e.g. Cas 6)

Summary

- Is there an RT–Cas1-Cas1 CRISPR acquisition complex that could acquire spacers directly from RNA and be expressed in *E. coli*?
- Can this RT–Cas1-Cas1 system acquire spacers directly from RNA?
- Can this system be used to quantitatively record transcriptional events in the cell?
- Does it reveal and describe complex cell behaviours?
- Does the use of this system exceed the abilities of RNA–seq?

References

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