



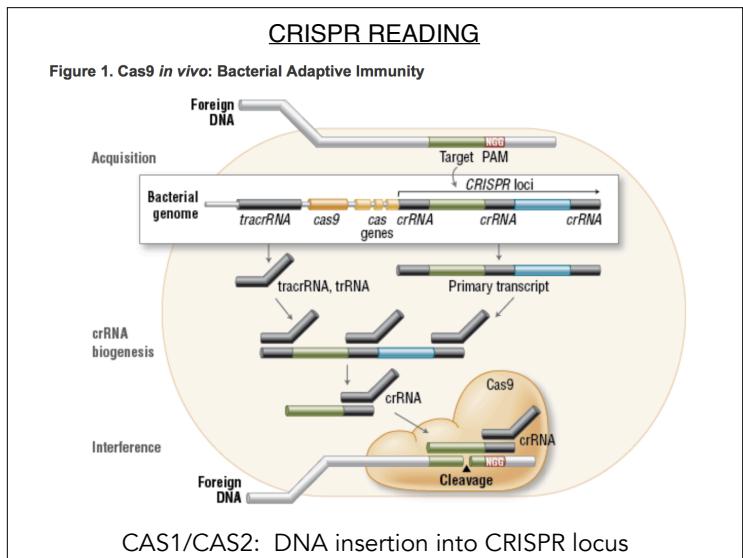
## Past and current questions? - hand in

Mojica et al. were the first to realize that ... bacterial and archaeal sequences were functionally related (27).

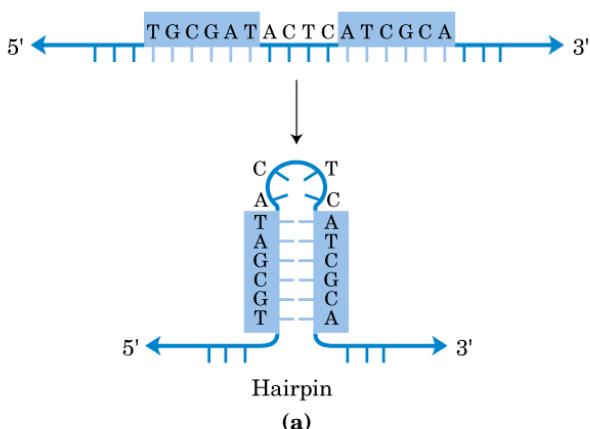
## common characteristics

- i) located in intergenic regions
  - ii) multiple (highly) conserved short direct repeats
  - iii) repeats are interspersed with non-conserved sequences
  - iv) a common sequence located on one side of the repeat cluster (four conserved genes).
  - v) conserved in (all) archaea and ~50% of bacteria (not eukaryotes)

**CRISPR: clustered regularly interspaced short palindromic repeats,**



palindrome

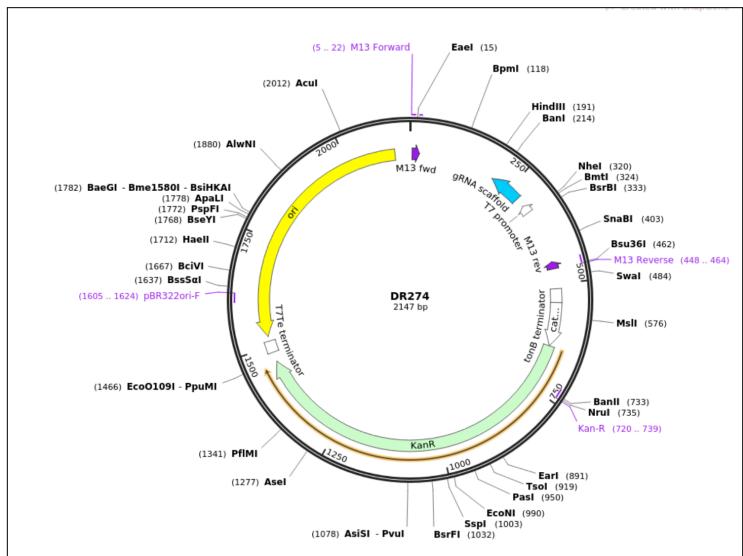


The diagram shows the repeat unit structure with a pink box labeled "Repeat unit (21-40 bp)" and a blue box labeled "Spacer (20-58 bp)". Below, two DNA sequences are aligned:

- E. coli (1987):** CGGTTTATCCCCGCTGCGCGGGAACTC
- H. mediterranei (1993):** GTTACAGACGAACCTAGTTGGGTTGAAGC

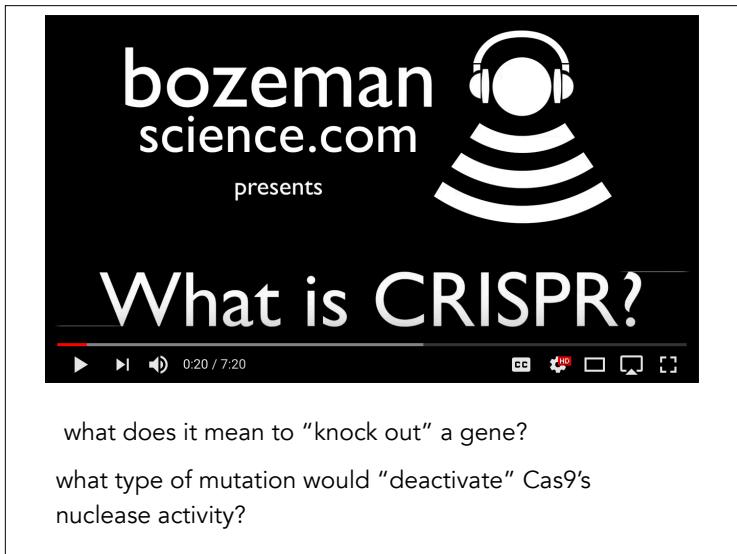
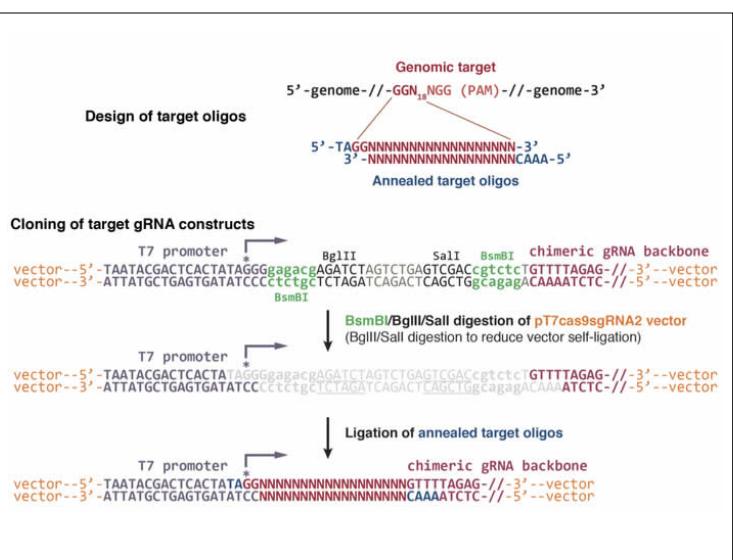
Differences are indicated by arrows and boxes:

- A yellow arrow points to a G in the E. coli sequence.
- A yellow arrow points to a G in the H. mediterranei sequence.
- A yellow box highlights a C in the E. coli sequence.
- A yellow box highlights a T in the H. mediterranei sequence.
- A yellow box highlights a C in the E. coli sequence.
- A yellow box highlights a T in the H. mediterranei sequence.
- A yellow box highlights a C in the E. coli sequence.
- A yellow box highlights a G in the H. mediterranei sequence.
- A yellow box highlights a C in the E. coli sequence.
- A yellow box highlights a G in the H. mediterranei sequence.
- A yellow box highlights a C in the E. coli sequence.
- A yellow box highlights a T in the H. mediterranei sequence.
- A yellow box highlights a C in the E. coli sequence.
- A yellow box highlights a G in the H. mediterranei sequence.
- A yellow box highlights a C in the E. coli sequence.
- A yellow box highlights a T in the H. mediterranei sequence.
- A yellow box highlights a T in the E. coli sequence.
- A yellow box highlights an A in the H. mediterranei sequence.
- A yellow box highlights a T in the E. coli sequence.
- A yellow box highlights an A in the H. mediterranei sequence.

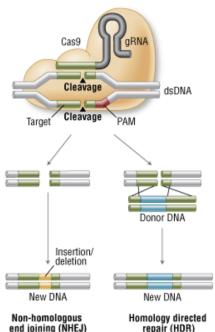


## Choosing a Target Sequence for CRISPR/Cas9 Gene Editing

CRISPR/Cas9 gene targeting requires a custom single guide RNA (sgRNA) that contains a targeting sequence (crRNA sequence) and a Cas9 nuclelease-recruiting sequence (tracrRNA). The crRNA region (shown in red below) is a 20-nucleotide sequence that is homologous to a region in your gene of interest and will direct Cas9 nuclease activity.



A. Genome Engineering With Cas9 Nuclease



## Mendel - chapter 15

Sir Paul Nurse: on Mendel

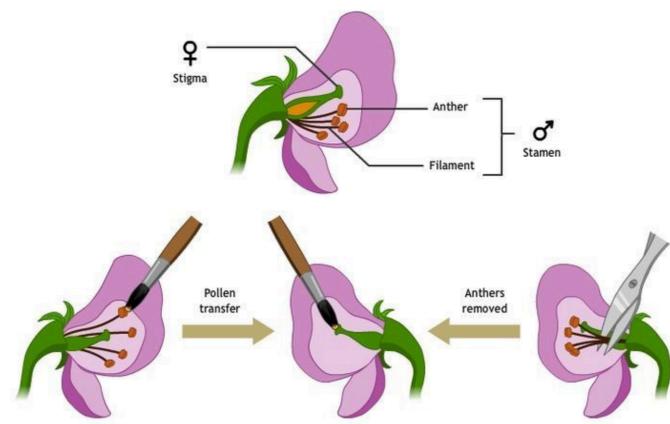
Mendel's findings were ignored



## HOW MENDEL MADE HYBRIDS:



From: The Cartoon Guide to Genetics by Larry Gonick & Mark Wheelis

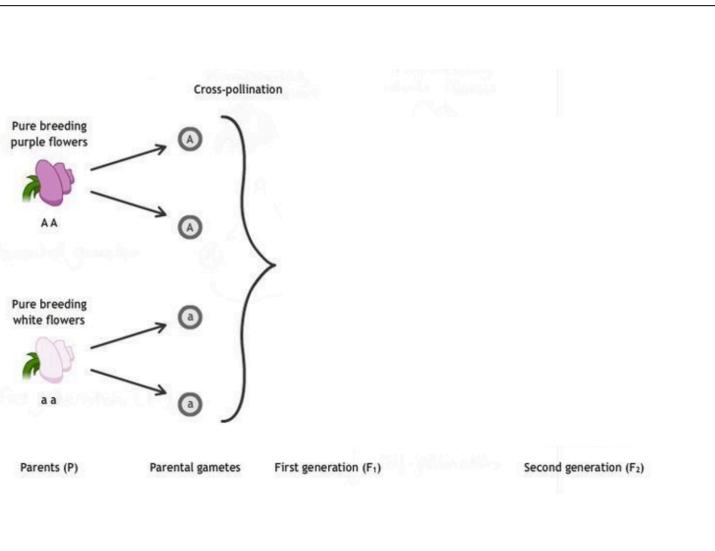


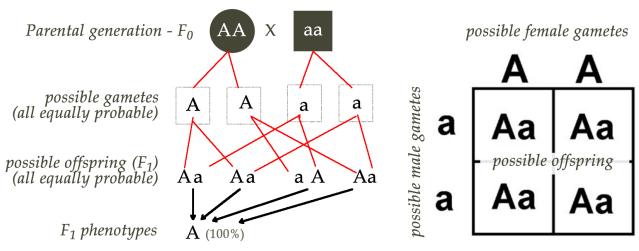
© 2005-2011 The University of Waikato | www.biotechlearn.org.nz

## Mendel - chapter 15

- true breeding lines / discrete traits (independent)

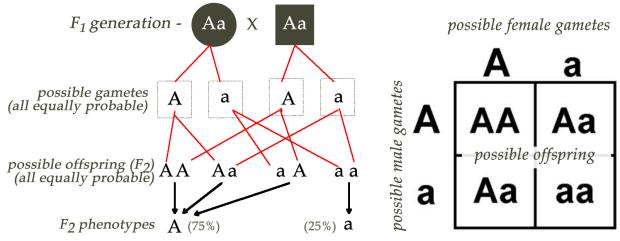
	Seed Shape	Seed Color	Seed Coat Color	Pod Shape	Pod Color	Flower Position	Plant Height
P	Round X Wrinkled	Yellow X Green	Gray X White	Smooth X Constricted	Green X Yellow	Axial X Terminal	Tall X Short





What percentage of phenotypes do you expect?

$F_1$  cross (between any two offspring) produces  $F_2$  generation (as below)



What percentage of phenotypes do you expect?  
why are the numbers not exact?

### Next: Chi square analysis

how do we know that the numbers we observe are consistent with our hypothesis, when chance plays such an integral role.