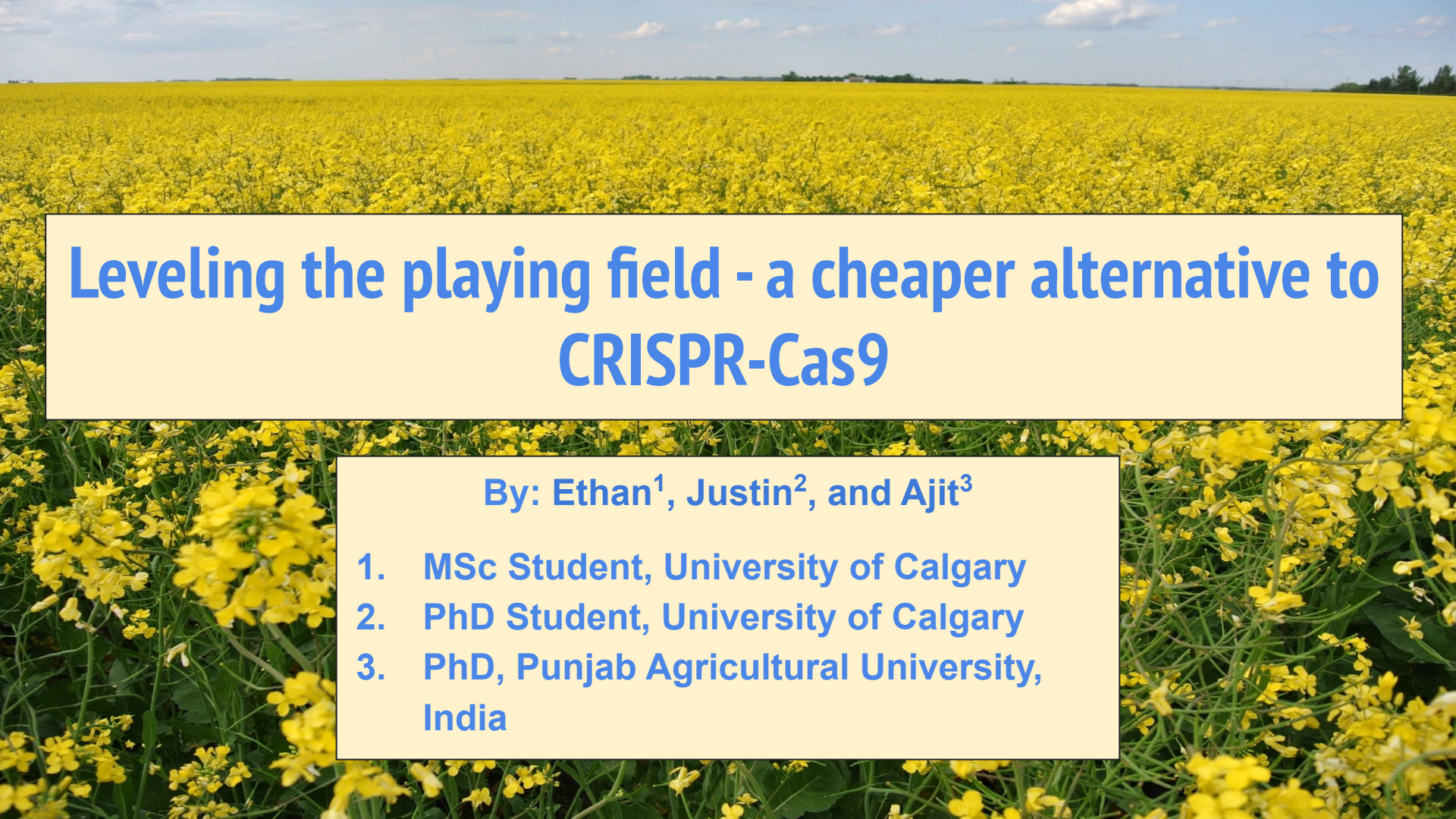

Leveling the playing field - a cheaper alternative to CRISPR-Cas9

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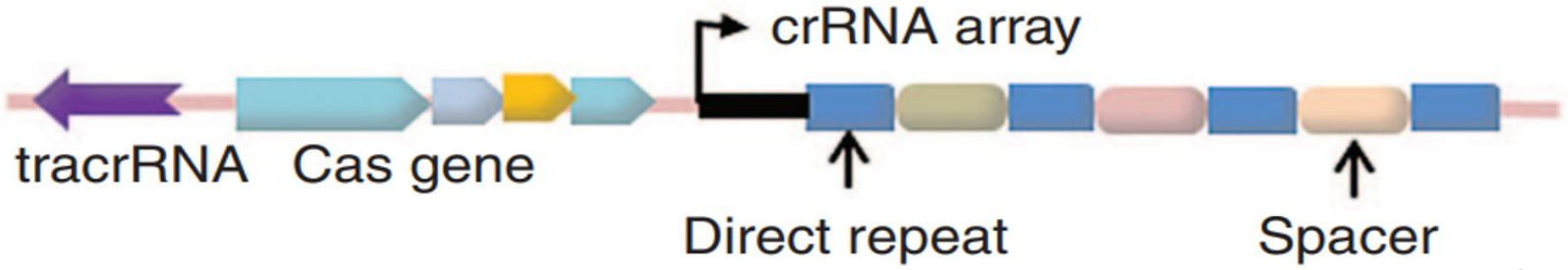
CRISPR-Cas systems are the leading method for gene editing

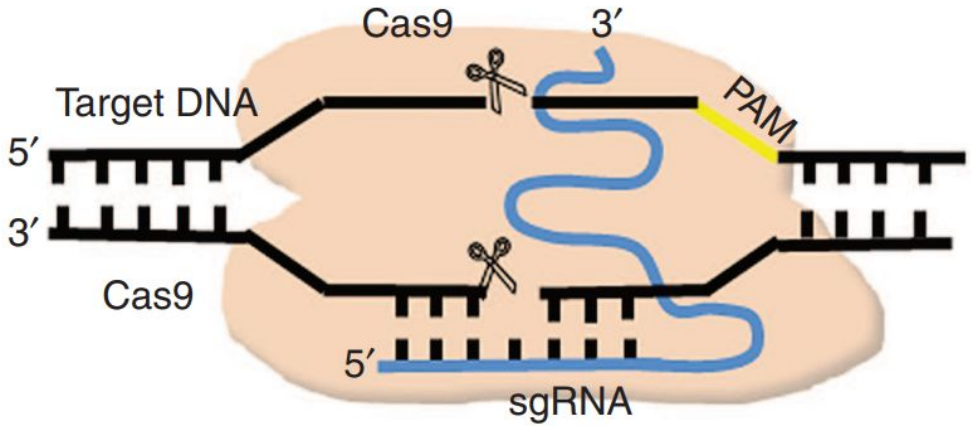
- Developed by Emmanuelle Charpentier and Jennifer Doudna in 2012
- CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats
- Form of adaptive immunity in prokaryotic organisms



Dai et al. 2016

CRISPR-Cas9 enables sequence specific editing

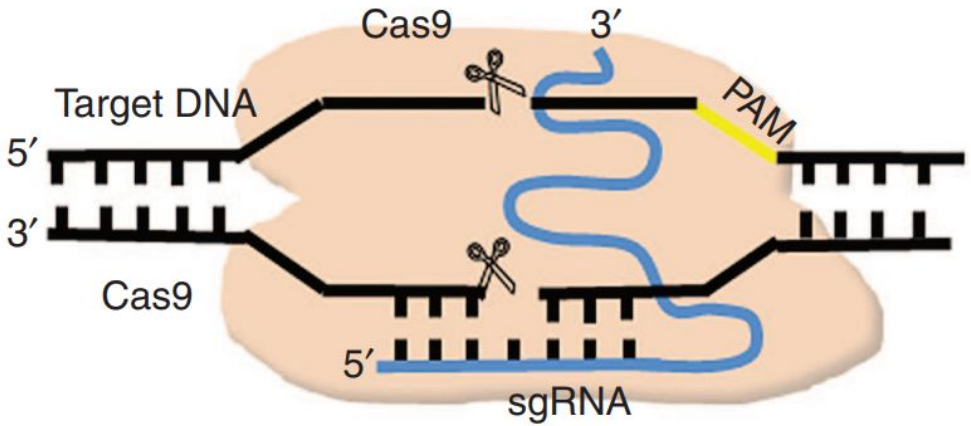
- CRISPR-Cas9 recognizes, binds and cuts DNA complementary to CRISPR sequence
- Cas9 coupled with a single guide RNA (sgRNA) can be programmed to create a double stranded break in a specific piece of DNA



Dai et al. 2016

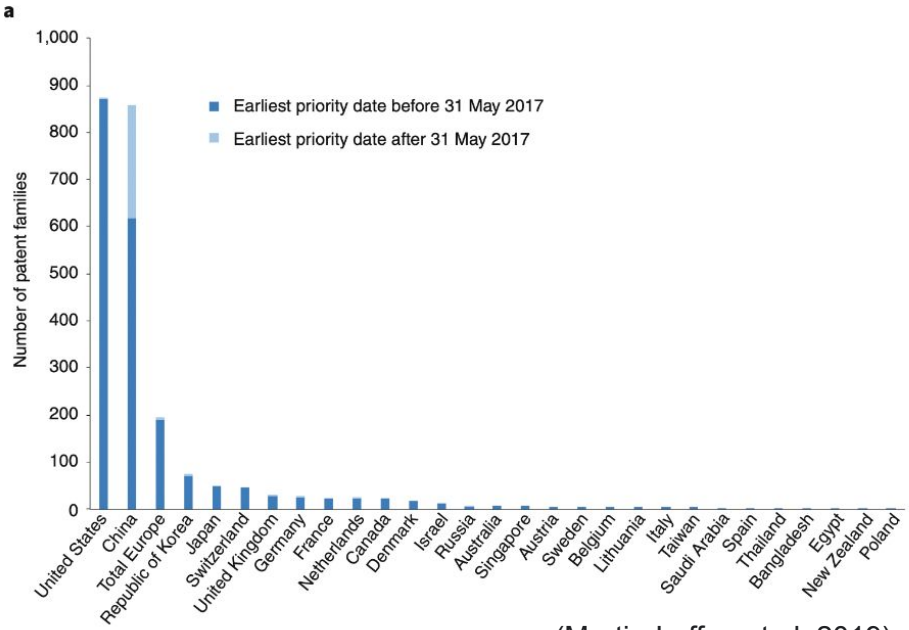
CRISPR-Cas9 enables sequence specific editing

- sgRNA consists of:
  - Palindromic sequence (scaffold) □ Interacts with Cas9
  - Template spacer sequence (crRNA) □ Customizable to target
- Cas9 requires a protospacer adjacent motif (PAM) site located 3' of crRNA
  - Cas9 recognizes 5'-NGG-3' PAM sites



Dai et al. 2016

CRISPR-Cas9 dominates the patent landscape for gene editing



(Martin-Laffon et al. 2019)

- In 2021 the US Patent and Trademark Office had around 6000 CRISPR patent applications
- In 2020, 17% of CRISPR patents were related to plant modification and 13% were related to animal modification

Who owns CRISPR-Cas9?

Two teams of researchers developed CRISPR-Cas9 around the same time

- ➔ Dr. Jennifer Doudna and Dr. Emmanuelle Charpentier (UC Berkeley)
  - ◆ Patent covers the use of CRISPR-Cas9 in any living cell

- ➔ Dr. Feng Zhang (Broad institute)
  - ◆ Patent covers the use of CRISPR-Cas9 in eukaryotic cells

Who got there first?

- Both groups filed their applications months apart in 2012
- UC Berkeley owns the patents in Europe
  - The Broad institute patents were invalidated due to technical issues
- The Broad institute owns the patents in the US
- Ownership of CRISPR-Cas9 has not yet been established in Canada
  - Both groups have filed patents with the Canadian Patent Office

Patent landscape of CRISPR-Cas9 makes it expensive to use

- The original patent dispute between UC Berkley and the Broad Institute from 2012 is still ongoing
- Over 11,000 patent claims have been filed
- Licencing fees to use CRISPR-Cas9 commercially range from $10,000 to $1,000,000

Companies in 2021 Founded on CRISPR-Cas9 Patents From the Original IP Dispute		
Jennifer Doudna University of California, Berkeley	**Feng Zhang** MIT-Harvard Broad Institute	**Emmanuelle Charpentier**
Caribou Biosciences	Sherlock Biosciences	CRISPR Therapeutics
Mammoth Biosciences	Arbor Biotechnologies	ERS Genomics
Intellia Therapeutics	Editas Medicine*	
Scribe Therapeutics	Beam Therapeutics	

* Jennifer Doudna was an original co-founder of Editas but is no longer involved after a reported falling out with Zhang.

Patent landscape of CRISPR-Cas9 for plants and agriculture

- There are often royalties associated with products developed with CRISPR-Cas9
- Depending on the specific use of Cas9 multiple licences may be necessary for a simple edit in a plant (Kock 2021)

Institution/ Patent holder	Surrogate company	Licensee	Field of application	Type of license*
Broad Institute, Harvard University & MIT (F. Zhang)	—	Bayer-Monsanto[12]	Agricultural applications (seed development)	Non-exclusive
		BASF[13]	Agricultural applications	
		Corteva Agriscience (DuPont Pioneer)[14]	Agricultural applications	
		Syngenta[15]	Agricultural applications	
	Pairwise[16]	—	Plant-based applications (fruits and vegetables)	
		Bayer-Monsanto[17]	Agricultural applications (in corn, soybean, cotton, wheat and canola)	Exclusive
University of California, Berkeley (J. Doudna)	Caribou Biosciences	Corteva Agriscience (DuPont Pioneer)[18]	Agricultural applications (major row crops) Other agricultural and industrial applications	Exclusive Non-exclusive
		Genus[19]	Livestock	Exclusive
		Regional Fish Institute[20]	Non-mammalian marine animals for agricultural purposes	Non-exclusive Asia Pacific
		TreeCo[21]	Trees	Exclusive
University of Vienna (E. Charpentier)	ERS Genomics	Evolva[21]	Food products (yeast and fungal engineering)	Non-exclusive
		Corteva Agriscience (DuPont Pioneer)[23]	All agricultural uses and applications in plants	Exclusive

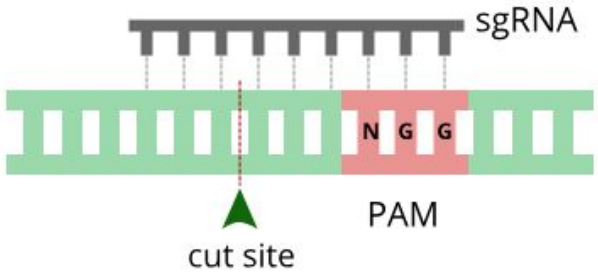
https://cban.ca/wp-content/uploads/Patents-on-Genome-Editing-cban-March-2022.pdf

MAD7 is a royalty-free endonuclease

- Engineered and released by Inscripta® in 2017
- Modified Cas12a endonuclease originating from the bacteria *Eubacterium rectale*
- Several improvements made to increase efficiency and decrease off-target editing

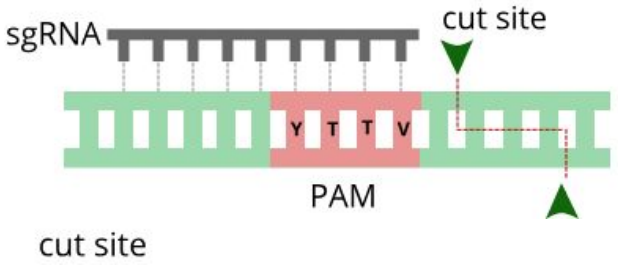
Cas9

- Multiple patent claims
- Royalties
- Expensive licencing fees
- Total sgRNA length = 100bp
- PAM site = 5’-NGG-3’
- Targets restricted to coding regions



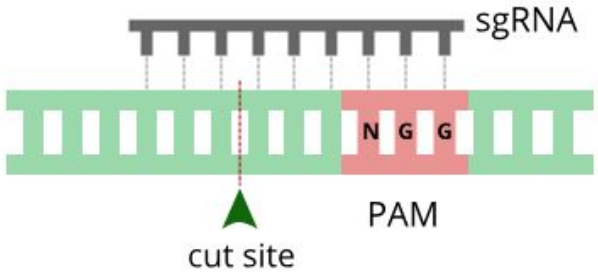
MAD7

- Clear ownership
- Royalty-free
- Reasonable licencing fees
- Total sgRNA length = 43bp
- PAM site = 5’-YTTV-3’
- More targets available in noncoding regions



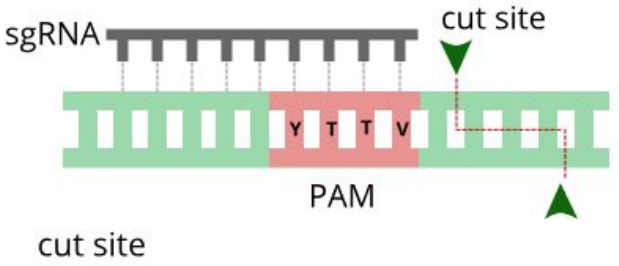
Cas9

- Creates blunt ends on cut site
- Cuts 3-5 base pairs upstream of PAM site
- Cuts within guide sequence



MAD7

- Creates 4bp overhangs on cut site
- Variable cut site
- Cuts outside of guide sequence



Conclusion

- MAD7 PAM site allows for greater targeting power
- Increased efficiency and decreased off-targeting
- MAD7 is a cheaper option to increase access to gene editing compared to Cas9