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Gene editing: Technical considerations

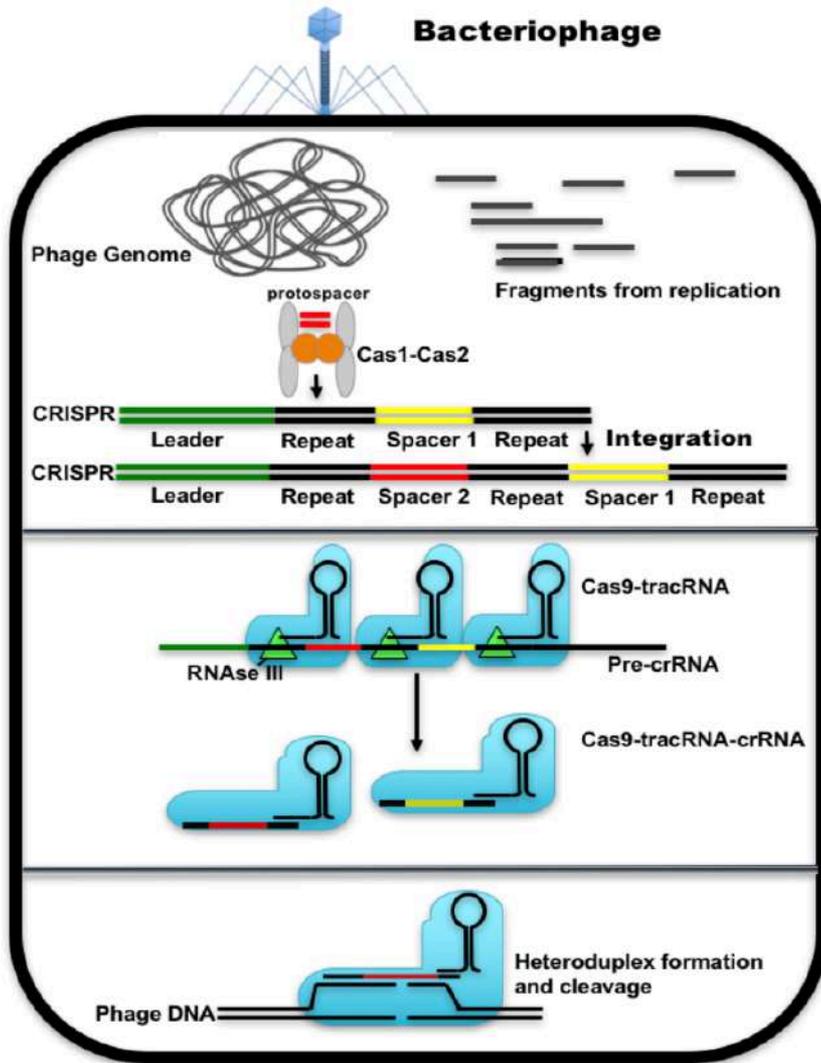
ARRIGE annual meeting: 14th November 2019

Dr Gaetan Burgio, MD, PhD,
The John Curtin School of Medical Research
The Australian National University, Canberra, Australia



@GaetanBurgio

CRISPR/Cas surveillance system



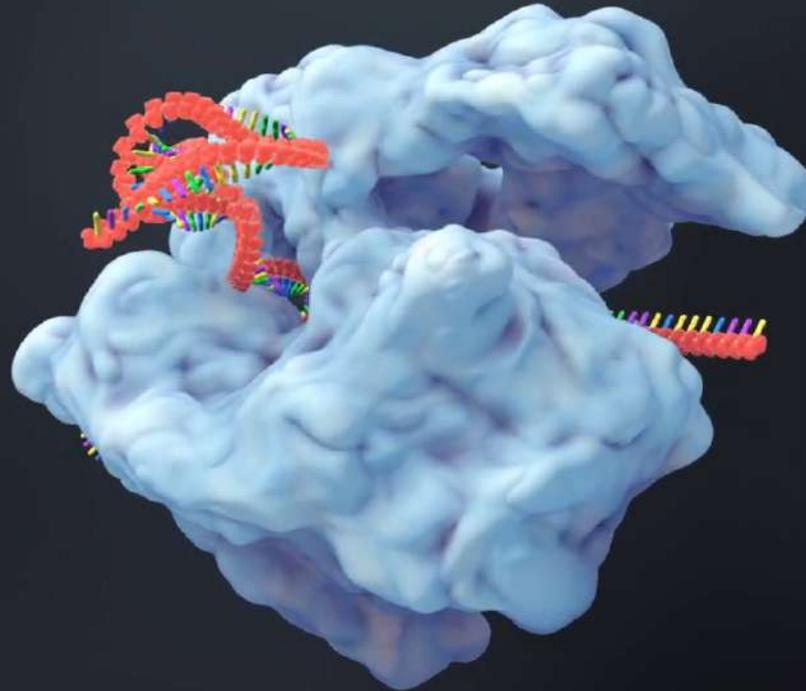
1. Adaptation: Creation of a genetic memory of past phage infections.

2. Processing: Production of mature CRISPR RNAs (crRNAs) from CRISPR arrays

3. Site specific targeting of phage nucleic acids via effector proteins

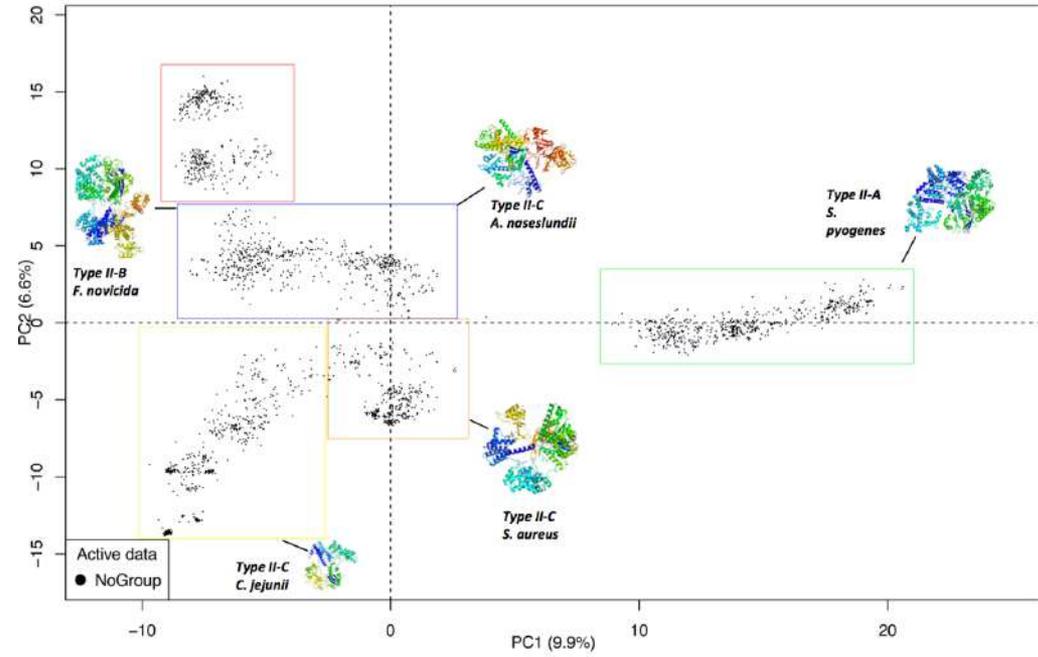
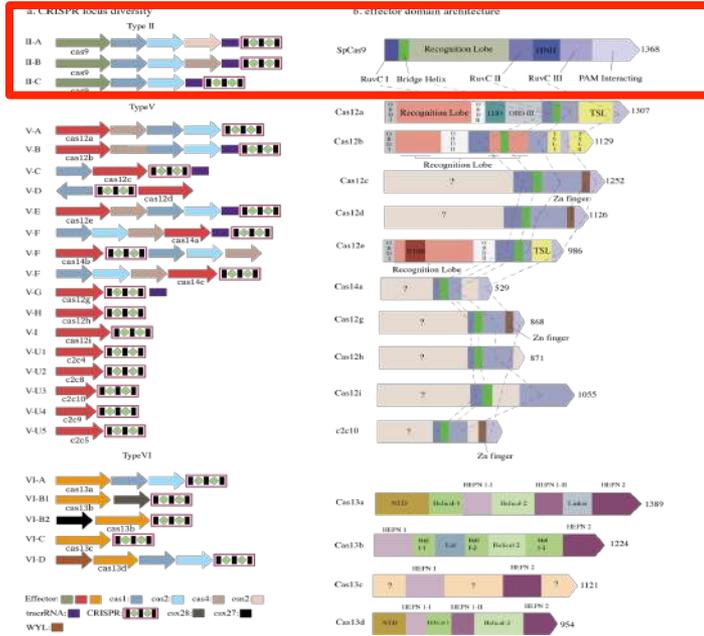


What is CRISPR-Cas9?





1/ CRISPR highly diverse family of anti phage defence systems



Alexander McKay

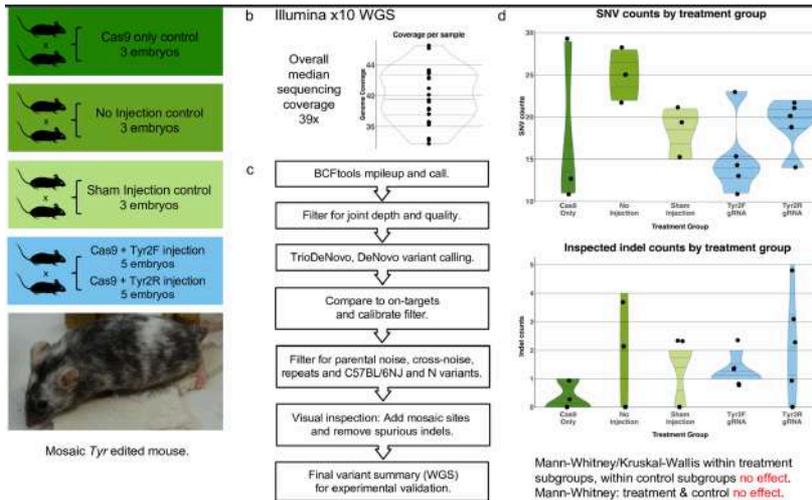
Hajizadeh-Dastjerdi et al. Biodrugs (in Press)

Anti-viral defence systems are highly diverse: 50 described + 33 subtype CRISPR systems

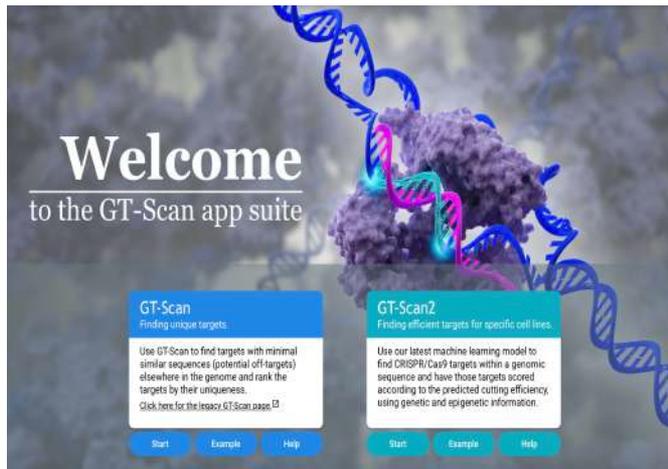
There are no perfect CRISPR system but could be optimised for specific applications



2/ Off target effects are not such a concern

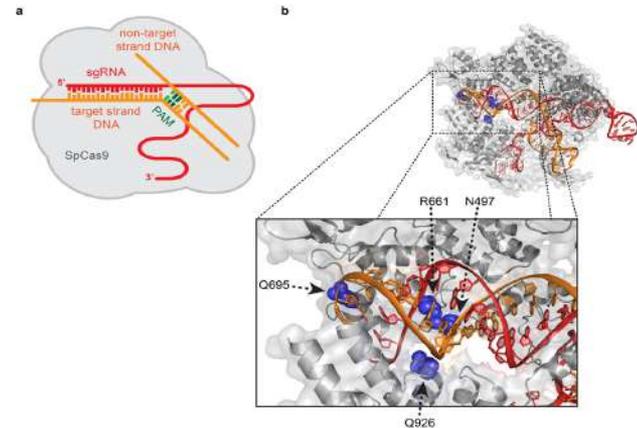


Lyer et al. Plos Genetics 14(7):e1007503



OFF target effects are predictable using machine learning

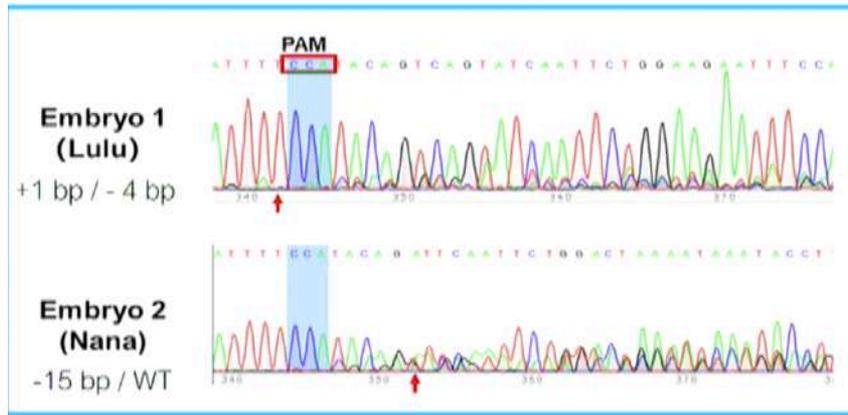
There are few off Target effects: Off targets sequences similar to the target DNA



Cas9 enzyme have been optimised to reduce off targets

3/ Mosaicism and On target effects are largely undetected

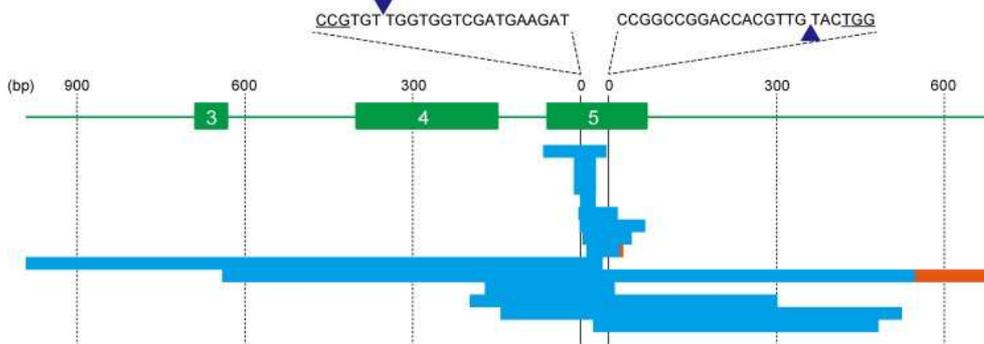
Edited Blastocysts



1_9736	1	9736	GTCCAGGACAGCCAGGGCTACACAGAGAGCTAGCTAACTTCGTATAGCATACATTAT
2_6192	2	6192	GTCCAGGACAGCCAGGGCTACACAGAGAAAACCTGTCTCGAAAAGCCAAAAA
3_4314	3	4314	GTCCAGGACAGCCAGGGCTATGGTGGCGACTCGGATGTTGAAACACAGCATGTCTGT
4_783	4	783	GTCCAGGACAGCCAGGGCTACACAGAGAAAACCTGTCCAGGGGAGTAAGGAGTCATGC
5_710	5	710	GTCCAGGACAGCCAGGGCTACACAGAGTAAGGAGTCATGCGGGGAGTACCGATCTCGT
6_603	6	603	GTCCAGGACAGCCAGGGCTGGAGTGGTGGAGTAAGGAGTCATGCGGGGAGTACCGATC
7_536	7	536	GTCCAGGACAGCCAGGGCTACAGAGTAAGGAGTCATGCGGGGAGTACCGATCTCGTAT
8_487	8	487	GTCCAGGACAGCCAGGGCTGAGGTCCAGGAGAGCTCTGCCACACAGCGCCACAC
9_417	9	417	GTCCAGGACAGCCAGGGCTAAGAAGTGGGAGTGGTGAAGGAGTCATGCGGGGAG
10_313	10	313	GTCCAGGACAGCCAGGGCTAAGCTGGAGCAGGCTGAAAGCACCATCAGGGAGCTCCGG
11_282	11	282	GTCCAGGACAGCCAGGGCTAGGGGCGAGTGTACGTGAAAGAGTAAGGAGTCATGCGGG
12_251	12	251	GTCCAGGACAGCCAGGGCTACACAGAGAAAACCTGTCTTGGGTTACGGGTGGAGGGG
13_227	13	227	GTCCAGGACAGCCAGGGCTAGTAAGGAGTCATGCGGGGAGTACCGATCTCGTATGCC
14_200	14	200	GTCCAGGACAGCCAGGGCTATACAGAGAAAACCTGTCTTAAAAAACAACAAAAACAA

Nikki Ross, Huiming Yang

Difficult to control the delivery of CRISPR reagent during early development



Ayabe et al. J Reproduction and Dev 2019 65(1): 1-5

Once the cut in the DNA is generated => Difficult to control its repair



4/ The CRISPR field has this constant cycle of discoveries – obstacles - resolution

1 *Nature Methods* publishes paper on off-target effects.

2 bioRxiv study suggests humans have preexisting immunity to Cas9.

3 *Nature Methods* retracts paper.

4 FDA places Crispr Therapeutics sickle cell program on clinical hold.

5 Two *Nature Methods* papers cause cancer scare.

■ Crispr Therapeutics
■ Editas Medicine
■ Intellia Therapeutics





STAT

BIOTECH

CRISPR-Edited Cells Linked to Cancer Risk in 2 Studies

The preliminary findings raise questions about one of the ways this tech edits genomes

By Sharon Begley, STAT on June 12, 2018



Thursday, November 14, 2019

[in depth](#) | [pointed remarks](#) | [about](#)

Does CRISPR cause cancer?

by Xavier Symons | 16 Jun 2018 | 1 comment



ARTICLE

Cell Stem Cell

Precise Gene Editing Preserves Hematopoietic Stem Cell Function following Transient p53-Mediated DNA Damage Response

Graphical Abstract

Authors

Giulia Schirolli, Anastasia Conti, Samuele Ferrari, ..., Pietro Genovese, Luigi Naldini, Raffaella Di Micco

Correspondence

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In Brief

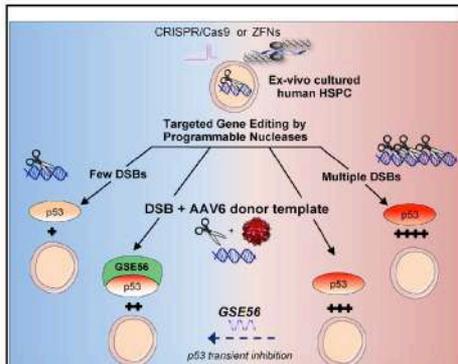
Precise gene editing has the potential to treat immune and hematological diseases. Genovese, Naldini, Di Micco, and colleagues now show that gene-editing procedures are well tolerated by hematopoietic stem cells and provide molecular evidence of the feasibility of

STAT+

IN THE LAB

CRISPR is ascending again, after scientists find 'elegant' fix for cancer worry

By SHARON BEGLEY @sxbegle / MARCH 21, 2019



6/ first clinical trials seems to show safety of the approach

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CRISPR-Edited Stem Cells in a Patient with HIV and Acute Lymphocytic Leukemia

Lei Xu, M.D., Ph.D., Jun Wang, M.D., Ph.D., Yulin Liu, B.S., Liangfu Xie, B.S., Bin Su, Ph.D., Danlei Mou, M.D., Ph.D., Longteng Wang, B.S., Tingting Liu, M.D., Xiaobao Wang, B.S., Bin Zhang, M.D., Ph.D., Long Zhao, Ph.D., Liangding Hu, M.D., et al.

Los Angeles Times

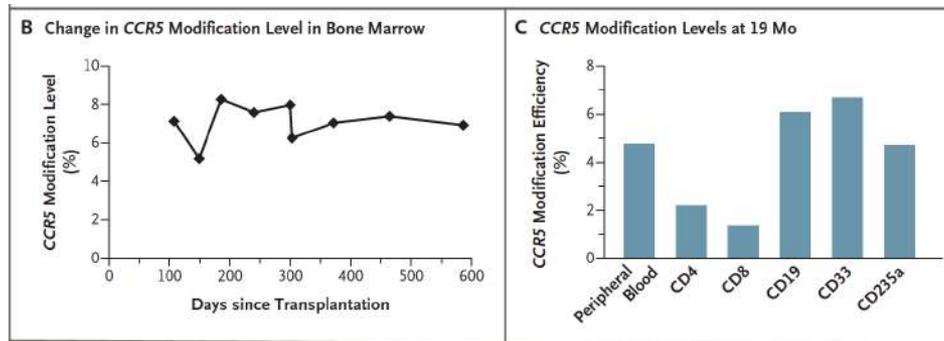
Doctors use CRISPR gene editing in cancer patients, a first in the U.S.



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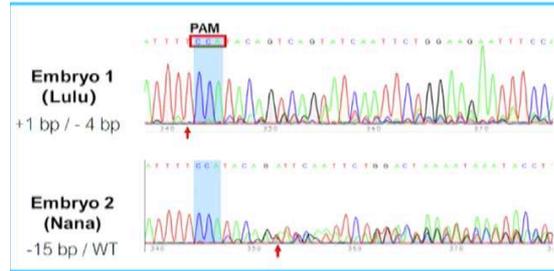
Efficiency in editing start from 18% down to ~ 5%

WGS no detectable off target effects. Despite high coverage (1000x), be cautious as the number of edited cells were low

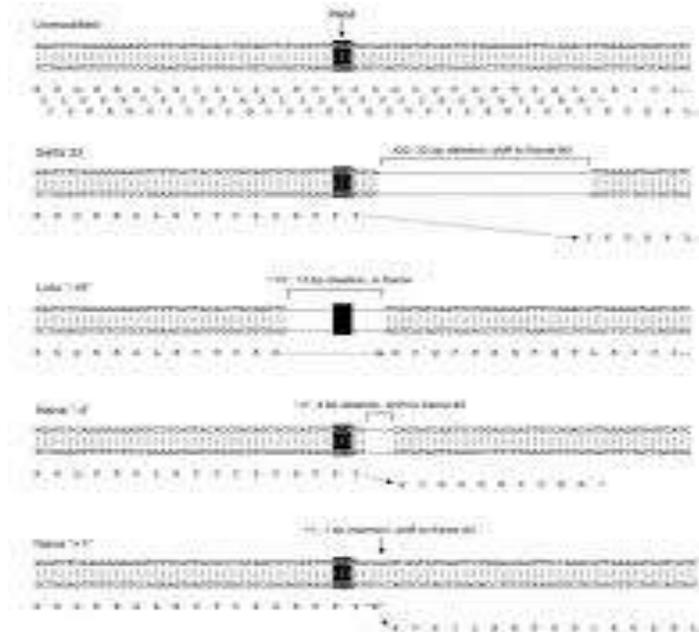


7/ Naivety of the approach has driven the Human Germline editing efforts From He and Rebrikov

Edited Blastocysts



Comparison of CCR5 alleles and their effects on protein coding



In conclusion :

Where the field is going:

Avoid double stranded break of the DNA

Improving the technology and exploring the diversity of
CRISPR

The CRISPR field is fast moving and this shouldn't be
underestimated

While a lot was discussed on the fact that the CRISPR
technology is not ready to ensure safety, there are/will be
improvement and the use of CRISPR technology for clinical
applications or food production

Acknowledgments

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Fei-Ju Li

Sadaf Ilyas



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