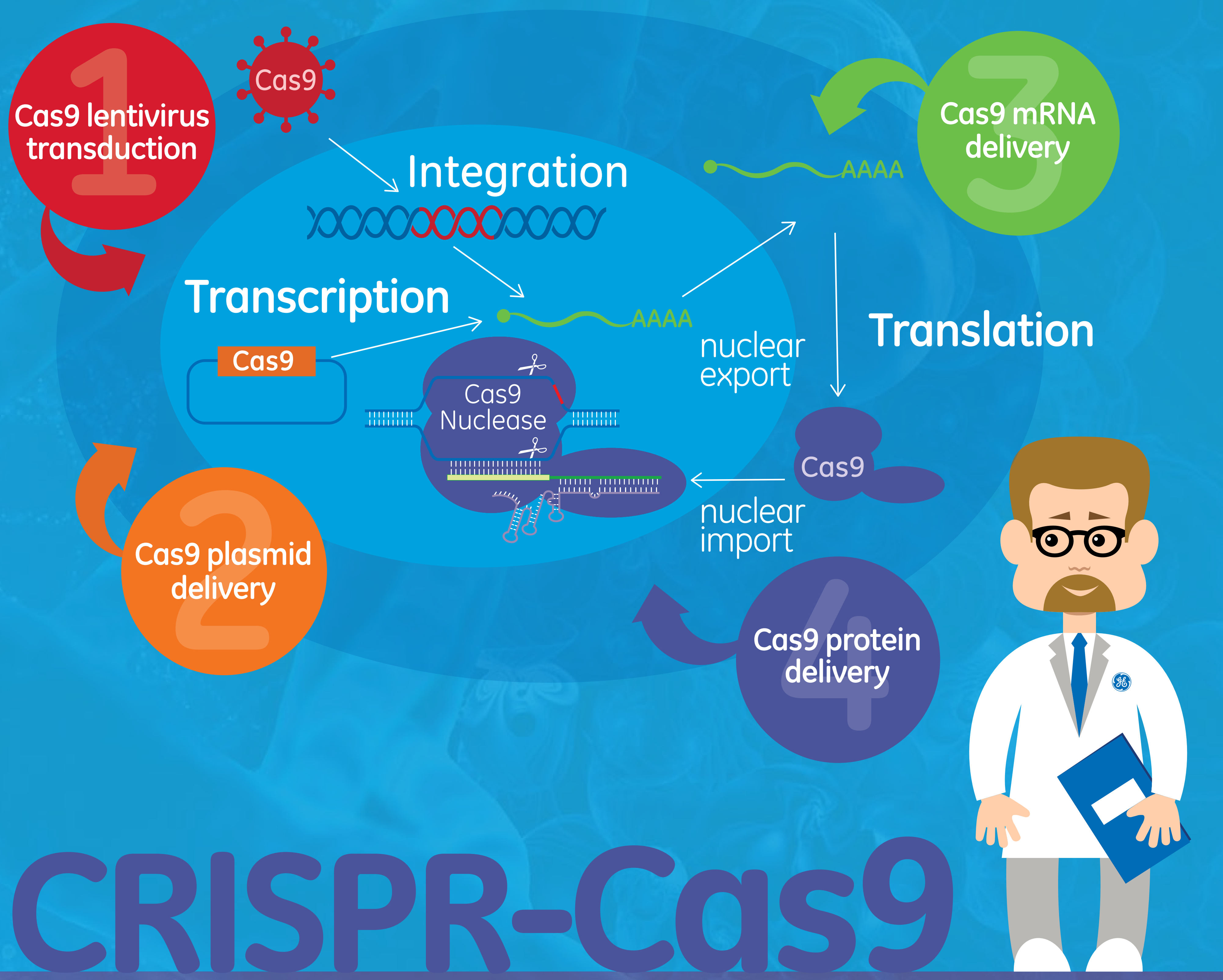
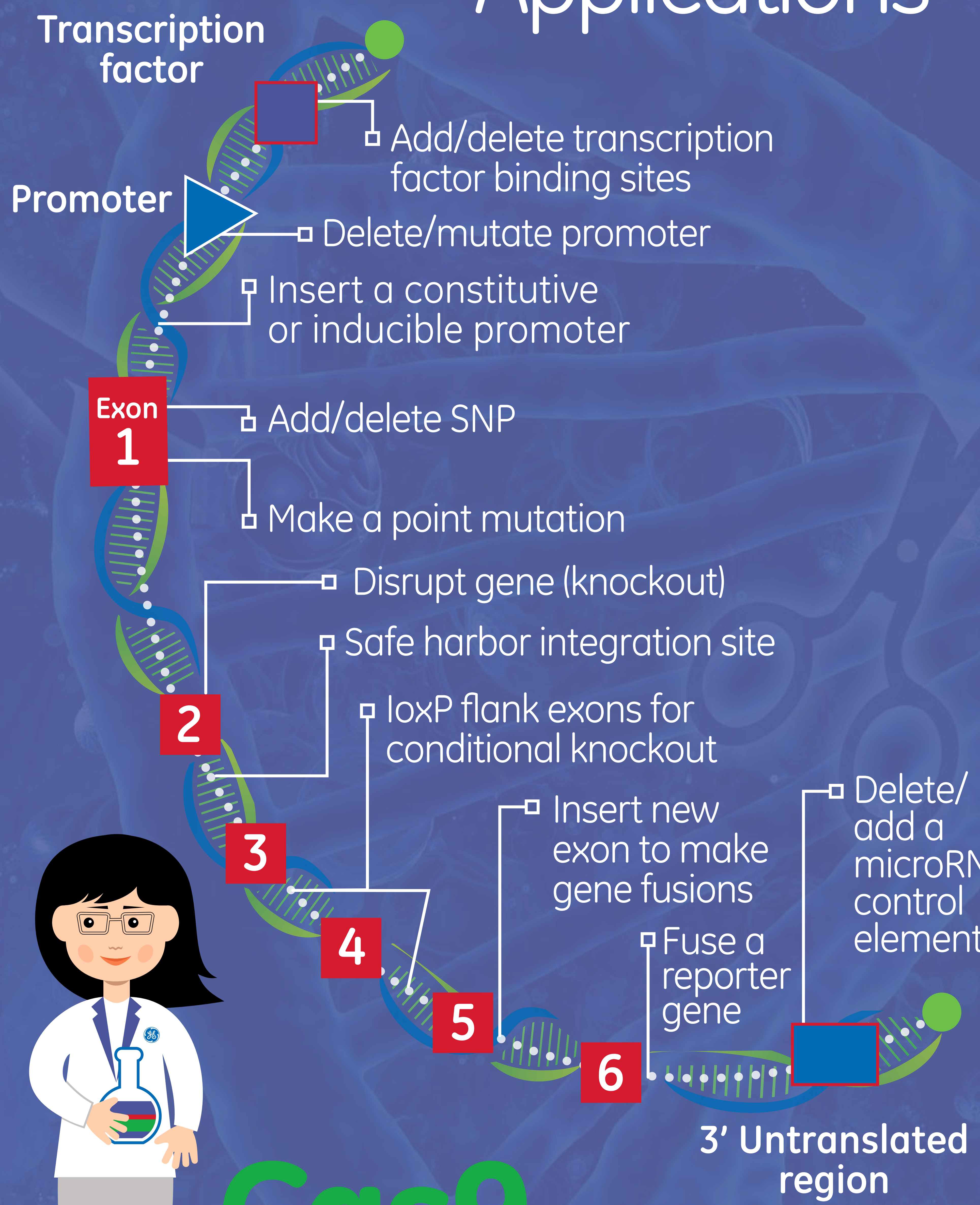
GE Healthcare

4 ways to express Dharmacon™ Cas9 nuclease



CRISPR-Cas9 Applications



Transcription factor

Add/delete transcription factor binding sites

Promoter

Delete/mutate promoter

Insert a constitutive or inducible promoter

Exon 1

Add/delete SNP

Make a point mutation

Disrupt gene (knockout)

Safe harbor integration site

2

loxP flank exons for conditional knockout

3

Insert new exon to make gene fusions

4

5

Fuse a reporter gene

6

Delete/add a microRNA control element

3’ Untranslated region

Cas9 Timeline

1987 Clustered regularly interspaced short palindromic repeats discovered in *E. coli*[1]

2000 CRISPR discovered in other bacteria & archaea[2]

2002 *Cas* genes discovered[3,4]

2005 CRISPR sequences discovered to come from extrachromosomal DNA (phage, plasmid)[5,6]

2007 CRISPR-Cas discovered to provide adaptive immunity in prokaryotes[7]

2011 CRISPR-Cas mechanism includes tracrRNA[8]

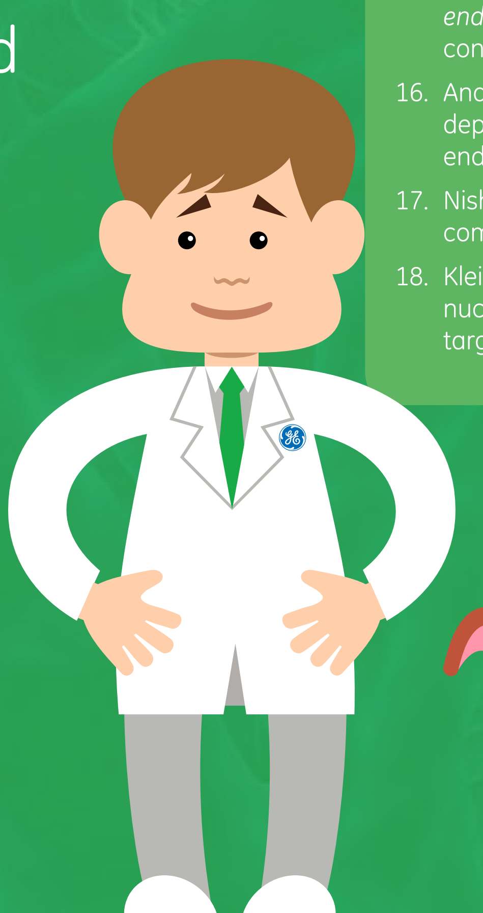
Cas9 is only *cas* gene necessary for bacterial defense mechanism[9]

2012 CRISPR-Cas9 first used in mammalian cells[10]

2013 Deactivated-Cas9 developed to repress/activate endogenous genes[11,12,13,14]

2014 Cas9 crystal structure solved[15,16,17]

2016 High-fidelity Cas9[18]



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CRISPR-Cas9 Fun Facts & Trivia

Extremely **muscled dogs** have been created by disrupting both copies of the **myostatin gene**. *(MIT Technology Review)*

Science magazine named **CRISPR** the 2015 “Breakthrough of the year”

October 2015, researchers modified **over 60 genes in pigs** with the goal of a steady supply of human donor organs. *(Nature)*

Therapeutic application areas announced in **2016**: HIV, Duchenne’s muscular, dystrophy, Beta thalassemia, Retinitis pigmentosa.

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