CENTURY THERAPEUTICS

CRISPR Genome editing components used for ex-vivo genome-editing of allogeneic cell therapy products

Their clearance and their effects

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CASSS CGTP 2025
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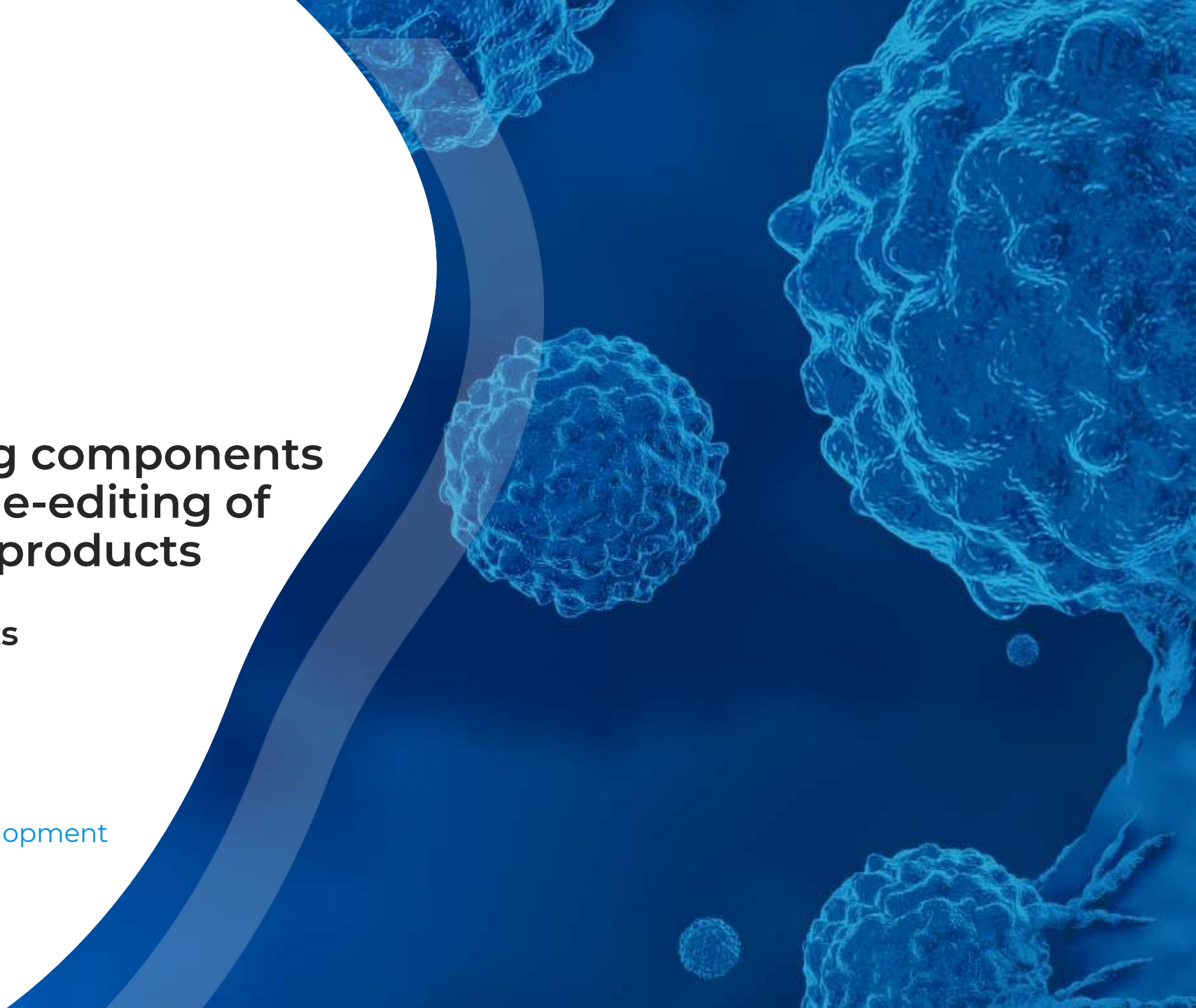
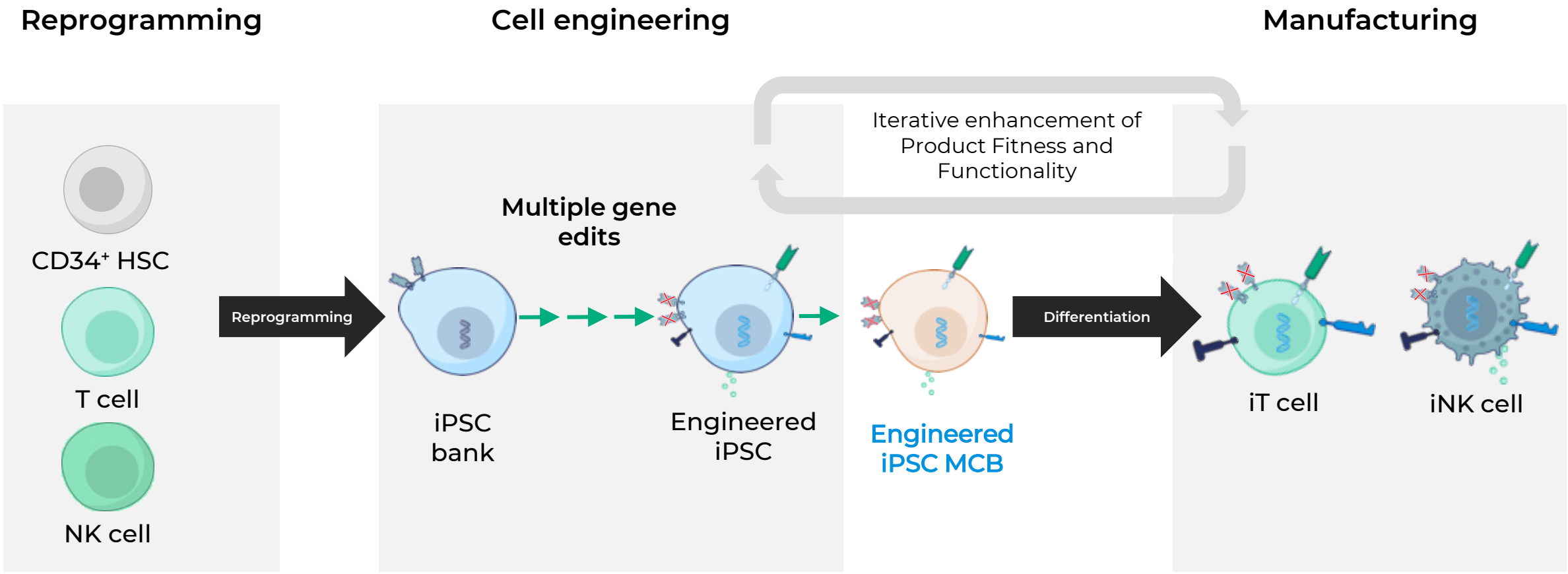


Century's end-to-end platform has the key components to realize potential of iPSCs



Where are genome editing (GE) components introduced?

- **Clonal iPSC-derived allogeneic cell therapy genetic engineering is performed during cell line development and the time in culture post-genetic engineering is substantially longer.**

- **Post-genetic engineering time in culture is typically short for autologous and donor derived allogeneic cell therapies.**

- **GE components are typically introduced once in iPSC-derived products compared to every time for autologous products**
  - The <u>risk</u> of these residuals may <u>not be the same</u> across modalities

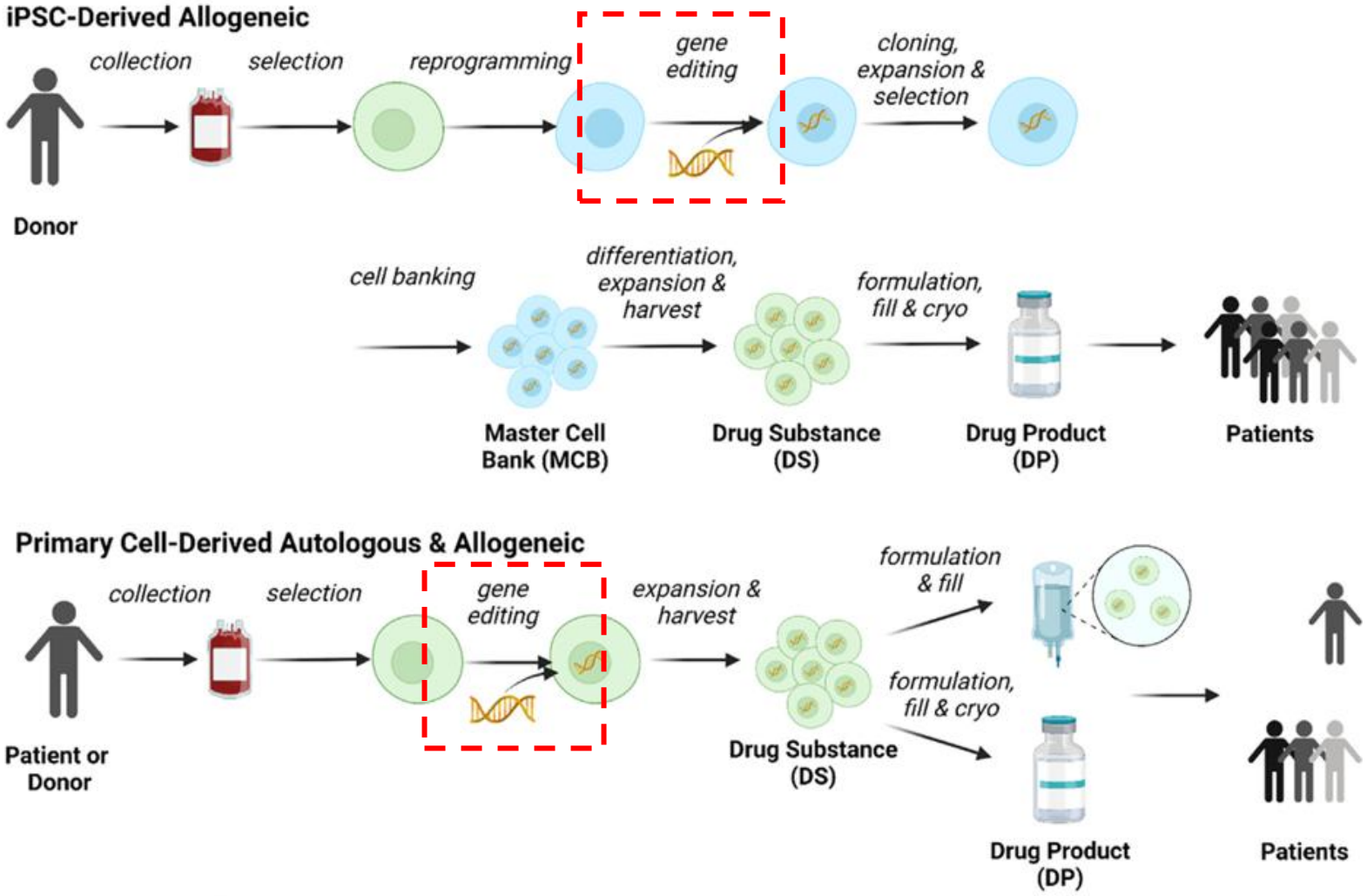


Figure from: Dashnau JL, Xue Q, Nelson M, Law E, Cao L, Hei D. A risk-based approach for cell line development, manufacturing and characterization of genetically engineered, induced pluripotent stem cell-derived allogeneic cell therapies. Cytotherapy. 2023 Jan;25(1):1-13. doi: 10.1016/j.jcyt.2022.08.001. Epub 2022 Sep 13. PMID: 36109321.

FDA guidance on residual genome editing (GE) components?

FDA Guidance

Release testing of ex vivo-modified human GE DPs should include evaluation of on-target editing efficiency and the total number (or frequency) of genome-edited cells. Additional characterization of the editing events occurring at the on-target site should also be performed. Assessments of off-target editing frequency, intrachromosomal and interchromosomal rearrangements, and residual GE components may also need to be included for release of the DP based on the outcomes of nonclinical studies. We also recommend that the number of edited cells or the frequency of GE be monitored during stability testing of ex vivo-modified human GE DP.

Excerpt from: U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research (CBER) (2024) Human Gene Therapy Products Incorporating Human Genome Editing; Guidance for Industry

Allogeneic cell therapy testing recommendations are more extensive but agnostic to donor vs. iPSC-derived therapies

- **By nature, allogeneic cell therapies are designed to be administered to multiple patients as opposed to the individualized medicine approach of autologous therapies leading to additional considerations**

- **As compared to donor-derived allogeneic therapies, iPSC-derived therapies have these additional considerations:**
  - Administered to even more patients
  - Manufacturing process substantially longer
  - Demonstration of genomic stability expected
  - But importantly GE components are only added during cell line development

FDA Guidance

Please note that if the ex vivo-modified human GE DP is an allogeneic human cell product, where a product lot is meant to treat multiple patients, additional DP testing and establishment of acceptance criteria may be appropriate. For example, additional adventitious agent testing, stringent acceptance criteria for the number of potentially alloreactive lymphocytes and absence of aberrant growth (i.e., if the DP is an allogeneic T cell product) should be included in lot release testing. Additional information on allogeneic products, including donor eligibility and testing recommendations for cell banks originating from allogeneic cells or tissues, are discussed in the GT CMC Guidance (Ref. 3)

Additional in-process, lot release, and characterization testing may be needed for more complex products (e.g., products incorporating multiple rounds of genome editing or the creation of multiple cell banks). Also, the timing and type of testing may depend on when the GE process is performed in manufacturing. For example, if a genome edited MCB is used to produce the DP without additional GE steps, some testing may be able to be performed on the MCB.

Excerpt from: U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research (CBER) (2024) Human Gene Therapy Products Incorporating Human Genome Editing; Guidance for Industry

Allogeneic cell therapy testing recommendations are more extensive but agnostic to donor vs. iPSC-derived therapies

FDA acknowledges that the context around the GE process can result in changes to the testing strategy.

FDA Guidance

Please note that if the ex vivo-modified human GE DP is an allogeneic human cell product, where a product lot is meant to treat multiple patients, additional DP testing and establishment of acceptance criteria may be appropriate. For example, additional adventitious agent testing, stringent acceptance criteria for the number of potentially alloreactive lymphocytes and absence of aberrant growth (i.e., if the DP is an allogeneic T cell product) should be included in lot release testing. Additional information on allogeneic products, including donor eligibility and testing recommendations for cell banks originating from allogeneic cells or tissues, are discussed in the GT CMC Guidance (Ref. 3)

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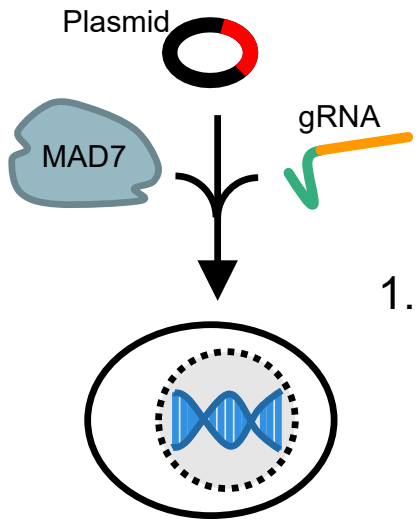
What are the genome editing components used by Century?

- **As part of cell-line development, three genome-editing components are introduced into our iPSCs**

1. Various CRISPR nucleases
2. gRNA

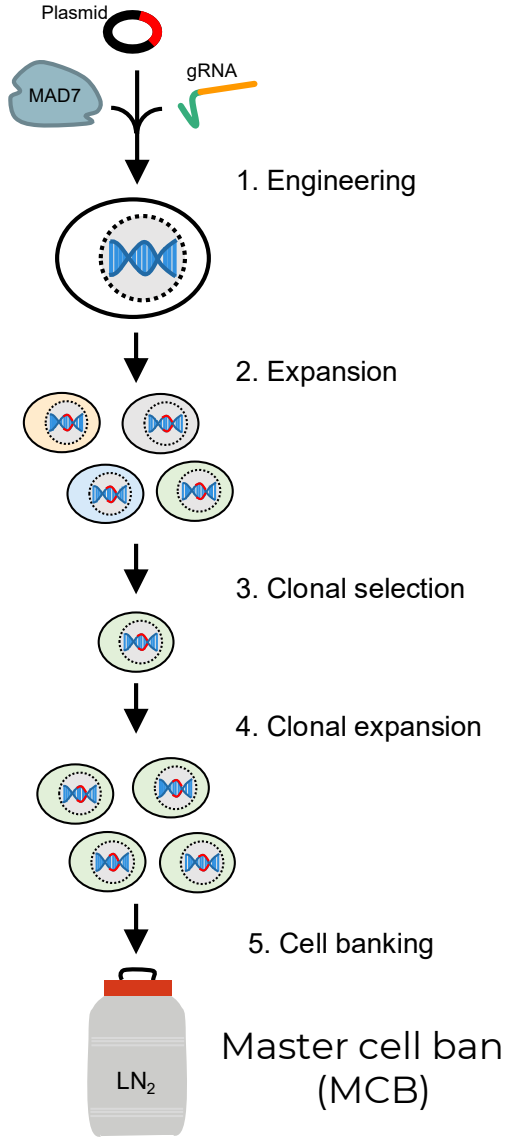
} Ribonucleoprotein (RNP) complex

3. Plasmid DNA with transgene



1. Engineering

Cells move to long-term culture after genome editing



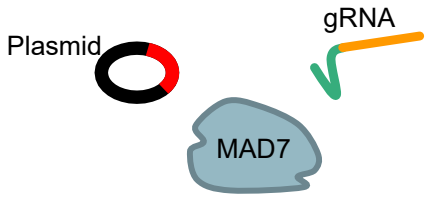
Genome editing components introduced

Minimum of 29 doublings (~10 passages)[1] but in practice approximately 30 passages are needed

Testing for Genome editing components

[1]Pakzad M, Hassani SN, Abbasi F, et al (2022) A Roadmap for the Production of a GMP-Compatible Cell Bank of Allogeneic Bone Marrow-Derived Clonal Mesenchymal Stromal Cells for Cell Therapy Applications. Stem Cell Rev Rep 18:2279–2295. https://doi.org/10.1007/s12015-022-10351-x

How to test genome editing components for future products?



1

Redesign new assays for each component

Not scalable & Testing Required

2

Universal assays that detect fluctuating components

Fairly scalable & Testing Required

3

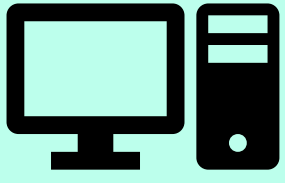
“Validate out” the risk for residual components

Highly scalable & No Routine Testing

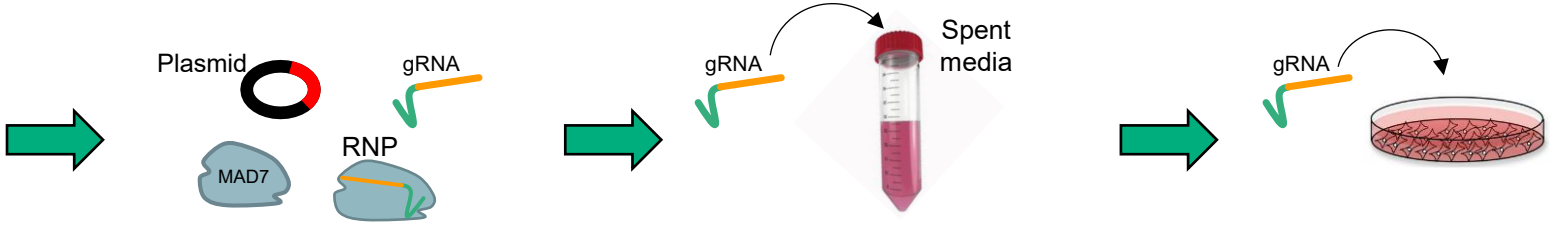
CENTURY THERAPEUTICS

Century follows a sequence of procedures to demonstrate that active engineering residuals are not present in our platform

1. **Modeling analysis of retained residuals**
   - Extracellular retention in cell media
   - Intracellular retention
2. **Determining requirements for genome editing using CRISPR**
3. **Stability of genome editing components in cell culture mimicking conditions**
4. **Stability of genome editing components during *ex vivo* engineering of iPSCs**



1. Theoretical Risk

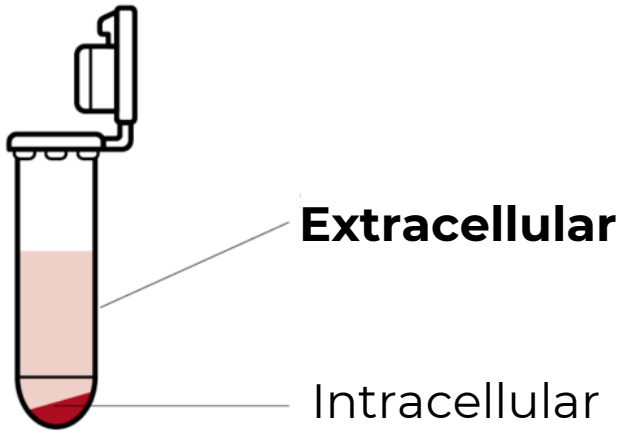


2. Genome editing potential

3. Stability in mimicking conditions

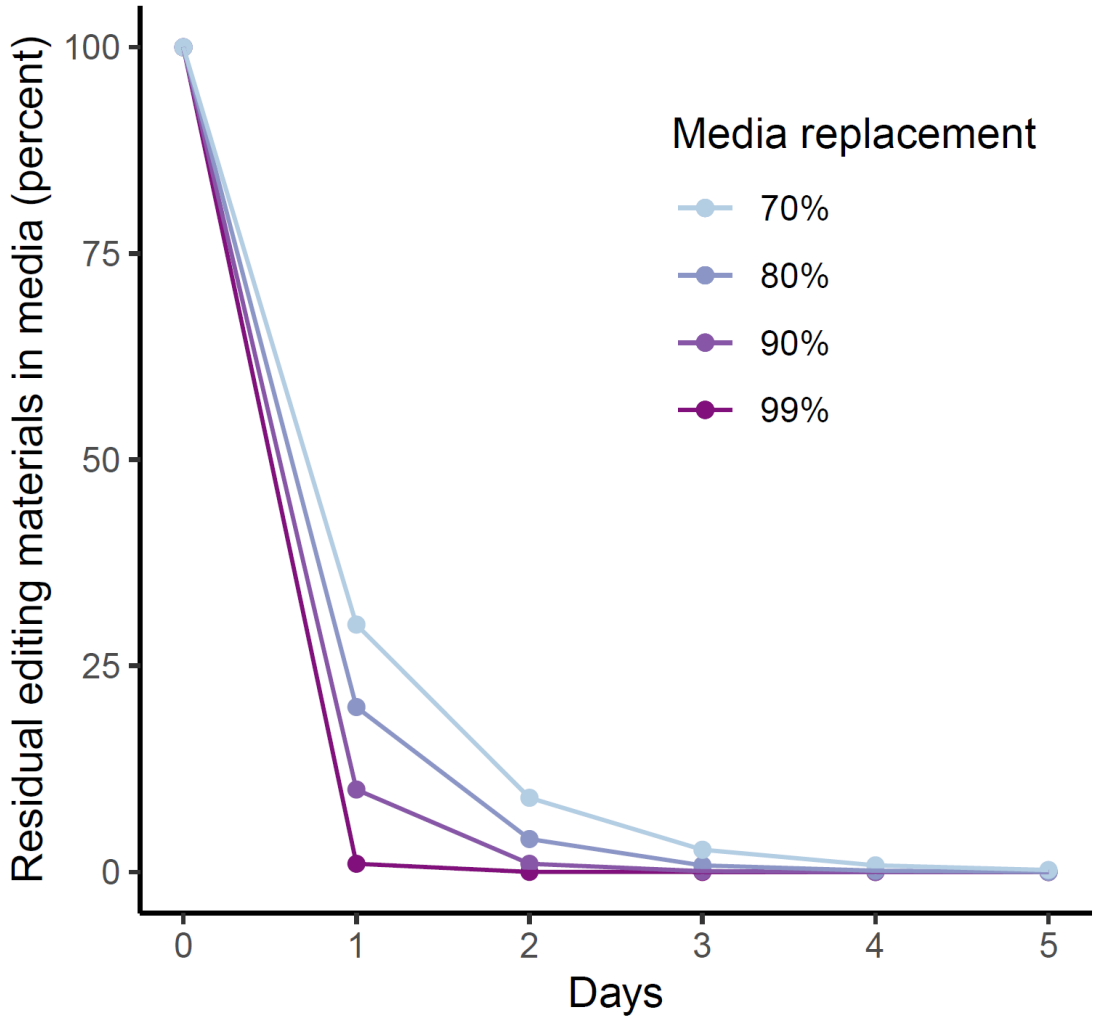
4. Stability in culture

Modeling analysis of retained residuals - extracellular

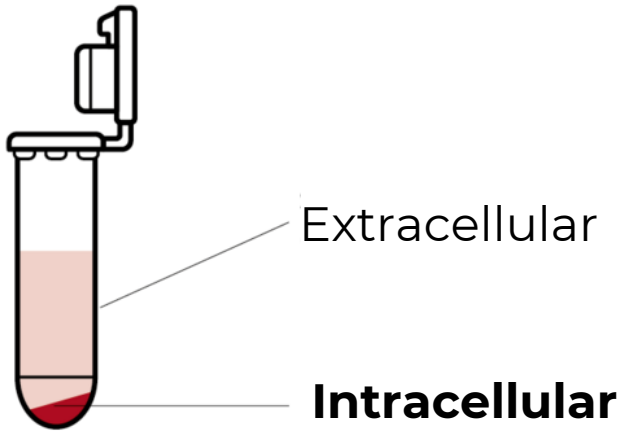


- Cells receive a full media changes during cell line development

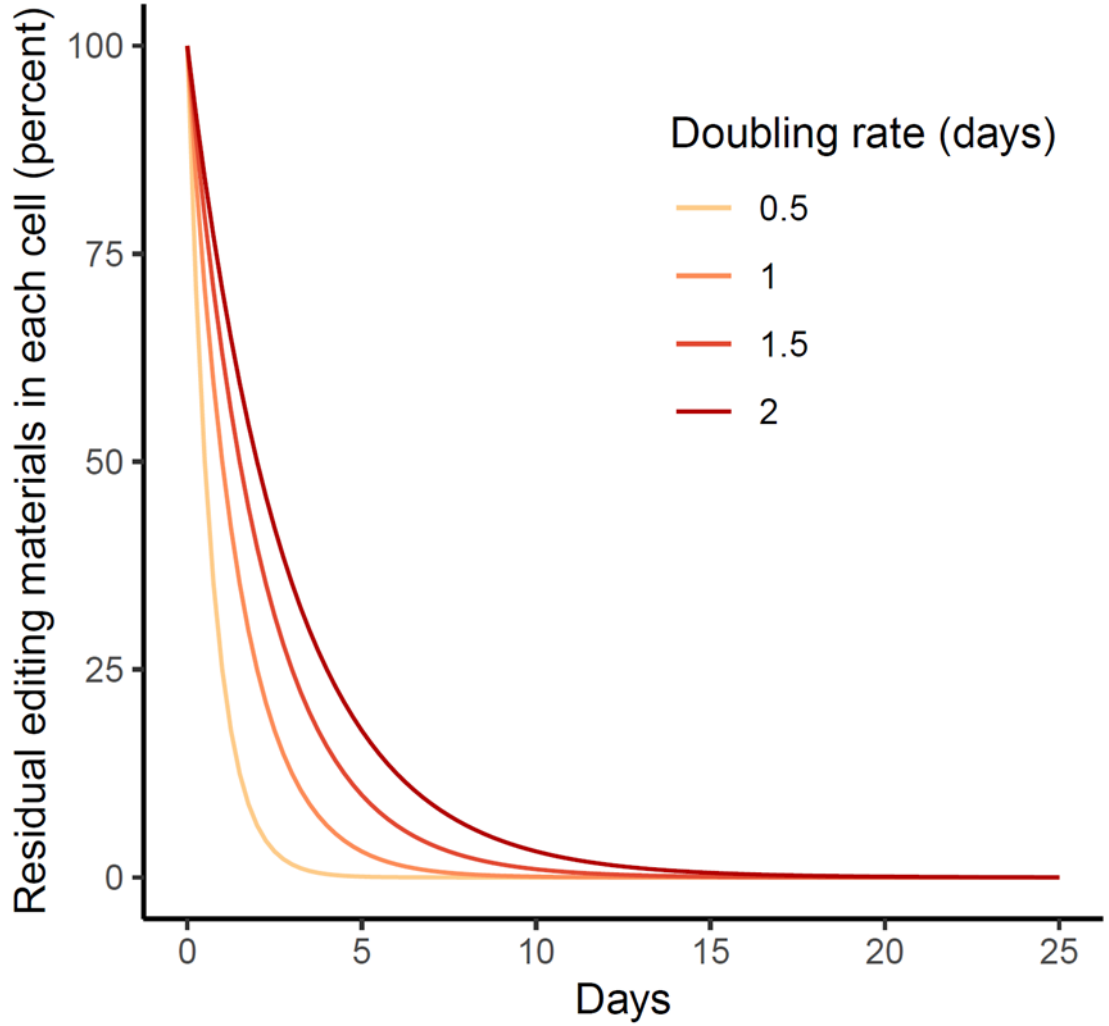
- Daily media changes of at least 70% result in nearly all extracellular gene editing components being washed out after 5 days (<0.24% remaining)



Modeling analysis of retained residuals - intracellular



- Per cell residuals drop exponentially with cell growth
- Even with a doubling time of 2 days, the per cell intracellular residual material nears zero after approximately 20 days



Century follows a sequence of procedures to demonstrate that active engineering residuals are not present in our platform

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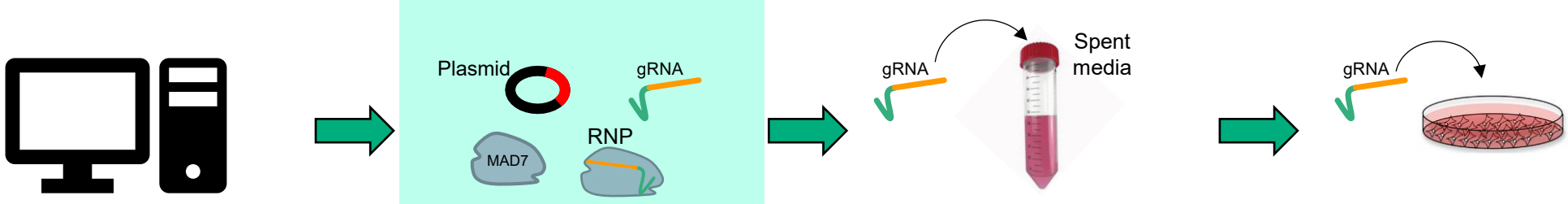

1. Theoretical Risk

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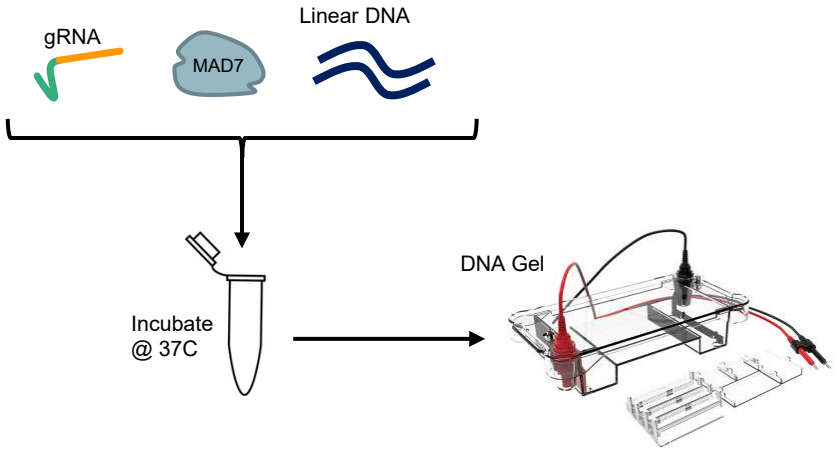
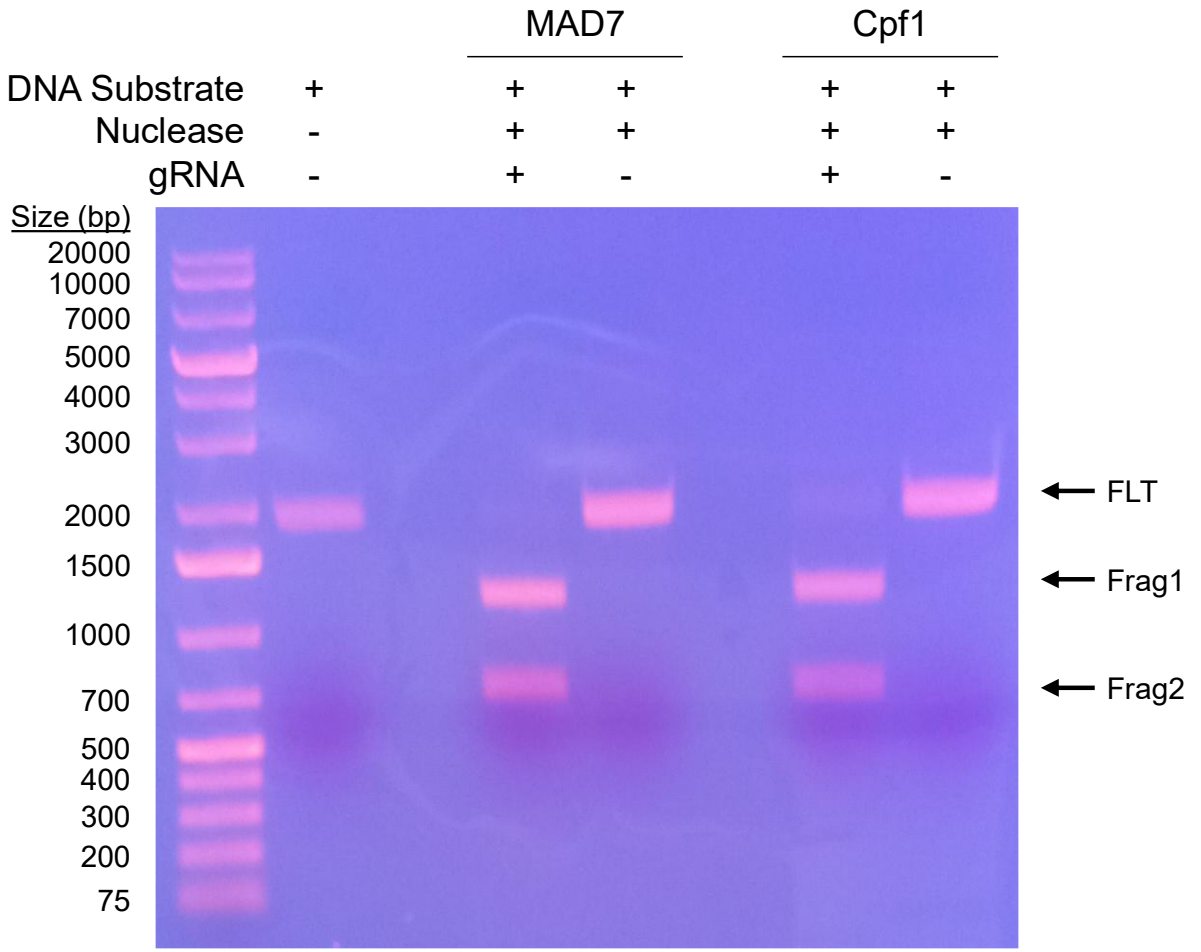
4. Stability in culture

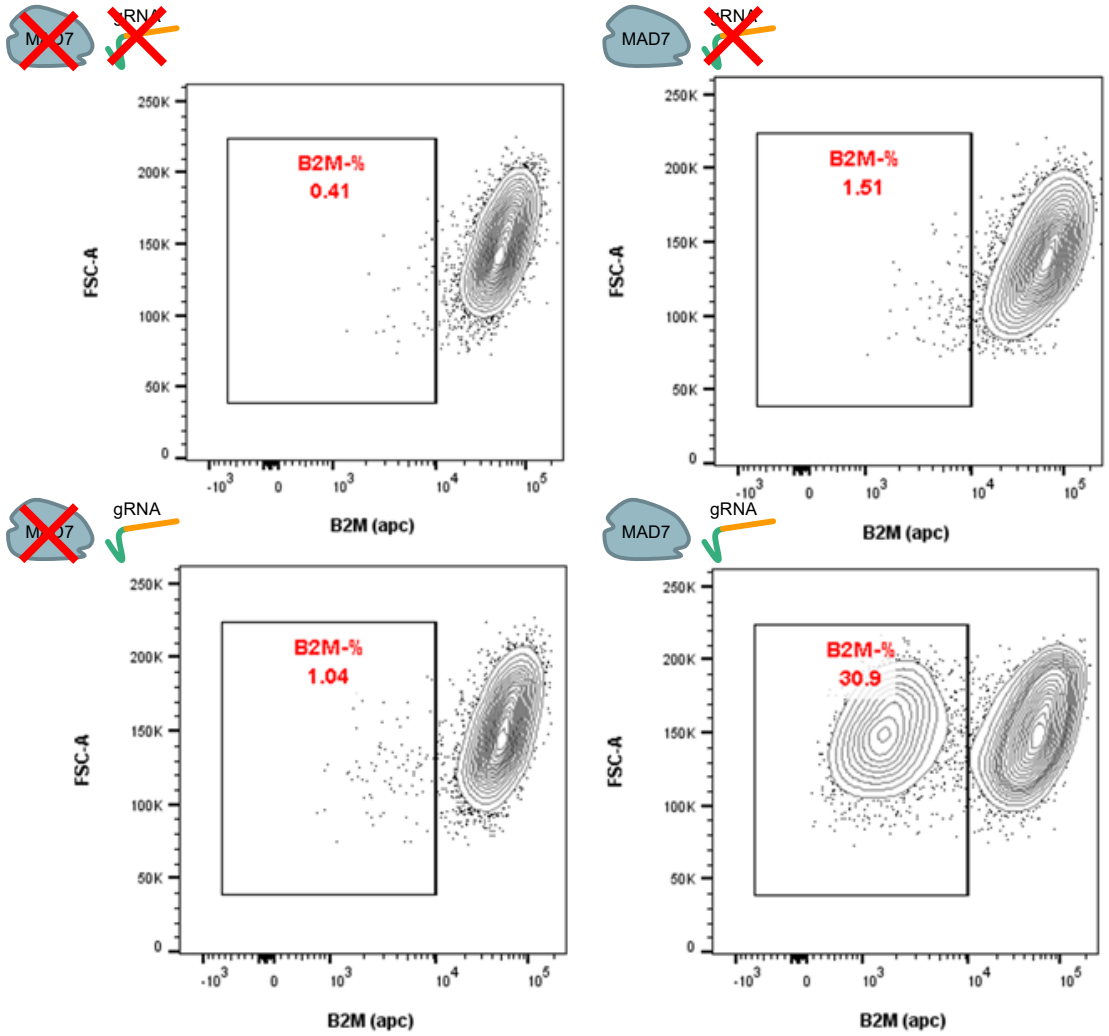
MAD7 CRISPR editing requires gRNA and Nuclease

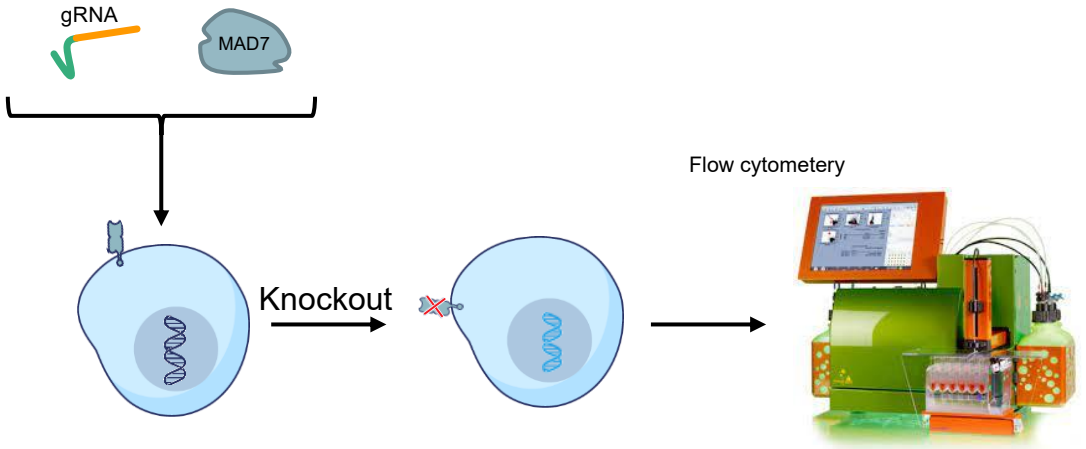




- MAD7 and Cpf1 require gRNA to create a double stranded DNA break *in vitro*
  - Together gRNA and nuclease form an active Ribonucleoprotein (RNP) complex

MAD7 CRISPR editing requires gRNA and Nuclease





- RNP complex is required for gene editing *ex vivo*
- Residual testing on one component is sufficient to rule out the possibility of continued target action
- This does not rule out any off-target action

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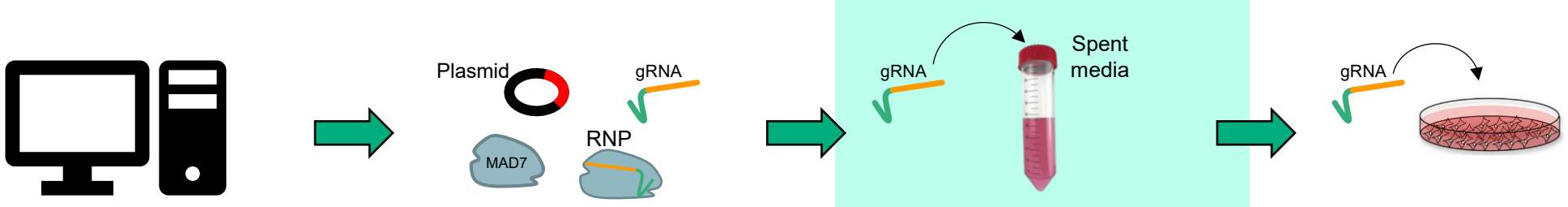

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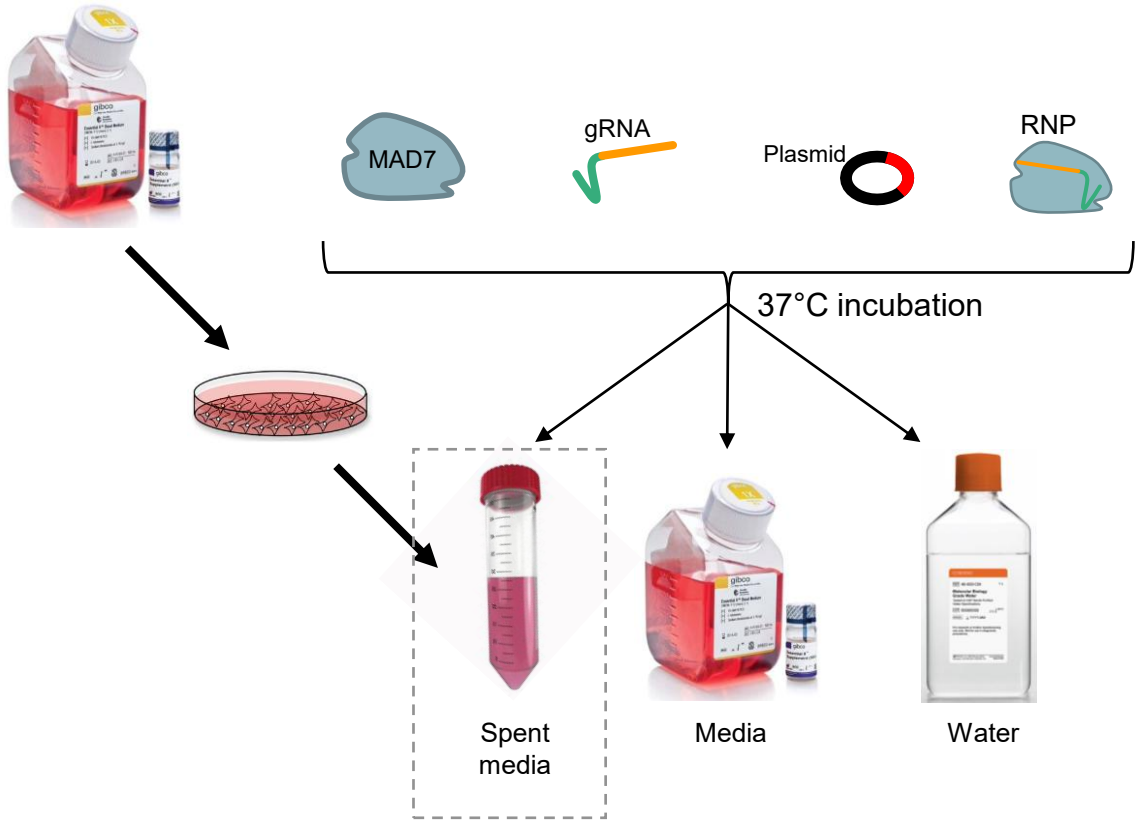
CENTURY THERAPEUTICS

Stability of genome editing components in cell culture mimicking conditions

- **The stability of genome editing components was evaluated in 3 condition**
  - Spent media (media previously cultured with iPSCs for 1 day)
  - Media
  - Molecular grade water

- **Testing was performed in a range of 4 hours to 50 days at 37°C**

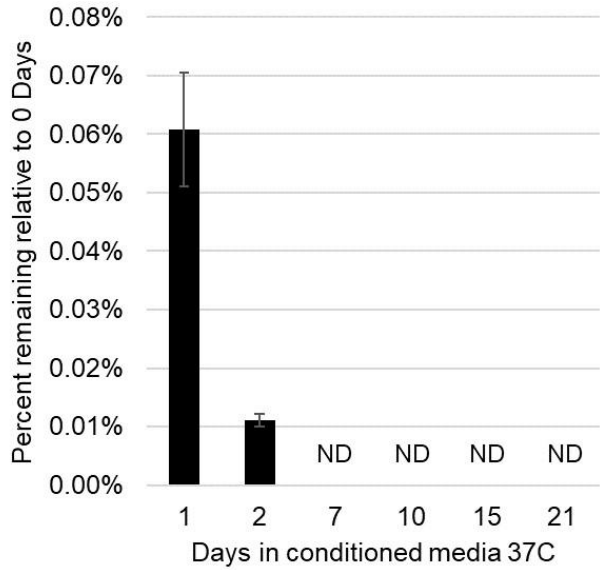
- **The spent media results will be discussed as they most closely resemble conditions to which the engineering components will be exposed.**



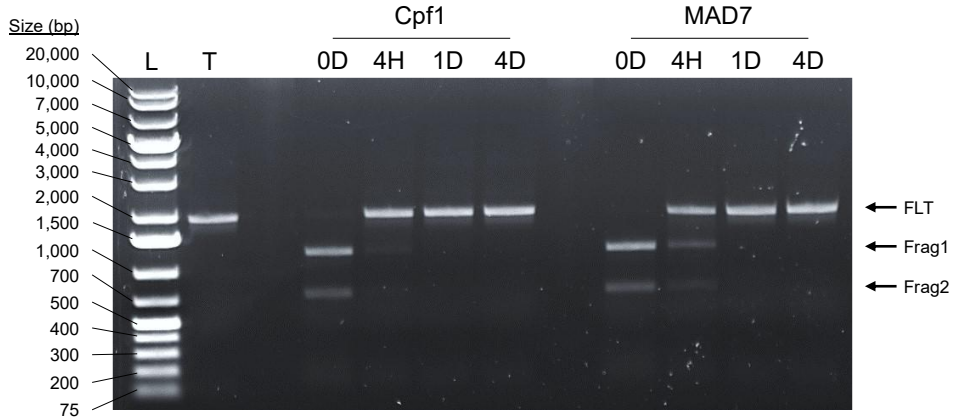
Nuclease, gRNA and RNP complex are short-lived in spent media

- **gRNA was detectable for less than 7 days**
  - Detected by ddPCR
- **RNP complex and Nuclease alone (not shown) were found to have activity for less than 1 day (1D)**

gRNA



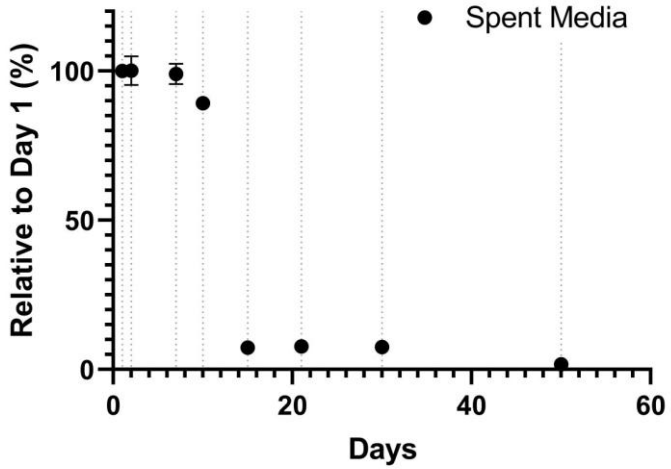
RNP



Plasmid DNA is stable in spent media

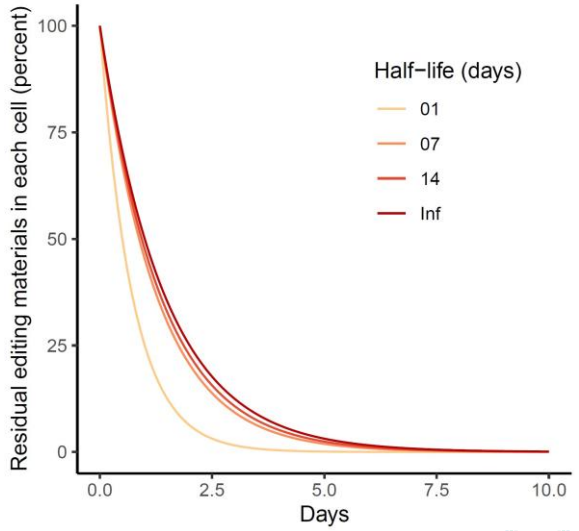
- **Plasmid DNA was detectable for at least 50 days**
  - Detected by ddPCR

Plasmid



- **Introducing degradation into the model shows an enhanced decline in engineering residuals per cell**

Model



Most components required for genome editing are short-lived

Stability of CRISPR genome editing components at 37°C in cell culture mimicking conditions

		Nuclease						RNP						gRNA			Plasmid		
		Cpf1			MAD7			Cpf1			MAD7								
	Condition	S	M	W	S	M	W	S	M	W	S	M	W	S	M	W	S	M	W
Days (D)	0 D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1 D	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
	7 D	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
	21 D	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-
	30 D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
	50 D																+	+	-

Short active life
&
Required for genome editing

Longer lived
&
Incomplete for genome editing

Detection of each material is indicated for the indicated duration in days (D). Detection or no detection is indicated by a + or – respectively:

- Spent media (S)
- Media (M)
- Nuclease free water (W)

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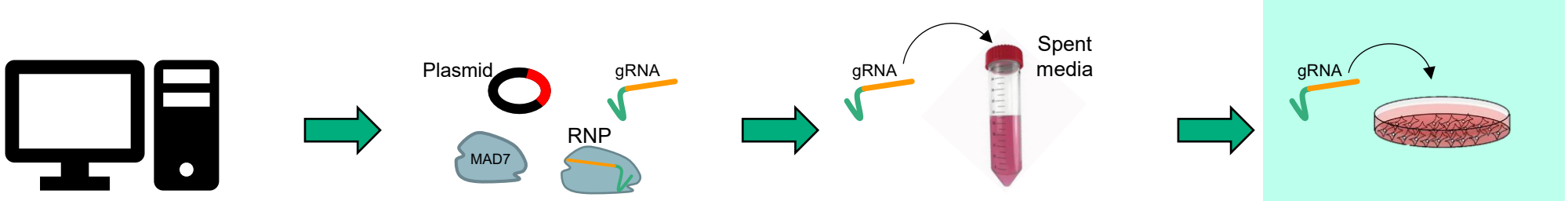

1. Theoretical Risk

2. Genome editing potential

3. Stability in mimicking conditions

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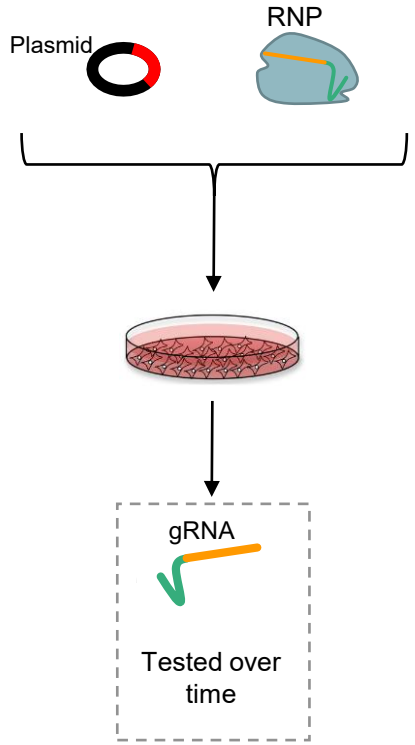
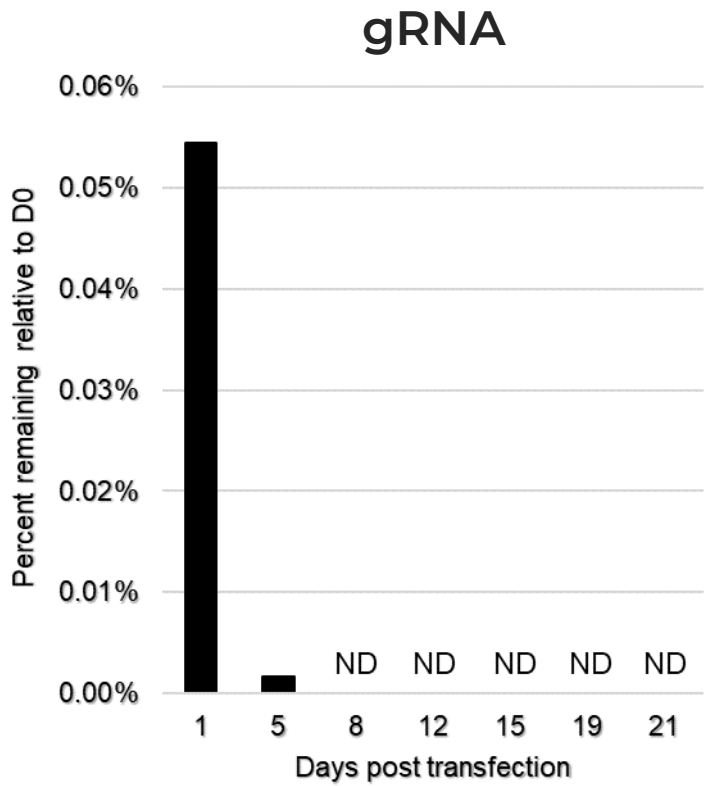
Stability of genome editing components in *ex vivo* genetic engineering

- **Ex vivo genetic engineering was performed following our clinical cell line development procedure**
  - Residual gRNA was detected post-engineering
  - gRNA is most stable of the non plasmid components and has the most sensitive analytical test (ddPCR)



gRNA stability during ex vivo engineering of iPSCs

- **Following our cell engineering protocol gRNA was found at detectable levels for <8 days**
  - In line with our findings, LiveFISH using dCAS9 shows gRNA stability >3 days in culture[1]
- **Single cell cloning alone require approximately 1 month of culture time**
  - From literature, a minimalistic clonal MCB that occurs no losses could be established in 29 doublings (~29 days of culture)[2]

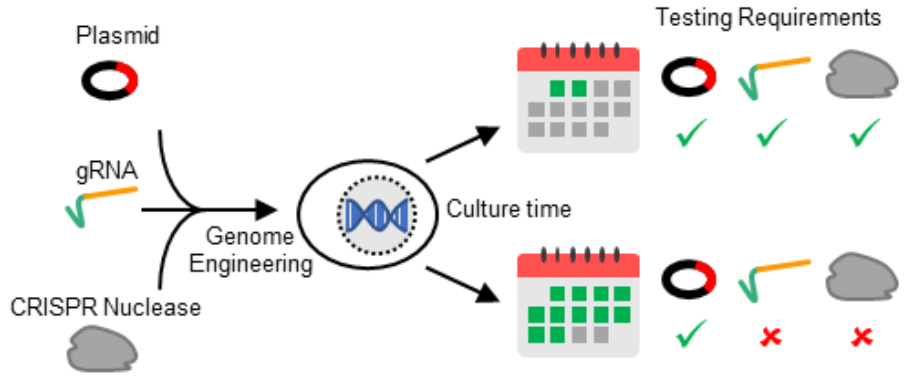


[1]Wang H, Nakamura M, Abbott TR, et al (2019) CRISPR-mediated live imaging of genome editing and transcription. Science (1979) 365:1301–1305. https://doi.org/10.1126/science.aax7852

[2]Pakzad M, Hassani SN, Abbasi F, et al (2022) A Roadmap for the Production of a GMP-Compatible Cell Bank of Allogeneic Bone Marrow-Derived Clonal Mesenchymal Stromal Cells for Cell Therapy Applications. Stem Cell Rev Rep 18:2279–2295. https://doi.org/10.1007/s12015-022-10351-x

Conclusion

- **Clonal iPSC-derived allogeneic cell therapies require months of culture time to generate appreciable amounts of cell product**
- **gRNA, active RNP complex, and CRISPR nucleases are stable for less than 2 weeks**
- **Plasmid DNA is significantly more stable than the other components used and thus should still be tested**
  - Genomic integration of unwanted plasmid sequences is also an outcome that justifies residual plasmid testing



- **Clonal iPSC-derived allogeneic cell therapies have exceedingly low risk for retaining gRNA, active RNP complex and/or CRISPR nucleases due to long culture times**
- **Companies should consider their process and the stability of their components to evaluate the risk of component retention**
- **For Century Therapeutics cell line development and manufacturing strategy, measurement of genome editing residuals other than plasmid is unnecessary**

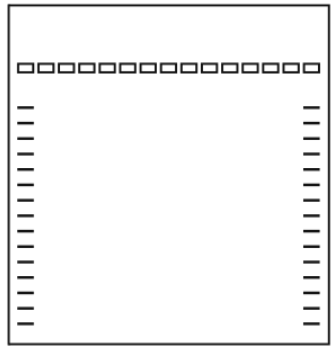
Century Therapeutics has an extensive genome monitoring process

PacBio WGS

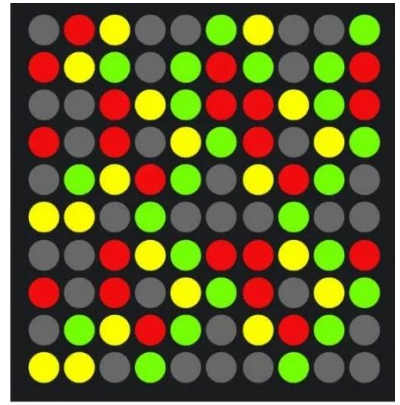
> Release testing of ex vivo-modified human GE DPs should include evaluation of on-target editing efficiency and the total number (or frequency) of genome-edited cells. Additional characterization of the editing events occurring at the on-target site should also be performed. Assessments of off-target editing frequency, intrachromosomal and interchromosomal rearrangements, and residual GE components may also need to be included for release of the DP based on the outcomes of nonclinical studies. We also recommend that the number of edited cells or the frequency of GE be monitored during stability testing of ex vivo-modified human GE DP.

U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research (CBER) (2024) Human Gene Therapy Products Incorporating Human Genome Editing; Guidance for Industry

PCRs

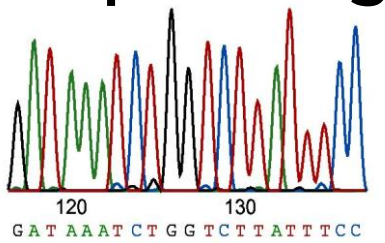


Micro Array



Optical Genome Mapping

Sanger Sequencing



Illumina WGS & TLA

Acknowledgments

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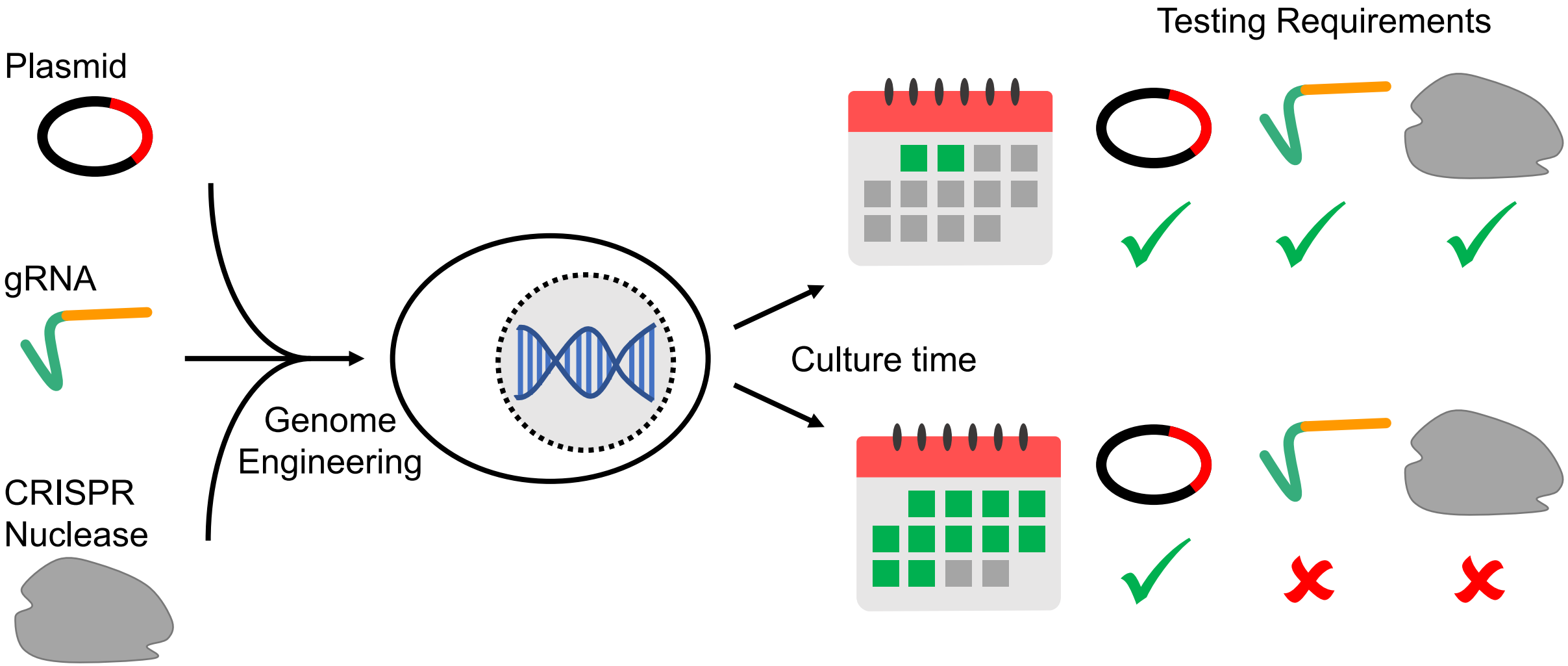
- Hunter Hoffman for the CRISPR nuclease cutting assay and MAD7 aliquots
- Rebecca Genovese for cell line generation and timepoint sampling
- Justin Bianchini for the B2M knockout study
- Damien Fink & Jennifer Dashnau for directional oversight

More details on this work can be found in:
Chialastri, A., Hoffman, H., Fink, D., & Dashnau, J. L. (2024). Clearance of residual genome editing components used for ex vivo genome-editing of allogeneic cell therapy products. Cytotherapy, 26(11), 1341–1352. https://doi.org/10.1016/j.jcyt.2024.06.005

Questions?


Testing Requirements
Plasmid
gRNA
CRISPR
Nuclease
Genome
Engineering
Culture time

Backup Slides

gRNA sequence does not significantly impact RNP complex stability