Treatment of Spinal Muscular Atrofi Disease with CRISPR Technology

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Spinal Musculer Atrofi, otozomal is an autsomal recessive disease. 5q11.2-13.3 on the SMN1 gene. It is located orn the arm.SMA is generally examined in 4 subgroups.

Tip I: It was defined by research from Werding and Hoffman towards the end of the 19th century [1] [2].Therefore, it also known as Werding Hoffman disease. The SMA disease group is the most severe disease group is the most severe disease group. It shows life 6 months after birth. Babies cannot raise their heads, sit and walk without support, have difficulty coughing and respiratory infections for a year at most. [3] [4]

Tip II: It also passes as an intermediate type of SMA and disease begins to ocur in the 7-8 of the baby is normal. As in Type I, the rapid deterioration process does not ocur here. Babies can crawl, stand and sit without support. The patients are susceptible to respiratory tract infections. Spinal curvature, hand, foot and chest Wall [3]abnormalities are common. Movement limitation may be oserved due to tendon contraction in the joints.

Tip III : It is the childhood mild form of SMA. It was described by Kugekberg-Walender syndrome. [1] The disease begins to appear after 18 months of age. It is are very mild and mat not be understood It can move slowly in the child's first sitting. In later periods, as the disease progresses, there may be difficulties in situations that require movement such as slaowin down in running and gettin up due to weakness in the hip, leg and hand muscles. A wheelchair may be needed at the ages of 20-30. Durin this peiod, spinal curvatures and skeletal disorders begin to become prominent. Respiratory distress is milder than Type I and Type II. The lifw span is normal

Tip IV: This type is know as adult SMA. It starts in the 30-40s and can be observed in older ages.Its begining and its progress is insidious. [3] The disease is not differentiated from Type III disease in terms of clinical, EMG and histological findings. In adult type SMA, weakness occurs in th legs n arms. Weakness spreads slowly. The life span of the patient is not affected or may be affeceted very little.

SMN Gene

The delection in the SMN gene in %95 of the patienst suggest, that the gene responsible for the disease [5] is SMN, and the gene consists of 9 exons of 20 kb [6]In total, the gene sequence oncodes 294 amino acids. [7] [8] [9] [2]The exon regions are called 2a and 2b. 31 polymorphisms have been identified in the SMN gene, 20 of wich are located in the promoter region, 1 in exon 2a in intron 1, and 15 in exon 3 in intron 6. Individuals with no SMA phenotype but with homozgous exon 7 and exon 8 deletion has been determined.

CRISPR-Cas9 technology was used fort he study on exon 7. CRISPR-Cas techonology has a great importance for biology and medicak science recently. It has taken its place in the World of science as a very ideal, fast and economical method fort he gene regions to be studied Short DNA sequences of pathogen origin are stored in the

CRISPR sequences of the host genome in infected cells in the form of "spacer". In the CRISPR-Cas system, the copy of the palindromic repeat sets of DNA is called "trans-activating RNA (tracr RNA) and the copy of the" spacer "regions CRISPR RNA (crRNA). [10] The Clusterd Regularly Interspaced Palindromic Reapeats (CRISPR) - Cas system, a genome sequencing tool based on the Watson-Crick DNA model principles, initiated a breakthrough in biological sciences. [10] [11] The CRISPR sequence is copied and the crRNA turns into small RNAs containing a single "spacer". The spacer sequences in CrRNA bind to the Cas protein structure and the tracrRNA molecule to form effector complexes that recognize the foreign DNA or RNA sequence by base pairing. They are helicases that can bind to RNA copied from palindromic repeat sets of DNA or whose RNA "spacers" cut paired DNA. TracrRNA and crRNA combine, forming the guided RNA (sgRNA) that activates the use of Cas9 to induce double-stranded DNA cutting at the proto-spacer adjacent motif [protospacer adjacent motifs (PAM)] regions.

Targetable nucleases, which allow scientists to theoretically target and alter any gene in any organism, greatly allow genome analysis of gene therapy. Zinc finger nucleases (ZFN), transcription activator-like effector nucleases [transcription activator-like Effector nucleases (TALENS)] and endonucleases, also known as meganucleases, are tools used for genome engineering. The newest genome editing added to these systems is the CRISPR- Cas9 system. Nuclease driven by small RNAs containing approximately 20 nucleotides is used to bind to the targeted DNA

1.1. Prime Editing

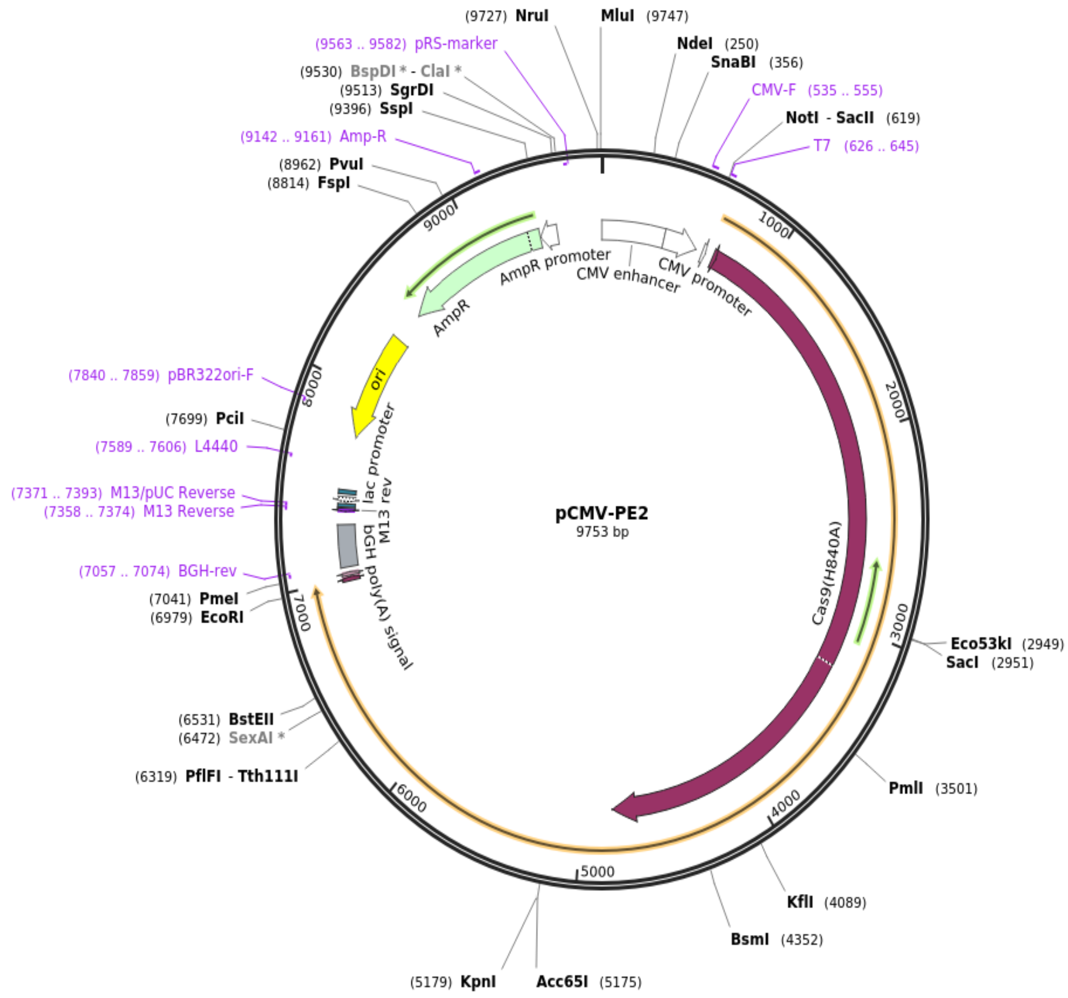
Prime editing combines a nicking-Cas9–reverse transcriptase fusion protein (PE2) with a prime editing guide RNA (pegRNA) containing the desired edit. The pegRNA-spacer guides the formation of a nick in the targeted DNA strand. The pegRNA-extension binds to this nicked strand and instructs the synthesis of an edited DNA flap. This edited flap is then integrated by DNA repair mechanisms, which can be enhanced by simultaneous nicking of the non-edited strand4. Prime editing has been applied in human cancer cell lines and plant cells, but not in human disease models4,5,6. We set out to develop and test prime editing in primary patient-derived organoids.

1.1. Herpes virus

Herpes viruses are large DNA viruses that cause common life-long infections. Adults can carry more than one herpesvirus. The Herpesvirus family consists of three subfamilies, Alpha-Beta and Gammaherpesvirinae. Alphaheherpesvirinae subfamily includes herpes simplex virus type 1 and type 2 (HSV-1 and 2) and varicella zoster virus (VZV). HSV-1 causes cold sores and herpes simplex keratitis, a common cause of corneal blindness. HSV-2 is responsible for genital herpes. Primary infection with VZV results in chickenpox. Reactivation can lead to herpes zoster or shingles. Subfamily Betaherpesvirinae includes human cytomegalovirus, which causes serious complications in immunocompromised individuals.

Antiviral agents target viral DNA polymerase during the infection productive (lytic) phase, however herpesviruses are characterized by their ability to create a calm and latent state. Herpesviruses cannot actively replicate their viral genome with viral DNA polymerases, and they inactivate antiviral treatments targeting these polymerases.

An adaptive immune system has been developed by systems associated with archaea and bacteria, CRISPR-Cas repeats that use short RNA molecules to induce the degradation of foreign nucleic acids of viruses. The CRISPR \ Cas9 system is designed to induce robust RNA guided genome modifications in human cells. By expressing a bacterial Cas9 nuclease with a guideRNA (gRNA), Cas9 can be directed almost anywhere in the genome and induce cleavage of double-stranded DNA (dsDNA) at the target site. The cleaved DNA is then repaired by mammalian DNA repair mechanisms, which are inherently error-prone, thus inducing insertions and deletions (indels) and mutations in the target site. With the advancement of molecular biology and CRISPR-Cas9 technology, research can produce complete knockouts for any gene, induce specific regulation / mutagenesis of loci and multiple alleles at once.


Created with SnapGene®
pCMV-PE2
9753 bp
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MluI (9747)
(9563 .. 9582) pRS-marker
NdeI (250)
SnaBI (356)
(9530) BspDI * - ClaI *
(9513) SgrDI
(9396) SspI
CMV-F (535 .. 555)
NotI - SacII (619)
T7 (626 .. 645)
(9142 .. 9161) Amp-R
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AmpR promoter
AmpR
CMV enhancer
CMV promoter
ori
(7840 .. 7859) pBR322ori-F
(7699) PciI
(7589 .. 7606) L4440
lac promoter
M13 rev
(7371 .. 7393) M13/pUC Reverse
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bGH poly(A) signal
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(7041) PmeI
(6979) EcoRI
Cas9(H840A)
Eco53kI (2949)
SacI (2951)
(6531) BstEII
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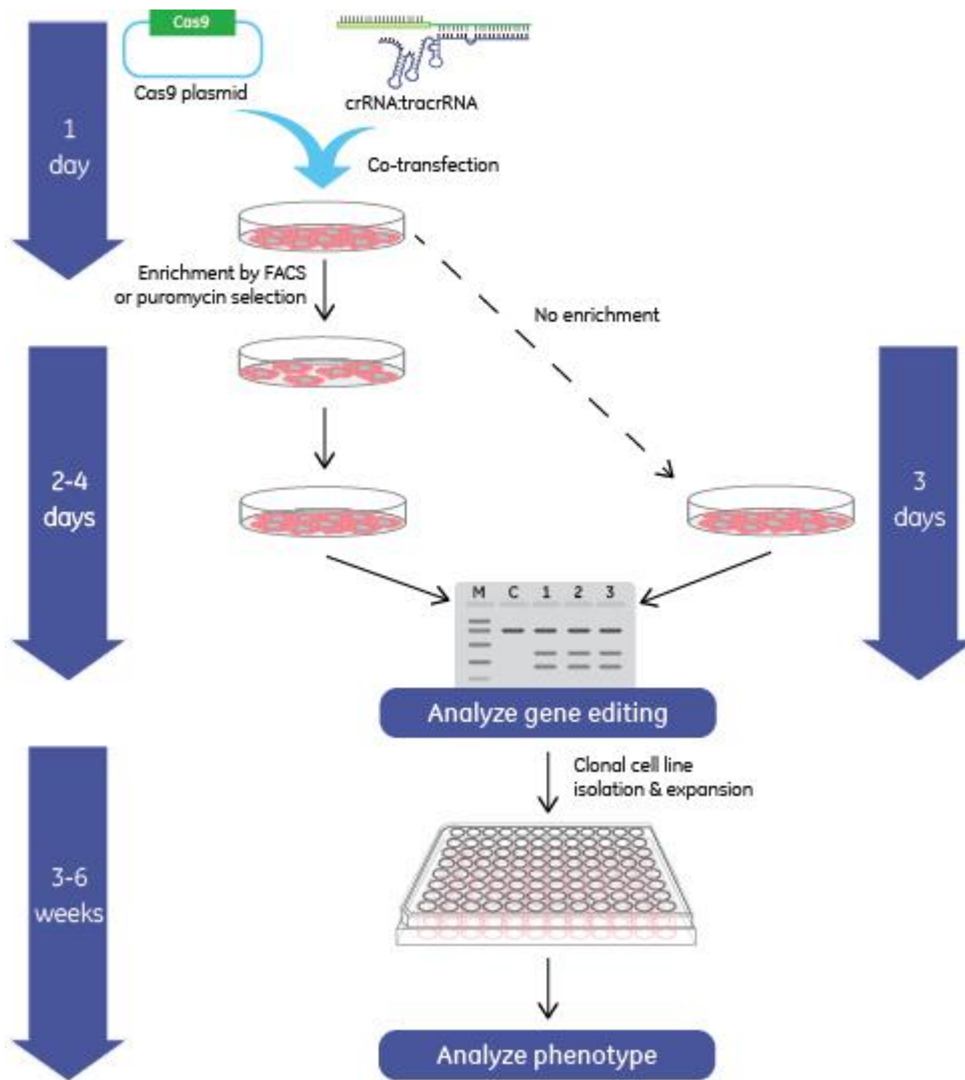

Figure: Gene knockout workflow using Cas9 expression plasmid and synthetic crRNA: tracrRNA

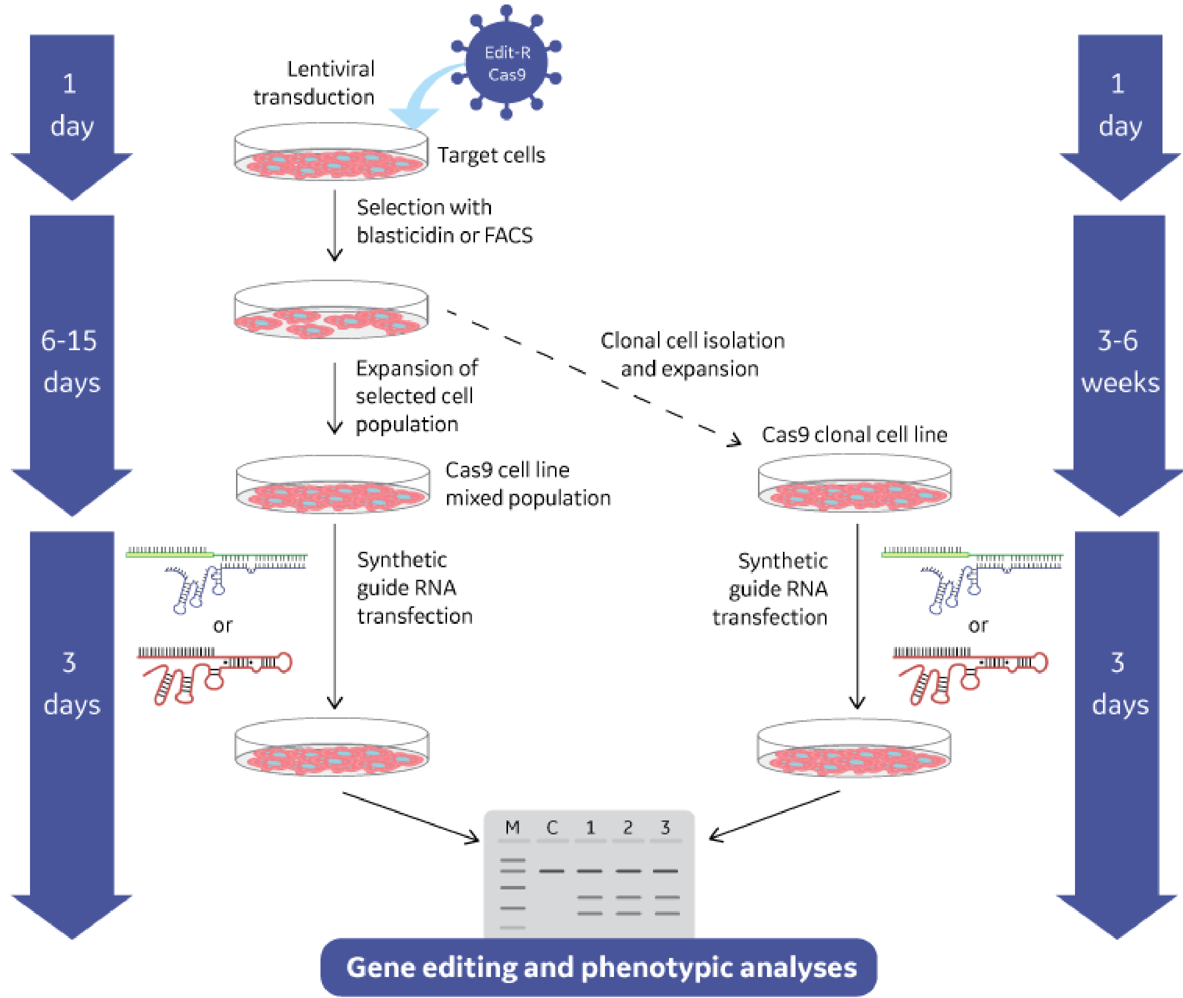

Figure: The structure should be provided as a gene knockout workflow using lentiviral Cas9 expression particles and synthetic crRNA: tracrRNA

Kaynakça

[1] S. Y. v. Ö. A, «Spinal Müsküler Atrofiler.,» *Ç. Ü. Arşiv Kaynak Tarama Dergisi,* cilt 5, no. 19, p. 202, 1996.

[2] M. T. L, «he Spinal Muscular Atrophies.,» *Curr Neurology,* cilt 4(3), no. 5, pp. 55-71, 1994.

[3] O. S. a. W. RB., «SMN Genetic testing and risk assessment for spinal muscular atrophy (SMA),» *Hum Genet,* cilt 477, no. 500, p. 111, 2002.

[4] D. R. F. G. e. a. Bradley WG, «Neurology in Clinical Practice Volume II.3th. Ed,» %1 içinde *Butterworth Heinemann.*, 2000, pp. 1997-2004.

[5] W. B., «n update of the mutation spectrum of the survival motor neuron gene (SMN1) in autosomal recessive spinal muscular atrophy (SMA),» %1 içinde *Hum Mutat ;* , 2000, pp. 15:228-37..

[6] h. (. t. 09.2011). [Çevrimiçi].

[7] L. C. B. D. ,. J. L. T. B. A. A. E. Strasswimmer J, «Identification of survival motor neuron as a transcriptional activator-binding protein,» *Human Mol Genet,* cilt 8, no. 26, p. 1219, 1999.

[8] N. S. C.-D. C. e. a. Frugier T, The molecular bases of spinal muscular atropy urr Opin Genet Dev, 2002; 12:294-98..

[9] h. (. E. t. 06.2011). [Çevrimiçi].

[10] Y. X. G. D. Chen S, «CRISPR-cas targeting,» y. Viruses., 2018.

[11] Doudna JA, «Charpentier E. Genome editing.,» *Science,* p. 346(6213):, 2014.

[12] M. J. Segal DJ, «Genome engineering at the dawn of the golden age,» *nnu Rev Genomics Hum Genet,* cilt 14, no. 35, p. 158, 2013.

[13] M. P. M. U. e. a. Parsons DW, «An 11 base pair duplication in exon 6 of the SMN gene produces a type I spinal muscular atrophy (SMA) phenotype: further evidence for SMN as the primary SMA-determining gene,» *Hum Mol Genet,* cilt 5, no. 32, p. 1727, 1996.