Creation of RUES2 cell lines carrying targeted modifications at the *HTT* gene

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Abstract

Huntington's disease (HD) is an autosomal dominant neurodegenerative condition caused by >36 repeat expansions of CAG trinucleotides in the huntingtin gene (*HTT*). Longer repeat expansions are associated with greater severity and earlier onset.

Although animal models for HD in different species have provided insights into its pathogenesis and enabled the generation of potential therapies, these have shown limited efficacy when tested in clinical trials[1]. Thus, there is an unmet need for physiologically relevant *in vitro* platforms to test and de-risk therapeutic approaches before testing them on animal models and/or transferring them into clinical evaluation.

In collaboration with CHDI, we have used our expertise in human induced pluripotent stem cells (iPSCs) to generate a portfolio of genetically engineered cell lines carrying targeted modifications at the *HTT* gene, to develop a powerful HD platform.

We applied CRISPR/Cas9 technology to the RUES2 embryonic stem cell line (hESCs) (*HTT* CAG22/CAG20) in two steps: generating intermediate clones carrying a landing pad, and then, retargeting to substitute with the final modified *HTT* sequence (fig. 1).

Due to the presence of a single nucleotide polymorphism (SNP) in *HTT* exon 1 in the RUES2 hESC line, the two parental alleles were independently targeted. Consequently, we have generated at least two independent hESC clones with 16 different *HTT* genotypes. The clones were qualified based on a range of tests, including microbiology, growth profile, cell line identity, karyology, array GH and pluripotency assessment to ensure high quality (fig. 2).

These genetically engineered cell lines can act as a robust HD platform for research, disease modeling and drug discovery, helping to bridge the translational gap in HD drug discovery. We are also developing additional hESC lines with different *HTT* genotypes of biological interest, facilitating further research and validation of potential therapeutic targets.

Methods

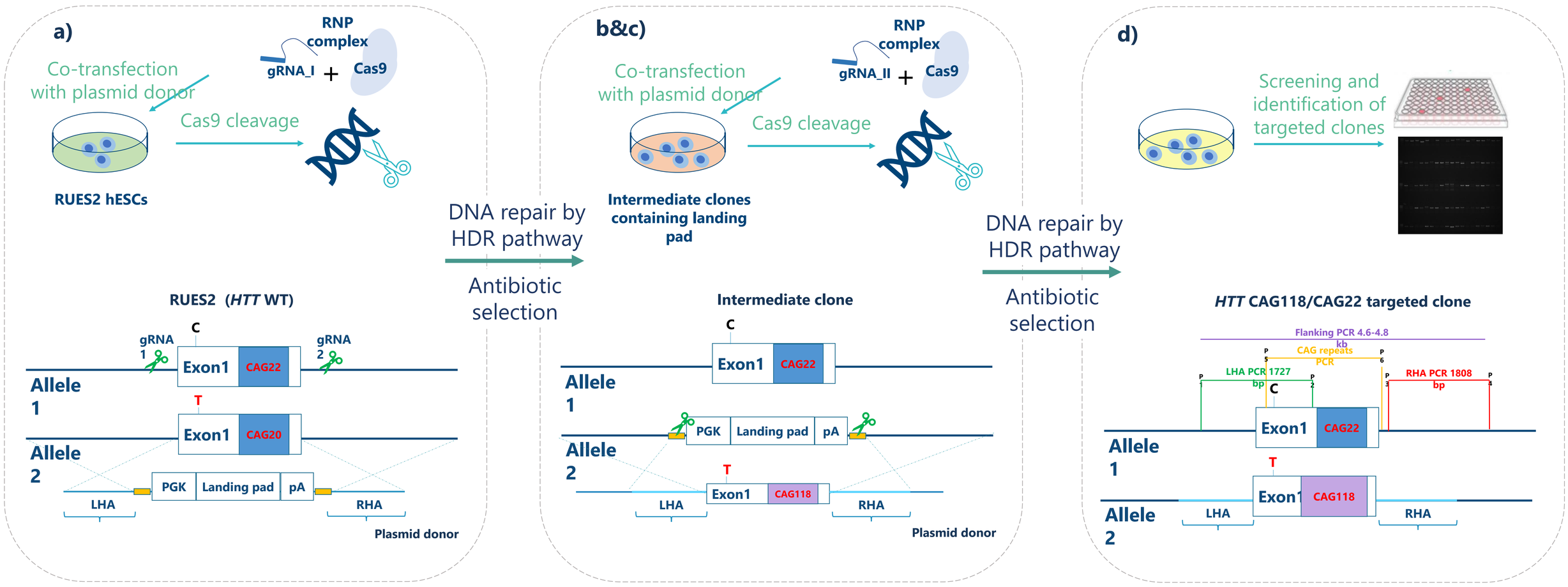
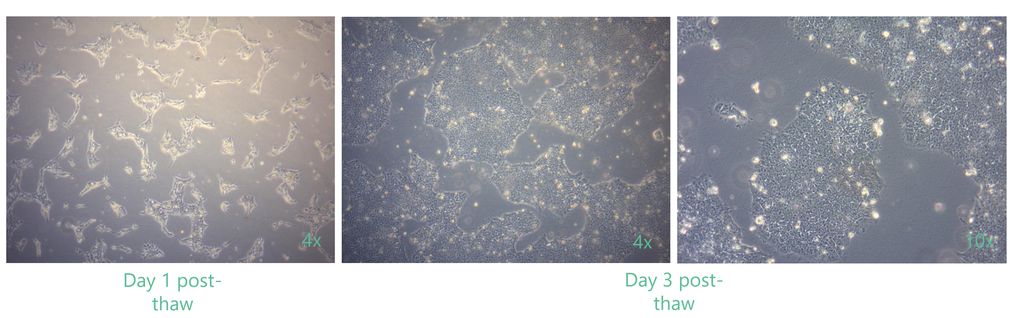


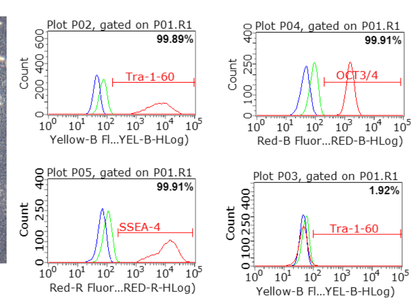
Figure1. a) Nucleofection of RUES2 WT hESC line with RNP complex and donor-targeting vector. **b)** Intermediate clones containing a landing pad. **c)** Nucleofection with RNP complex to substitute landing pad with *HTT* sequence of interest. **d)** Genotyping of targeted clones. gRNA= guide RNA; HDR=homology-directed repair; LHA= left homology arm; RHA= right homology arm; RNP= ribonucleoprotein.

Results

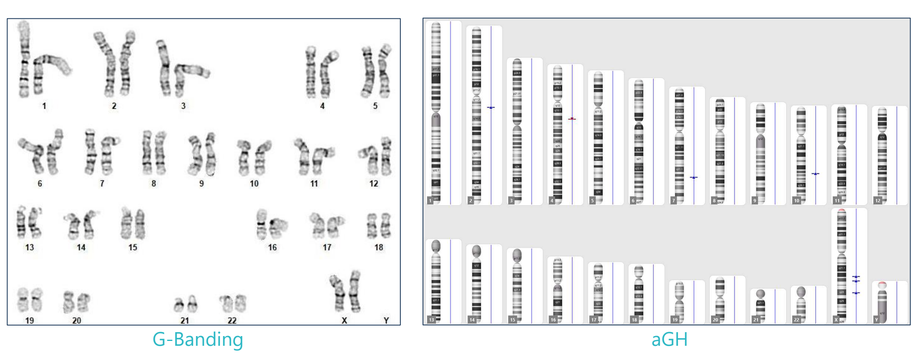
1. Morphology assessment



2. Pluripotency assessment



3. Karyology



4. Cell line identity

5. Confirmation of targeting and CAG sizing analysis

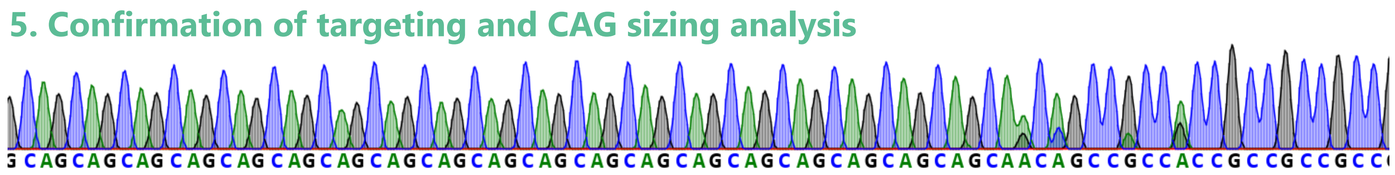


Figure 2. Representative results of a *HTT* targeted clone qualification assays. 1. Representative bright field images to assess morphology after banking. **2.** Pluripotency-associated marker assessment by flow cytometry. **3.** Karyology analysis by G-Banding (left) and aGH (right). **4.** Short tandem repeats (STR) analysis to confirm cell line identity. **5.** Sanger sequencing to show section of CAG repeat region.

RUES2 Cell lines with corresponding *HTT* gene modification

Cell line	Genotype	Clones *
HTT Null	*HTT*-/-	CHDI-90004567
		CHDI-90004568
		CHDI-90004569
	HTT WT/-	CHDI-90004286
		CHDI-90004287
		CHDI-90004288
WT-retargeted	CAG22/CAG20	CHDI-90004563
		CHDI-90004564
	CAG20/CAG22	CHDI-90004529
		CHDI-90004530
"High Normal"	CAG35/CAG22	CHDI-90003875
		CHDI-90003876
Long repeats	CAG98/CAG22	CHDI-90004609
	CAG76/CAG22	CHDI-90004610
	CAG118/CAG20	CHDI-90004561
		CHDI-90004562
	CAG118/CAG22	CHDI-90004531
		CHDI-90004532
Interrupted long repeats	CAG59CAACAG59/20	CHDI-90005180
		CHDI-90005178
		CHDI-90005179
	CAG59CAACAG59/22	CHDI-90005175
		CHDI-90005176
		CHDI-90005177
Duplicated CAACAG after long repeats	CAG118(CAACAG)2CCA/20	CHDI-90005613
		CHDI-90005614
	CAG118(CAACAG)2CCA/22	CHDI-90005278
		CHDI-90005279
		CHDI-90005280
Absence of CAACAG after long repeats	CAG118(CAACAG)0CCG/20	CHDI-90005576
		CHDI-90005577
	CAG118(CAACAG)0CCG/22	CHDI-90005615
		CHDI-90005616
Mutated uORF	CAG22(uORF ATG>CGA)/20	CHDI-90006276
		CHDI-90006277
	CAG56(uORF ATG>CGA)/20	CHDI-90006279
		CHDI-90006280
	CAG118(uORF ATG>CGA)/20	CHDI-90006281
		CHDI-90006282

*Please contact Richard Chen at CHDI for availability of these cell lines.

Conclusion

Using CRISPR-Cas9 technology we can generate isogenic pairs of RUES2 hESC lines that carry different CAG repeat lengths, serving as a powerful resource to understand HD biology. These lines can be further differentiated to different neuronal cell types including striatal neurons, astrocytes and cortical excitatory neurons. These cells can be used in 2D monoculture or more complex co-culture models to better understand the disease mechanism and phenotype as well as for pre-clinical validation of targets.

References

1 Ferguson MW, Kennedy CJ, Palpagama TH, Waldvogel HJ, Faull RLM, Kwakowsky A. Current and Possible Future Therapeutic Options for Huntington's Disease. J Cent Nerv Syst Dis. 2022 May 21;14:11795735221092517. doi: 10.1177/11795735221092517. PMID: 35615642; PMCID: PMC9125092.

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