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Modeling the CHARGE Syndrome in Human Fibroblast Cells using CRISPR/Cas9 Genetic Technology

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Outline:

1. **Background:**
   - *What is CHARGE Syndrome?*
   - *What is CHD7 gene?*
2. **Rationale:** *What are the reasons for doing this research?*
3. **Goal:** *What is the main objective?*
4. **Key Method:** *What is CRIPSR/CAS9?*
5. **Results:** Data presentation
6. **Summary**
7. **Acknowledgements**

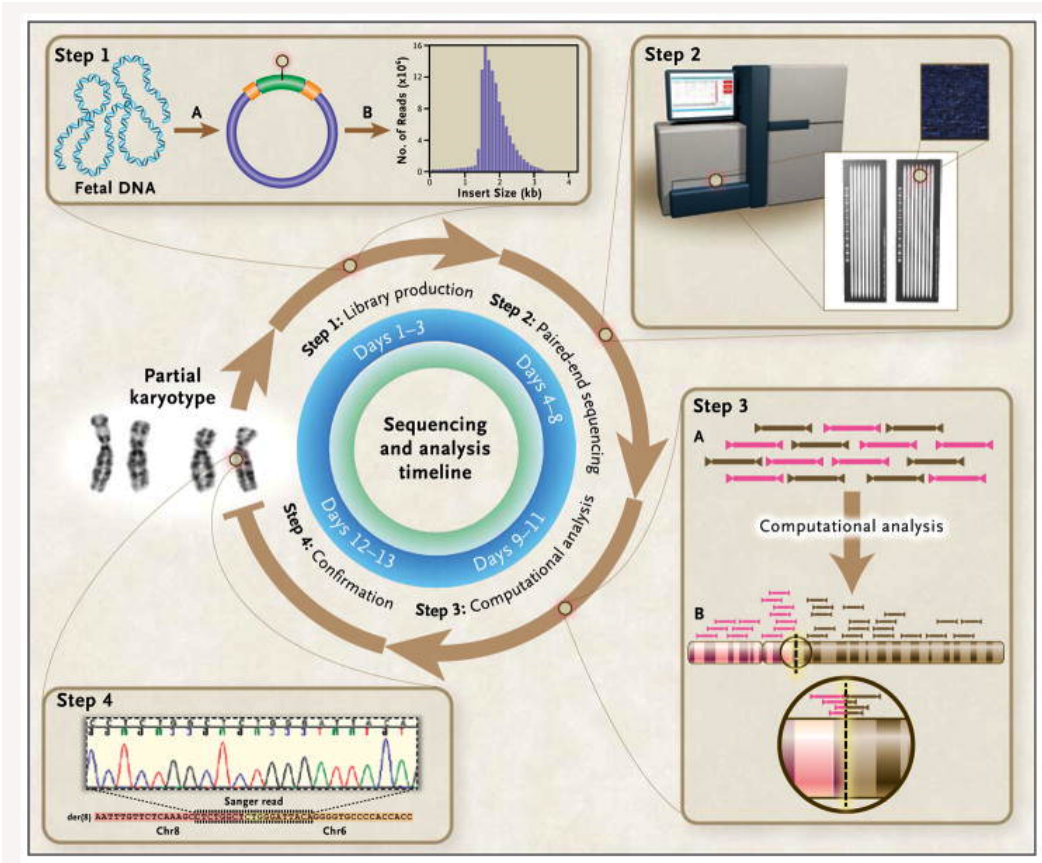
What is CHARGE Syndrome?

- CHARGE syndrome is *a cluster of life-threatening genetic disorders* that affects multiple organs; typically, children born with CHARGE syndrome show several phenotypes:

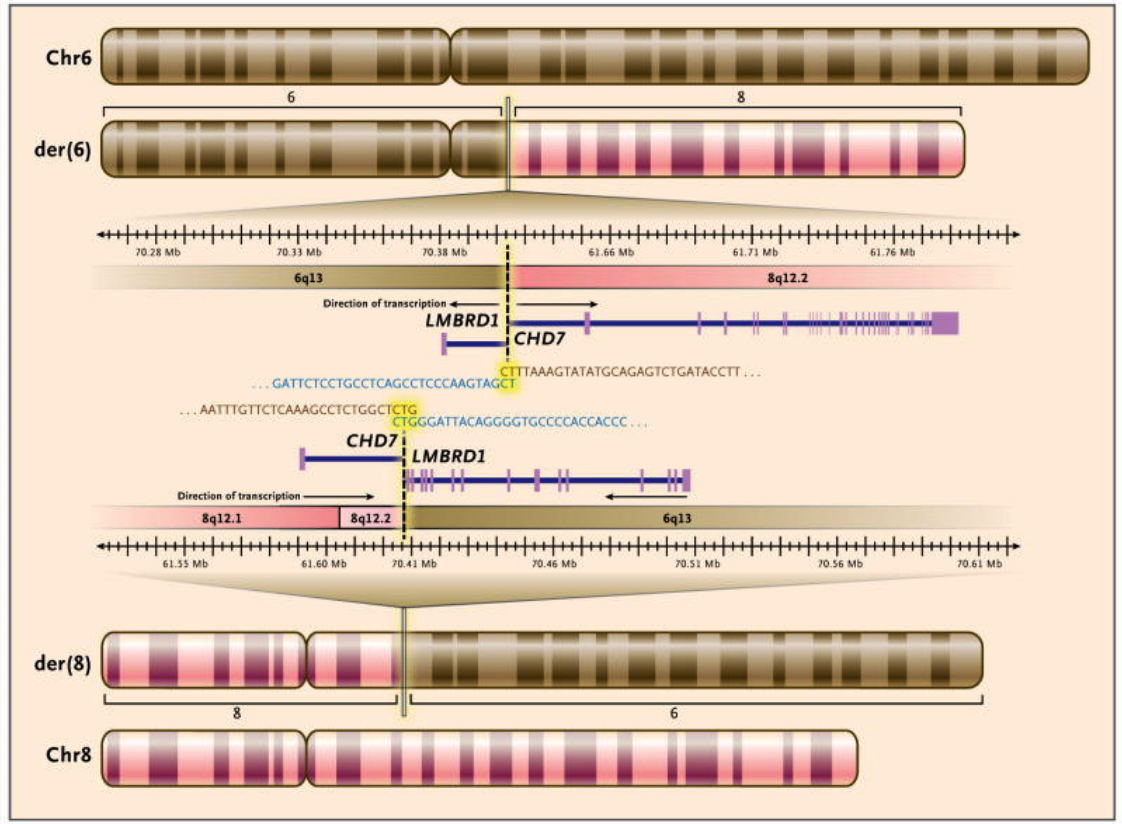
  Coloboma (*a hole in the eye*),
  Heart defect,
  Atresia choanae (*back of nasal passage is blocked*),
  Retarded growth and development,
  Genital hypoplasia,
  Ear anomalies/deafness

An example of CHARGE syndrome diagnosis *in vivo* coupled with DNA sequencing

Nature Genetics **36**, 955 - 957 (2004)



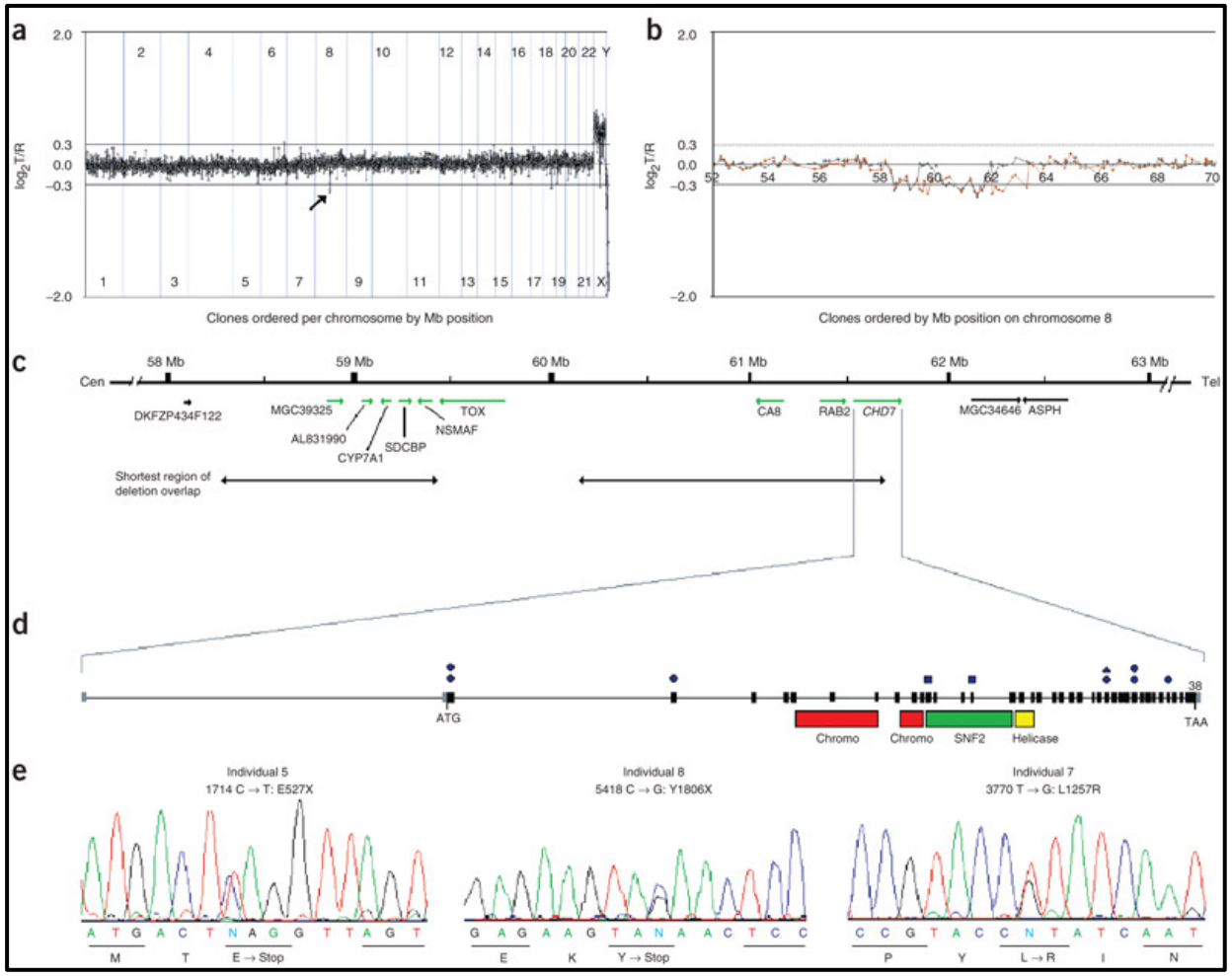
Deep Sequencing and Analysis Timeline



Sequence-Based Delineation of a Balanced *De Novo* *Translocation*

CHARGE syndrome is a neglected disease. Thus, we understand very little about the function of CHD7 protein.

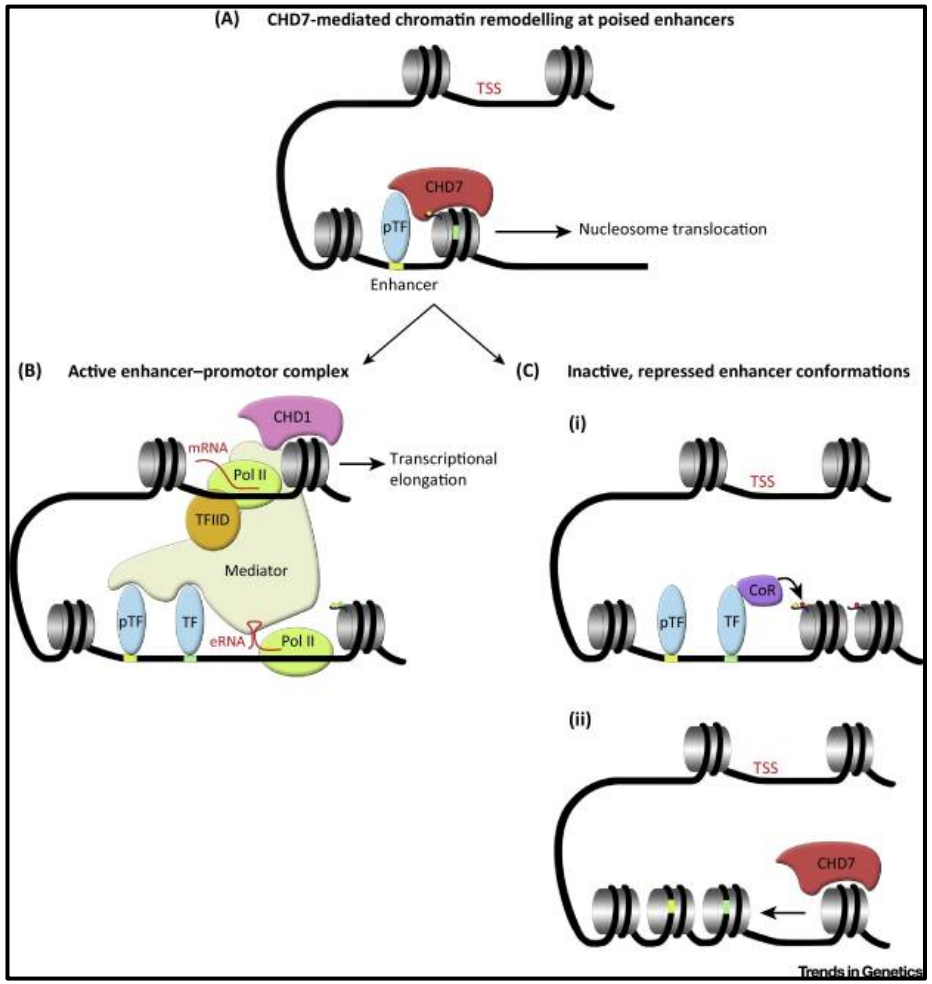
Mutations in a chromodomain gene family cause CHARGE syndrome



Nature Genetics **36**, 955 - 957 (2004)

Dominant *loss-of-function* mutations in the gene encoding chromodomain helicase DNA binding protein 7 (CHD7), an ATP-dependent chromatin remodeler, is the main cause of CHARGE syndrome.

Proposed Model of CHD7 Action at Enhancers



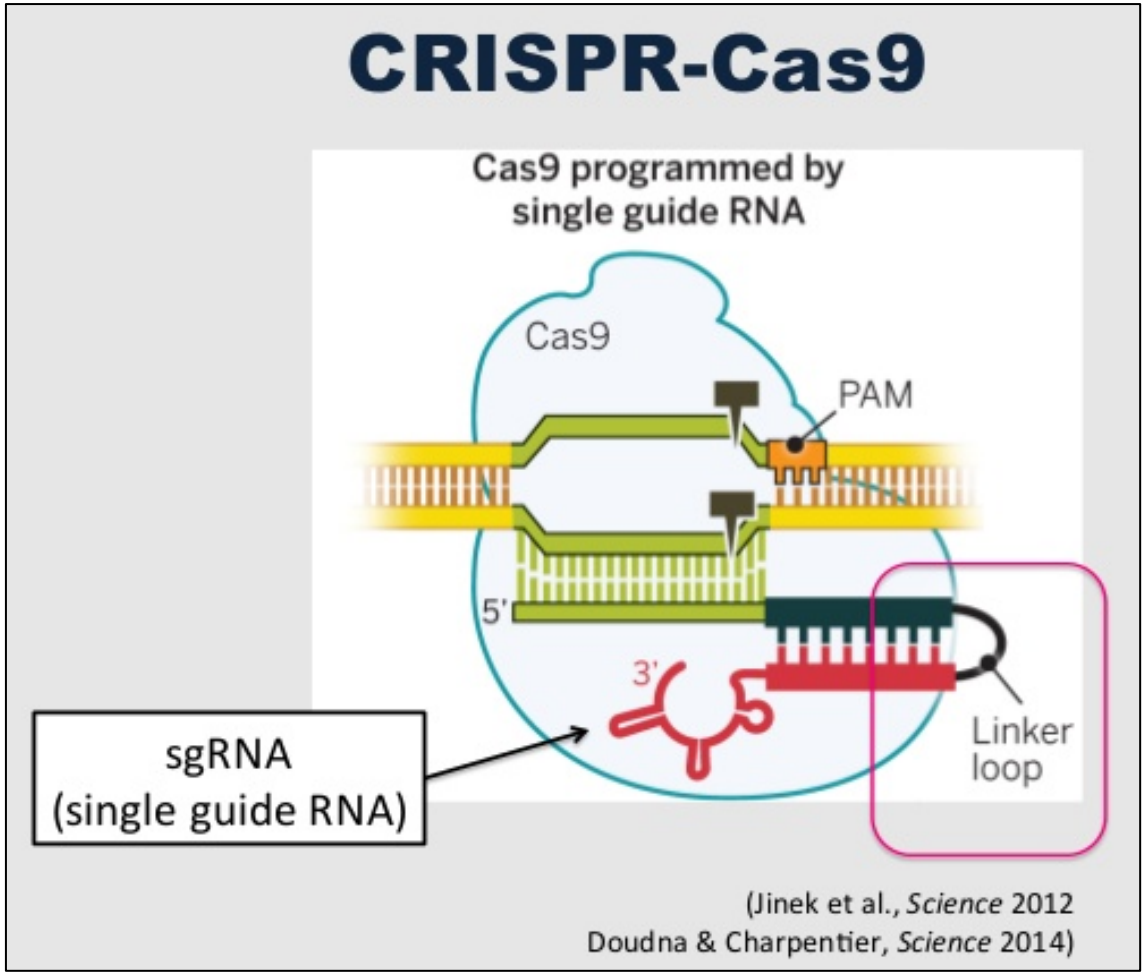
Trends Genet. 2015 Oct; 31(10): 600–611.

Proposed functions of CHD7

1. Remodel chromatin
2. May bind to DNA directly
3. May repress the action of transcription factors including p53
4. Mutation of CHD7 might affect lifespan stem cells

To address this deficiency in our knowledge, we proposed to use CRISPR/Cas9 to model CHARGE disease-in-a-dish.

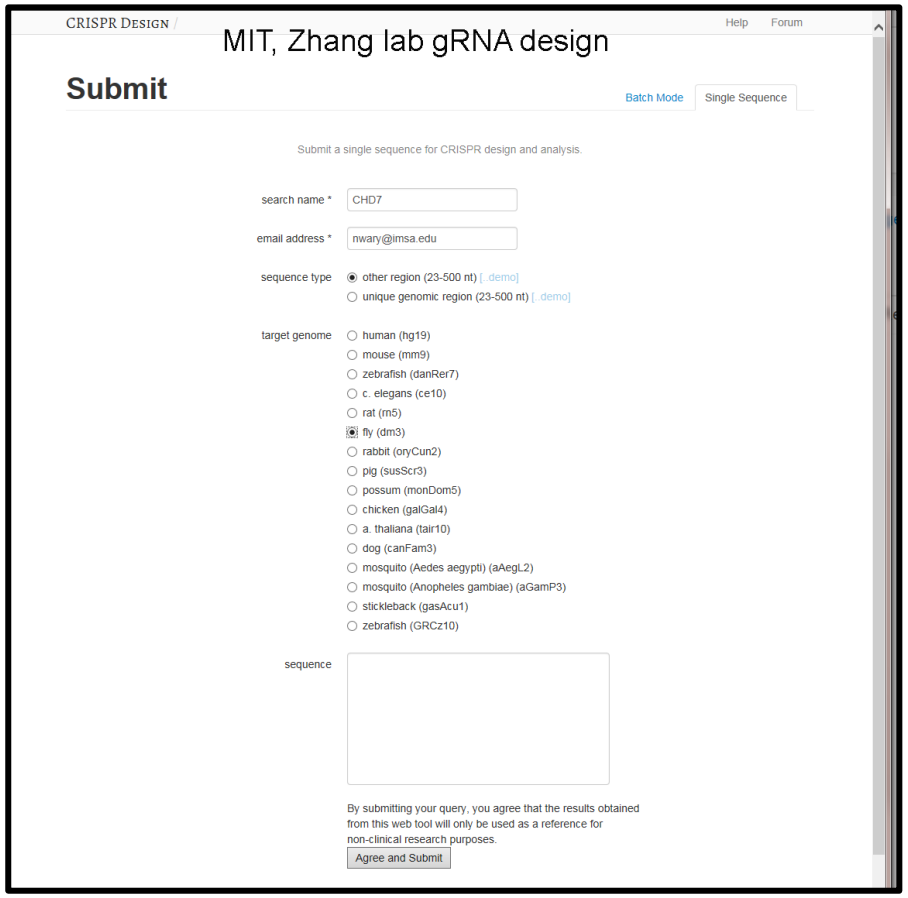
How does CRISPR/Cas9 work?

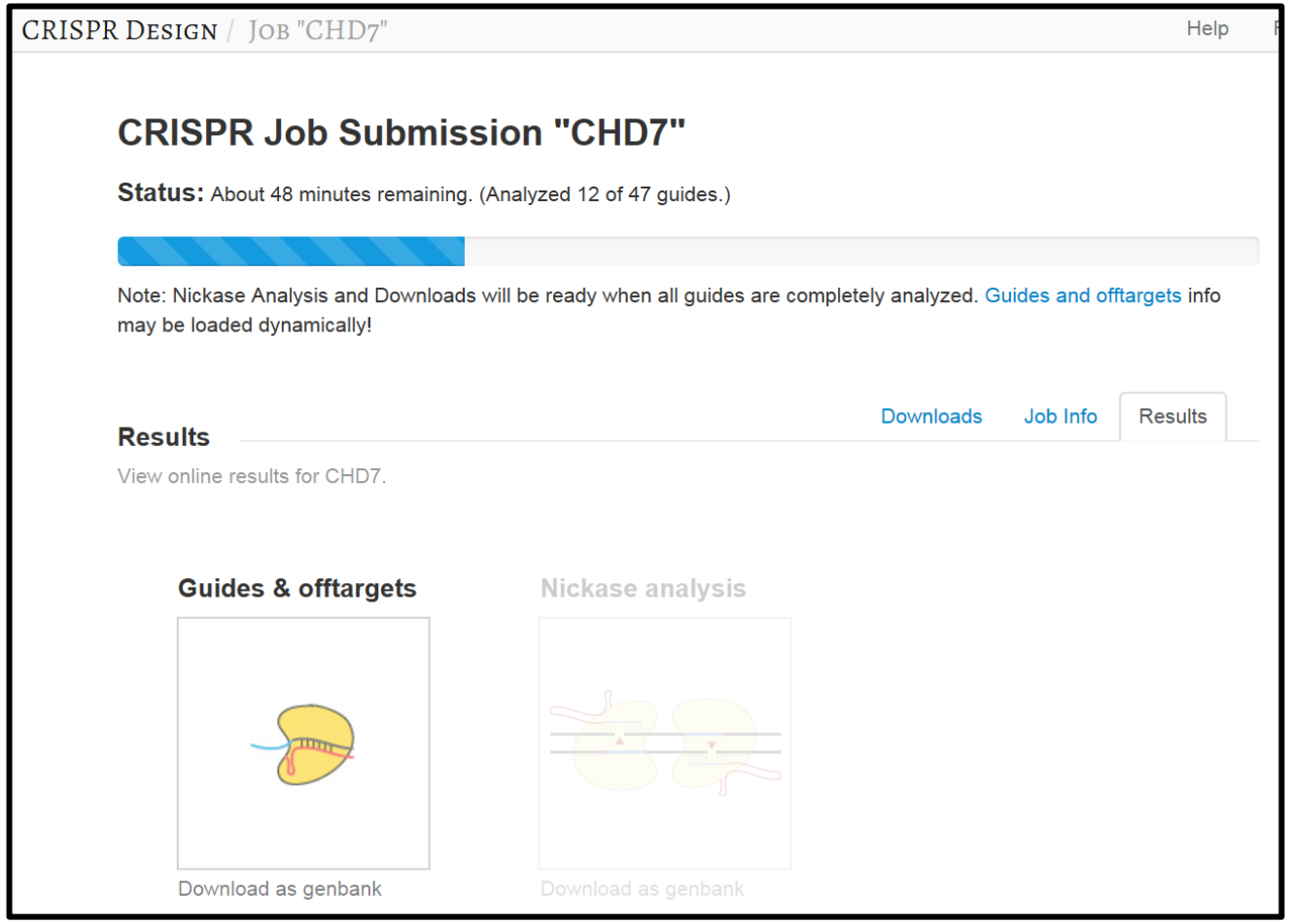


Emmanuelle Charpentier (left), Jennifer Doudna (right)

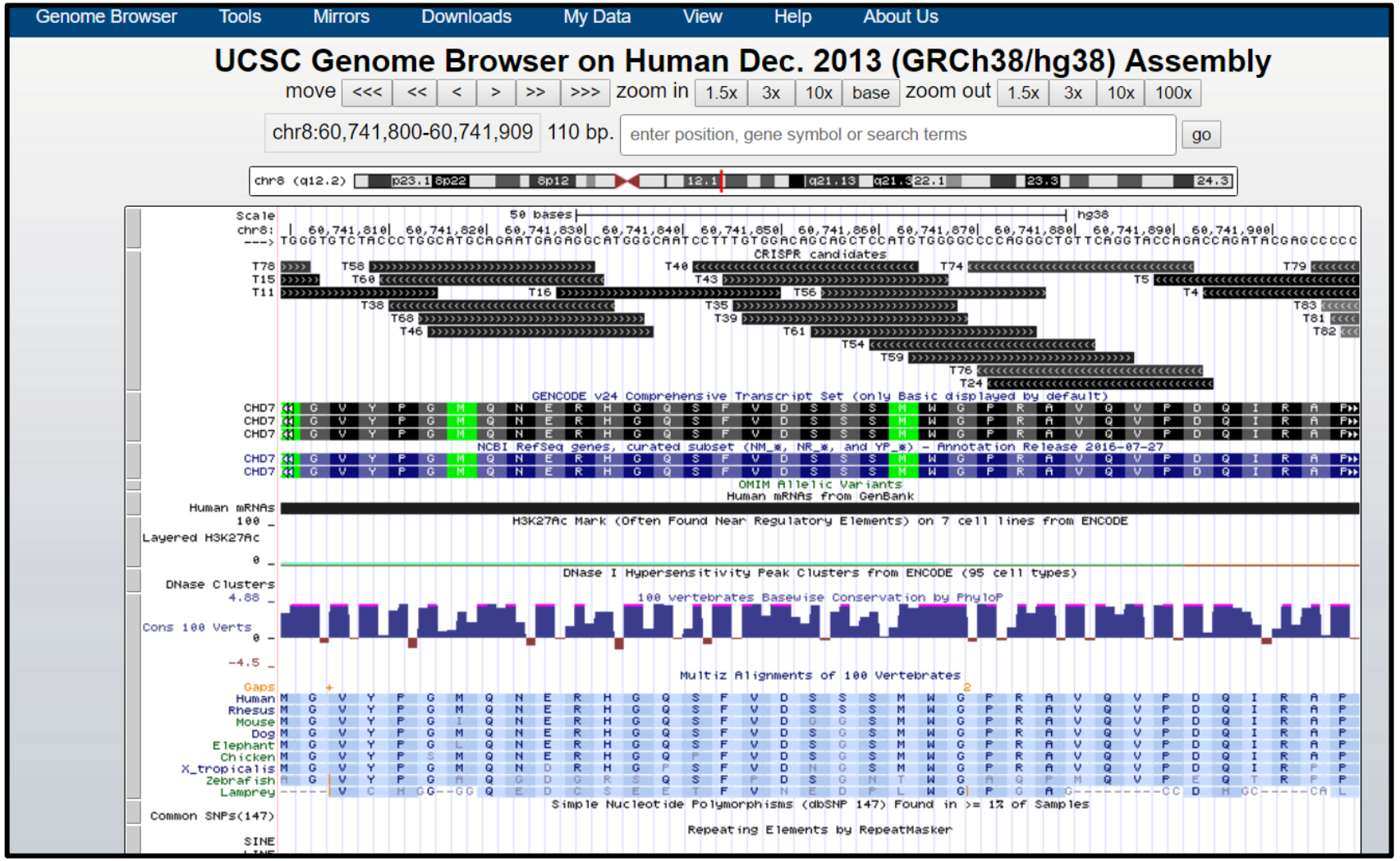
Designing CHD7 gRNA for CRISPR/Cas9

Select 23-250 nucleotide and search for gRNA using the MIT Zhang lab software





An example of designing sgRNAs using the UCSC genome browser

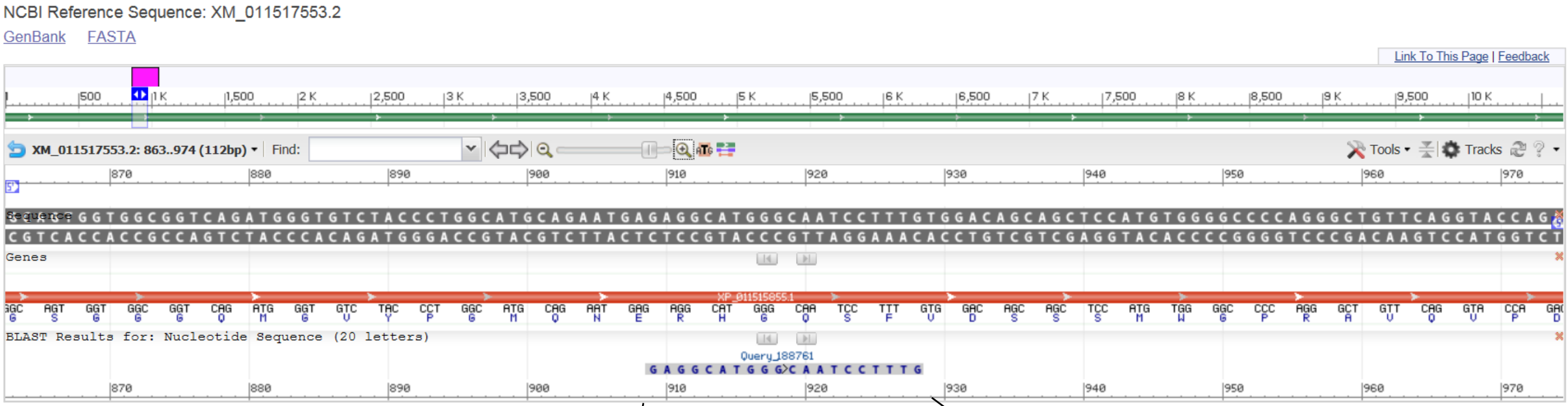


CHD7 exon-1

GTACAAAAAAGCAGAAGGGCCGTCAAGGCCCACCATGGCAGATCCAGGAATGATGAGTCTTTTTGGCGAG
GATGGGAATATTTTCAGTGAAGGTCTTGAAGGCCTCGGAGAATGTGGTTACCCGGAAAATCCAGTAAATC
CTATGGGTCAGCAAATGCCAATAGACCAAGGCTTTGCCT***CTTTACAGCCATCCCTTCATCATC***CTTCAAC ***For:***
TAATCAAAATCAAACAAAGCTGACACATTTTGATCACTATAATCAGTATGAACAACAAAAGATGCATCTG
ATGGATCAGCCGAACAGAATGATGAGCAACACCCCTGGGAACGGACTCGCGTCTCCGCACTCGCAGTATC
ACACCCCTCCCGTTCCTCAGGTGCCCCATGGTGGCAGTGGTGGCGGTCAGATGGGTGTCTACCCTGGCAT
GCAGAATGA**GAGGCATGGGCAATCCTTTG**TGGACAGCAGCTCCATGTGGGGCCCCAGGGCTGTTCAGGTA
CCAGACCAGATACGAGCCCCCTACCAGCAGCAGCAGCCACAGCCGCAGCCACCGCAGCCGGCTCCGTCGG
GGCCCCCTGCACAGGGCCACCCTCAGCACATGCAGCAGATGGGCAGCTATATGGCACGTGGGGATTTTTC
CATGCAGCAGCATGGTCAGCCACAGCAGAGGATGAGCCAGTTTTCCCAAGGCCAAGAGGGCCTCAATCAG
GGAAATCCTTTTATTGCCACCTCAGGACCTGGCCACTTGTCCCACGTGCCCCAGCAGAGTCCCAGCATGG
CACCTTCCTTGCGTCACTCGGTGCAGCAGTTCCATCACCACCCCTCTACTGCTCTCCATGGAGAATCCGT
TGCCCACAGTCCCAGATTCTCCCCGAATCCTCCCCAACAAGGGGCTGTTAGGCCGCAAACCCTTAACTTT
AGTTCTCGGAGCCAGACAGTCCCCTCT***CCTACTATAAACAACTCAGGGCAG***TATTCTCGATATCCTTACA ***Rev:***
GTAACCTAAATCAGGGATTAGTTAACAATACAGGGATGAATCAAAATTTAGGCCTTACAAATAATACTCC
AATGAATCAGTCCGTACCAAGATACCCCAATGCTGTAGGATTCCCATCAAACAGTGGTCAAGGACTAATG
CACCAGCAGCCCATCCACCCCAGTGGCTCACTTAACCAAATGAACACACAAACTATGCATCCTTCACAGC

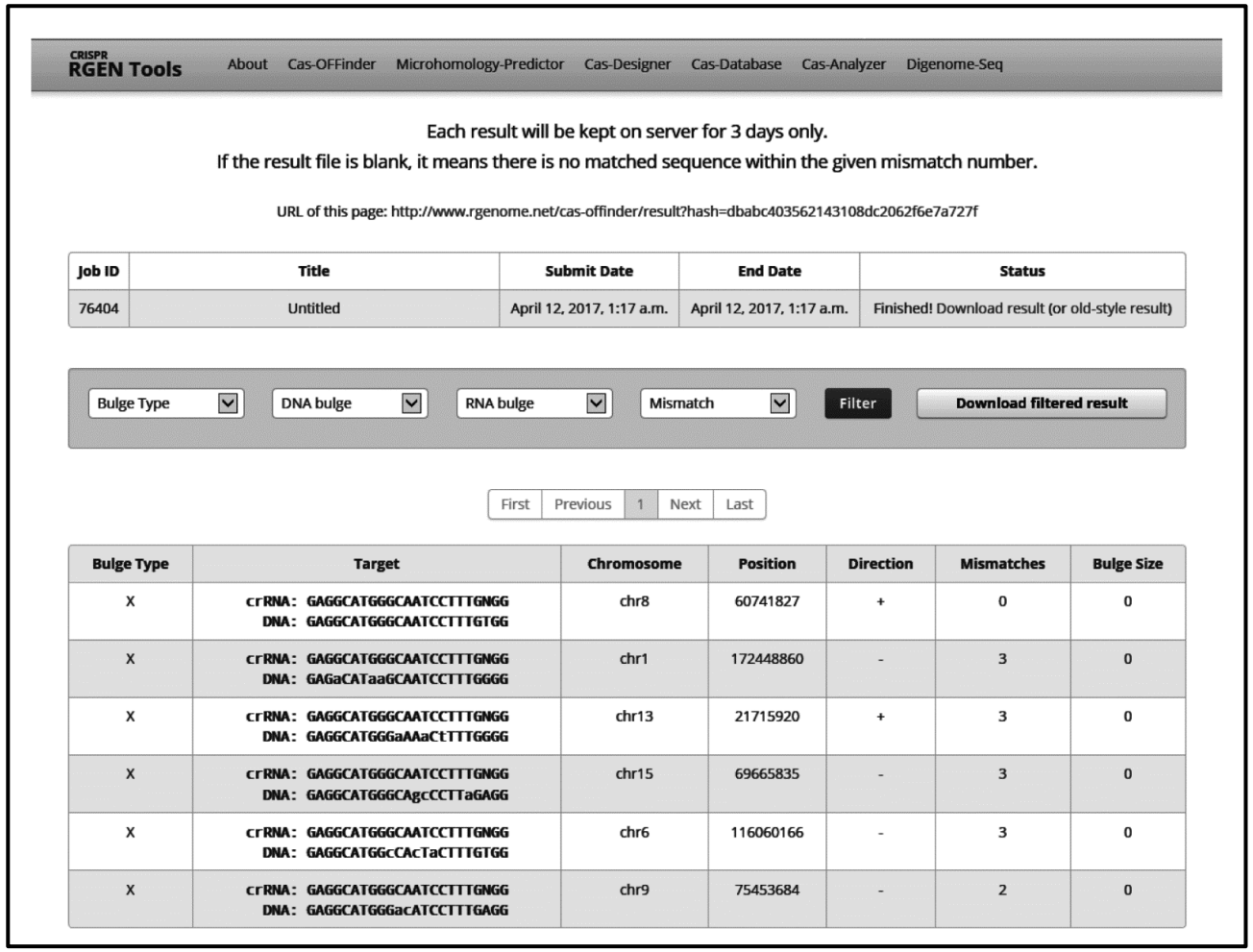
CHD7 Target 1- gRNA:	**396**	**GAGGCATGGGCAATCCTTTG**
CHD7 Target 2- gRNA:	**1234**	**GCACTTCCCGGCCTGACTTG**
CHD7 Target 3- gRNA	**1322**	**GTGCCATGGGAATCGGACAG**

Making sure that our gRNA matches the target sequence



GAGGCATGGGCAATCCTTTG Pam sequence

However, if we introduce 3 nucleotide mismatches, there could be 5 off-target effects



CRISPR RGEN Tools | About | Cas-OFFinder | Microhomology-Predictor | Cas-Designer | Cas-Database | Cas-Analyzer | Digenome-Seq

Each result will be kept on server for 3 days only.
If the result file is blank, it means there is no matched sequence within the given mismatch number.

URL of this page: http://www.rgenome.net/cas-offinder/result?hash=dbabc403562143108dc2062f6e7a727f

Job ID	Title	Submit Date	End Date	Status
76404	Untitled	April 12, 2017, 1:17 a.m.	April 12, 2017, 1:17 a.m.	Finished! Download result (or old-style result)

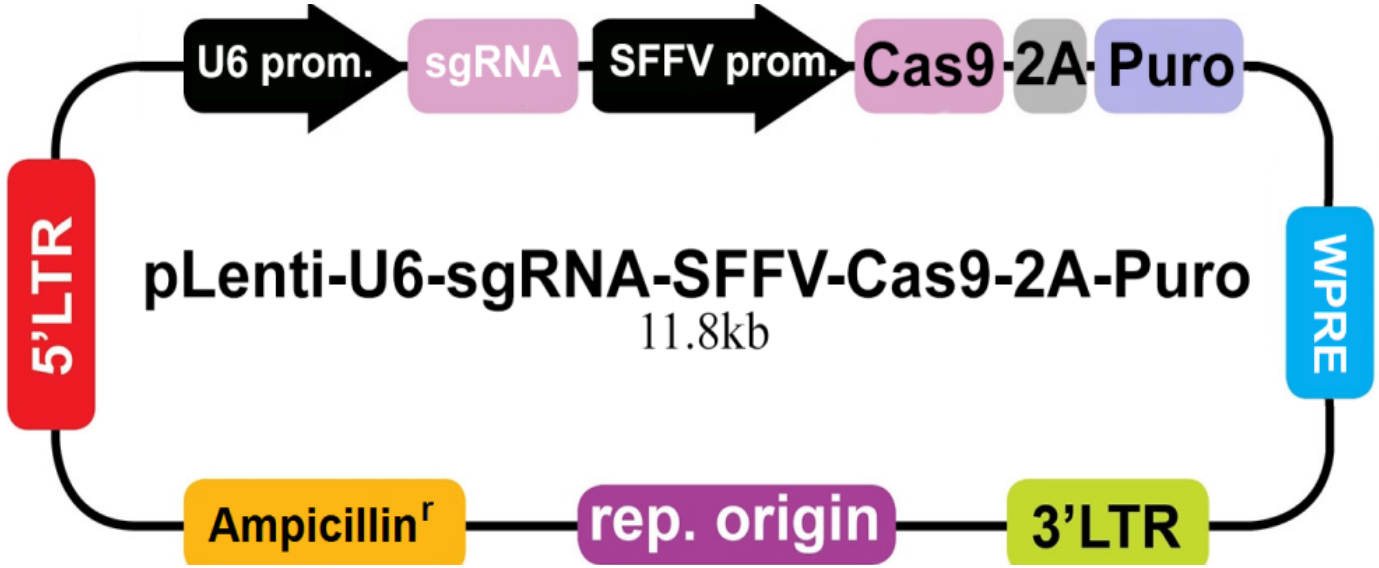
Bulge Type | DNA bulge | RNA bulge | Mismatch | Filter | Download filtered result

First | Previous | 1 | Next | Last

Bulge Type	Target	Chromosome	Position	Direction	Mismatches	Bulge Size
X	crRNA: GAGGCATGGGCAATCCTTTGNGG DNA: GAGGCATGGGCAATCCTTTGTGG	chr8	60741827	+	0	0
X	crRNA: GAGGCATGGGCAATCCTTTGNGG DNA: GAGaCATaaGCAATCCTTTGGGG	chr1	172448860	-	3	0
X	crRNA: GAGGCATGGGCAATCCTTTGNGG DNA: GAGGCATGGGaAAaCtTTTGGGG	chr13	21715920	+	3	0
X	crRNA: GAGGCATGGGCAATCCTTTGNGG DNA: GAGGCATGGGCAgcCCTTaGAGG	chr15	69665835	-	3	0
X	crRNA: GAGGCATGGGCAATCCTTTGNGG DNA: GAGGCATGGcCAcTaCTTTGTGG	chr6	116060166	-	3	0
X	crRNA: GAGGCATGGGCAATCCTTTGNGG DNA: GAGGCATGGGacATCCTTTGAGG	chr9	75453684	-	2	0

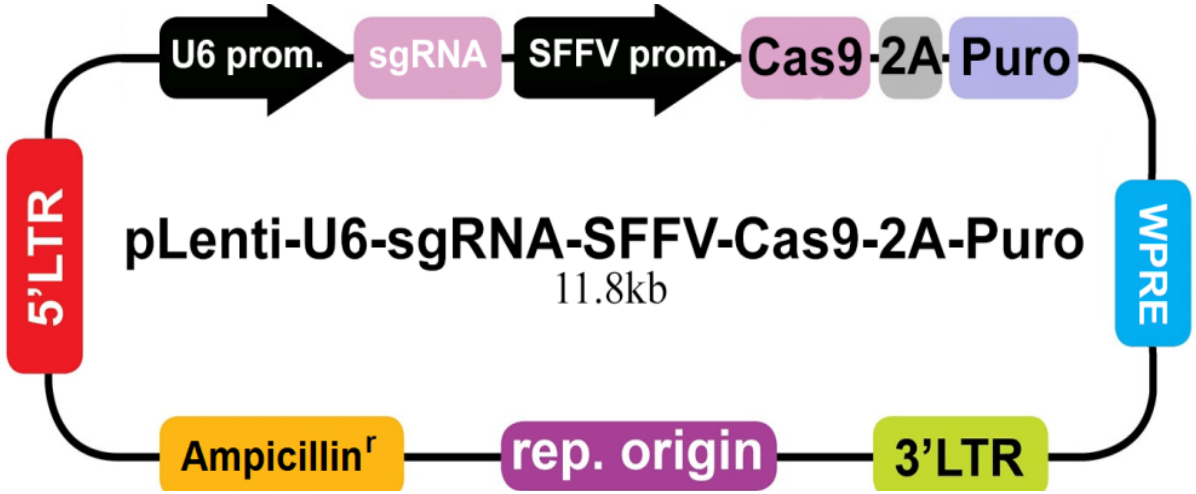
sgRNAs to CHD7 exon-1 in all-in-one pLentivector

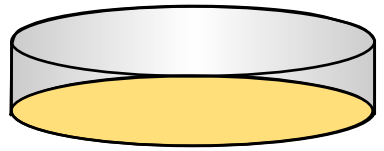
CHD7 Target 1- gRNA:	396	GAGGCATGGGCAATCCTTTG
CHD7 Target 2- gRNA:	1234	GCACTTCCCGGCCTGACTTG
CHD7 Target 3- gRNA	1322	GTGCCATGGGAATCGGACAG



Vector Name	pLenti-U6-sgRNA-SFFV-Cas9-2A-Puro
VectorType	Lentiviral Vector
Antibiotic Information	Bacterial: Ampicillin Mammalian: Puromycin

Preparation of DNAs (sgRNAs) in pLentivirus vector





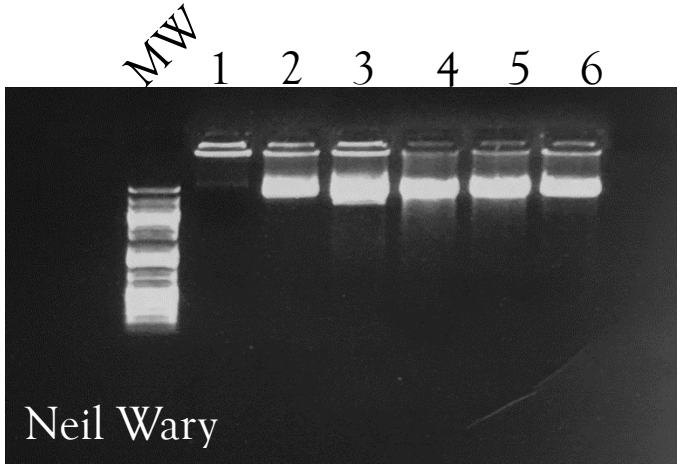
LB plate

Transform *E Coli* and grow in an ampicillin containing LB plates

Pick transformed *E. coli* colony from plates

Grow *E coli* in 100ml LB medium culture overnight

Prepare DNAs, quantify and check it in a agarose gel



Generating lentivirus all-in-one CHD7 sgRNA

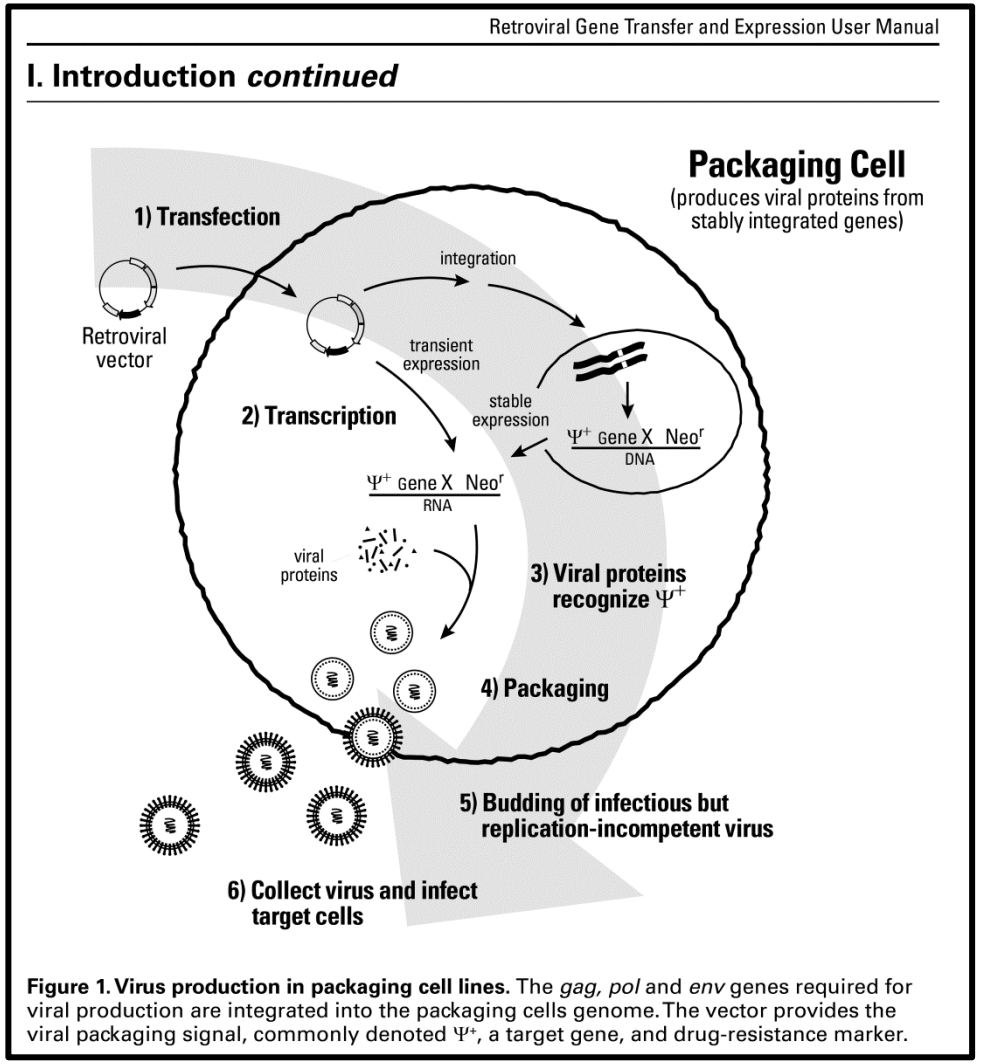
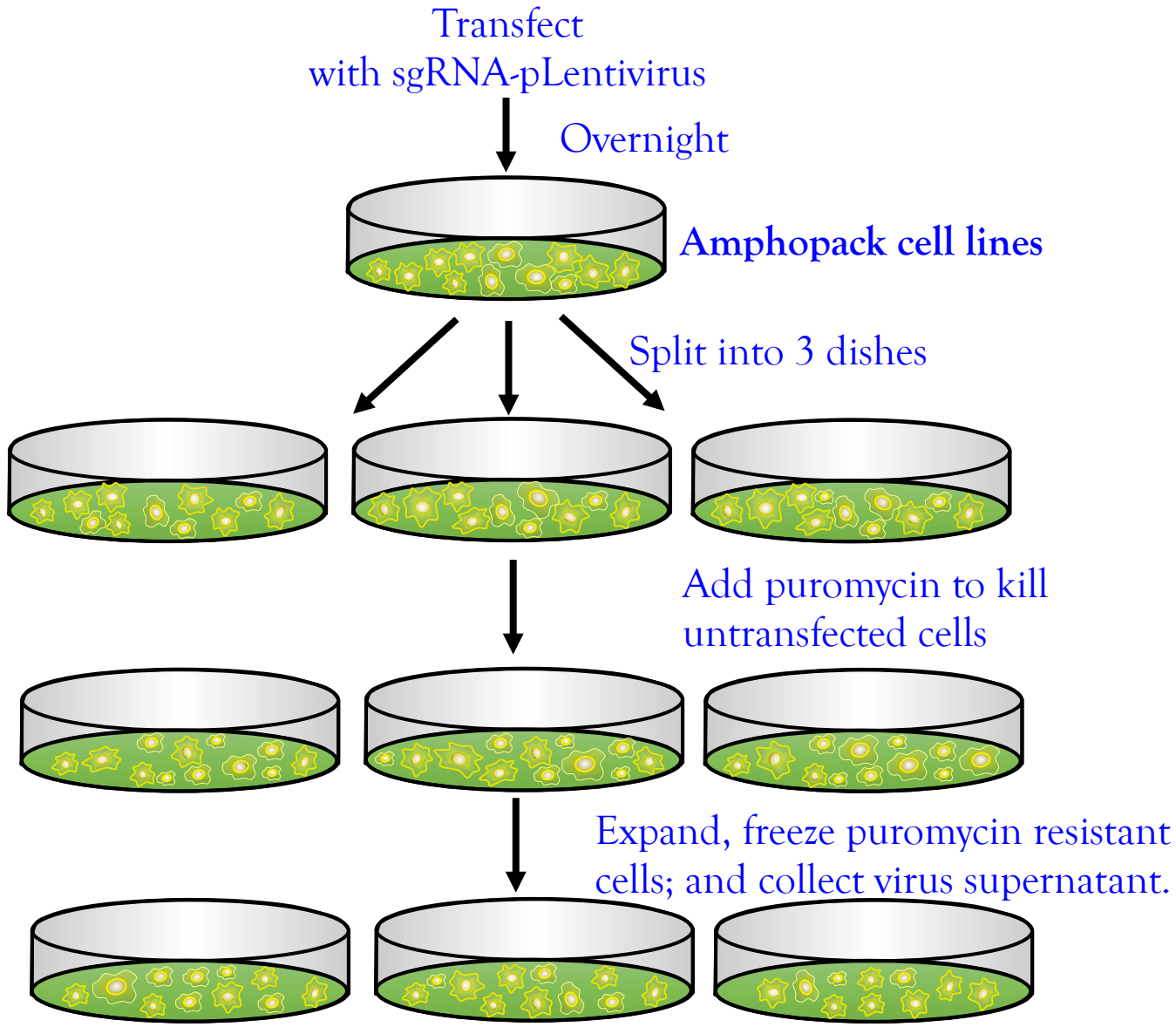
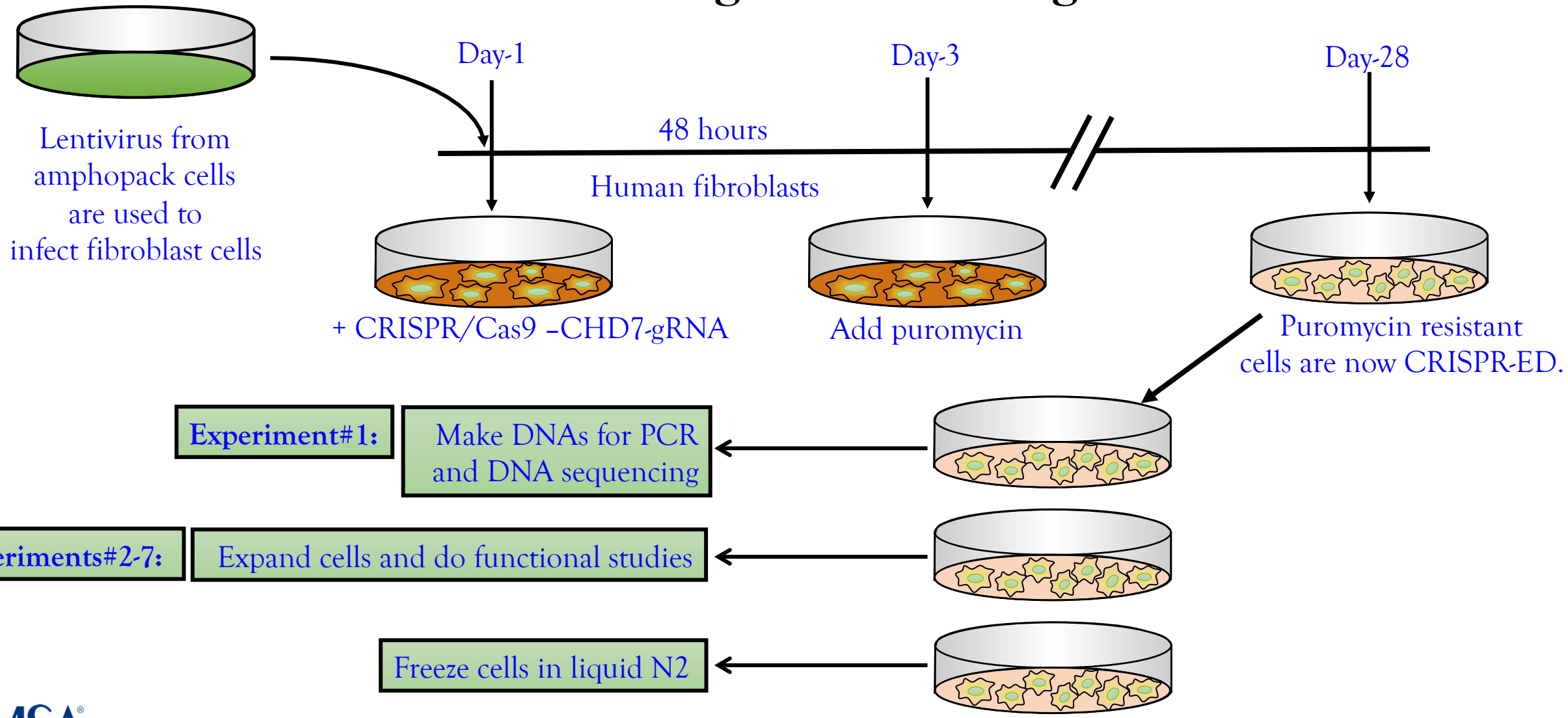


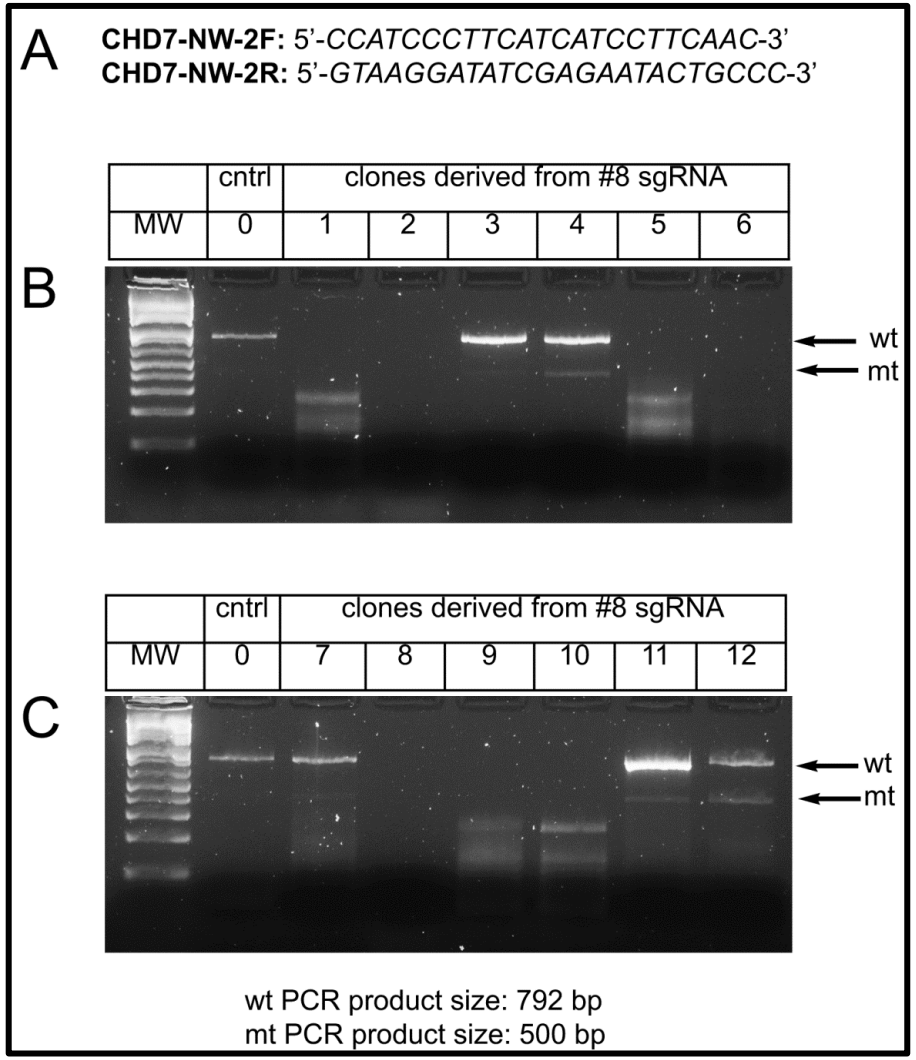
Figure 1. Virus production in packaging cell lines. The *gag, pol* and *env* genes required for viral production are integrated into the packaging cells genome. The vector provides the viral packaging signal, commonly denoted Ψ+, a target gene, and drug-resistance marker.



Normal human fibroblast cells infected with Lentivirus driving the *CHD7* sgRNA



Diagnostic test for CHD7 gene editing

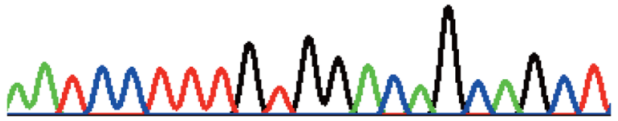


An example of *CHD7* gene editing DNA sequencing result

A

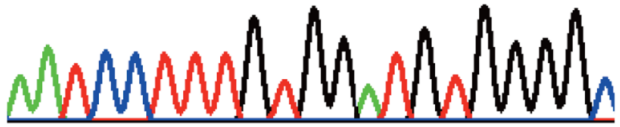
CTATGGGTCAGCAAATGCCAATAGACCAAGGCTTTGCCT***CTTTACAGCCATCCCTTCATCATC***CTTCAAC
TAATCAAAATCAAACAAAGCTGACACATTTTGATCACTATAATCAGTATGAACAACAAAAGATGCATCTG
ATGGATCAGCCGAACAGAATGATGAGCAACACCCCTGGGAACGGACTCGCGTCTCCGCACTCGCAGTATC
ACACCCCTCCCGTTCCTCAGGTGCCCCATGGTGGCAGTGGTGGCGGTCAGATGGGTGTCTACCCTGGCAT
GCAGAATGA**GAGGCATGGGCAATCCTTTGTGG**ACAGCAGCTCCATGTGGGGCCCCAGGGCTGTTCAGGTA
CCAGACCAGATACGAGCCCCCTACCAGCAGCAGCAGCCACAGCCGCAGCCACCGCAGCCGGCTCCGTCGG
GGCCCCCTGCACAGGGCCACCCTCAGCACATGCAGCAGATGGGCAGCTATATGGCACGTGGGGATTTTTC
CATGCAGCAGCATGGTCAGCCACAGCAGAGGATGAGCCAGTTTTCCCAAGGCCAAGAGGGCCTCAATCAG
GGAAATCCTTTTATTGCCACCTCAGGACCTGGCCACTTGTCCCACGTGCCCCAGCAGAGTCCCAGCATGG
CACCTTCCTTGCGTCACTCGGTGCAGCAGTTCCATCACCACCCCTCTACTGCTCTCCATGGAGAATCCGT
TGCCCACAGTCCCAGATTCTCCCCGAATCCTCCCCAACAAGGGGCTGTTAGGCCGCAAACCCTTAACTTT
AGTTCTCGGAGCCAGACAGTCCCCTCT***CCTACTATAAACAACTCAGGGCAG***TATTCTCGATATCCTTACA

B WT



C WT **AATCCTTTGTGGACAGCAGCT**

D MT

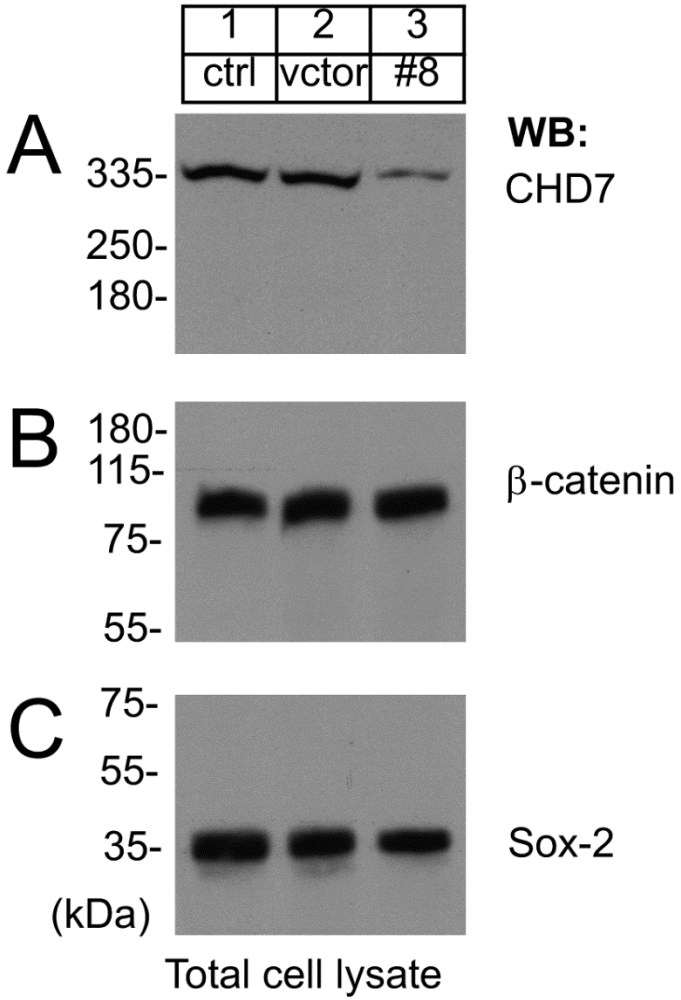


E MT **AATCCTTTGTGG/15 nt del**

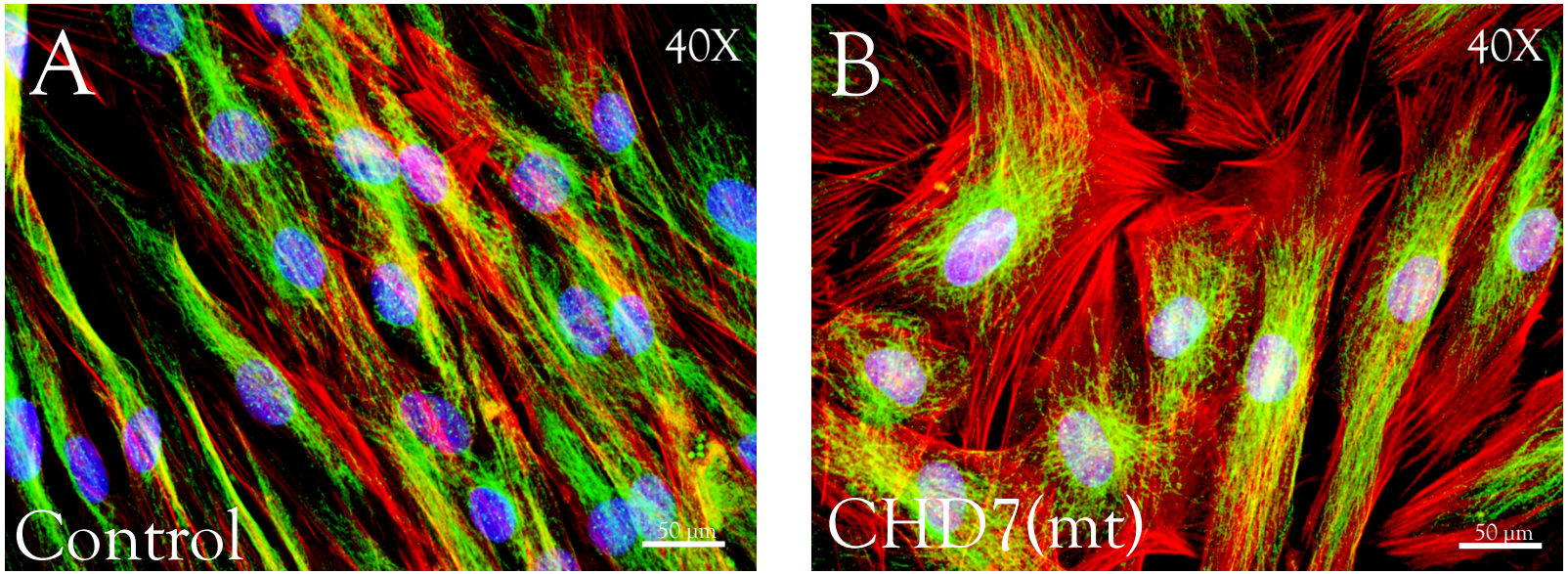
WT: wild-type

MT: mutant

Validation of *CHD7* gene editing by Western blot



An example of CHD7 mutant fibroblast cells



Green: Tubulin; **Red**: actin: **Blue**: DAPI (nucleus)

Summary:

Here, we:

1. Generated CRISPR/Cas9 lentivirus using three different sgRNAs.
2. Produced human fibroblast cells, in which CHD7 gene is mutated or deleted.
3. Used DNA sequencing method to identify and characterize the exact mutation.
4. Gearing up to carry-out functional study of this mutant gene using genome edited fibroblast cells.

Future Study:

These novel cell lines should become useful to screen drugs that could either increase (agonist) or decrease (antagonist) the expression of CHD7 – in the long run may aid in improving the lives of CHARGE children.

References

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