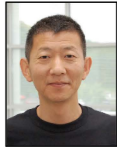


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Our service provides expertise to generate induced pluripotent stem cells (iPSC) by reprogramming and to perform iPSC gene editing by CRISPR/Cas9. We serve the UW-Madison human stem cell community, allowing scientists to focus their time and resources on the biological study and therapeutic applications of PSCs.

To learn more, visit  
<https://www.waisman.wisc.edu/ipsc-services>

To request services, please send email to  
[pjiang39@wisc.edu](mailto:pjiang39@wisc.edu)

## iPSC Reprogramming

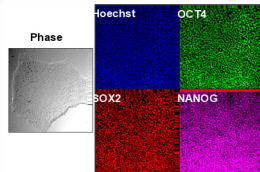
Banking and isolation of source material  
-- Fibroblasts from skin biopsies  
-- Peripheral blood mononuclear cells (PBMC) from fresh blood draws

Reprogramming to generate iPSC with Yamanaka factors and non-integrating methods (Sendai viruses) using two types of somatic cells  
-- Fibroblasts  
-- PBMCs

## iPSC characterization

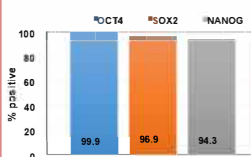
### Qualitative analysis

- Morphology via phase contrast
- Stem cell markers via immunofluorescence



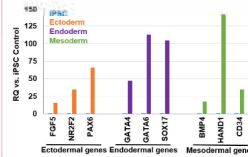
### Quantitative analysis

- Antibody staining for OCT4, SOX2, & NANOG
- High content image collection on Perkin-Elmer Operetta
- >10,000 cells counted to generate stem cell percentage



### Trilineage potential analysis

- Directed differentiation or embryoid body formation
- Custom qPCR array for germ layer markers



### WiCell characterization – 3 clones per line

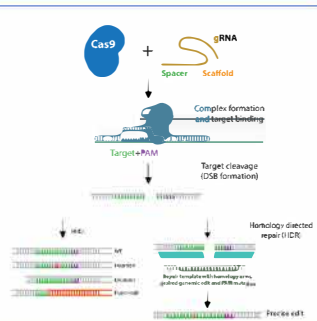
- Karyotyping
- Short Tandem Repeat (STR) profiling
- Sterility testing
- Mycoplasma testing
- Banking and distribution

Line	Cell Type	Genotype	STR	Y	Sex	Age	Source
1	Fibroblast	46,XY	158-162	158-162	Male	35	Human
2	Fibroblast	46,XY	158-162	158-162	Male	35	Human
3	Fibroblast	46,XY	158-162	158-162	Male	35	Human

## iPSC Deliverables

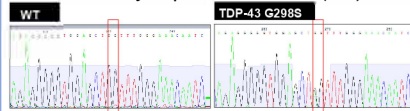
- 6 iPSC clones for each line
- Mycoplasma testing of starting material and final cell lines
- Frozen vials of resultant clones
- Qualitative stem cell analysis via immunofluorescence
- Quantitative stem cell analysis via high content image screening
- Optional – tri-lineage potential
- Optional – WiCell characterization

## CRISPR/Cas9

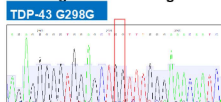


## Gene SNP modification

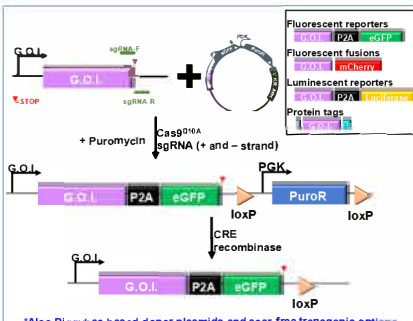
A G298S mutation in TDP-43 causes the neurodegenerative disease Amyotrophic lateral sclerosis (ALS)



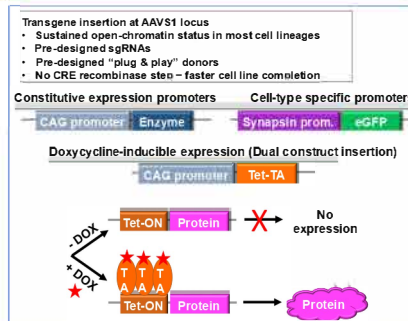
Using CRISPR/Cas9, we can repair the mutation and generate an isogenic control



## Endogenous transgenes



## Safe harbor transgenes



## CRISPR Deliverables

- CRISPR/Cas9 system design & preparation
- ≥3 clones for SNP modifications
- 1 sequenced homozygous and heterozygous for transgenes
- Off-target analysis at top 5 predicted loci for each gRNA
- Mycoplasma & karyotype testing
- Multiple vials of each clone
- All documents and sequences for publication