FINAL SCRIPT: APPROVED FOR FILMING

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Title: *Saccharomyces cerevisiae* Models of Alzheimer's Disease to Screen Genes, Mutations, and Chemicals Affecting Amyloid Beta Production by γ-Secretase

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**
   Videographer: Please film the shots labeled SCREEN as backup
   SCREEN: 2.11.2, 4.1.1

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 30
Number of Shots: 59

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1. **Eugene Futai:** We are reconstituting human γ-secretase in yeast to investigate how FAD-related mutations impact its activity and whether suppressor mutations or chemical modulators can restore cleavage and reduce pathogenic Aβ42 levels.

    1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:5.1* *Videographer's Note : last take best*

What technologies are currently used to advance research in your field?

    1.2. **Eugene Futai:** We combine functional analysis using mammalian cell and yeast systems with structural analysis via cryo-EM to study the mechanism of γ-secretase modulation.

        1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-*camera.*
        Videographer's Note : Last take best. Slated as 1.1.2

What significant findings have you established in your field?

    1.3. **Ying Dai:** We analyzed FAD mutations using γ-secretase reconstitution in yeast and identified suppressors that restore the activity of FAD mutants and reduce Aβ42 levels, mimicking the effect of γ-secretase modulators.

        1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Videographer's Note : Last two takes were good*

What advantage does your protocol offer compared to other techniques?

    1.4. **Mio Shiina:** Our protocol enables rapid, scalable functional screening of γ-secretase activity in yeast, allowing analysis of human mutations and modulators without relying on complex mammalian systems.

        1.4.1. INTERVIEW: Named Talent says the statement above in an interview-sty two takes le shot, looking slightly off-camera. *Suggested B.roll:5.6* *Videographer's Note : first and last best*

What research questions will your laboratory focus on in the future?

    1.5. **Mio Shiina:** We will focus on identifying new genetic or chemical modulators and inhibitors of γ-secretase, elucidating mechanisms of Aβ42 production, and expanding yeast-based reconstitution systems for other human intramembrane proteases.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Videographer's Note : First and last best*

Videographer: Obtain headshots for all authors available at the filming location.

2. Measuring γ-Secretase Activity in Yeast Using a Reporter Assay

Demonstrator: Mio Shiina

2.1. To begin, transform the plasmids into the yeast PJ-69-4A *(P-J-sixty-nine-four-A)* strain using the standard lithium acetate method to express γ-secretase *(gamma-secretase)* and its substrate **[1-TXT]**.

2.1.1. WIDE: Talent holding a plate with the transformed yeast. **TXT: Select transformants on synthetic complete medium without Leu, Trp and Ura**

2.2. Streak the transformed yeast strains onto SC-LWHUAde *(S-C-L-W-H-U-Aid)* medium also lacking histidine and adenine to assess substrate cleavage based on growth **[1]**. Incubate the plates at 30 degrees Celsius for 3 days **[2]**.

2.2.1. Talent streaking yeast onto SC-LWHUAde agar plates.

2.2.2. Shot of plates being placed inside an incubator set to 30 degrees Celsius.

2.3. Evaluate colony size to assess expression of HIS3 *(H-I-S-Three)* and ADE2 *(A-D-E-Two)* genes **[1]**.

2.3.1. Talent inspecting yeast plates and comparing colony sizes.
Videographer's Note : two different frames in one take
AND
TEXT ON PLAIN BACKGROUND:
Classify colony growth as:
+++ : Wild-type growth with colonies > than 1 mm
++ : Partial growth with colonies > than 0.5 mm
+ : Colonies < 0.5 mm
– : No growth

Video Editor: Please play both shots side by side

2.4. To assess substrate cleavage via β *(beta)*-galactosidase activity, grow the yeast strains in SC-LWU *(S-C-L-W-U)* liquid medium at 30 degrees Celsius until the culture reaches a concentration of 10^7 cells per milliliter **[1-TXT]**. Pipette out 1.5 milliliters of the culture into centrifuge tubes **[2]** then centrifuge the tubes at 21,100 *g* for 3 minutes at 4 degrees Celsius in a 1.5-milliliter tube **[3]**.

2.4.1. Talent placing culture tubes in a shaker. **TXT: Culture OD_{600}: 0.8 - 1.0**
Videographer's Note : two takes with different angles

2.4.2. Talent pipetting out 1.5 mL culture into tubes.

2.4.3. Talent placing the tubes in a centrifuge.

2.5. Wash the pellet with 100 microliters of ice-cold water **[1]** and resuspend it in 30 microliters of D-buffer containing 1 millimolar dithiothreitol and protease inhibitor cocktails made with water and dimethyl sulfoxide **[2]**.

2.5.1. Talent pipetting water into the tube with pellet.

2.5.2. Shot of the pellet being resuspended in D-buffer with additives.
Videographer's Note : extreme closeup. Audio slate only

2.6. Now add an acid-washed glass bead so that it is just below the surface of the solution **[1]**. Vortex the mixture ten times for 1 minute each at maximum speed **[2-TXT]**.

2.6.1. Talent dropping the bead into the tube.
Videographer's Note : extreme closeup. Audio slate only two takes

2.6.2. Talent vigorously vortexing with tube placed on ice between intervals. **TXT: Place on ice in between mixing for 1 min intervals**

2.7. With a hot 25-gauge needle, make a hole in the bottom of the tube **[1].** Centrifuge the lysed cells again at 865 *g* for 2 minutes then again 21,100 *g* for 10 minutes at 4 degrees Celsius **[2]**. Collect the supernatant as the crude lysate **[3]**.

2.7.1. Shot of a hole being made at the bottom of the tube with a heated 25 G needle.

2.7.2. Talent placing tube into centrifuge. *Videographer's Note :* two takes

2.7.3. Talent pipetting the clear supernatant into a fresh tube. *Videographer's Note :* two takes

2.8. Next, combine 5 to 10 microliters of crude lysate with 0.4 milliliters of Z-buffer **[1]**. Then add 80 microliters of 0.4% o-nitrophenyl-β-D-galactopyranoside solution and vortex the mixture **[2]**. Incubate at 37 degrees Celsius until the solution turns pale yellow due to nitrophenol formation **[3]**.

2.8.1. Talent pipetting lysate and Z-buffer into a tube.

2.8.2. Talent adding ONPG solution then mixing by vortexing.

2.8.3. Talent placing cuvette into 37 degrees Celsius water bath.
Videographer's Note : shot together with 2.8.2

2.9. Stop the reaction by adding 0.2 milliliters of 1 molar sodium carbonate solution **[1]**. Measure absorbance at 420 nanometers to determine β-galactosidase activity **[2]**.

2.9.1. Talent adding sodium carbonate to reaction mixture and vortexing.

2.9.2. Talent pipetting the solution in a cuvette and into a spectrophotometer.
Videographer's Note : Two takes

2.10. Quantify protein concentrations in crude lysates using a protein assay kit based on the Bradford method with bovine serum albumin as a standard **[1]**. Prepare the dye reagent by diluting one part of the stock with four parts of double-distilled deionized water **[2]**.

2.10.1. Talent arranging BSA standards, lysate samples and a 96-well plate for assay.

2.10.2. Talent preparing dye reagent.

2.11. Mix 1 to 5 microliters of each lysate or standard with 100 microliters of diluted dye in a microtiter plate well **[1]**. After incubating the plate at room temperature for 5 minutes, measure absorbance at 595 nanometers to determine protein concentration using the standard curve **[2]**.

2.11.1. Talent pipetting lysate into wells.

2.11.2. Talent placing the plate in a spectrophotometer.
Videographer's Note : Two takes and screen
AND
SCREEN: Excel file showing calculation for determination of protein concentration

Videographer: Please film the screen as backup

3. Measurement of Aβ Production By *In Vitro* Microsome Assay

Demonstrator: Mio Shiina

3.1. Start by combining two microsomes, each containing 40 to 80 micrograms of protein, with γ-buffer in a final volume of 12.5 microliters **[1-TXT]**. Pipette an equal volume of 2% CHAPSO *(Chap-so)* in γ-buffer before incubating for 1 hour **[2]**.

3.1.1. Talent pipetting microsomes and buffer into reaction tube. **TXT: Microsomes: APPC55 or γ-secretase**
Videographer's Note : **changed zoom at the end for closer shot**

3.1.2. Talent adding CHAPSO solution, mixing gently and placing tube on shaker or incubator.

3.2. To start the reaction, dilute the mixture four-fold to a final volume of 100 microliters with γ-buffer containing protease inhibitor mix **[1]**. Incubate the reaction at 37 degrees Celsius for 24 hours **[2-TXT]**.

3.2.1. Talent pipetting dilution buffer into reaction tube and mixing contents.
Videographer's Note : two frames

3.2.2. Talent placing tubes in 37 degrees Celsius incubator. **TXT: If necessary, add 0.1% PC, 10 µM L685, 458 DAPT or γ-secretase inhibitors and γ-secretase modulators**
Videographer's Note : **two takes slight frame adjustment**

3.3. Pipette 500 microliters of chloroform-methanol mixture in a 2 to 1 ratio to stop the reaction **[1]** and incubate for 1 hour, vortexing intermittently **[2]**.

3.3.1. Talent adding chloroform/methanol mixture.

3.3.2. Talent vortexing tubes.

3.4. Next, add 900 microliters of methanol and mix thoroughly **[1]**. Centrifuge the tubes at 21,100 *g* for 15 minutes to pellet amyloid-β peptides **[2]**.

3.4.1. Talent adding methanol and mixing thoroughly.

3.4.2. Talent placing the tubes in a centrifuge.

3.5. Wash the pellet with 500 microliters of chloroform-methanol-water mixture in a 1 to 2 to 0.8 ratio and centrifuge again **[1]**. After air-drying the pellet, suspend it in 40 microliters of SDS sample buffer **[2]** then boil for 10 minutes **[3]**.

3.5.1. Talent adding 500 µL of chloroform-methanol-water mix to the pellet.

3.5.2. Shot of the pellet being resuspended in 40 µL of SDS sample buffer.
Videographer's Note : Extreme closeup audio slate only

3.5.3. Talent boiling samples in heat block or water bath. *Videographer's Note :* Two takes second closer in

3.6. Now run the samples on a 16.5% Tris-Tricine *(Tris-Try-seen)* polyacrylamide gel to analyze amyloid-β production **[1-TXT]**.

3.6.1. Talent loading gel and starting electrophoresis on 16.5% gel. **TXT: Use 10% Tris-Tricine with 8M Urea (pH 8.45) for specific identification of amyloid- β**

Videographer's Note : **two takes second extreme closeup up audio**

3.7. Immunoblot with the 82E1 *(Eight-Tow-E-One)* primary antibody specific to the aspartic acid-1 site of human amyloid-β **[1]**. Use horseradish peroxidase-conjugated secondary antibody against mouse immunoglobulin G for detection **[2]**.

3.7.1. Talent incubating membrane with primary antibody 82E1.

3.7.2. Talent transferring the membrane into HRP-conjugated secondary antibody.

3.8. For chemiluminescent detection, prepare the substrate by mixing equal amounts of solution A and B **[1]** and incubate the membrane in the prepared solution for 1 minute **[2]**. Develop the blot and quantify the signal intensity using a luminescent image analyzer **[3]**.

3.8.1. Talent mixing chemiluminescent reagents A and B. Ok

3.8.2. Talent immersing membrane briefly in the mixture. Ok

3.8.3. Talent placing the membrane in a luminescent image analyzer. Ok

4. Identifying Modulatory Mutations in γ-Secretase or APP

Demonstrator: Ying Dai

4.1. Design primers for error-prone polymerase chain reaction that incorporate 40 base pair overlapping sequences from the gene of interest at the 5-prime ends of both forward and reverse primers **[1]**.

4.1.1. SCREEN: The primer sequences are being input into oligo design software.
Videographer: Please film the screen as backup

4.2. Now, prepare a 50-microliter error-prone polymerase chain reaction **[1]**. Run the thermal cycler for the specified number of cycles **[2]**.

4.2.1. Talent assembling PCR reaction in PCR tubes.
Videographer's Note : Two shots one closer
AND
TEXT ON PLAIN BACKGROUND:
PCR Reaction Mixture
50 ng of template DNA
0.2 mM dGTP
1 mM dATP/dTTP/dCTP

3 mM $MgCl_2$
0.5 mM $MnCl_2$
1.25 U Taq polymerase
400 nM primers

Use 1X Taq buffer
Final volume: 50 µL
Video Editor: Please play both shots side by side

4.2.2. TEXT ON PLAIN BACKGROUND: shot placing tube into pcr
94 °C for 5 min
30 cycles of 94 °C for 30 s
55 °C for 30 s
72 °C for 120 s (1 min/kb)
72 °C for 10 min

4.3. Transform 4 micrograms of mutagenized DNA fragments along with 4 micrograms of linearized plasmid into the PJ69-4A *(P-J-sixty-nine-four-A)* yeast strain expressing familial Alzheimer's disease APP or PS1 mutations **[1]**. Then select transformants on SC-LWU plates **[2]**.

4.3.1. Talent pipetting DNA into yeast cells for transformation and plating on SC-LWU.
*Videographer's Note : S*hot out of order taking in 2 separate takes. 4.3.1a 4.3.1b use from 13:45:41 for 4.3.1a. Third take 4.3.2 should be the best

4.3.2. Talent taking out plates from the incubator ~~and labeling SC-LWU plates with transformants.~~

4.4. Count the colonies on SC-LWU plates **[1]**. Replicate them by pressing the original plate onto a velvet-covered block **[2]** then press a fresh SC- LWHUAde plate medium lacking histidine and adenine plate to transfer colonies **[3]**.

4.4.1. Talent counting colonies.and labeling SC-LWU plates with transformants.
*Videographer's Note : F*rom 4.3.2 two takes pen wasn't working

4.4.2. Shot of the plate being pressed onto velvet-covered block.

4.4.3. Talent transferring colonies onto new plate using velvet technique.

4.5. To recover plasmid DNA from positive clones, inoculate the strains in 3 milliliters of SC-LWU medium **[1]**. Incubate overnight at 30 degrees Celsius until optical density at 600 nanometers exceeds 1 **[2]**.

4.5.1. Talent inoculating the strains in 3 mL SC-LWU medium.

4.5.2. Shot of the culture being placed at 30 °C.

4.6. Centrifuge the cells in a 15-milliliter tube at 2,100 *g* for 10 minutes at 4 degrees Celsius **[1]**. Then wash the pellet with 0.8 milliliters of ice-cold water **[2]**.

4.6.1. Shot of the tube being placed in a centrifuge.

4.6.2. Talent adds 0.8 mL of ice-cold water to the pellet.
Videographer's Note : Use from 14:25:10 this also includes 4.7.1

4.7. Transfer the suspension into a new 1.5-milliliter tube before centrifuging again for 3 minutes **[1]**. Resuspend the pellet in 0.3 milliliters of lysis solution **[2]**.

4.7.1. Talent transferring the suspension to microcentrifuge tube.

4.7.2. Talent adding lysis solution to the pellet.

4.8. Add 0.3 milliliters of phenol-chloroform-isoamyl alcohol mixture in a 25 to 24 to 1 ratio and 0.3 grams of glass beads to lyse the cells **[1]**. Vortex the mixture at maximum speed for 10 minutes, then centrifuge **[2-TXT]**.

4.8.1. Talent adding extraction solvent and glass beads.

4.8.2. Talent vortexing vigorously. **TXT: Centrifugation: 21,000 x *g*, 10 min, RT**

4.9. Transfer the upper aqueous phase to a new tube **[1]**. Mix it with 30 microliters of 3 molar sodium acetate and 700 microliters of 100% ethanol to precipitate DNA **[2]**.

4.9.1. Talent pipetting clear aqueous layer without disturbing interphase.
Videographer's Note : Extreme closeup up audio slate only. Did second take later on. Please use second take.

4.9.2. Talent adding sodium acetate and ethanol to precipitate DNA.
Videographer's Note : Shot out of sequence

4.10. After centrifuging the DNA pellet as before, wash it with 70 % ethanol, air-dry **[1]** and vortex to resuspend the DNA in 15 microliters of TE buffer **[2]**.

4.10.1. Talent adding 70% ethanol to the DNA pellet.

4.10.2. Talent vortexing dried pellet in TE buffer.

4.11. Transform the purified plasmid DNA into *Escherichia coli* DH5α *(D-H-Five-Alpha)* using high-efficiency transformation techniques **[1-TXT]**.

4.11.1. Shot of a plate with transformed *E.coli.* **TXT: If targeting specific protein regions, design Quikchange or inverse PCR primers**

Results

5. Representative Results

5.1. The double mutant F411Y/S438P *(F-Four-eleven-Y-S-four-three-Eight-P)* supported robust yeast growth in the absence of nicastrin, forming colonies larger than 1 millimeter **[1]**, and showed full β-galactosidase activity comparable to wild-type PS1 *(P-S-One)* **[2]**.

5.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the sector labeled "F411Y/S438P no NCT" in the circle and mark the large white colonies in that quadrant of the agar plate.*

5.1.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the two tallest bars labeled "PS1 WT" and "F411Y/S438P"*

5.2. S438P *(S-four-three-Eight-P)* showed weak growth while F411Y *(F-Four-eleven-Y)* showed none **[1]**, and S438P *(S-four-three-Eight-P)* showed low β-galactosidase activity while F411Y *(F-Four-eleven-Y)* showed no activity **[2]**.

5.2.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the sectors labeled "F411Y no NCT" and "S438P no NCT"*

5.2.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the two shortest bars labeled "S438P" and "F411Y"*

5.3. Rescreening cells from the PS1 F411Y mutant reconfirmed F411Y/S438P as the dominant active clone, with 40 positive hits **[1]**, while only 1 additional clone showed alternative triple mutations **[2]**.

5.3.1. LAB MEDIA: Table 5. *Video editor: Highlight the first row under section 1 showing "F411Y/S438P (the original mutant)"*

5.3.2. LAB MEDIA: Table 5. *Video editor: Highlight the second row under section 1 showing "V236M/F428Y/F411Y" with just 1 clone.*

5.4. Screening from the S438P mutant revealed multiple combinations with low clone counts, except for V236M/S438P *(V-Two-Three-Six-M-S-four-three-Eight-P)* which appeared in 13 positive clones, indicating S438P's dominant role over other mutations **[1]**.

5.4.1. LAB MEDIA: Table 5. Video editor: Highlight row "V236M/S438P"

5.5. The L30F/T164A Aph1a *(L-thirty-F-T-Sixteen-Four-A-A-P-H-1-A)* mutant increased total amyloid-beta production under both phosphatidylcholine-positive and phosphatidylcholine-negative conditions **[1]**, particularly enhancing aggregation-prone Aβ42 and Aβ43 species in the phosphatidylcholine-negative condition **[2]**.

5.5.1. LAB MEDIA: Figure 2C upper bar graph. *Video editor: Highlight the black bars labeled "L30F/T164A" in both PC– and PC+ groups*

5.5.2. LAB MEDIA: Figure 2C lower bar graph. *Video editor: Highlight black bars for "L30F/T164A" under PC– for Aβ42 and Aβ43*

5.6. Phosphatidylcholine enhanced amyloid-beta production for both wild-type and mutant forms **[1]**, while γ-secretase inhibitor L685,458 blocked Aβ generation completely **[2]**.

5.6.1. LAB MEDIA: Figure 2C western blot (upper). *Video editor: Highlight lanes 3 and 7 (+PC) showing thick Aβ40 bands*

5.6.2. LAB MEDIA: Figure 2C western blot (lower).

Pronunciation Guide:

Saccharomyces cerevisiae
Pronunciation link: https://www.merriam-webster.com/dictionary/Saccharomyces
IPA: /ˌsækəˈroʊmɪˌsiːz/ səˌriːˈvɪsiaɪ/
Phonetic Spelling: sak-uh-roh-my-seez ser-ee-viss-ee-eye

Alzheimer's
Pronunciation link: https://www.merriam-webster.com/dictionary/Alzheimer%27s
IPA: /ˈɑːltshaɪmərz/, /ˈɔːltshaɪmərz/, /ˈæltshaɪmərz/
Phonetic Spelling: allts-hy-mers or alts-hy-mers

Amyloid
Pronunciation link: https://www.merriam-webster.com/dictionary/amyloid
IPA: /ˈæməˌlɔɪd/
Phonetic Spelling: am-uh-loid

γ-Secretase (gamma-secretase)
Pronunciation link: https://www.merriam-webster.com/dictionary/secretase
IPA: /ˈsɛkrəˌteɪs/
Phonetic Spelling: gam-uh-sek-ruh-tayss

Cryo-EM (Cryo-electron microscopy)
Pronunciation link: https://www.merriam-webster.com/dictionary/cryo-EM
IPA: /ˌkraɪoʊ iːˈɛm/
Phonetic Spelling: kry-oh-ee-em

Dithiothreitol
Pronunciation link: https://www.merriam-webster.com/dictionary/dithiothreitol
IPA: /daɪˌθaɪoʊˈθriːɪtɔːl/
Phonetic Spelling: dye-thigh-oh-three-it-all

Dimethyl sulfoxide (DMSO)
Pronunciation link: https://www.merriam-webster.com/dictionary/dimethyl%20sulfoxide
IPA: /daɪˈmɛθəl ˈsʌlfɒksaɪd/
Phonetic Spelling: dye-meth-uhl sull-fok-side

o-Nitrophenyl-β-D-galactopyranoside (ONPG)
Pronunciation link: No confirmed link found
IPA: /ˌoʊ naɪtroʊˈfɛnəl ˌbeɪtə di gəˌlæktəˈpaɪrəˌnəsaɪd/
Phonetic Spelling: oh-ny-troh-fen-uhl bay-tuh-dee guh-lack-toh-pie-ruh-nuh-side

Tris-Tricine
Pronunciation link: No confirmed link found
IPA: /trɪs ˈtraɪsiːn/
Phonetic Spelling: tris-try-seen

Immunoblot
Pronunciation link: https://www.merriam-webster.com/dictionary/immunoblot
IPA: /ɪˈmjuːnəˌblɑt/
Phonetic Spelling: ih-myoo-noh-blot

Horseradish peroxidase
Pronunciation link: https://www.merriam-webster.com/dictionary/peroxidase
IPA: /ˈhɔrsˌrædɪʃ pəˈrɑksɪˌdeɪs/
Phonetic Spelling: horse-rad-ish puh-rock-sih-dayss

Chemiluminescent
Pronunciation link: https://www.merriam-webster.com/dictionary/chemiluminescence
IPA: /ˌkɛmiˌluːməˈnɛsənt/
Phonetic Spelling: keh-mee-loo-muh-ness-ent

Escherichia coli
Pronunciation link: https://www.merriam-webster.com/dictionary/Escherichia%20coli
IPA: /ˌɛʃəˈrɪkiə ˈkoʊˌlaɪ/
Phonetic Spelling: esh-uh-rik-ee-uh koh-lie

Nicastrin
Pronunciation link: No confirmed link found
IPA: /ˌnaɪˈkæstrɪn/
Phonetic Spelling: nigh-kass-trin

Phosphatidylcholine
Pronunciation link: https://www.merriam-webster.com/dictionary/phosphatidylcholine
IPA: /ˌfɑsfəˌtaɪdəlˈkoʊˌliːn/
Phonetic Spelling: foss-fuh-tie-dul-koh-leen