Mouse Genotyping Protocol

Dasgupta Laboratory - Emory University

SCD1, SCD2, Cdh5-Cre, and tdTomato Reporter Lines

Protocol Overview

This protocol describes comprehensive genotyping procedures for transgenic mouse lines maintained in the Dasgupta Laboratory. Each mouse line requires specific primers and PCR conditions to accurately determine genotypes.

General Principles:

- Always include positive and negative controls
- Run wild-type and floxed/transgene primers in separate reactions unless noted
- Use fresh reagents and maintain sterile technique
- Document all results in laboratory notebook and database

🧬 Mouse Lines Covered

- **SCD1 Floxed** - Conditional knockout allele
- **SCD2 Floxed** - Conditional knockout allele
- **Cdh5-Cre** - Endothelial cell-specific Cre
- **tdTomato Reporter** - Rosa26-LSL-tdTomato

⏱️ Timeline

- **Day 1:** DNA extraction (~2-3 hours)
- **Day 2:** PCR setup and run (~3-4 hours)
- **Day 2:** Gel electrophoresis (~1 hour)
- **Total:** 2 days

⚠️ Important Notes:

- Tail samples are clipped and stored in -20°C freezer (arrivals bin)
- Change pipette tips between each sample to prevent cross-contamination

- Keep all reagents on ice during setup
- Store DNA samples at 4°C (not -20°C) for accessibility

Required Materials

Equipment

- Heat blocks (95°C) - holds 12 tubes each
- Thermal cycler (PCR machine)
- Centrifuge (max speed ~13,000 rpm)
- Vortex mixer
- Microwave for gel preparation
- Gel electrophoresis apparatus
- UV transilluminator or blue light imager
- Pipettes (P20, P200, P1000)
- Multichannel pipette (optional, for large batches)

DNA Extraction Reagents

- 25 mM NaOH/EDTA solution
- 40 mM Tris-HCl (pH neutralization)
- 1.5 mL microcentrifuge tubes
- Cap locks for heat block tubes
- Sterile pipette tips
- Mouse tail tissue samples (from -20°C freezer)

PCR Reagents

- 2X PCR Master Mix (Taq polymerase, dNTPs, buffer)
- Primers (10 µM working stocks) - see Mouse Lines section
- Nuclease-free water
- PCR tubes or 96-well PCR plates
- Positive control DNA (known genotypes)
- Negative control (water)

Gel Electrophoresis

- Agarose powder
- 1X TAE buffer
- Ethidium bromide or safe DNA stain
- 6X loading dye
- 100 bp DNA ladder
- Gel casting tray and combs
- UV protection equipment (face shield, gloves)

Standard PCR Reaction (25 µL total)

Component	Volume	Final Concentration
2X PCR Master Mix	12.5 µL	1X
Forward Primer (10 µM)	1.0 µL	0.4 µM
Reverse Primer (10 µM)	1.0 µL	0.4 µM
Template DNA	1.0 µL	~50-100 ng
Nuclease-free water	9.5 µL	-
Total	**25 µL**	

DNA Extraction (Tail Digestion)

> **Note:** Tail samples are clipped in the latter half of the week and stored in the arrivals bin in the -20°C freezer.

Step 1: Preheat Heat Blocks

Mark Done

Preheat the heat blocks to **95°C**.

- Takes approximately 10-20 minutes
- Each heat block holds 12 tubes - plan accordingly
- Start preheating while organizing samples

Step 2: Add NaOH/EDTA Solution

Mark Done

1. Add **100 µL of 25 mM NaOH/EDTA** to each tube containing tail tissue
2. Ensure the tail is fully submerged in the solution using pipette tips

> **CRITICAL:**
> If a pipette tip touches the inside of the tube or the tail, it MUST be discarded and replaced before moving to the next tube.

Step 3: Incubate at 95°C

Mark Done

1. Put cap locks on every tube
2. Place tubes in the preheated heat blocks
3. Let sit for **1 hour**
4. Set a timer to avoid over-digestion

Step 4: Cool Samples

Mark Done

Remove tubes from heat blocks and allow to cool for **10-60 seconds** before removing cap locks.

This cooling period allows tubes to relieve pressure from temperature change and prevents them from popping open and splashing solution.

Step 5: Add Tris-HCl (Neutralization)

Mark Done

Add **100 µL of 40 mM Tris-HCl** to each tube.

> **IMPORTANT:**
> If the pipette tip touches the inside of the tube or the solution, it MUST be discarded and replaced before moving to the next tube.

Step 6: Vortex

Mark Done

Vortex the tubes thoroughly to mix the neutralized solution.

Step 7: Centrifuge

Mark Done

Centrifuge tubes at **max speed for 5 minutes**.

> **BE CAREFUL:**

When removing tubes, all tissue is at the bottom. Jostling them too much can reverse the effects of centrifugation.

Step 8: Transfer Supernatant

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1. Prepare and label new eppendorf tubes corresponding to each sample
2. Carefully pipette out **150 µL of supernatant** from each tube into its respective new tube
3. Be careful NOT to pick up any particulate matter (this will destroy sample over time)
4. The pipette tip MUST be changed between each sample

Step 9: Store DNA Samples

Mark Done

Store the DNA samples in the **4°C freezer** in their respective boxes.

Note: DNA is stored at 4°C (not -20°C) for easy access and to prevent freeze-thaw cycles.

PCR Setup and Master Mix

PCR Setup Tips:

- Prepare master mix for all reactions plus 10% extra
- Keep all reagents on ice
- Add DNA template last
- Mix thoroughly but gently
- Spin down briefly before placing in thermocycler

Master Mix Preparation

Master Mix Strategy: Prepare a master mix containing all components except the DNA template. This ensures consistency across samples and reduces pipetting errors.

Example: For 10 samples + 2 controls = 12 reactions total

Add 10% extra: 12 × 1.1 = **13.2 reactions** (round to 13)

Component	1 rxn	13 rxns
2X Master Mix	12.5 µL	162.5 µL
Forward Primer (10 µM)	1.0 µL	13 µL

Component	1 rxn	13 rxns
Reverse Primer (10 µM)	1.0 µL	13 µL
Nuclease-free water	9.5 µL	123.5 µL
Master Mix Total	**24 µL**	**312 µL**

Aliquot 24 µL master mix into each PCR tube, then add 1 µL DNA template (total 25 µL)

PCR Setup Procedure

Mark Done

1. Thaw all reagents on ice (2X Master Mix, primers, DNA)
2. Label PCR tubes with sample IDs
3. Calculate master mix volumes (number of reactions + 10%)
4. In a clean tube, combine:
   - 2X PCR Master Mix
   - Forward primer
   - Reverse primer
   - Nuclease-free water
5. Mix master mix thoroughly by pipetting up/down or vortexing gently
6. Aliquot 24 µL of master mix into each labeled PCR tube
7. Add 1 µL of DNA template to each tube (change tip between samples)
8. Include controls:
   - Positive control: Known genotype DNA
   - Negative control: Water instead of DNA
9. Cap tubes and spin down briefly (quick spin, 2-3 seconds)
10. Place tubes in thermocycler
11. Start appropriate PCR program (see Mouse Lines section)

Pro Tips:
• For multiple genotypes, prepare separate master mixes for each primer set
• Keep master mix on ice while aliquoting to tubes
• Work quickly but carefully to minimize time at room temperature
• Double-check that thermocycler program is correct before starting

SCD1 Floxed | SCD2 Floxed | Cdh5-Cre | tdTomato Reporter

SCD1 Floxed Allele Genotyping

Purpose:

To identify mice carrying the floxed SCD1 allele (conditional knockout)

Reaction A: Floxed Allele Detection

Forward (T-SCD1 PrA):	`5'-CAACTCAGGGTTTCCCTA-3'`
Reverse (Neo 7):	`5'-ATAGCAGGCATGCTGGGGAT-3'`
Expected Product:	**>1000 bp** (floxed)

Reaction B: Wild-Type Allele Detection

Forward (T-SCD1 PrA):	`5'-CAACTCAGGGTTTCCCTA-3'`
Reverse (T-SCD1 PrB):	`5'-GCCATGGCTACTCAGTGA-3'`
Expected Product:	**~700 bp** (wild-type)

PCR Cycling Conditions

Step	Temperature	Time	Cycles
Initial Denaturation	95°C	2 min	1
Denaturation	95°C	30 sec	35 cycles
Annealing	58°C	45 sec	
Extension	72°C	1 min	
Final Extension	72°C	10 min	1
Hold	4°C	∞	

Interpretation:

- **FL/FL:** Band >1000 bp (Rxn A), no band (Rxn B)

- **+/+:** No band (Rxn A), band ~700 bp (Rxn B)
- **FL/+:** Band >1000 bp (Rxn A), band ~700 bp (Rxn B)

Important Notes:

- Run Reactions A and B **separately**
- Include wild-type control in both reactions
- Floxed band is large (>1000 bp) - run gel longer if needed

SCD2 Floxed Allele Genotyping

Purpose:

To identify mice carrying the floxed SCD2 allele

Reaction A: Floxed Allele Detection

Forward (Scd2-A):	`5'-TGGGTAGTCAGGGATCAAGG-3'`
Reverse (Scd2-C):	`5'-ATAGCAGGCATGCTGGGAT-3'`
Expected Product:	**~250 bp** (floxed)

Reaction B: Wild-Type Allele Detection

Forward (Scd2-A):	`5'-TGGGTAGTCAGGGATCAAGG-3'`
Reverse (Scd2-B):	`5'-ATCTCGACACGGCACAGAG-3'`
Expected Product:	**~500 bp** (wild-type)

PCR Cycling Conditions

Step	Temperature	Time	Cycles
Initial Denaturation	95°C	2 min	1

Step	Temperature	Time	Cycles
Denaturation	95°C	20 sec	
Annealing	65°C	30 sec	40 cycles
Extension	72°C	1 min 10 sec	
Final Extension	72°C	5 min	1
Hold	4°C	∞	

Interpretation:

- **FL/FL:** Band ~250 bp (Rxn A), no band (Rxn B)
- **+/+:** No band (Rxn A), band ~500 bp (Rxn B)
- **FL/+:** Band ~250 bp (Rxn A), band ~500 bp (Rxn B)

Important Notes:

- Run Reactions A and B as **2 separate reactions**
- SCD2 floxed band (~250 bp) is smaller than SCD1 (>1000 bp)
- Include appropriate controls for both reactions

Cdh5-Cre (VE-Cadherin-Cre) Genotyping

Purpose:

To identify mice carrying the Cdh5-Cre transgene (endothelial cell-specific Cre recombinase expression)

Transgene Detection

Forward (15623):	`5'-AGGCAGCTCACAAAGGAACAAT-3'`
Reverse (15625):	`5'-TCGTTGCATCGACCGGTAA-3'`
Expected Product:	**~300 bp** (transgene)

Internal Positive Control

Control F (oIMR7338):	`5'-CTAGGCCACAGAATTGAAAGATCT-3'`
Control R (oIMR7339):	`5'-GTAGGTGGAAATTCTAGCATCATCC-3'`
Expected Product:	**324 bp** (always present)

Special Protocol: Touchdown PCR

This protocol uses a touchdown approach where the annealing temperature decreases by 0.5°C per cycle during the first 10 cycles (65°C down to 60°C). This improves specificity.

PCR Cycling Conditions (Touchdown Protocol)

Step	Temperature	Time	Cycles
Initial Denaturation	94°C	2 min	1
Touchdown Phase			
Denaturation	94°C	30 sec	10 cycles
Annealing	65°C (-0.5°C/cycle)	30 sec	
Extension	68°C	30 sec	
Standard Phase			
Denaturation	94°C	30 sec	28 cycles
Annealing	60°C	30 sec	
Extension	72°C	30 sec	
Final Extension	72°C	2 min	1
Hold	4°C	∞	

Interpretation:

- **Cre/+:** Two bands at ~300 bp and 324 bp

- **+/+:** Single band at 324 bp (internal control only)

Important Notes:

- Internal control confirms PCR reaction worked
- If no 324 bp band appears, PCR failed - do not interpret transgene results
- Can run as single reaction with all 4 primers (multiplex)

tdTomato Reporter Genotyping

Purpose:

To identify mice carrying the Rosa26-LSL-tdTomato reporter allele. This reporter is useful for visualizing Cre activity via tdTomato fluorescence (bright red under fluorescence microscopy).

Mutant (tdTomato) Allele Detection

Forward (oIMR9105):	`5'-CTGTTCCTGTACGGCATGG-3'`
Reverse (oIMR9103):	`5'-GGCATTAAAGCAGCGTATCC-3'`
Expected Product:	**196 bp** (mutant)

Wild-Type Allele Detection

Forward (oIMR9020):	`5'-AAGGGAGCTGCAGTGGAGTA-3'`
Reverse (oIMR9021):	`5'-CCGAAAATCTGTGGGAAGTC-3'`
Expected Product:	**297 bp** (wild-type)

Multiplex Option:

Can run as single multiplex reaction with all 4 primers. Both bands will appear in heterozygous mice, making genotype interpretation straightforward.

PCR Cycling Conditions (Touchdown Protocol - Recommended)

Step	Temperature	Time	Cycles
Initial Denaturation	94°C	2 min	1
Touchdown Phase			
Denaturation	94°C	30 sec	10 cycles
Annealing	65°C (-0.5°C/cycle)	30 sec	
Extension	68°C	30 sec	
Standard Phase			
Denaturation	94°C	30 sec	28 cycles
Annealing	60°C	30 sec	
Extension	72°C	30 sec	
Final Extension	72°C	2 min	1
Hold	4°C	∞	

Alternative Protocol (Yoshida Lab)

Step	Temperature	Time	Cycles
Initial Denaturation	94°C	5 min	1
Denaturation	94°C	45 sec	38 cycles
Annealing	60°C	45 sec	
Extension	72°C	1 min	
Final Extension	72°C	7 min	1
Hold	4°C	∞	

Interpretation:

- **Td/Td:** Band at 196 bp only

- **Td/+:** Bands at both 196 bp and 297 bp
- **+/+:** Band at 297 bp only

Important Notes:

- Homozygous tdTomato mice are bright red under fluorescence microscopy
- Useful for Cre-reporter experiments to visualize Cre activity
- Can run as single multiplex reaction with all 4 primers

Gel Electrophoresis

Agarose Gel Preparation (2%)

Mark Done

2% Agarose Gel (Standard for Genotyping)

2% gels provide good resolution for DNA fragments from ~100 bp to ~1500 bp, making them ideal for genotyping where bands range from 196 bp to >1000 bp.

1. Weigh **2 g agarose powder**
2. Add to **100 mL 1X TAE buffer** in Erlenmeyer flask
3. Microwave in 30-second intervals, swirling between heating, until fully dissolved
   - Total time usually 2-3 minutes
   - Watch carefully to prevent boil-over
4. Cool to ~60°C (comfortable to touch flask)
5. Add **5 µL ethidium bromide** (10 mg/mL) or equivalent DNA stain
   - Alternative: Use safer DNA stains like SYBR Safe or GelRed
6. Pour into gel casting tray with comb in place
7. Allow to solidify (30-45 minutes at room temperature)
8. Remove comb carefully
9. Place gel in electrophoresis chamber

Running the Gel

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1. Fill electrophoresis chamber with **1X TAE buffer** (just covering gel)
2. Mix **5 µL PCR product** with **1 µL 6X loading dye**
3. Load samples into wells
   - Load entire 6 µL mixture

- Pipette slowly to avoid sample escaping well

4. Load **5 µL of 100 bp DNA ladder** in first and/or last lane
5. Run gel at **95V for 35 minutes** at room temperature
   - DNA migrates toward positive (red) electrode
   - Can adjust time based on separation needed
6. Image gel on UV transilluminator or blue light system

> **Timing Adjustments:**
> • For SCD1 (>1000 bp band): May need 40-45 minutes for good separation
> • For tdTomato and SCD2: 30-35 minutes usually sufficient
> • Monitor dye front - stop before it runs off gel

Gel Imaging and Documentation

Mark Done

> **SAFETY:**
> • Use UV protection equipment (face shield, gloves)
> • Ethidium bromide is a mutagen - handle with care
> • Dispose of gels in appropriate hazardous waste container

1. Transfer gel to imaging system
2. Capture high-quality image for records
   - Use consistent exposure settings
   - Ensure ladder bands are visible
   - All sample lanes should be clearly visible
3. Label lanes clearly on saved image
4. Save image with informative filename:
   - Format: `YYYYMMDD_MouseLine_SampleIDs.jpg`
   - Example: `20251026_SCD1_Flox_M001-M012.jpg`
5. Store in laboratory shared drive
6. Backup regularly

Interpretation of Results

> **General Guidelines:**
> • Always compare to positive and negative controls
> • Band intensity can vary but should be clearly visible
> • Non-specific bands may occasionally appear - focus on expected sizes

• If no bands appear in any sample including controls, PCR failed

Expected Band Sizes Summary

Mouse Line	Genotype	Expected Bands (bp)
SCD1	FL/FL	>1000 (flox), no WT band
	FL/+	>1000 (flox), ~700 (WT)
	+/+	~700 (WT only)
SCD2	FL/FL	250 (flox), no WT band
	FL/+	250 (flox), ~500 (WT)
	+/+	~500 (WT only)
Cdh5-Cre	Cre/+	300 (transgene), 324 (control)
	+/+	324 (control only)
tdTomato	Td/Td	196 (mutant only)
	Td/+	196 (mutant), 297 (WT)
	+/+	297 (WT only)

Complex Genotypes

For mice carrying multiple transgenes (e.g., **SCD1-FL/FL; Cdh5-Cre; tdTomato**):

• Run separate PCR reactions for each genotype
• Record all results in laboratory database
• Verify expected genotypes based on breeding scheme
• Example complex genotype: $SCD1^{FL/FL}$; $SCD2^{FL/FL}$; $Cdh5\text{-}Cre^{+/+}$; $tdTomato^{+/+}$

Troubleshooting Guide

No Bands in Any Sample (Including Controls)

Possible Causes:

- PCR failed to amplify
- DNA degraded or insufficient
- Primers degraded or incorrect
- Thermocycler malfunction

Solutions:

- Check thermocycler program ran correctly
- Prepare fresh PCR master mix
- Extract fresh DNA
- Order new primers
- Check reagent expiration dates

Weak or Faint Bands

Possible Causes:

- Low DNA concentration
- Insufficient PCR cycles
- PCR inhibitors in DNA prep

Solutions:

- Use more template DNA (2-3 µL instead of 1 µL)
- Extract DNA again with fresh reagents
- Dilute DNA 1:10 (may dilute out inhibitors)
- Increase cycle number by 3-5 cycles

Non-Specific Bands or Smearing

Possible Causes:

- Annealing temperature too low
- Too many PCR cycles
- Primer degradation or dimer formation

Solutions:

- Increase annealing temperature by 2°C

- Decrease cycle number
- Order fresh primers
- Optimize $MgCl_2$ concentration

Internal Control Fails (Cre Genotyping)

Possible Causes:

- DNA quality issue
- PCR setup error

Solutions:

This indicates PCR failure - do not interpret transgene results! Re-extract DNA, check all primer stocks, and repeat PCR with fresh reagents.

Unexpected Genotype Results

Possible Causes:

- Sample mix-up
- Contamination
- Mouse pedigree error

Solutions:

- Repeat genotyping from new tissue sample
- Verify mouse ID matches cage card
- Check breeding records
- Consider re-genotyping parents

Record Keeping Requirements

Laboratory Notebook

- Date of genotyping
- Mouse ID numbers
- Tissue sample type and collection date

- DNA extraction method and date
- PCR primer set and lot numbers
- PCR conditions (if different from protocol)
- Gel image with sample IDs
- Interpretation of results

Database Entry

- Mouse ID
- Genotype for each allele
- Date genotyped
- Technician initials
- Link to gel image file

Breeding Records

- Confirmed genotypes of parents
- Offspring genotypes
- Mendelian ratios (expected vs. observed)
- Any unexpected results flagged for follow-up

⚠️ Safety and Compliance

- Always wear gloves when handling mice and biological samples
- Use aseptic technique for tissue collection
- Dispose of biohazardous waste according to institutional guidelines
- Follow IACUC-approved protocols for all animal procedures
- NaOH is caustic - handle with care and use appropriate PPE
- Work in designated areas for pre-PCR and post-PCR to avoid contamination

↑ Back to Top

About This Protocol

Comprehensive genotyping protocol for SCD1, SCD2, Cdh5-Cre, and tdTomato mouse lines. Developed and optimized for routine colony management.

Quick Links

Protocol Overview

Troubleshooting

Contact

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