This is the protocol developed by Dr. Howard Rockman’s laboratory at Duke University with generous help from Ms. Barbara Williams. This is a critical breeding procedure to ensure viability and fertility of the CSQ mice for studying chronic heart failure and its related diseases, such as cardiac cachexia.

**Procedures**

1. Breed CSQ mice with pure wild type DBA/2J mice (The Jackson Laboratories). The offspring are usually only about 30% positive.

2. Keep all the mice on a 0.2% metoprolol (Sigma M5391) in drinking water and replace it weekly. This solution lasts up to two weeks, but the bottles and stoppers can get a bit gross if they are not cleaned weekly. **IMPORTANT:** Keep the positive mice and the breeders with pups on this their entire lives if you do not intend to use them for experiments. For experiments, remove the beta-blocker solution prior to birth.

3. Use 250 ml amber bottles with black stopper since Metoprolol solution is light sensitive. Do not cover the bottles with aluminum foil or anything else since mice will chew whatever they have around them. The amber glass is enough for light blocking even though it is not 100%. The solution is VERY bitter. Mice can get used to it, but they do not drink as much as they would drink normal water.

4. Fill the bottles half full and then add if needed since the drug is fairly expensive. Check the cage daily for dead animals and low food.

5. Start breeding while they are in quarantine since CSQ mice are sensitive and can die quickly. It is OK to set up one male to one female, but a harem with two females to one male is the best.

6. Keep the positive mice and the breeders with pups on 0.2% metoprolol solution throughout their entire lives if they are not used for experiments.

7. Replace 0.2% metoprolol solution with regular water prior to birth and use these mice for experimental purposes. They will usually live 6 mon-1.5 yrs with medication—without medication, they will only live 6-9 months on average and will not breed well at all.

**Reagents**

<table>
<thead>
<tr>
<th>0.2% metoprolol (125 ml)</th>
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<tr>
<td><strong>Reagent</strong></td>
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<td>Metoprolol</td>
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Weigh and dissolve 250 mg of metoprolol in 125 ml of ddH2O.