Figure 2

Microinject CRISPR injection mix into both gonad arms of Day 0 about 30 worms (P₀) and single onto individual MYOB plates. Transfer injected worms onto new plates (1 worm per plate) Day 1 and allow to continue producing progeny (F_1) . Transfer between 50 to 100 Rol F₁ worms onto new individual Days 3 to 4 plates (1 worm per plate) and allow to produce progeny (F2). Prepare single worm lysates for singled F₁ rollers. Perform PCR, Day 6 restriction digestion and agarose gel electrophoresis. Transfer 8 to 12 non-Rol, non-Dpy positive F₂ worms onto new Day 7 plates (1 worm per plate) and allow to produce progeny (F₃). Prepare single worm lysates for singled F₂ worms. Perform PCR, Day 9 restriction digestion and agarose gel electrophoresis. Perform worm lysis, PCR and PCR cleanup for homozygous F₃ Day 10 worms. Measure DNA concentration. Set up sequencing reactions, send samples for sequencing Day 11 and 12 and analyze sequencing results.