Deletions

```
4696
|
ACC TAT AGC TAC TAC ACG AAT GGC GTG GGA GTC ACT  wt

ACC TAT AGC TAC TAC ACG -AT GGC GTG GGA GTC ACT (7)

ACC TAT AGC TAC TAC ACG AA- GGC GTG GGA GTC ACT (7)

ACC TAT AGC TAC TAC ACG A-T GGC GTG GGA GTC ACT (4)

ACC TAT AGC TAC TAC -CG AAT GGC GTG GGA GTC ACT

ACC TAT AGC TAC TAC ACG --T GGC GTG GGA GTC ACT (2)

ACC TAT AGC TAC TAC A-- AAT GGC GTG GGA GTC ACT (3)

ACC TAT AGC TAC TAC --G AAT GGC GTG GGA GTC ACT

ACC TAT AGC TAC TAC A-- -AT GGC GTG GGA GTC ACT (4)

ACC TAT AGC TAC TAC ACG AA- --C GTG GGA GTC ACT

ACC TAT AGC TAC TAC ACG AA- --- GTG GGA GTC ACT (2)

ACC TAT AGC TAC TAT AC- --- GGC GTG GGA GTC ACT

ACC TAT AGC TAC TAC ACG --- --C GTG GGA GTC ACT

ACC TAT AGC TAC TA- --- --T GGC GTG GGA GTC ACT (3)

ACC TAT AGC TAC TAC --- --- GGC GTG GGA GTC ACT

ACC TAT AGC TAC TAC ACG AA- --- --G GGA GTC ACT

ACC TAT AGC TAC TAC ACG --- --- -TG GGA GTC ACT

ACC TAT AGC TAC T-- --- --- GGC GTG GGA GTC ACT (2)

ACC TAT AGC TAC TAC --- --- --- GTG GGA GTC ACT (3)*

ACC TAT AGC TAC TAC A-- --- --- -TG GGA GTC ACT

ACC TAT AGC TA- --- --- --- -GC GTG GGA GTC ACT
```

```
ACC TAT AGC TAC TAC A-- --- --- --- GGA GTC ACT

ACC TAT AGC TAC T-- --- --- --- --G GGA GTC ACT

ACC TAT AGC --- --- --- --- --- GTG GGA GTC ACT

ACC TAT AGC --- --- --- --- --- GTG GGA GTC ACT

ACC TAT AGC TAC TAC --- -- (803 bp Δ) - --- ---
```

Insertions

```
4696
|
ACC TAT AGC TAC TAC ACG AAT GGC GTG GGA GTC ACT  wt

                          |A
ACC TAT AGC TAC TAC ACG AAT GGC GTG GGA GTC ACT (4)

                        |C
ACC TAT AGC TAC TAC ACG AAT GGC GTG GGA GTC ACT (6)

                         |CG
ACC TAT AGC TAC TAC ACG AAT GGC GTG GGA GTC ACT

                          |AA
ACC TAT AGC TAC TAC ACG AAT GGC GTG GGA GTC ACT

                         |CGA
ACC TAT AGC TAC TAC ACG AAT GGC GTG GGA GTC ACT (2)

                          |CGAA
ACC TAT AGC TAC TAC ACG AAT GGC GTG GGA GTC ACT (18)†

                      |C
ACC TAT AGC TAC TAC ACG A-T GGC GTG GGA GTC ACT

                        |(200 bp)‡
ACC TAT AGC TAC TAC A-G AAT GGC GTG GGA GTC ACT

                          |GT
ACC TAT AGC TAC TAC A-- -AT GGC GTG GGA GTC ACT

                        |TGTAGTCCCACG
ACC TAT AGC TAC TAC A-- -AT GGC GTG GGA GTC ACT
```

```
                        |CATGTTTCGCATGTTACTAC
ACC TAT AGC TAC TAC --- -AT GGC GTG GGA GTC ACT

                        |TACTACACCT
ACC TAT AGC TAC TAC --- -AT GGC GTG GGA GTC ACT

                          |TCCCAC
ACC TAT AGC TAC TAC AC- --- GGC GTG GGA GTC ACT

                           |G
ACC TAT AGC TAC TAC --- --T GGC GTG GGA GTC ACT (6)

                        |GTGGCTATAGCTACG
ACC TAT AGC TAC TAC --- --T GGC GTG GGA GTC ACT

                           |TACAC
ACC TAT AGC TAC TAC AC- --- -GC GTG GGA GTC ACT

                             |GTCACT
ACC TAT AGC TAC TAC ACG A-- --- GTG GGA GTC ACT

                        |GTGGGCGTG
ACC TAT AGC TAC TAC --- --- GGC GTG GGA GTC ACT

                        |GGCGTAGTAGCTACTACGTAGTGACTACTACAC
ACC TAT AGC TAC TAC --- --- GGC GTG GGA GTC ACT

                          |GGCGTAGGAGTCATGTAGTACCAC
ACC TAT AGC TAC TAC AC- --- --- GTG GGA GTC ACT

                        |G
ACC TAT AGC TAC --- --- --T GGC GTG GGA GTC ACT

                        |ACA
ACC TAT AGC TAC --- --- --- -GC GTG GGA GTC ACT

                        |TA
ACC TAT AGC TA- --- --- --- -GC GTG GGA GTC ACT

                      |G
ACC TAT AGC --- --- --- --T GGC GTG GGA GTC ACT

                        |(399 bp)**
ACC TAT AGC TAC TA- --- --- --- --G GGA GTC ACT

                               |TGT
```

```
ACC TAT AGC TAC TAC A-- --- --- --- --A GTC ACT

                                |GCTAGAGATC
ACC TAT AGC TAC TAC A-- --- --- --- --- --- ---

                            |TATTGCCTGAC
ACC TAT AGC TAC TAC --- --- (44 bp Δ)-- --- ---

                                |TGAA
ACC TAT AGC TAC TAC ACG A-- --- (915 bp Δ)- ---
```

†The 4-base fill-in and blunt join of the overlap created by ZFN cleavage.
‡This insertion corresponds to sequences in the 18S rRNA gene.
****** This insertion matches sequences from the histone gene cluster.

FIGURE S1.—NHEJ mutations at *ry* from *lig4*$^{+}$ flies. Sequences of the ZFN-induced mutants in the *ry* gene. The wild type sequence is shown above the deletions and the insertions for comparison. The first position in the sequence is numbered from start of transcription. ZFN recognition sequences are in red; insertions and substitutions are in blue. All these sequences were isolated independently – i.e., they came from different heat-induced parents. When a mutant sequence was isolated more than once, the number of observations is shown in parentheses to the right. Many of the short insertions are partially homologous to *ry* sequences in the immediate vicinity of the ZFN cut. *A 9-bp deletion found more commonly from *lig4*$^{-}$ parents.