**Court lab protocol**
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# Protocol for oligo recombineering to make a point mutation

This protocol is intended to be a "crib sheet" for those that already are recombineering savy. For further details on all steps, see the "Step-by-step ssDNA protocol".

**1.** Design construction such that the MMR system can be avoided.

**2.** Electroporate ~100ng of salt-free oligo into 50$\mu$l of freshly prepared electocompetent, recombineering-proficient cells. For optimal results, be sure that the cells and oligo were properly mixed.

**3.** After the 1ml L broth is added and the mix is transferred to a sterile culture tube, outgrow the cell for 30 min – 2 hours at 32° with rolling or shaking. The proper length of outgrowth depends on the details of the construction.

**3.** After outgrowth, make 10-fold serial dilutions in TMG, minimal salts or similar osmotically balanced medium. Plate 0.1ml of dilutions on L plates (normally: $10^{-4}$, $10^{-5}$) to determine viable cells (~$10^{8}$/ml are expected although in some genetic backgrounds the viability can be lower) and on the appropriate plate for recombinants. For example if the recombinants are non-selective, you need only the L plates. If MMR is avoided and all other factors are optimized, you can expect as high as 50% of the colonies will be recombinant. Plates should be incubated at 32° to prevent further expression of the Red functions.

**4.** Screen for recombinants via colony PCR, colony size, or whatever appropriate means. An illustration of how PCR can be used to find the recombinant can be found in the "Step-by-step ssDNA protocol" as well as in the FAQ on the website.

**5.** Once a candidate colony is found, go back to the master plate, streak the colony out for single colonies and repeat the PCR screen on pure individual colonies.

**6.** Always sequence final constructs to be certain the oligo did not introduce any extraneous mutations.