Leslie Cytron Characterization of New Rat Reporter Strain: Le-*Rosa26*<sup>em1(CAG-LSL-TdTomato)Rrrc</sup> July 29, 2021

The purpose of this project is to characterize a new, genetically engineered transgenic rat strain, Le-Rosa26<sup>em1(CAG-LSL-TdTomato)Rrrc</sup>, that expresses fluorescent TdTomato protein in the presence of Cre recombinase protein. The transgene contains a regulatory element (SV40 STOP) that normally prevents expression of TdTomato. However, when Cre recombinase is present, it recognizes the *loxP* sites that flank the SV40 STOP element, and a Cre recombinase-mediated recombination event removes the regulatory sequence. That allows expression of the *TdTomato* gene, which is under control of the ubiquitously expressed CAG promoter. The TdTomato red fluorescent protein is expressed as a result. To confirm that the rat strain expresses TdTomato only in the presence of Cre recombinase, female hemizygous Le-Rosa26em1(CAG-LSL-TdTomato)Rrrc rats were mated with male hemizygous Wistar-Tq(CAG-NCre)81Jmsk transgenic rats that express Cre recombinase ubiquitously. Offspring were genotyped using PCR assays to detect both transgenes. In order to visualize the presence or lack of TdTomato expression in animals of all possible genotypes, fluorescence microscopy was performed. As expected, only those animals that carried both transgenes (double hemizygous) had expression of TdTomato while animals with all other genotypes (no transgenes or singly hemizygous) did not. TdTomato expression was robust: it could be seen with the naked eye as a noticeable red tint in the skin and eyes of double hemizygous rats. Gross examination of whole organs by fluorescence microscopy showed TdTomato expression in ovary/testis, kidney, spleen, stomach, liver, small intestine, skeletal muscle, heart, thymus, eye, lung, and brain tissues. These results validate the Le-Rosa26em1(CAG-LSL-TdTomato)Rrrc rat model phenotypically and establish it as an effective reporter strain. Thus, the model can be used with other Cre-expressing rat strains to label cells and tissues with TdTomato for future research in a wide variety of biomedical fields.