Appendix-2A. Transposon library sampling for microarray and data analysis

Each transposon insertion queries a T7 RNA polymerase promoter (PT7) that is used to generate a unique transcript for each mutant. Each genomic sequence adjacent to Tn insertion is sampled in 3 steps:

Polyadenylation: PolyA tails were added to fragmented genomic DNA using terminal transferase (TdT) as follows: 1.5 µg of DNA fragments were incubated for 30 min at 37°C in a total reaction volume of 50 µl containing 40 U TdT (New England BioLabs), $CoCl_2$ 0.25 mM, and dATP 0.4 mM. Terminal transferase was subsequently inactivated at 70°C for 10 min and the tailed product was purified using the QIAquick PCR purification kit (Qiagen).

A two steps PCR was used to amplify the polyA-tailed DNA fragments containing the insert PT7 and the flanking inserted region: In the first PCR reaction, 50 ng of purified polyA-tailed DNA was used as template for a PCR reaction using primer DOPR2 (CAACGCAGACCGTTCCGTGGCA), and a primer designed to anneal to the polyA-tail ($CCT_{24}VN$). The reaction mixture consisted of 1 x PCR buffer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 0.05 U *Taq* polymerase (Promega), and 0.2 µM of each forward and reverse primer in a total reaction volume of 25 µl. The PCR reaction was performed under the following conditions: initial denaturation at 94°C for 1 min followed by 30 cycles with denaturation at 94°C for 10 s, annealing at 50°C for 10 s, and extension at 72°C for 5 s. The last cycle was followed by a final extension for 3 min at 72°C. In the second amplification step, a nested PCR was performed using 1 µl of the amplified product from the initial PCR in a total volume of 50 µl. Internal primer KAN2FP1-B (GTCCACCTACAACAAAGCTCTCATCAACC) and primer $CCT_{24}VN$ were used under identical cycling conditions as during the initial PCR reaction. PCR products were analyzed on 1% agarose gels.

cDNA synthesis and labeling: 8 µl of the nested PCR reaction were used as template for a 20 µl *in vitro* transcription reaction using the AmpliScribe T7 transcription kit (Epicentre). The RNA was purified through RNeasy spin columns (Qiagen) and used as template to synthesize labeled cDNA by incorporation of Cy5-dCTP or Cy3-dCTP (GE Healthcare) using SuperScript II reverse transcriptase (Invitrogen). The labeled cDNA was purified through a spin column (Qiagen). 2 µg of labeled cDNA from the output library (Cy3 label) and the input library (Cy5 label) were mixed 1:1 and hybridized to slides containing a *Salmonella* ORF microarray printed in triplicate [20]. Signal intensities were quantified using the QuantArray 3.0 software package, and normalized based on total signal in each channel. The ratio of the signal from tumor and from normal tissue was compared to the input library. The Tables present data filtered to show only the largest changes, which was arbitrarily set at a threshold of a 2.25-fold change [$Log_2(2.25)= -1.5$] to show a few of the most dramatic changes.