http://www.vcru.wisc.edu/simonlab/sdata/MK000056.pdf

MK000056=Transposon Display

Markers: Several: td-ACA073; td-ACA104; td-ACA128; td-ACA143; td-ACT069; td-ACT082; td-ACT099; td-ACT121; td-AGT084; td-CAG177; td-CAT171; td-CAT182; td-CAT213; td-CTA069; td-CTA119; td-CTT069; td-CTT088; td-CTT102; td-GAA205; td-GAT101; td-GAT120; td-GTA076; td-GTT068

Type: Dominant in 493×QAL

Description: Transposon display, in this example ACA is shown as the three selective nucleotides, but may be any of the various combinations listed here.

Reference: The *DcMaster* Transposon Display maps polymorphic insertion sites in the carrot (*Daucus carota* L.) genome. Dariusz Grzebelus; Barbara Jagosz; Philipp W. Simon. Gene 390 (2007) 67–74

Adapter sequences: SplA1 .....5'-CGAATCGTAACCGTTCGTACGAGAATGTCCTCTCCAACGAGCCAAGG-3'

SplA2 .....5'-TACCTTGGCTCGTTTTTTTTTGCAAAAA-3'

Digestion and Ligation: Approximately 200 ng of DNA was completely digested in 37 °C for 3 h in a 20 µl reaction mixture containing 5 u MseI (New England Biolabs) and 1× NEB buffer 2. MseI-compatible splinkrette type adaptors, modified from the adaptor sequences originally published by Devon et al. (1995) were ligated to restriction fragments using T4 DNA ligase (Promega). Adaptors were obtained through annealing of the two single stranded oligonucleotides shown above. Ligation products were 5-fold diluted and subjected to the first round of PCR amplification

Preamplification Primers: DcMtd1m: ..... 5'-TATCAAAAAGCTGCTGTTGTGGTTG-3'
tdP1..... 5'-CGAATCGTAACCGTTCGTACGAGAA-3'

PCR Reaction: The 10 µl reaction mixture consisted of 1 µl restriction/ligation DNA fragments, 2 mM MgCl2, 250 µM dNTPs, 1 µM each primer, 0.5 U TAQ, and 1 µl 10× PCR buffer (Promega).

PCR Program: Cycling conditions were as followed: the initial denaturation at 94 °C/30 s, and then 40 cycles of 55 °C/30 s, and 68 °C/60 s, followed by the final elongation at 68 °C/5 min. Subsequently, the reaction mixture was diluted 1:50 and used as template for the second round of PCR amplification

Selective Aplification Primers: DcMtd2m..... 5'-GCTGCTGTTGTGGTTGGCAAC-3'
tdMse-ACA ..... 5'-TCCAACGAGCCAAGGTAAACA-3'

PCR Reaction: The 10 µl selective amplification mixture consisted of 1 µl diluted preamplification products, 2 mM MgCl2, 250 µM dNTPs, 0.5 µMeach primer, 0.5UTAQand 1 µl 10× PCR buffer (Promega).

PCR Program: Selective amplification cycling conditions started with denaturation at 94 °C/2 min followed by 5 cycles of touch down PCR (94 °C/30 s, 60 °C/30 s decreased by 1.0 °C per each subsequent cycle, and 68 °C/60 s), then 35 cycles of 94 °C/30 s, 55 °C/30 s, and 68 °C/60 s. Amplification was completed with the final elongation step at 68 °C/5 min and then kept at 4 °C.

Screening Method: Products amplified in the course of selective amplification were separated on 6% denaturing polyacrylamide gels and visualized as described for AFLP products by Briard et al. (2000).

Product Sizes: The products used for mapping in 493×QAL and their sizes are:

ACA	73, 104, 128, 143 b.p.
ACT	69, 82, 99, 121 b.p.
AGT	84 b.p.
CAG	177 b.p.
CAT	171, 182, 213 b.p.
CTA	69, 119 b.p.
CTT	69, 88, 102 b.p.
GAA	205 b.p.
GAT	101, 120 b.p.
GTA	76 b.p.
GTT	68 b.p.

Example: Schematic representation of the DcMaster Transposon Display system. Genomic DNA is digested with MseI, Splinkrette-type adaptors are ligated to restriction fragments, and two rounds of PCR amplification are performed resulting in the amplification of a subset of products containing the 5′ terminal region of DcMaster with the adjacent genomic flanking region of different lengths depending on the distance from the insertion site to the nearest MseI restriction site. Thick lines—genomic DNA, letters

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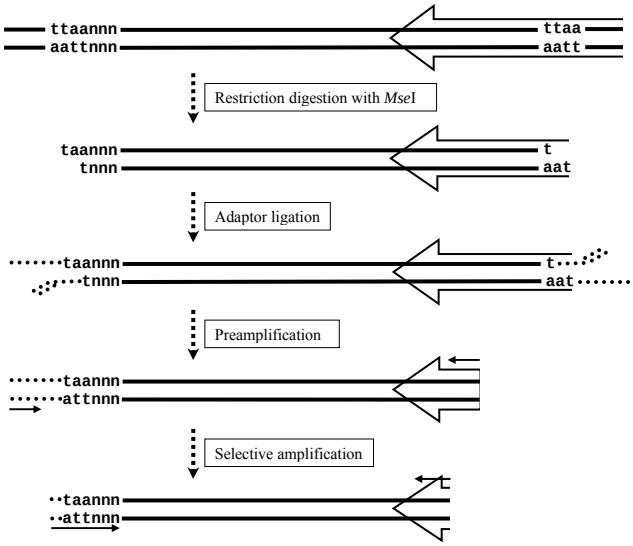
'ttaa'—MseI restriction site, letters 'nnn'—three selective nucleotides, large open arrow—5′ terminal region of DcMaster, dotted lines— adaptors, small arrows— primer annealing sites. The scheme is not drawn to scale.



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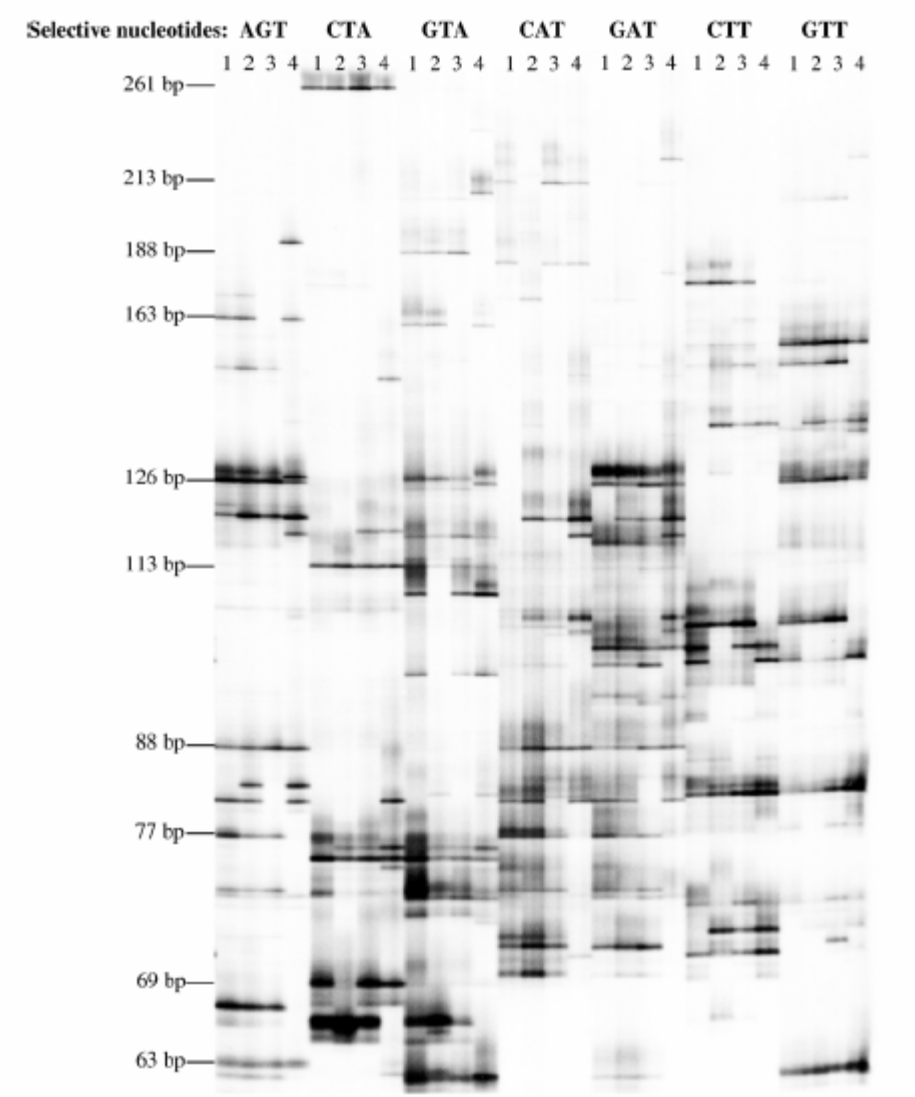
An example of the DcMaster Transposon Display profile obtained with seven selective primer combinations. Lanes: 1 – (QAL x B493)F2#5, 2 – (QAL x B493)F2#6, 3 – (QAL x B493)F2#7, 4 – (QAL x B493)F2#8



Genbank reference:

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Sequence Information: DNA sequences of 100 bp long portion of eight specific and two non-specific *DcM*TD amplicons and consensus terminal inverted repeat sequences for three putative *DcMaster* subfamilies. Thick gray line - primer anealing site, black arrow – *DcMaster* TIR, TSD – target site duplication, DcM-a – *DcMaster*-a terminus and the sequence flanking insertion.