

<b>Pool Name</b>	<b>Strains</b>	<b>Geographical origin</b>	<b>Reference</b>
Wi	wi1, wi3, wi15, wi18,wi41.5, wi45, wi68,wi77,wi83,wi98	Wolfskill Orchard, CA	Sergey Nuzhdin
We1	we4, we10, we11, we25, we44, we47, we50, we60, we67, we80	Raleigh, NC	Greg Gibson
We2	we13, we17, we28, we33, we37, we63, we70, we75, we88, we91	Raleigh, NC	Greg Gibson
NB	NB1, NB6, NB7, NB8, NB12, NB13, NB14, NB16	New Buffalo, MI	Bettina Harr
CSW	3B, 6D, 11D, 20C, 23D, 25C, 29B, 36D	Countryside Winery, Blountville, TN	Lev Yampolsky
Zmel	zmel58, zmel81, zmel125, zmel131, zmel145, zmel159, zmel178, zmel191, zmel196	Zimbabwe	Charles Aquadro
ZW	ZW104, ZW109, ZW122, ZW140, ZW141, ZW142, ZW144, ZW149, ZW155, ZW156, ZW177, ZW183	Zimbabwe	Peter Andolfatto
KY	KY01, KY10, KY12, KY16, KY20, KY23, KY24, KY38, KY42, KY91, KY106	Kenya	Peter Andolfatto
MW	MW7, MW8, MW11, MW12, MW14, MW15, MW27, MW28, MW35, MW56, MW60	Malawi	—

Table S1: Summary of strains used.

Flybase ID	Primer	Sequence (5' → 3')	Primer	Sequence (5' → 3')	Primer	Sequence (5' → 3')
FBt0018877	18877_FL	AACGTCGATTTTGATTGTACT	18877_R	AGGATGGTGTGCCTTTGGTACT	18877_L	TCCTCACATGATTAGTGAGGGTTG
FBt0018878	18878_FL	TACTGTTTGCCTGGGTTTGTAA	18878_R	ATAAGGAAAGGCCCAACAAACAA	18878_L	TCCTCACATGATTAGTGAGGGTTG
FBt0018879	18879_FL	ACAGCCGAAACTGAGAAAAGAG	18879_R	AATACTTITGTGAGAAAGAAAAA	18879_L	GTCCTCACATGATTAGTGAGGGTTG
FBt0019079	19079_FL	GGTGCCTAAAGAAGGGCTAAAGAA	19079_R	ATGCGAAATTTTATTGAACGGCT	19079_L	GGAATACAGATCTGGGTATTCGC
FBt0019133	19133_FL	ACGTTTAACGTGGCCTAAAGAAAT	19133_R	TTTTCACTGGGAATTGGTACTC	19133_L	TCCTCACATGATTAGTGAGGGTTG
FBt0019158	19158_FL	GAAGTTGGGTGCAACTTTGAAIG	19158_R	AAGGATTAGAGTGAATGGCTC	19158_L	TCCTCACATGATTAGTGAGGGTTG
FBt0019165	19165_FL	ACACCAAATAAGCCGTAGGTTI	19165_R	TGATTGTAACGGCAAACAAACCC	19165_L	GGAATACAGATCTGGGTATTCGC
FBt0019312	19312_FL	ATTGACACACTTTGCTGTTGC	19312_R	TCGACTCCCAATTACAGTTAGCG	19312_L	TCCTCACATGAGTAGTGAGGGTTG
FBt0019315	19315_FL	GTGCGCTTTGCTGTCCAACTATG	19315_R	GTTCCCTTAAGTGGGAGTGAAG	19315_L	TCCTCACATGATTAGTGAGGGTTG
FBt0019378	19378_FL	AGAACAAAGTGTGCCAAGCAGT	19378_R	CGCAGAGTAAATTAAATGACTTGCCCTC	19378_L	TCCTCACATGATTAGTGAGGGTTG
FBt0019388	19388_FL	TGTCATTCATAATTGGCCAG	19388_R	ATTGGGCACATCTGAATAAGACCC	19388_L	CACITGACAACCCTAAGAACCC
FBt0019410	19410_FL	AGCAATCATCGAAATCGAAAAAA	19410_R	CCAACCAGACTAGTGACGAATGG	19410_L	GGAATACAGATCTGGGTATTCGC
FBt0019426	19426_FL	AAAGTCGGACCAACTGAGGCAACT	19426_R	AAAATAAGCAAAAGTGACACGGCG	19426_L	TCCTCACATGATTAGTGAGGGTTG
FBt0019604	19604_FL	GAGGCCATTCATGTCACACACAAG	19604_R	AACAGGAAACCAAAACAAAGGAACC	19604_L	GATTAAAAAGGAGGGTTTCGCC
FBt0020056	20056_FL	GATTAGCATCACATGGATTGCG	20056_R	GGAGGCTGCCATCCTATTTCTA	20056_L	TCAAAGGATIGCAAACACTTCGAT
FBt0020057	20057_FL	ATTCTGTGCAAGAGGGTTGIG	20057_R	CCCATATCACATTAGCTGATTCTC	20057_L	TCCTCACATGATTAGTGAGGGTTG
FBt0020125	20125_FL	GGGCACAGCACTGGAGAAATAATA	20125_R	GGAAACTTACCCACACATTGCG	20125_L	GGAATACAGATCTGGGTATTCGC
FBt0020149	20149_FL	TCGCCCTCCATTAACTTGATT	20149_R	GGAAAGGGTGTGTCAGCAAATGTG	20149_L	GGAATACAGATCTGGGTATTCGC

Table S2: Summary of primers used in this study. The primer label ‘\* FL’ corresponds to a ‘Flanking’ primer, 5’ of the insertion (see also **Materials and Methods**); the label ‘\* L’ corresponds to a ‘Left’ primer found in the interior of the insertion; the label ‘\*\_R’ corresponds to a ‘Right’ primer found 3’ of the insertion.