

**Additional file 1.** List of the strains used in this study.

<b>Yeast Strain</b>	<b>Genotype / Plasmid added</b>	<b>Source</b>
MAE	MAE	Lallemand
MAE <i>mks1</i> Δ	MAE CRISPR/Cas9 <i>mks1</i> Δ	Garrigós et al., 2023
eMAE 8-1a	MAE evolved in SD+AEC after 8 transfers, culture 1 colony a	This study
eMAE 8-1b	MAE evolved in SD+AEC after 8 transfers, culture 1 colony b	This study
eMAE 8-1c	MAE evolved in SD+AEC after 8 transfers, culture 1 colony c	This study
eMAE 8-1d	MAE evolved in SD+AEC after 8 transfers, culture 1 colony d	This study
eMAE 8-1e	MAE evolved in SD+AEC after 8 transfers, culture 1 colony e	This study
eMAE 8-2a	MAE evolved in SD+AEC after 8 transfers, culture 2 colony a	This study
eMAE 8-2b	MAE evolved in SD+AEC after 8 transfers, culture 2 colony b	This study
eMAE 8-2c	MAE evolved in SD+AEC after 8 transfers, culture 2 colony c	This study
eMAE 8-2d	MAE evolved in SD+AEC after 8 transfers, culture 2 colony d	This study
eMAE 8-2e	MAE evolved in SD+AEC after 8 transfers, culture 2 colony e	This study
eMAE 29-1a	MAE evolved in SD+AEC after 29 transfers, culture 1 colony a	This study
eMAE 29-1b	MAE evolved in SD+AEC after 29 transfers, culture 1 colony b	This study
eMAE 29-1c	MAE evolved in SD+AEC after 29 transfers, culture 1 colony c	This study
eMAE 29-1d	MAE evolved in SD+AEC after 29 transfers, culture 1 colony d	This study
eMAE 29-1e	MAE evolved in SD+AEC after 29 transfers, culture 1 colony e	This study
eMAE 29-2a	MAE evolved in SD+AEC after 29 transfers, culture 2 colony a	This study
eMAE 29-2b	MAE evolved in SD+AEC after 29 transfers, culture 2 colony b	This study
eMAE 29-2c	MAE evolved in SD+AEC after 29 transfers, culture 2 colony c	This study
eMAE 29-2d	MAE evolved in SD+AEC after 29 transfers, culture 2 colony d	This study
eMAE 29-2e	MAE evolved in SD+AEC after 29 transfers, culture 2 colony e	This study
TAE	TAE	Lallemand

TAE <i>mks1</i> Δ	TAE CRISPR/Cas9 <i>mks1</i> Δ	Garrigós et al., 2023
eTAE 8a	TAE evolved in SD+AEC after 8 transfers, colony a	This study
eTAE 8b	TAE evolved in SD+AEC after 8 transfers, colony b	This study
eTAE 8c	TAE evolved in SD+AEC after 8 transfers, colony c	This study
eTAE 8d	TAE evolved in SD+AEC after 8 transfers, colony d	This study
eTAE 8e	TAE evolved in SD+AEC after 8 transfers, colony e	This study
eTAE 29b	TAE evolved in SD+AEC after 29 transfers, colony b	This study
eTAE 29d	TAE evolved in SD+AEC after 29 transfers, colony d	This study
eTAE 29l	TAE evolved in SD+AEC after 29 transfers, colony l	This study
eTAE 29q	TAE evolved in SD+AEC after 29 transfers, colony q	This study
eTAE 29t	TAE evolved in SD+AEC after 29 transfers, colony t	This study
EAE	EAE	Lallemant
EAE <i>mks1</i> Δ	EAE CRISPR/Cas9 <i>mks1</i> Δ	Garrigós et al., 2023
eEAE 8a	EAE evolved in SD+AEC after 8 transfers, colony a	This study
eEAE 8b	EAE evolved in SD+AEC after 8 transfers, colony b	This study
eEAE 8c	EAE evolved in SD+AEC after 8 transfers, colony c	This study
eEAE 8d	EAE evolved in SD+AEC after 8 transfers, colony d	This study
eEAE 8e	EAE evolved in SD+AEC after 8 transfers, colony e	This study
eEAE 29j	EAE evolved in SD+AEC after 29 transfers, colony j	This study
eEAE 29k	EAE evolved in SD+AEC after 29 transfers, colony k	This study
eEAE 29n	EAE evolved in SD+AEC after 8 transfers, colony n	This study
eEAE 29o	EAE evolved in SD+AEC after 29 transfers, colony o	This study
eEAE 29s	EAE evolved in SD+AEC after 29 transfers, colony s	This study
C9	C9 Mat a <i>ho::loxP</i>	Walker et al., 2003
C9 <i>lys20</i> Δ	C9 <i>lys20::loxP</i>	This study

C9 <i>lys21</i> Δ	C9 <i>lys21::loxP</i>	This study
C9 <i>lys20</i> Δ <i>lys21</i> Δ	C9 <i>lys20::loxP lys21::loxP</i>	This study
C9 <i>rtg2</i> Δ	C9 <i>rtg2::loxP</i>	This study
C9 vector	C9 with plasmid pCUP1pNuiHA kanMX CEN	This study
C9 pLYS21 – MAE	C9 with plasmid pLYS21 – MAE	This study
C9 pLYS21 – eMAE 8-1b	C9 with plasmid pLYS21 – eMAE 8-1b	This study
C9 pLYS21 – TAE	C9 with plasmid pLYS21 – TAE	This study
C9 pLYS21 – eTAE 29I	C9 with plasmid pLYS21 – eTAE 29I	This study
C9 pLYS20 – EAE	C9 with plasmid pLYS20 – EAE	This study
C9 pLYS20 – eEAE 29j	C9 with plasmid pLYS21 – eEAE 29j	This study
C9 pRTG2 – MAE	C9 with plasmid pRTG2 – MAE	This study
C9 pRTG2 – eMAE 29-2c	C9 with plasmid pRTG2 – eMAE 29-2c	This study
C9 pRTG2 – TAE	C9 with plasmid pRTG2 – TAE	This study
C9 pRTG2 – eTAE 29I	C9 with plasmid pRTG2 – eTAE 29I	This study
C9 <i>lys20</i> Δ vector	C9 <i>lys20</i> Δ with plasmid pCUP1pNuiHA kanMX CEN	This study
C9 <i>lys20</i> Δ pLYS20 – EAE	C9 <i>lys20</i> Δ with plasmid pLYS20 – EAE	This study
C9 <i>lys20</i> Δ pLYS20 – eEAE 29j	C9 <i>lys20</i> Δ with plasmid pLYS20 – eEAE 29j	This study
C9 <i>lys21</i> Δ vector	C9 <i>lys21</i> Δ with plasmid pCUP1pNuiHA kanMX CEN	This study
C9 <i>lys21</i> Δ pLYS21 – MAE	C9 <i>lys21</i> Δ with plasmid pLYS21 – MAE	This study
C9 <i>lys21</i> Δ pLYS21 – eMAE 8-1b	C9 <i>lys21</i> Δ with plasmid pLYS21 – eMAE 8-1b	This study
C9 <i>lys21</i> Δ pLYS21 – TAE	C9 <i>lys21</i> Δ with plasmid pLYS21 – TAE	This study
C9 <i>lys21</i> Δ pLYS21 – eTAE 29I	C9 <i>lys21</i> Δ with plasmid pLYS21 – eTAE 29I	This study
C9 <i>lys20</i> Δ <i>lys21</i> Δ vector	C9 <i>lys20</i> Δ <i>lys21</i> Δ with plasmid pCUP1pNuiHA kanMX CEN	This study

C9 <i>lys20</i> Δ <i>lys21</i> Δ pLYS20 – EAE	C9 <i>lys20</i> Δ <i>lys21</i> Δ with plasmid pLYS20 – EAE	This study
C9 <i>lys20</i> Δ <i>lys21</i> Δ pLYS20 – eEAE 29j	C9 <i>lys20</i> Δ <i>lys21</i> Δ with plasmid pLYS20 – eEAE 29j	This study
C9 <i>lys20</i> Δ <i>lys21</i> Δ pLYS21 – MAE	C9 <i>lys20</i> Δ <i>lys21</i> Δ with plasmid pLYS21 – MAE	This study
C9 <i>lys20</i> Δ <i>lys21</i> Δ pLYS21 – eMAE 8-1b	C9 <i>lys20</i> Δ <i>lys21</i> Δ with plasmid pLYS21 – eMAE 8-1b	This study
C9 <i>lys20</i> Δ <i>lys21</i> Δ pLYS21 – TAE	C9 <i>lys20</i> Δ <i>lys21</i> Δ with plasmid pLYS21 – TAE	This study
C9 <i>lys20</i> Δ <i>lys21</i> Δ pLYS21 – eTAE 29l	C9 <i>lys20</i> Δ <i>lys21</i> Δ with plasmid pLYS21 – eTAE 29l	This study
C9 <i>rtg2</i> Δ vector	C9 <i>rtg2</i> Δ with plasmid pCUP1pNuiHA kanMX CEN	This study
C9 <i>rtg2</i> Δ pRTG2 – MAE	C9 <i>rtg2</i> Δ with plasmid pRTG2 – MAE	This study
C9 <i>rtg2</i> Δ pRTG2 – eMAE 29-2c	C9 <i>rtg2</i> Δ with plasmid pRTG2 – eMAE 29-2c	This study
C9 <i>rtg2</i> Δ pRTG2 – TAE	C9 <i>rtg2</i> Δ with plasmid pRTG2 – TAE	This study
C9 <i>rtg2</i> Δ pRTG2 – eTAE 29l	C9 <i>rtg2</i> Δ with plasmid pRTG2 – eTAE 29l	This study