Supplementary Table. Newly cloned and existing ADH genes used in the ancestral reconstruction
Abbreviation/Strain/Designation/GenBank accession number/source

KwADH1: *Kluyveromyces wickerhamii* ADH1 (AY216989) This study
KwADH3: *Kluyveromyces wickerhamii* ADH3 (AY216990) This study
KwADH4: *Kluyveromyces wickerhamii* ADH4 (AY216991) This study
SbADH1: *Saccharomyces bayanus* ADH1 (AY216992) This study
SbADH2: *Saccharomyces bayanus* ADH2 (AY216993) This study
SbADH3: *Saccharomyces bayanus* ADH3 (AY216994) This study
SbADH5: *Saccharomyces bayanus* ADH5 (AY216995) This study
SkADH1: *Saccharomyces kluyveri* ADH1 (AY216996) This study
SkADH2: *Saccharomyces kluyveri* ADH2 (AY216997) This study
SkADH3: *Saccharomyces kluyveri* ADH3 (AY216998) This study
SkADH4: *Saccharomyces kluyveri* ADH4 (AY216999) This study
SpADH1: *Saccharomyces pastorianus* ADH1 (AY217000) This study
SpADH2: *Saccharomyces pastorianus* ADH2 (AY217001) This study
SpADH3: *Saccharomyces pastorianus* ADH3 (AY217002) This study
SpADH5: *Saccharomyces pastorianus* ADH5 (AY217003) This study

ScADH1: *Saccharomyces cerevisiae* ADH1 (P00330)
ScADH2: *Saccharomyces cerevisiae* ADH2 (P00331)
ScADH3: *Saccharomyces cerevisiae* ADH3 (P07246)
ScADH5: *Saccharomyces cerevisiae* ADH5 (P38113)
KmADH1: *Kluyveromyces marxianus* ADH1 (X60224)
KmADH2: *Kluyveromyces maxianus* ADH2 (AAF91235.1)
KlADH1: *Kluyveromyces lactis* ADH1 (P20369)
KlADH2: *Kluyveromyces lactis* ADH2 (X64397)
KlADH3: *Kluyveromyces lactis* ADH3 (X62766)
KlADH4: *Kluyveromyces lactis* ADH4 (X62767)
PSADH1: *Pichia stipitis* ADH1 (AF008245)
PsADH2: *Pichia stipitis* ADH2 (AF008244)

Adh sequences from this study have been deposited in Genbank under accession numbers AY216989-AY217003.

Table 2. Gene and Protein Sequences of Reconstructed Yeast Alcohol Dehydrogenases

MKD: *Saccharomyces cerevisiae* Ancestor (M168/K211/D236)
Gene:
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
GTTGGAATACAAAGATATTCCAGTTCCAAAGCCAAAGCCAAACGAATTGTTG
ATCAACGTTAAATACTCTGGTGTCTGTCACACTGACTTGCACGCTTGGCACGG
TGACTGGCCATTGCCCACTAAGCTACCATTAGTCGGTGGTCACGAAGGTGCC
GGTGTCGTTGTCGCCATGGGTGAAAACGTTAAGGGCTGGAAGATCGGTGACT
ACGCCGGTATCAAATGGTTGAACGGTTCTTGTATGGCCTGTGAATACTGTGAA
TTGGGTAACGAATCCAACTGTCCTCACGCTGACTTGTCTGGTTACACCCACGA
CGGTTCTTTCCAACAATACGCTACCGCTGACGCTGTTCAAGCCGCTCACATTC
CTCAAGGTACCGACTTGGCCGAAGTTGCTCCCATCTTGTGTGCTGGTATCACC
GTCTACAAGGCTTTGAAGTCTGCTAACTTGATGGCCGGTCACTGGGTTGCTAT
CTCCGGTGCTGCTGGTGGTCTAGGTTCTTTGGCTGTTCAATACGCCAAGGCTA
TGGGTTACAGAGTCTTGGGTATTGACGGTGGTGAAGGTAAGGAAGAATTATT
CAAGTCTTTGGGTGGTGAAGTCTTCATTGACTTCACTAAGGAAAAGGACATT
GTCGGTGCTGTTATCAAGGCCACTGACGGTGGTGCTCACGGTGTCATCAACGT
TTCCGTTTCCGAAGCCGCTATTGAAGCTTCTACCAGATACTGTAGAGCTAACG
GTACTGTTGTTTTGGTCGGTTTGCCAGCTGGTGCCAAGTGTTCTTCTGATGTCT
TCAACCAAGTCGTCAAGTCCATCTCTATTGTTGGTTCTTACGTCGGTAACAGA
GCTGACACCAGAGAAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCC
AATCAAGGTTGTCGGCTTGTCTAACTTGCCAGAAATTTACGAAAAGATGGAA
AAGGGTCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAA
Protein:
MSIPETQKGVIFYESNGKLEYKDIPVPKPKPNELLINVKYSGVCHTDLHAWHGD
WPLPTKLPLVGGHEGAGVVVAMGENVKGWKIGDYAGIKWLNGSCMACEYCEL
GNESNCPHADLSGYTHDGSFQQYATADAVQAAHIPQGTDLAEVAPILCAGITVY
KALKSANLMAGHWVAISGAAGGLGSLAVQYAKAMGYRVLGIDGGEGKEELFK
SLGGEVFIDFTKEKDIVGAVIKATDGGAHGVINVSVSEAAIEASTRYCRANGTVV
LVGLPAGAKCSSDVFNQVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVG
LSNLPEIYEKMEKGQIVGRYVVDTSK

MKN: *Saccharomyces cerevisiae* Ancestor (M168/K211/N236)
Gene:
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
GTTGGAATACAAAGATATTCCAGTTCCAAAGCCAAAGCCAAACGAATTGTTG
ATCAACGTTAAATACTCTGGTGTCTGTCACACTGACTTGCACGCTTGGCACGG
TGACTGGCCATTGCCCACTAAGCTACCATTAGTCGGTGGTCACGAAGGTGCC
GGTGTCGTTGTCGCCATGGGTGAAAACGTTAAGGGCTGGAAGATCGGTGACT
ACGCCGGTATCAAATGGTTGAACGGTTCTTGTATGGCCTGTGAATACTGTGAA
TTGGGTAACGAATCCAACTGTCCTCACGCTGACTTGTCTGGTTACACCCACGA
CGGTTCTTTCCAACAATACGCTACCGCTGACGCTGTTCAAGCCGCTCACATTC
CTCAAGGTACCGACTTGGCCGAAGTTGCTCCCATCTTGTGTGCTGGTATCACC
GTCTACAAGGCTTTGAAGTCTGCTAACTTGATGGCCGGTCACTGGGTTGCTAT
CTCCGGTGCTGCTGGTGGTCTAGGTTCTTTGGCTGTTCAATACGCCAAGGCTA
TGGGTTACAGAGTCTTGGGTATTGACGGTGGTGAAGGTAAGGAAGAATTATT
CAAGTCTTTGGGTGGTGAAGTCTTCATTGACTTCACTAAGGAAAAGGACATT
GTCGGTGCTGTTATCAAGGCCACTAACGGTGGTGCTCACGGTGTCATCAACGT
TTCCGTTTCCGAAGCCGCTATTGAAGCTTCTACCAGATACTGTAGAGCTAACG
GTACTGTTGTTTTGGTCGGTTTGCCAGCTGGTGCCAAGTGTTCTTCTGATGTCT
TCAACCAAGTCGTCAAGTCCATCTCTATTGTTGGTTCTTACGTCGGTAACAGA
GCTGACACCAGAGAAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCC

AATCAAGGTTGTCGGCTTGTCTAACTTGCCAGAAATTTACGAAAAGATGGAA
AAGGGTCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAA
Protein:
MSIPETQKGVIFYESNGKLEYKDIPVPKPKPNELLINVKYSGVCHTDLHAWHGD
WPLPTKLPLVGGHEGAGVVVAMGENVKGWKIGDYAGIKWLNGSCMACEYCEL
GNESNCPHADLSGYTHDGSFQQYATADAVQAAHIPQGTDLAEVAPILCAGITVY
KALKSANLMAGHWVAISGAAGGLGSLAVQYAKAMGYRVLGIDGGEGKEELFK
SLGGEVFIDFTKEKDIVGAVIKATNGGAHGVINVSVSEAAIEASTRYCRANGTVV
LVGLPAGAKCSSDVFNQVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVG
LSNLPEIYEKMEKGQIVGRYVVDTSK

MRD: *Saccharomyces cerevisiae* Ancestor (M168/R211/D236)
Gene::
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
GTTGGAATACAAAGATATTCCAGTTCCAAAGCCAAAGCCAAACGAATTGTTG
ATCAACGTTAAATACTCTGGTGTCTGTCACACTGACTTGCACGCTTGGCACGG
TGACTGGCCATTGCCCACTAAGCTACCATTAGTCGGTGGTCACGAAGGTGCC
GGTGTCGTTGTCGCCATGGGTGAAAACGTTAAGGGCTGGAAGATCGGTGACT
ACGCCGGTATCAAATGGTTGAACGGTTCTTGTATGGCCTGTGAATACTGTGAA
TTGGGTAACGAATCCAACTGTCCTCACGCTGACTTGTCTGGTTACACCCACGA
CGGTTCTTTCCAACAATACGCTACCGCTGACGCTGTTCAAGCCGCTCACATTC
CTCAAGGTACCGACTTGGCCGAAGTTGCTCCCATCTTGTGTGCTGGTATCACC
GTCTACAAGGCTTTGAAGTCTGCTAACTTGATGGCCGGTCACTGGGTTGCTAT
CTCCGGTGCTGCTGGTGGTCTAGGTTCTTTGGCTGTTCAATACGCCAAGGCTA
TGGGTTACAGAGTCTTGGGTATTGACGGTGGTGAAGGTAAGGAAGAATTATT
CAGATCTTTGGGTGGTGAAGTCTTCATTGACTTCACTAAGGAAAAGGACATT
GTCGGTGCTGTTATCAAGGCCACTGACGGTGGTGCTCACGGTGTCATCAACGT
TTCCGTTTCCGAAGCCGCTATTGAAGCTTCTACCAGATACTGTAGAGCTAACG
GTACTGTTGTTTTGGTCGGTTTGCCAGCTGGTGCCAAGTGTTCTTCTGATGTCT
TCAACCAAGTCGTCAAGTCCATCTCTATTGTTGGTTCTTACGTCGGTAACAGA
GCTGACACCAGAGAAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCC
AATCAAGGTTGTCGGCTTGTCTAACTTGCCAGAAATTTACGAAAAGATGGAA
AAGGGTCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAA
Protein:
MSIPETQKGVIFYESNGKLEYKDIPVPKPKPNELLINVKYSGVCHTDLHAWHGD
WPLPTKLPLVGGHEGAGVVVAMGENVKGWKIGDYAGIKWLNGSCMACEYCEL
GNESNCPHADLSGYTHDGSFQQYATADAVQAAHIPQGTDLAEVAPILCAGITVY
KALKSANLMAGHWVAISGAAGGLGSLAVQYAKAMGYRVLGIDGGEGKEELFR
SLGGEVFIDFTKEKDIVGAVIKATDGGAHGVINVSVSEAAIEASTRYCRANGTVV
LVGLPAGAKCSSDVFNQVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVG
LSNLPEIYEKMEKGQIVGRYVVDTSK

MRN: *Saccharomyces cerevisiae* Ancestor (M168/R211/N236)
Gene:
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
GTTGGAATACAAAGATATTCCAGTTCCAAAGCCAAAGCCAAACGAATTGTTG
ATCAACGTTAAATACTCTGGTGTCTGTCACACTGACTTGCACGCTTGGCACGG
TGACTGGCCATTGCCCACTAAGCTACCATTAGTCGGTGGTCACGAAGGTGCC
GGTGTCGTTGTCGCCATGGGTGAAAACGTTAAGGGCTGGAAGATCGGTGACT
ACGCCGGTATCAAATGGTTGAACGGTTCTTGTATGGCCTGTGAATACTGTGAA
TTGGGTAACGAATCCAACTGTCCTCACGCTGACTTGTCTGGTTACACCCACGA
CGGTTCTTTCCAACAATACGCTACCGCTGACGCTGTTCAAGCCGCTCACATTC
CTCAAGGTACCGACTTGGCCGAAGTTGCTCCCATCTTGTGTGCTGGTATCACC
GTCTACAAGGCTTTGAAGTCTGCTAACTTGATGGCCGGTCACTGGGTTGCTAT

CTCCGGTGCTGCTGGTGGTCTAGGTTCTTTGGCTGTTCAATACGCCAAGGCTA
TGGGTTACAGAGTCTTGGGTATTGACGGTGGTGAAGGTAAGGAAGAATTATT
CAGATCTTTGGGTGGTGAAGTCTTCATTGACTTCACTAAGGAAAAGGACATT
GTCGGTGCTGTTATCAAGGCCACTAACGGTGGTGCTCACGGTGTCATCAACGT
TTCCGTTTCCGAAGCCGCTATTGAAGCTTCTACCAGATACTGTAGAGCTAACG
GTACTGTTGTTTTGGTCGGTTTGCCAGCTGGTGCCAAGTGTTCTTCTGATGTCT
TCAACCAAGTCGTCAAGTCCATCTCTATTGTTGGTTCTTACGTCGGTAACAGA
GCTGACACCAGAGAAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCC
AATCAAGGTTGTCGGCTTGTCTAACTTGCCAGAAATTTACGAAAAGATGGAA
AAGGGTCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAA
Protein:
MSIPETQKGVIFYESNGKLEYKDIPVPKPKPNELLINVKYSGVCHTDLHAWHGD
WPLPTKLPLVGGHEGAGVVVAMGENVKGWKIGDYAGIKWLNGSCMACEYCEL
GNESNCPHADLSGYTHDGSFQQYATADAVQAAHIPQGTDLAEVAPILCAGITVY
KALKSANLMAGHWVAISGAAGGLGSLAVQYAKAMGYRVLGIDGGEGKEELFR
SLGGEVFIDFTKEKDIVGAVIKATNGGAHGVINVSVSEAAIEASTRYCRANGTVV
LVGLPAGAKCSSDVFNQVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVG
LSNLPEIYEKMEKGQIVGRYVVDTSK

MTD: *Saccharomyces cerevisiae* Ancestor (M168/T211/D236)
Gene:
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
GTTGGAATACAAAGATATTCCAGTTCCAAAGCCAAAGCCAAACGAATTGTTG
ATCAACGTTAAATACTCTGGTGTCTGTCACACTGACTTGCACGCTTGGCACGG
TGACTGGCCATTGCCCACTAAGCTACCATTAGTCGGTGGTCACGAAGGTGCC
GGTGTCGTTGTCGCCATGGGTGAAAACGTTAAGGGCTGGAAGATCGGTGACT
ACGCCGGTATCAAATGGTTGAACGGTTCTTGTATGGCCTGTGAATACTGTGAA
TTGGGTAACGAATCCAACTGTCCTCACGCTGACTTGTCTGGTTACACCCACGA
CGGTTCTTTCCAACAATACGCTACCGCTGACGCTGTTCAAGCCGCTCACATTC
CTCAAGGTACCGACTTGGCCGAAGTTGCTCCCATCTTGTGTGCTGGTATCACC
GTCTACAAGGCTTTGAAGTCTGCTAACTTGATGGCCGGTCACTGGGTTGCTAT
CTCCGGTGCTGCTGGTGGTCTAGGTTCTTTGGCTGTTCAATACGCCAAGGCTA
TGGGTTACAGAGTCTTGGGTATTGACGGTGGTGAAGGTAAGGAAGAATTATT
CACTTCTTTGGGTGGTGAAGTCTTCATTGACTTCACTAAGGAAAAGGACATTG
TCGGTGCTGTTATCAAGGCCACTGACGGTGGTGCTCACGGTGTCATCAACGTT
TCCGTTTCCGAAGCCGCTATTGAAGCTTCTACCAGATACTGTAGAGCTAACGG
TACTGTTGTTTTGGTCGGTTTGCCAGCTGGTGCCAAGTGTTCTTCTGATGTCTT
CAACCAAGTCGTCAAGTCCATCTCTATTGTTGGTTCTTACGTCGGTAACAGAG
CTGACACCAGAGAAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCA
ATCAAGGTTGTCGGCTTGTCTAACTTGCCAGAAATTTACGAAAAGATGGAAA
AGGGTCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAA
Protein:
MSIPETQKGVIFYESNGKLEYKDIPVPKPKPNELLINVKYSGVCHTDLHAWHGD
WPLPTKLPLVGGHEGAGVVVAMGENVKGWKIGDYAGIKWLNGSCMACEYCEL
GNESNCPHADLSGYTHDGSFQQYATADAVQAAHIPQGTDLAEVAPILCAGITVY
KALKSANLMAGHWVAISGAAGGLGSLAVQYAKAMGYRVLGIDGGEGKEELFT
SLGGEVFIDFTKEKDIVGAVIKATDGGAHGVINVSVSEAAIEASTRYCRANGTVV
LVGLPAGAKCSSDVFNQVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVG
LSNLPEIYEKMEKGQIVGRYVVDTSK

MTN: *Saccharomyces cerevisiae* Ancestor (M168/T211/N236)
Gene:
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
GTTGGAATACAAAGATATTCCAGTTCCAAAGCCAAAGCCAAACGAATTGTTG

ATCAACGTTAAATACTCTGGTGTCTGTCACACTGACTTGCACGCTTGGCACGG
TGACTGGCCATTGCCCACTAAGCTACCATTAGTCGGTGGTCACGAAGGTGCC
GGTGTCGTTGTCGCCATGGGTGAAAACGTTAAGGGCTGGAAGATCGGTGACT
ACGCCGGTATCAAATGGTTGAACGGTTCTTGTATGGCCTGTGAATACTGTGAA
TTGGGTAACGAATCCAACTGTCCTCACGCTGACTTGTCTGGTTACACCCACGA
CGGTTCTTTCCAACAATACGCTACCGCTGACGCTGTTCAAGCCGCTCACATTC
CTCAAGGTACCGACTTGGCCGAAGTTGCTCCCATCTTGTGTGCTGGTATCACC
GTCTACAAGGCTTTGAAGTCTGCTAACTTGATGGCCGGTCACTGGGTTGCTAT
CTCCGGTGCTGCTGGTGGTCTAGGTTCTTTGGCTGTTCAATACGCCAAGGCTA
TGGGTTACAGAGTCTTGGGTATTGACGGTGGTGAAGGTAAGGAAGAATTATT
CACTTCTTTGGGTGGTGAAGTCTTCATTGACTTCACTAAGGAAAAGGACATTG
TCGGTGCTGTTATCAAGGCCACTAACGGTGGTGCTCACGGTGTCATCAACGTT
TCCGTTTCCGAAGCCGCTATTGAAGCTTCTACCAGATACTGTAGAGCTAACGG
TACTGTTGTTTTGGTCGGTTTGCCAGCTGGTGCCAAGTGTTCTTCTGATGTCTT
CAACCAAGTCGTCAAGTCCATCTCTATTGTTGGTTCTTACGTCGGTAACAGAG
CTGACACCAGAGAAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCA
ATCAAGGTTGTCGGCTTGTCTAACTTGCCAGAAATTTACGAAAAGATGGAAA
AGGGTCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAA
Protein:
MSIPETQKGVIFYESNGKLEYKDIPVPKPKPNELLINVKYSGVCHTDLHAWHGD
WPLPTKLPLVGGHEGAGVVVAMGENVKGWKIGDYAGIKWLNGSCMACEYCEL
GNESNCPHADLSGYTHDGSFQQYATADAVQAAHIPQGTDLAEVAPILCAGITVY
KALKSANLMAGHWVAISGAAGGLGSLAVQYAKAMGYRVLGIDGGEGKEELFT
SLGGEVFIDFTKEKDIVGAVIKATNGGAHGVINVSVSEAAIEASTRYCRANGTVV
LVGLPAGAKCSSDVFNQVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVG
LSNLPEIYEKMEKGQIVGRYVVDTSK

RKD: *Saccharomyces cerevisiae* Ancestor (R168/K211/D236)
Gene:
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
GTTGGAATACAAAGATATTCCAGTTCCAAAGCCAAAGCCAAACGAATTGTTG
ATCAACGTTAAATACTCTGGTGTCTGTCACACTGACTTGCACGCTTGGCACGG
TGACTGGCCATTGCCCACTAAGCTACCATTAGTCGGTGGTCACGAAGGTGCC
GGTGTCGTTGTCGCCATGGGTGAAAACGTTAAGGGCTGGAAGATCGGTGACT
ACGCCGGTATCAAATGGTTGAACGGTTCTTGTATGGCCTGTGAATACTGTGAA
TTGGGTAACGAATCCAACTGTCCTCACGCTGACTTGTCTGGTTACACCCACGA
CGGTTCTTTCCAACAATACGCTACCGCTGACGCTGTTCAAGCCGCTCACATTC
CTCAAGGTACCGACTTGGCCGAAGTTGCTCCCATCTTGTGTGCTGGTATCACC
GTCTACAAGGCTTTGAAGTCTGCTAACTTGAGAGCCGGTCACTGGGTTGCTAT
CTCCGGTGCTGCTGGTGGTCTAGGTTCTTTGGCTGTTCAATACGCCAAGGCTA
TGGGTTACAGAGTCTTGGGTATTGACGGTGGTGAAGGTAAGGAAGAATTATT
CAAGTCTTTGGGTGGTGAAGTCTTCATTGACTTCACTAAGGAAAAGGACATT
GTCGGTGCTGTTATCAAGGCCACTGACGGTGGTGCTCACGGTGTCATCAACGT
TTCCGTTTCCGAAGCCGCTATTGAAGCTTCTACCAGATACTGTAGAGCTAACG
GTACTGTTGTTTTGGTCGGTTTGCCAGCTGGTGCCAAGTGTTCTTCTGATGTCT
TCAACCAAGTCGTCAAGTCCATCTCTATTGTTGGTTCTTACGTCGGTAACAGA
GCTGACACCAGAGAAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCC
AATCAAGGTTGTCGGCTTGTCTAACTTGCCAGAAATTTACGAAAAGATGGAA
AAGGGTCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAA
Protein:
MSIPETQKGVIFYESNGKLEYKDIPVPKPKPNELLINVKYSGVCHTDLHAWHGD
WPLPTKLPLVGGHEGAGVVVAMGENVKGWKIGDYAGIKWLNGSCMACEYCEL
GNESNCPHADLSGYTHDGSFQQYATADAVQAAHIPQGTDLAEVAPILCAGITVY
KALKSANLRAGHWVAISGAAGGLGSLAVQYAKAMGYRVLGIDGGEGKEELFKS

LGGEVFIDFTKEKDIVGAVIKATDGGAHGVINVSVSEAAIEASTRYCRANGTVVL
VGLPAGAKCSSDVFNQVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVGL
SNLPEIYEKMEKGQIVGRYVVDTSK

RKN: *Saccharomyces cerevisiae* Ancestor (R168/K211/N236)
Gene:
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
GTTGGAATACAAAGATATTCCAGTTCCAAAGCCAAAGCCAAACGAATTGTTG
ATCAACGTTAAATACTCTGGTGTCTGTCACACTGACTTGCACGCTTGGCACGG
TGACTGGCCATTGCCCACTAAGCTACCATTAGTCGGTGGTCACGAAGGTGCC
GGTGTCGTTGTCGCCATGGGTGAAAACGTTAAGGGCTGGAAGATCGGTGACT
ACGCCGGTATCAAATGGTTGAACGGTTCTTGTATGGCCTGTGAATACTGTGAA
TTGGGTAACGAATCCAACTGTCCTCACGCTGACTTGTCTGGTTACACCCACGA
CGGTTCTTTCCAACAATACGCTACCGCTGACGCTGTTCAAGCCGCTCACATTC
CTCAAGGTACCGACTTGGCCGAAGTTGCTCCCATCTTGTGTGCTGGTATCACC
GTCTACAAGGCTTTGAAGTCTGCTAACTTGAGAGCCGGTCACTGGGTTGCTAT
CTCCGGTGCTGCTGGTGGTCTAGGTTCTTTGGCTGTTCAATACGCCAAGGCTA
TGGGTTACAGAGTCTTGGGTATTGACGGTGGTGAAGGTAAGGAAGAATTATT
CAAGTCTTTGGGTGGTGAAGTCTTCATTGACTTCACTAAGGAAAAGGACATT
GTCGGTGCTGTTATCAAGGCCACTAACGGTGGTGCTCACGGTGTCATCAACGT
TTCCGTTTCCGAAGCCGCTATTGAAGCTTCTACCAGATACTGTAGAGCTAACG
GTACTGTTGTTTTGGTCGGTTTGCCAGCTGGTGCCAAGTGTTCTTCTGATGTCT
TCAACCAAGTCGTCAAGTCCATCTCTATTGTTGGTTCTTACGTCGGTAACAGA
GCTGACACCAGAGAAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCC
AATCAAGGTTGTCGGCTTGTCTAACTTGCCAGAAATTTACGAAAAGATGGAA
AAGGGTCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAA
Protein:
MSIPETQKGVIFYESNGKLEYKDIPVPKPKPNELLINVKYSGVCHTDLHAWHGD
WPLPTKLPLVGGHEGAGVVVAMGENVKGWKIGDYAGIKWLNGSCMACEYCEL
GNESNCPHADLSGYTHDGSFQQYATADAVQAAHIPQGTDLAEVAPILCAGITVY
KALKSANLRAGHWVAISGAAGGLGSLAVQYAKAMGYRVLGIDGGEGKEELFKS
LGGEVFIDFTKEKDIVGAVIKATNGGAHGVINVSVSEAAIEASTRYCRANGTVVL
VGLPAGAKCSSDVFNQVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVGL
SNLPEIYEKMEKGQIVGRYVVDTSK

RRD: *Saccharomyces cerevisiae* Ancestor (R168/R211/D236)
Gene:
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
GTTGGAATACAAAGATATTCCAGTTCCAAAGCCAAAGCCAAACGAATTGTTG
ATCAACGTTAAATACTCTGGTGTCTGTCACACTGACTTGCACGCTTGGCACGG
TGACTGGCCATTGCCCACTAAGCTACCATTAGTCGGTGGTCACGAAGGTGCC
GGTGTCGTTGTCGCCATGGGTGAAAACGTTAAGGGCTGGAAGATCGGTGACT
ACGCCGGTATCAAATGGTTGAACGGTTCTTGTATGGCCTGTGAATACTGTGAA
TTGGGTAACGAATCCAACTGTCCTCACGCTGACTTGTCTGGTTACACCCACGA
CGGTTCTTTCCAACAATACGCTACCGCTGACGCTGTTCAAGCCGCTCACATTC
CTCAAGGTACCGACTTGGCCGAAGTTGCTCCCATCTTGTGTGCTGGTATCACC
GTCTACAAGGCTTTGAAGTCTGCTAACTTGAGAGCCGGTCACTGGGTTGCTAT
CTCCGGTGCTGCTGGTGGTCTAGGTTCTTTGGCTGTTCAATACGCCAAGGCTA
TGGGTTACAGAGTCTTGGGTATTGACGGTGGTGAAGGTAAGGAAGAATTATT
CAGATCTTTGGGTGGTGAAGTCTTCATTGACTTCACTAAGGAAAAGGACATT
GTCGGTGCTGTTATCAAGGCCACTGACGGTGGTGCTCACGGTGTCATCAACGT
TTCCGTTTCCGAAGCCGCTATTGAAGCTTCTACCAGATACTGTAGAGCTAACG
GTACTGTTGTTTTGGTCGGTTTGCCAGCTGGTGCCAAGTGTTCTTCTGATGTCT
TCAACCAAGTCGTCAAGTCCATCTCTATTGTTGGTTCTTACGTCGGTAACAGA

GCTGACACCAGAGAAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCC
AATCAAGGTTGTCGGCTTGTCTAACTTGCCAGAAATTTACGAAAAGATGGAA
AAGGGTCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAA
Protein:
MSIPETQKGVIFYESNGKLEYKDIPVPKPKPNELLINVKYSGVCHTDLHAWHGD
WPLPTKLPLVGGHEGAGVVVAMGENVKGWKIGDYAGIKWLNGSCMACEYCEL
GNESNCPHADLSGYTHDGSFQQYATADAVQAAHIPQGTDLAEVAPILCAGITVY
KALKSANLRAGHWVAISGAAGGLGSLAVQYAKAMGYRVLGIDGGEGKEELFRS
LGGEVFIDFTKEKDIVGAVIKATDGGAHGVINVSVSEAAIEASTRYCRANGTVVL
VGLPAGAKCSSDVFNQVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVGL
SNLPEIYEKMEKGQIVGRYVVDTSK

RRN: *Saccharomyces cerevisiae* Ancestor (R168/R211/N236)
Gene:
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
GTTGGAATACAAAGATATTCCAGTTCCAAAGCCAAAGCCAAACGAATTGTTG
ATCAACGTTAAATACTCTGGTGTCTGTCACACTGACTTGCACGCTTGGCACGG
TGACTGGCCATTGCCCACTAAGCTACCATTAGTCGGTGGTCACGAAGGTGCC
GGTGTCGTTGTCGCCATGGGTGAAAACGTTAAGGGCTGGAAGATCGGTGACT
ACGCCGGTATCAAATGGTTGAACGGTTCTTGTATGGCCTGTGAATACTGTGAA
TTGGGTAACGAATCCAACTGTCCTCACGCTGACTTGTCTGGTTACACCCACGA
CGGTTCTTTCCAACAATACGCTACCGCTGACGCTGTTCAAGCCGCTCACATTC
CTCAAGGTACCGACTTGGCCGAAGTTGCTCCCATCTTGTGTGCTGGTATCACC
GTCTACAAGGCTTTGAAGTCTGCTAACTTGAGAGCCGGTCACTGGGTTGCTAT
CTCCGGTGCTGCTGGTGGTCTAGGTTCTTTGGCTGTTCAATACGCCAAGGCTA
TGGGTTACAGAGTCTTGGGTATTGACGGTGGTGAAGGTAAGGAAGAATTATT
CAGATCTTTGGGTGGTGAAGTCTTCATTGACTTCACTAAGGAAAAGGACATT
GTCGGTGCTGTTATCAAGGCCACTAACGGTGGTGCTCACGGTGTCATCAACGT
TTCCGTTTCCGAAGCCGCTATTGAAGCTTCTACCAGATACTGTAGAGCTAACG
GTACTGTTGTTTTGGTCGGTTTGCCAGCTGGTGCCAAGTGTTCTTCTGATGTCT
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GCTGACACCAGAGAAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCC
AATCAAGGTTGTCGGCTTGTCTAACTTGCCAGAAATTTACGAAAAGATGGAA
AAGGGTCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAA
Protein:
MSIPETQKGVIFYESNGKLEYKDIPVPKPKPNELLINVKYSGVCHTDLHAWHGD
WPLPTKLPLVGGHEGAGVVVAMGENVKGWKIGDYAGIKWLNGSCMACEYCEL
GNESNCPHADLSGYTHDGSFQQYATADAVQAAHIPQGTDLAEVAPILCAGITVY
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RTD: *Saccharomyces cerevisiae* Ancestor (R168/T211/D236)
Gene:
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
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ACGCCGGTATCAAATGGTTGAACGGTTCTTGTATGGCCTGTGAATACTGTGAA
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CGGTTCTTTCCAACAATACGCTACCGCTGACGCTGTTCAAGCCGCTCACATTC
CTCAAGGTACCGACTTGGCCGAAGTTGCTCCCATCTTGTGTGCTGGTATCACC

GTCTACAAGGCTTTGAAGTCTGCTAACTTGAGAGCCGGTCACTGGGTTGCTAT
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AGGGTCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAA
Protein:
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WPLPTKLPLVGGHEGAGVVVAMGENVKGWKIGDYAGIKWLNGSCMACEYCEL
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KALKSANLRAGHWVAISGAAGGLGSLAVQYAKAMGYRVLGIDGGEGKEELFTS
LGGEVFIDFTKEKDIVGAVIKATDGGAHGVINVSVSEAAIEASTRYCRANGTVVL
VGLPAGAKCSSDVFNQVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVGL
SNLPEIYEKMEKGQIVGRYVVDTSK

RTN: *Saccharomyces cerevisiae* Ancestor (R168/T211/N236)
Gene:
ATGTCTATCCCAGAAACTCAAAAAGGTGTTATCTTCTACGAATCTAACGGTAA
GTTGGAATACAAAGATATTCCAGTTCCAAAGCCAAAGCCAAACGAATTGTTG
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Protein:
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KALKSANLRAGHWVAISGAAGGLGSLAVQYAKAMGYRVLGIDGGEGKEELFTS
LGGEVFIDFTKEKDIVGAVIKATNGGAHGVINVSVSEAAIEASTRYCRANGTVVL
VGLPAGAKCSSDVFNQVVKSISIVGSYVGNRADTREALDFFARGLVKSPIKVVGL
SNLPEIYEKMEKGQIVGRYVVDTSK

Alcohol dehydrogenase families

Other "alcohol dehydrogenases" in Saccharomyces cerevisiae.
The alcohol dehydrogenases Adh 1 and Adh 2 are part of a large family of "long chain dehydrogenases". These have a molecular mass of approximately 35,000 Daltons, and generally require zinc for catalytic activity. They have been recruited repeatedly throughout the history of the biosphere, and the yeast genome contains a large number of these. Such recruitment is well known in molecular evolution, and the function of the recruited proteins can vary widely, even to comprise quite different substrates.

In *Saccharomyces cerevisiae*, only Adh 1 and Adh 2 are believed to be involved in the large scale fermentation of sugars to make ethanol, as shown by a large number of studies on metabolic regulation and flux analysis in yeast, as well as classical genetic analysis. Adh 3, in the mitochondria, may play a small role here, although this has not been supported well by genetic studies.

In *Saccharomyces cerevisiae*, five genes other than Adh 1 and Adh 2 carry the 'Adh' label. These are reviewed briefly below. None are involved in the large scale fermentation of sugars to make ethanol. More detailed information can be obtained from the SGD database.

ADH6 is a NAPDH dependent enzyme, and can be ignored as an enzyme important in the degradation of sugars. It appears that Adh6 is an aldehyde reductase with substrate preference towards cinnamaldehyde with little or no oxidation of ethanol (Larroy, et al. 2002a). Adh7 is a close relative of Adh6, has a similar substrate specificity towards cinnamaldehyde and a lower oxidation of ethanol (Larroy, et al 2002b).

A summary of the substrate specificity of Adh 6 is given below.

Substrate specificity of Adh 6

Reduction	**Relative Activity**	**Oxidation**	**Relative Activity**
Cinnamaldehyde	100	Cinnamyl alcohol	100
Sinapaldehyde	3	Coniferyl alcohol	27
Coniferaldehyde	10	Benzyl alcohol	45
Benzaldehyde	72	Ethanol	4
Veratraldehyde	82	Propanol	7
Anisaldehyde	72	Butanol	24
Vanillin	57	Pentanol	89
Propanal	21	Hexanol	154
Butanal	57	Octanol	52
Pentanal	109	2-Methylpropanol	1
Hexanal	89	2-(S)-Methylbutanol	7
Heptanal	88	3-Methylbutanol	15
Octanal	84	2-Butanol	2
2-Methylpropanal	67	2-Propanol	ND
2-Methylbutanal	92	1,2-Propanediol	ND
3-Methylbutanal	84	1,2-Butanediol	ND
trans-2-Nonenal	39	1,3-Butanediol	2

4-Hydroxynonenal	30	1,4-Butanediol	4
Furfural	27	2,3-Butanediol	ND
Methylglioxal	ND	Eugenol	ND
Acetone	ND		
Cyclohexanone	ND		

Substrate	***K*m (mM)**	***k*cat (min-1)**	***k*cat/*K*m (mM-1·min-1)**
Cinnamaldehyde	0.172±0.012	18400±600	107200±8600
Veratraldehyde	0.073±0.003	15800±140	217800±9470
Hexanal	0.152±0.017	21270±920	139950±16780
Pentanal	0.060±0.009	22700±190	380300±57830
3-Methylbutanal	0.129±0.035	14000±670	109000±30440
NADPH	0.029±0.003		
Cinnamyl alcohol	0.436±0.029	884±90	2028±247
Hexanol	9.1±2.4	640±92	71±24
*Cinnamyl alcohol	0.700±0.120	4200±60	6000±1000
*NADP+	0.061±0.002		

* Activities measured in 0.1 M glycine (pH 10.0).
Reduction activities were measured in 33mM sodium phosphate buffer (pH 7.0) containing 1mM substrate and 0.5mM NADPH. The activity towards cinnamaldehyde was taken as 100% with the specific activity being 183units/mg of protein. Oxidation activities were measured in 0.1M glycine at pH 10.0 containing 10mM substrate (except for octanol where 1mM was used) and 1.2mM NADP+. ND; not detected.
From Larroy *et al*. (2002a)

ADH5 represents a minor enzyme with the only function being attributed to the formation of long-chain alcohols. However, any of the six Adhs can perform this function. The function of Adh5 is largely uncharacterized, though it has been proposed to share a common ancestor with Adh1, from which it appeared to have diverged as part of a block duplication (Wolfe and Shields, 1997).

ADH4 is an iron dependent enzyme having no obvious homology to the long chain dehydrogenases. Based on sequence analysis, it appears that this enzyme originated from bacteria. Expression of the enzyme cannot normally be detected in laboratory strains of yeast.

ADH3 is interesting, in that it is activated under oxidative conditions, is mitochondrial, and has a growth defect on minimal medium +his/leu/ura. ADH3 probably functions to consume trace ethanol not utilized by ADH2. This activity may have been greater prior to the duplication of Adh1/2 and explains the rapid divergence of ADH5 from ADH1.

Alcohol dehydrogenases in other fungi
Some of the ADHs from *Kluyveromyces lactis*, also called "Adh", have numbers that follow the name (1 and 2, for example). These are not orthologs of their namesakes in *cerevisiae*. This can be seen from the tree in Figure 2, which shows that the *Kluyveromyces* paralogs diverged from each other after *Kluyveromyces* diverged from *Saccharomyces*.

In contrast, species now listed in the *Saccharomyces* genus that are closely related to *cerevisiae* have Adh 1 and Adh 2 paralogs that tree with their respective orthologs in *cerevisiae*. There is little doubt that the genome duplication predates the speciation event, and that the function in the *Saccharomyces* species is parallel.

Although beyond the scope of this paper, a larger view of the evolution of dehydrogenases in yeast-like fungi must consider the life styles of other species of *Saccharomyces*, as well as the lifestyles of species like *Kluyveromyces lactis*.

In *Kluyveromyces lactis*, the kinetic properties of some of the enzymes are known as well. Enzyme properties are shown for several of these in the tables below (From Bozzi *et al*. 1997).

Isozyme	Ethanol		
	K_m	K_{cat}	K_{cat}/K_m
Cytosolic			
KLADH1	2.7×10^4	2.5×10^5	9.3×10^3
KLADH2	2.3×10^4	2.8×10^4	1.2×10^3
Mitochondrial			
KLADH3	2.57×10^3	8.2×10^4	3.2×10^4
KLADH4	1.56×10^3	1.3×10^4	8.3×10^5

Isozyme	Acetaldehyde		
	K_m	K_{cat}	K_{cat}/K_m
Cytosolic			
KLADH1	1.2×10^3	3.6×10^5	3.0×10^5
KLADH2	1.7×10^3	3.3×10^4	2.0×10^4
Mitochondrial			
KLADH3	0.1×10^3	8.6×10^5	8.6×10^6
KLADH4	3.1×10^3	2.9×10^4	9.0×10^5

Isozyme	NAD^+		
	K_m	K_{cat}	K_{cat}/K_m
Cytosolic			
KLADH1	2.8×10^3	5.8×10^4	2.1×10^4
KLADH2	2.2×10^3	7.0×10^4	3.2×10^4
Mitochondrial			
KLADH3	0.31×10^3	6.1×10^4	1.9×10^5
KLADH4	0.20×10^3	1.1×10^3	5.5×10^5

Isozyme	$NADP^+$		
	K_m	K_{cat}	K_{cat}/K_m
Cytosolic			
KLADH1	n.i.	-	-
KLADH2	n.i.	-	-
Mitochondrial			
KLADH3	4.1×10^3	6.6×10^3	1.6×10^3
KLADH4	4.6×10^3	3.3×10^4	7.1×10^5

Isozyme	NADH		
	K_m	K_{cat}	K_{cat}/K_m
Cytosolic			
KLADH1	0.11×10^3	8.1×10^4	7.3×10^5
KLADH2	0.18×10^3	1.2×10^5	6.6×10^5
Mitochondrial			
KLADH3	0.02×10^3	6.0×10^5	3.0×10^7
KLADH4	0.03×10^3	2.3×10^4	7.6×10^5

Isozyme	NADPH		
	K_m	K_{cat}	K_{cat}/K_m
Cytosolic			
KLADH1	n.i.	-	-
KLADH2	n.i.	-	-
Mitochondrial			
KLADH3	0.12×10^3	2.4×10^4	2.0×10^5
KLADH4	0.95×10^3	2.1×10^4	2.2×10^4