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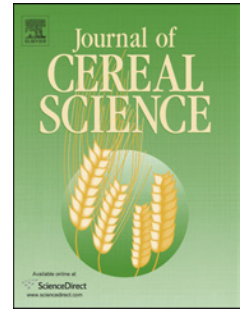http://researchrepository.murdoch.edu.au/4321/

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1   **QTLs for Malting Flavor Component Associated with Pre-harvest Sprouting**

2   **Susceptibility in Barley (*Hordeum vulgare* L.)**

3

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21   Abbreviation:

22   cM = Centimorgan

23   DH= Doubled Haploid

24   DHLs = Doubled Haploid Lines

25   LOX= Lipoxygenase

26   QTL= Quantitative Trait Loci

27   (S)-9-HPODE = (9S)-9-hydroperoxy-10E, 12Z-octadecadienoic acid

28   (S)-13-HPODE= (13S)-13-hydroperoxy-9Z, 11E-octadecadienoic acid

29   THOD = Trihydroxyoctadecenoic acid

30   9-HPL-like = Hydroperoxide Lyase-like.

Abstract

Lipoxygenase (LOX) is a key factor affecting quality of beer in terms of foam stability and flavour. Low LOX content is a desirable trait for malting quality. A doubled haploid (DH) population was made from a cross of Australian malting barley Stirling and Canadian malting barley Harrington and mapped with 513 molecular markers. The 120 DH lines with their parents were planted in field trials and harvested grains were micro-malted for analysis of LOX content in two consecutive years. LOX content was controlled by both genetic effects and environment conditions. Three QTLs were consistently detected. One QTL flanked by the markers E6216 and SCssr03907 at the telomere region of chromosome 5HL contributed 39% of genetic variation in LOX content. The second QTL close to the centromere region of chromosome 5H accounted for 17% of genetic variation. A minor QTL on chromosome 2H explained 6% of genetic variation but was significant in both years. The Australian variety Stirling contributed to higher LOX content for the three QTLs. The two QTLs mapped at chromosome 5H for LOX content coincided with the QTLs for seed dormancy/pre-harvest sprouting from the same population. The pre-harvest sprouting susceptible alleles were associated with low LOX content, which indicated that the low LOX QTL from the Canadian malting barleys are only useful in the barley growing areas where the pre-harvest sprouting risk is low. New genetic sources for low LOX should be exploited in different germplasm with different mechanisms.

Keywords

Lipoxygenase, Malting flavor, Quantitative trait loci, Seed dormancy

1. Introduction

Lipoxygenases (linoleate:oxygen oxidoreductase, LOX, EC 1.13.11.12) form a family of non-heme-iron-containing fatty acid dioxygenases that are widely distributed in plants and animals. The enzymes catalyze the peroxidation of polyunsaturated fatty acids containing a 1Z, 4Z-pentadiene system to yield the (S)-configured hydroperoxy fatty acids and are involved in the first reaction for synthesis of compounds derived from polyunsaturated fatty acids, collectively called oxylipins (Feussner and Wasternack, 2002). They are involved in several plant metabolic processes, such as seed development, germination, vegetative growth, wounding, stress responses, senescence and cell signaling (Porta and Rocha-Sosa, 2002). Many isoenzymes of LOX have been found in plants.

Three isoforms of LOX have been described in barley. Through lipoxygenase reactions, the fatty acids can be converted to a variety of secondary metabolites which are considered to play a role in staling and off-flavor formation in beer (Feussner et al., 2001; Kuroda et al., 2003a). Besides favouring the yeast performance during fermentation, lipids can negatively influence filtration rates, decrease foam stability, and may give rise to the development of off-flavor compounds (Doderer et al., 1992). A lipoxygenase (LOX1) in barley was first reported by Franke and Freshe in 1953. Subsequently a second isoenzyme (LOX2), which generally appears to develop only after germination, was documented (Yabuuchi, 1976; Baxter, 1982; Doderer et al., 1992). LOX-1 is already present in quiescent grains and catalyzes the formation of (9S)-9-hydroperoxy-10E, 12Z-octadecadienoic acid [(S)-9-HPODE]. LOX-2 is a germination-associated LOX isoform, which is formed during germination and catalyzes the formation of (13S)-13-hydroperoxy-9Z, 11E-octadecadienoic acid [(S)-13-HPODE] with linoleic acid as the substrate (van Mechelen et al., 1999; Holtman et al., 1997; Doderer et al., 1992). In mature plants its distribution is similar to that of LOX-1 with the highest activity in leaves and roots (Holtman et al., 1996). Expression of the third LOX isoform has been detected only after germination and is similar to that of LOX-2, although in mature vegetative tissues it is present only at low levels (van Mechelen et al., 1999). LOX-1 is of great interest because 9-HPOD

84   forms beer-deteriorating substances such as trans-2-nonenal and

85   trihydroxyoctadecenoic acid (THOD) during further reactions in the brewing process

86   (Kobayashi et al., 1993; Kuroda et al., 2002, 2003b). Trans-2-nonenal is known as a

87   major component of the cardboard flavour in aged beer (Meilgaard, 1975; Drost et al.,

88   1990). THOD is known to have an adverse effect on the quality of beer in terms of

89   foam stability and flavour (Bauer et al., 1977; Yabuuchi and Yamashita, 1979;

90   Kaneda et al., 2001; Kobayashi et al., 2002).

91   The genes encoding LOXs were cloned and then mapped to the LoxA locus on

92   chromosome 4H and the LoxC locus on chromosome 5H, respectively (van Mechelen

93   et al., 1995, 1999). LOX-1 provides the predominant LOX activity in malt and has a

94   relatively low pI compared to LOX-2 (Yang et al., 1993; Yang and Schwarz, 1995).

95   In contrast to these functional characterisations of LOX, little is known about its

96   genetic variation in barley. Recently, LOX-1 null mutants were explored to reduce

97   LOX content in new malting barley varieties (Hirota et al., 2006). Malting barley lines

98   with the mutant gene demonstrated significant improvement of flavour and foam

99   stability (Hirota et al., 2006).

100   The test of wort nonenal potential, which is widely used by brewers to estimate

101   quality of malting related to beer flavour stability, is a kind of forcing test to measure

102   the potential of wort to form 2(E)-nonenal. Recently a successful malt selection and

103   malting methods for improving the flavour stability of beer was identified, which

104   involved in the production of 2(E)-nonenal during mashing. LOXs, which are

105   nonheme ferrus proteins and catalyze the hydroperoxidation of polyunsaturated fatty

106   acids with a 1.4-cis–cis-pentadiene structure, play an important role in this process. It

107   was found that 2(E)-nonenal is produced by the cascade reaction of barley LOX and

108   malt 9-fatty acid hydroperoxide lyase-like activity (9-HPL-like activity) in malt

109   (Larsen et al. 2001; Founier et al., 2001). Thus, LOX activity has been chosen as an

110   indicator for beer quality. However, measurement of LOX activity is a time

111   consuming and labour intensive process as demonstrated in the material and methods

112   section in this paper. Furthermore, the requirement of malting barley grain before the

113 enzyme assay also restricts the use of the chemical LOX method for early generation

114 selection of LOX in breeding programs.

115   It has been a market mystery that the Canadian malting barley has better malting

116 flavour. In our barley breeding program, significant variation has been observed for

117 LOX content among barley varieties. In general, the Canadian malting barley varieties

118 and the varieties derived from the Canadian varieties showed lower LOX content

119 comparing to Australian malting barley varieties. In this study, we examined LOX

120 content in a DH population from a cross between Australian and Canadian malting

121 barley varieties and identified QTLs for LOX content which will provide tools for

122 marker assisted selection for low LOX in the breeding programs.

123

124 **2.** Experimental

125 *2.1. Plant material*

126   A population of 185 doubled haploid lines (DHLs) was generated by anther

127 culture from a Stirling/Harrington cross and kindly provided by Kirin Australia. This

128 population was previously used to map QTLs controlling seed dormancy/pre-harvest

129 sprouting. Details of the map construction and seed dormancy analysis were reported

130 in the previous publication (Bonnardeaux et al., 2008).

131

132 *2.2 .Field experiment*

133   The Stirling/Harrington DH population was planted in 2005 and 2006 at the

134 Wongan Hills Research Station, Department of Agriculture and Food WA (DAFWA),

135 188 km north-east of Perth. The field plot was planted in a randomised complete

136 block design with plots of 1 by 3 m^2. Control plots of Stirling (parental line) plants

137 were sown in the first and last rows (rows 1 and 19) and Hamelin (progeny of

138 Stirling/Harrington cross) control plots were sown in the middle of the field plot (row

139 11). The control varieties were used for spatial adjustment of the experimental data.

140 The second parental line, Harrington, was planted in an individual plot in the next

141 block in one season.

142

143 *2.3. Micro-malting process*

144   120 DH lines were sub-sampled from this population for micro-malting and QTL

145 analysis of LOX content. Barley samples were cleaned and sieved over a 2.2 mm

146 screen prior to micro-malting in a Joe White Systems micro-malting unit without the

147 use of additives. A standard malting schedule was used: Steeping - 19°C, 7h wet, 8h

148 air rest, 3h wet, 4h air rest, 1h wet. Germination was total 96 hours (48 hours at 18°C

149 followed by 48 hours at 16°C), moisture adjusted to 46% at 24 hours. Kilning was 2h

150 at 45°C, 3h at 50°C, 4h at 55°C, 3h at 60°C, 3h at 65°C, 3h at 70°C, 2h at 75°C, and

151 4h at 80°C. Malt rootlets were removed using a custom made rootlet removing

152 machine (Fraser Fabrications P/L, Malaga, Western Australia).

153

154   *2.4. Lipoxygenase Assay*

155   We used the Joe White Malting revised version of the Malt Lipoxygenase (LOX).

156   The assay was originally from Baxter (1982). All processes were completed on ice

157   unless otherwise indicated.

158

159   *2.4. 1. Preparation of substrate solution (2.5% linoleic acid)*

160   A 5 mL of 0.05 M-borate buffer (pH 9.0) was added to a volumetric flask (10 mL)

161   followed by adding 0.25 mL Tween20, 0.25 mL Linoleic acid and 0.65 mL 1M

162   NaOH . The contents were shaken gently in the ultrasonic bath with ice water until the

163   solution became clear, then distilled water was added to 10 mL.

164

165   *2.4. 2. Enzyme extraction from finished malt*

166   Finished malts were milled in a Retsch ZM200 centrifugal mill (Retsch GmBH,

167   Germany) with a 1.0 mm screen and 5 g of milled malt was transferred to 100 mL

168   flask. 50 mL of acetate buffer (pH5.0) containing 0.1 M NaCl was added and kept in

169   ice water bath for 15 min with occasional shaking. The resulting solution was

170   transferred into a 1.5 mL eppendorf tube and centrifuged for 5 min at 10,000 rpm. The

171   supernatant was subsequently transferred to a new eppendorf tube and stored on ice.

172

173   *2.4. 3. Enzyme assay*

174   The temperature of the cell holder and phosphate buffer (0.1M, pH 6.8) was

175   equilibrated to 25°C by water circulation. 100 µL enzyme extract and 2850 µL

176   phosphate buffer (0.1 M, pH 6.8) was added to 50 µL substrate solution, mixed,

177   returned to the cell holder and the absorbance recorded at 1min and 4min at 234 nm.

178   The blank absorbance was measured using 50 µL substrate solution and 2950 µL

179   phosphate buffer at 1 min.

180

181   *2.4. 4. Calculation and expression of results*

182   One unit of LOX activity represents an increase in absorbance at 234 nm of 1.0

183 per minute, per gram of malt on dry basis, as calculated using the following formula.

184

185 $$\text{LOX activity} = \frac{\text{Abs (4 min)-Abs (1 min)}}{3} \times \frac{1}{5 \times (100\text{-}M/100)} \times \frac{50{,}000}{B}$$

188

189 Abs (4 min): Absorbance at 234 nm at 4min of reaction

190 Abs (1 min): Absorbance at 234 nm at 1min of reaction

191 M: Moisture content of malt sample

192 B: Volume (µL) of enzyme extract of malt used

193

194 *2.5 . Map construction*

195   In addition to the previous SSR markers ((Bonnardeaux et al., 2008), 120 DH

196 lines and their parents were genotyped by Diversity Arrays Technology Pty Ltd

197 (http://www.diversityarrays.com) using the barley version 2.0 array. More than 900

198 DArT markers were scored in the population. After removing non-polymorphic and

199 low quality markers, 513 markers were used for map construction.

200 Software package Map Manager QTX (Manly et al., 2001) was used to determine

201 initial linkage groups using the Kosambi map function (Kosambi, 1944) and a search

202 and linkage criterion of P < 0.001. RECORD (Van Os et al., 2005) was used to

203 optimise the marker order of linkage groups and the final map was then defined by

204 Map Manager QTX.

205

206 *2.6. Data analysis and QTL mapping*

207   After removing the redundant markers, a framework map with 284 molecular

208 markers were used for QTL mapping. Software package Map Manager QTX was

209 initially used to map the QTL based on each year's data. The data collected over the

210 two years generated similar QTLs for LOX content. Final QTL analyses were

211 performed using the software package QTLNetwork (Yang et al., 2008) to combine

212 the data from the two years which were treated as an environmental factor. In this

213   software the QTL effects are estimated by the Monte Carlo Markov Chain

214   method/mixed linear model approach. Permutation tests (Doerge and Churchill, 1996)

215   were carried out using 1000 iterations at 1 centimorgan (cM) intervals. A minimum

216   separation of 10 cM ('filtration window') was used to define individual adjacent

217   QTLs. The QTL Network calculates a P value for significance and in the present study

218   a threshold of $P < 0.05$ was used to declare a significant QTL.

219   JMP software was used for statistical analysis (SAS Institute). Association of

220   different traits was calculated using stepwise regression analysis by the 'Fit-model'

221   function of JMP software (SAS Institute). A probability of $p<0.01$ was used to claim

222   association between traits.

3. Results

3.1. Genetic analysis of LOX content

The LOX and protein contents in the Stirling/Harrington DH population were assessed over 2 years. The LOX content varied from 8.9 to 48.1 U/g with average 22.1 U/g in 2005 trials while the variation was from 13.4 to 48.7 U/g with an average 26.5 U/g in 2006. The 2006 samples had significantly higher protein content compared to the 2005 samples ($p<0.01$). However, the protein content was not correlated to LOX content in both years ($R^2=0.02$ & 0.00 respectively in 2005 and 2006). The LOX content was significantly correlated each other in the two years (Fig. 1). The 2005 data could explain 39% of the variation of 2006 data for the LOX content. Thus, LOX content was controlled by genetic factors and impacted on by environment in the Stirling/Harrington DH population.

Fig. 2 shows the distribution of average LOX content in 2005 and 2006. A normal distribution pattern was observed, which suggested that multiple genes/QTLs control LOX content in the Stirling/Harrington DH population. Australian malting barley variety Stirling consistently showed higher LOX content.

3.2. Molecular linkage map

The molecular linkage maps included 513 markers. Except for chromosome 7H, a single linkage group was established for each chromosome (Fig. 3). Molecular markers for each chromosome varied from 33 on chromosome 1H to 104 on chromosome 2H. The 7 chromosomes covered 1043 cM with an average marker distance of 2 cM.

3.3. Genetic mapping of LOX content

Genomic scanning of the 7 chromosomes identified three significant quantitative trait loci (QTL) for LOX content. Two QTL for LOX content were mapped on chromosome 5H and the third mapped on chromosome 2H (Fig. 3). The most significant QTL was located on the telomere region of chromosome 5HL and flanked by the markers E6126 and SCssr03907. This QTL could explain 39% of the

253 phenotypic variation. The second locus was mapped close to the centromere region of

254 chromosome 5H and flanked by the markers Bpb9719 and Bpb6967. This QTL

255 explains 17% of the LOX content variation. The third QTL was mapped between the

256 markers Bpb8292 and Bpb4523 on chromosome 2H and accounted for 6% of LOX

257 content variation (Fig. 3; Table 1). There was no QTL x QTL or QTL x environment

258 interaction detected. Thus additive effects were the predominant factors controlling

259 LOX content. The Australian variety Stirling contributed to increasing LOX content.

260

261 *3.4. Association of LOX content with seed dormancy*

262 The seed dormancy was assessed in the Stirling/Harrington DH population in the

263 previous study (Bonnardeaux et al., 2008). The association of LOX content with seed

264 dormancy is shown in Fig. 4. The X-axis shows the average germination and the

265 Y-axis shows average LOX content of each DH line respectively. Linkage analysis

266 revealed that the individuals with higher germination rates or less dormancy have

267 lower LOX content (r=0.65***). The seed dormancy could explain 42% phenotypic

268 variation of the LOX content in the DH population (p<0.001).

269

270 **4. Discussion**

271 Due to the difficulty for the measurement of LOX activity, the early generation

272 selection of LOX in breeding programs is prohibitive. In the present study, the LOX

273 content was stable over two continued years and they were controlled by genetic

274 factor and impacted on by environment slightly in the Stirling/Harrington DH

275 population. Then the QTLs controlling LOX content were detected in the

276 Stirling/Harrington DH population. As these QTLs were consistently detected over

277 two years, they are considered to be authentic loci controlling the LOX content. The

278 molecular markers associated with the QTLs will provide an effective tool for

279 selection of low LOX in the breeding program through marker-assisted selection.

280 The previous research showed barley contains at least two distinct isoenzymes,

281 LOX-1 and LOX-2 (Yabuuchi, 1976; Baxter, 1982; Doderer et al., 1992; Yang et al.,

282 1993).   The two isozymes of seed LOX, were cloned and then mapped to the LoxA

283   locus on chromosome 4H and the LoxC locus on chromosome 5H, respectively (van

284   Mechelen et al., 1995, 1999). The product related to the third cDNA (loxB) has not

285   been identified so far. LOX-1 provides the predominant LOX activity in malt and has

286   a relatively low pI compared to LOX-2 (Yang et al., 1993; Yang and Schwarz, 1995).

287   The LOX-1 null mutants have been identified and a gene-specific marker has

288   provided the perfect tool for selecting low LOX malting barley varieties (Hirota et al.,

289   2006). In the present study, no QTL was detected on chromosome 4H, which

290   indicated that the structural gene of LOX-1 may or may not play a key role for

291   determining the LOX content in the present study. This phenomenon has been

292   observed in QTL mapping for a lot of traits including enzyme activity for

293   alpha-amylase and beta-glucanase (Li et al., 2009). One possibility is that both parents

294   have the same allele for the structural gene. We are in the process to survey the

295   sequence variation of LOX-1 in different barley varieties. Further research would

296   examine the combination of the low LOX QTLs in the present study with the LOX-1

297   null mutant gene to investigate the effect on malt and beer quality.

298   It has been a market mystery that the Canadian malting barley varieties especially

299   Harrington and its derived varieties have better malting and wort flavor. This is

300   consistent with our general observation that the Canadian malting barley varieties

301   have lower LOX content. The present study demonstrated that the two major QTLs

302   for low LOX content were mapped on chromosome 5H. Their locations differed from

303   that of the LoxC locus on chromosome 5H (van Mechelen et al., 1995, 1999). Instead

304   these two QTLs coincided with the two major QTLs for seed dormancy/pre-harvest

305   sprouting tolerance (Bonnardeaux et al., 2008). The non-dormancy or pre-harvest

306   sprouting susceptible alleles contributed to low LOX content (Fig 4). Previous

307   research showed that LOX is not directly involved in the induction of grain dormancy

308   as no significant differences were observed in lipoxygenase mRNA levels in

309   developing grains grown under dormant or non-dormant conditions (van Mechelen et

310   al., 1999). It is not clear if this association is due to pleiotrophic effects of the genes

311   controlling seed dormancy/pre-harvest sprouting tolerance or multiple gene clusters.

312   The recently developed new molecular markers targeted the specific chromosome

313   region will provide a tool to further dissect the chromosomal region (Zhang et al.,

314   2010). These QTL regions were also reported to control other hydrolytic enzyme

315   activity and malting quality (Li et al., 2009). In contrast, the pre-harvest sprouting

316   susceptible allele increased hydrolytic activity of alpha-amylase and beta-glucanase,

317   but decrease the LOX content in the present study. In the practical point view, the

318   association of low LOX content with pre-harvest sprouting susceptibility

319   demonstrated that the low LOX QTL from the Canadian malting barleys are only

320   useful in the barley growing areas where the pre-harvest sprouting risk is low. New

321   genetic sources for low LOX should be exploited in different germplasm with

322   different mechanisms

323

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Legends to Figures

Fig. 1

Leverage plot of LOX content in 2005 and 2006 in the Stirling/Harrington DH population. The X-axis shows the average LOX content in 2006 and the Y-axis shows the average LOX content in 2005.

Fig. 2

Distribution of average LOX content in 2005 and 2006 in the Stirling/Harrington DH population. The X-axis shows the average LOX content in 2005 and 2006, the Y-axis shows the Number of individuals.

Fig. 3

Molecular linkage map of SSR and DArT markers and the QTL for LOX content in Stirling/Harrington DH population. Chromosomes 2H,5H and 6H split into two fragments from the centromere regions and genetic distance between the two fragments is 0. The vertical bar indicated the QTL region associated with LOX content.

Fig. 4

Association of LOX content with seed dormancy in the Stirling/Harrington DH population. X-axis shows the average germination of individuals. Y-axis shows the average LOX content. The horizontal dashed line indicates the population average of LOX content. The oblique dashed lines show the linear model between seed dormancy and LOX content,

Table 1

Chromosomal locations, marker intervals, additive effects, heritability and positive parents for LOX content in the Stirling/Harrington DH population. The F value for genome-wide significant $P_{0.05}$ is 7.9.

QTL	Maker interval	Additive effect	Heritability	F value	Positive
Qtl2-1	Bpb8292-Bpb1098	1.70±0.30	0.06	13.7	Stirling
Qtl5-1	Bpb9719-Bpb6967	2.85±0.35	0.17	15.1	Stirling
Qtl5-2	E6216-SCssr03907	4.27±0.30	0.39	45.4	Stirling
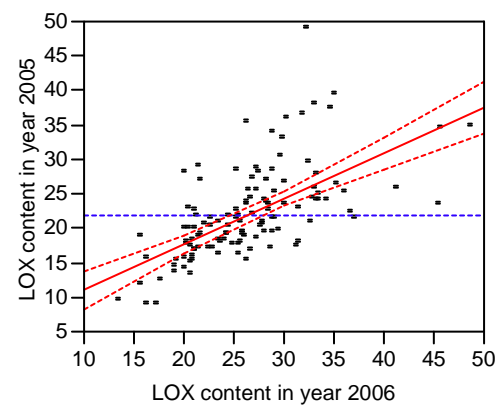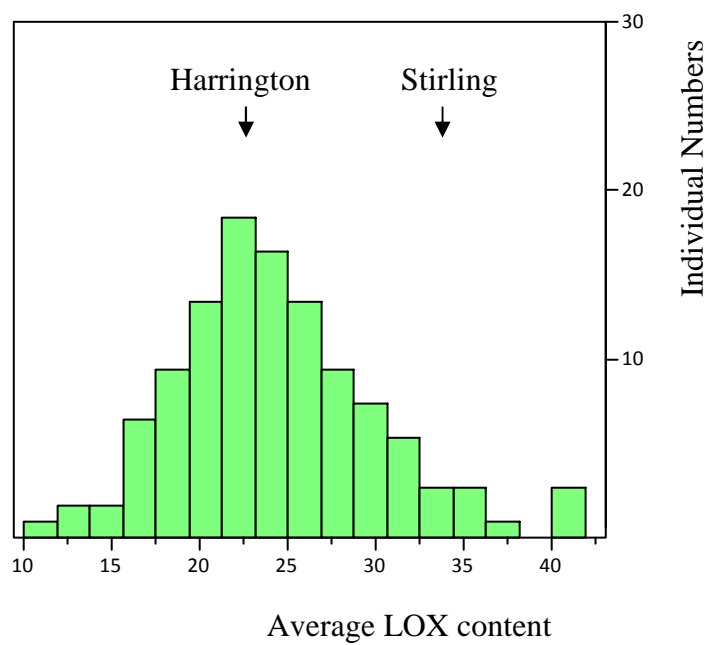
bPb_0663
bPb_3229
bPb_3865
bPb_4824
bPb_7350
bPb_3569
bPb_2415
bPb_0527
bPb_1814
bPb_2548
bPb_2965
bPb_5487
bPb_6825
bPb_6944
bPb_9878
bPb_0079
bPb_4602
GBM1233
BMAG10
GBM1043
bPb_1579
bPb_7695
bPb_1961
bPb_9110
Bmag0013
GBM1037
bPb_1609
bPb_6249
bPb_5312
EBmac0541
Hv13GEIII
bPb_4456
GBM1059
bPb_1829
bPb_1822
EBmac0708
bPb_0200
bPb_1928
bPb_7164
bPb_8322
bPb_8907
HVM0070b
bPb_4169
bPb_0361
GBM1238
bPb_5570
bPb_0848
bPb_6221
bPb_5256
bPb_7724
scssr25538
GBMS0068
GMS0061

Bmag0490
bPb_4216
bPb_5480
EBmac0683
bPb_9504
GBM1067
scind03751
Bmag0808
Bmag0333
bPb_1278
bPb_3268
bPb_3684
bPb_9039
EBmac0749a
EBmac0749b
EBmac0895
scind04312b
AWBMS0062a
Bmac344b
EBmag0769
bPb_0130
bPb_0365
bPb_3045
bPb_4183
bPb_4333
bPb_6973
GMS0089
bPb_3512
bPb_0513
bPb_1148
GBM1069
HVM0040a
Bmag0741
GBM1055
GBM1221
GBM1465
bPb_4699
GBM1501
EBmac0672
AWBMS0062b
GBMS0031
scind02622
Bmac0134
Bmag0641
Bmac0298
EBmac0635
EBmac0701
bPb_8164
bPb_9859
GBM1048
bPb_1166
bPb_6523
bPb_6107
bPb_5265
bPb_9668
GBM1015
Bmag0419b
bPb_3717
bPb_4765
Bmag0138a
AfR4a

bPb_2591
bPb_7676
bPb_1820
bPb_6183
bPb_5166
bPb_6363
bPb_5369
Bmag0005
Bmag0477
bPb_2795
bPb_6603
bPb_9317
EBmag0814
HVACLI
AWBMS0031
AWBMS0070
Bmac0047
Bmac0113c
Bmag0022b
Bmag0387
Bmag0751
bPb_0786
bPb_0949
scssr18076c
Bmac303
bPb_5584
Bmag0479a
bPb_1046
bPb_4210
bPb_8929

GBM1399
bPb_5381
bPb_8637
bPb_5532
bPb_9719
bPb_6967
bPb_1241
bPb_2425
bPb_8022
bPb_8101
bPb_5596
bPb_7395
bPb_0710
bPb_6135
bPb_7187
bPb_4744
bPb_3879
bPb_4334
D6
D7
D8
bPb_2325
bPb_7854
bPb_8731
bPb_9518
bPb_3241
bPb_4115
bPb_3114
bPb_4058
D5
bPb_3887
bPb_5845
bPb_6578
bPb_3945
bPb_2006
bPb_8341
bPb_4753
bPb_3138
bPb_0799
bPb_1719
bPb_1965
bPb_9868
bPb_4595
bPb_0835
bPb_4621
bPb_0877
E6041redo
bPb_8754
bPb_4971
bPb_2689
bPb_1217
E6102
E45_P6F
E6104
E6118
E6126
scssr09041a
bPb_7292
scssr03907
bPb_4725
bPb_5766
bPb_6124

bPb_0359
bPb_0386
bPb_3780
bPb_7068
bPb_7313
bPb_7165
Bmag500
bPb_3202
bPb_4246
bPb_5027
bPb_8708
EBmag0755B
bPb_3586
bPb_2751
bPb_7193
bPb_8135
bPb_9065
bPb_1009
bPb_3807
bPb_0597
bPb_3927
bPb_6002
GBM1021
GBM1075a
bPb_2464
bPb_7550
bPb_5822
BMAG173
bPb_1466
bPb_2592