

1 We would like to thank Reviewer #2 for her/his time, effort, and valuable comments. We have  
2 prepared a response taking into account all the points raised, as described below. We show  
3 the reviewer's comments in bold, while our responses are formatted as standard text. Line  
4 numbers refer to the original manuscript.

5

6 **Krauze at al. investigated the interplay between the microbial community and soil**  
7 **formation after glacier retreat at Maritime Antarctica (here: King George Island). This is**  
8 **a fascinating topic which fits very well to the scope of Biogeosciences. The paper is**  
9 **well written and the quality of English language is high (only spelling error I found is in**  
10 **line 364, ice fee instead of ice free).**

11

12 Thank you, we corrected the spelling error.

13

14 **All in all the manuscript is a good and inspiring read. Unfortunately some doubts on the**  
15 **experimental design and the drawn conclusions of the study arise while reading and**  
16 **cloud the pleasure remarkably. The absence of hypotheses is puzzling, especially since**  
17 **the chosen ecological model of very young soils, only a few decades after glacier**  
18 **retreat, should have led to several hypotheses around the topic of temporal dynamics**  
19 **of C and N accumulation and the involved microbial functional traits, thresholds or**  
20 **tipping points in the system regarding soil development etc. A study only aspiring an**  
21 **objective of "identifying processes" but not having any assumptions on which**  
22 **processes it should look, is probably not meeting contemporary standards of good**  
23 **scientific communication anymore.**

24

25 Thanks for your comment. In order to clarify the objectives of the study, we have reformulated  
26 the corresponding part in the introduction. In addition, we changed certain passages in the text  
27 that refer to "processes".

28 "We hypothesize that prokaryotic microorganisms initiate/drive soil properties changes (e.g.  
29 soil organic carbon accumulation, weathering) within decades after deglaciation. To test our  
30 hypothesis, we related the investigated soil properties to microbial community structure and  
31 microbial abundances in the recently (< 50 years) deglaciated foreland and compared it with  
32 an older soil located behind a lateral moraine (> 70 years) of the Ecology Glacier." (L. 78ff)

33 We updated the Conclusions section of our manuscript addressing the hypothesis, and a  
34 potential setup and study design to further study the investigated processes.

35 "However, we conclude that prokaryotic microorganisms initiate measurable changes of soil  
36 properties such as pH at a very early stage (within decades) before the soil surface is colonized  
37 by pioneer plants or soil horizons other than C horizons are detectable, and thereby promote  
38 weathering processes." (L. 426ff)

39 "To further verify our conclusions and illuminate the microbial processes driving soil formation,  
40 in future studies multiple comparable setups (freshly deglaciated material vs. older, more  
41 matured soil close to the foreland) could be studied and include metagenomic and  
42 metatranscriptomic analyses." (L. 428)

43

44 **Further, the experimental design is challenged by a very low repetition number (only**  
45 **one field repetition per age class). In Antarctica, harsh conditions melt down inotially**  
46 **planned sampling schemes for sure, but only 4 soils in single replication in close**  
47 **vicinity to a research station, at least raises the question, why there was not a valid**  
48 **experimental design possible.**

49  
50 In this study, we do not present four age classes represented by one profile each. By sampling  
51 three profiles in close proximity in the recently deglaciated area we tried to capture the potential  
52 heterogeneity of these soils, and compare these younger soils with an older soil just behind  
53 the lateral moraine.

54  
55 To clarify this, we rephrased a sentence in the Introduction section:

56  
57 “To capture the heterogeneity of the soil landscape, three soils in close proximity (maximum  
58 distance of 150 m) formed on the same substrate and in a similar topographic position but with  
59 differing vegetation cover were sampled. These soils, which represent a recently deglaciated  
60 area, were compared with an older soil that had formed on a similar substrate that had been  
61 deglaciated for at least 70 years.” (L. 81ff)

62  
63 Additionally, we rephrased a sentence in the Material and Methods section:

64  
65 “The profiles KGI A, B, and C are located within 150 m distance on the same substrate  
66 deglaciated since 1979.” (L. 117)

67  
68 In addition, by changing the date we corrected a related mistake in the caption of Fig. 1:

69  
70 “A, B, and C are located in the glacier foreland deglaciated since 1979.”  
71

72 **The authors additionally should make the age determination of their sites much more**  
73 **transparent as well as the data of vegetation on the sites should be reported in full**  
74 **detail. For instance, the authors discuss the role of vegetation for the microbial**  
75 **community in their manuscript, but write simply “mosses, 5% coverage” in the table.**  
76 **This is an almost useless information, as some Antarctic moss species are nourished**  
77 **by aeolian input without interfering much with the soil, while other moss species deeply**  
78 **penetrate into soil with their rhizoids.**

79 Many thanks for this comment. Additional  $^{14}\text{C}$  datings were done.

80 To determine the age of the soil organic carbon, two samples with the highest SOC contents  
81 were chosen for AMS  $^{14}\text{C}$  dating (KGI C 0 – 1 cm in the glacier foreland and KGI D 0 - 3 cm  
82 from behind the lateral moraine). KGI C 0 – 1 cm was the only sample from the foreland with  
83 detectable SOC. The samples were pre-treated with the acid/alkali/acid method and the alkali  
84 soluble organics (also known as the humin fraction, i.e. the oldest portion of soil organic matter)  
85 and were measured by Beta Analytic, Inc. in Florida, USA.

86  
87 KGI C (Beta – 570459); IRMS  $\delta^{13}\text{C}$ : -27.9 ‰  
88 (92.2%) 1992 - 1995 cal AD (-43 - -46 cal BP)  
89 (3.2%) 1957 cal AD (-8 cal BP)

90  
91 KGI D (Beta – 570458) IRMS  $\delta^{13}\text{C}$ : -26.1 ‰  
92 (95.4%) 1954 - 1956 cal AD (-5 - -7 cal BP)

93  
94 The results show with a probability of 92.2% that the humin fraction of soil organic matter in  
95 profile KGI C was formed after the melting of the glacier in 1979, whereas the humin fraction  
96 of the upper 3 cm in profile KGI D (with a probability of 95.4%) is dated 1954 - 1956 cal AD.

97 The SOC in the lower part of the KGI D may even be older. Therefore, the SOC at KGI D was  
98 already formed at a time when the present foreland of the Ecology Glacier and with this the  
99 sites of KGI A, B, C were still under the glacier.

100  
101 We included these results in a table in the supplementary (Tab. S1) and integrated the new  
102 information on the potential age of the sites in 3.1 Soil classification and soil properties in the  
103 Results section:

104  
105 “<sup>14</sup>C dating showed that the humin fraction of soil organic matter in profile KGI C was formed  
106 after the melting of the glacier in 1979 (92.2% 1992 - 1995 cal AD (-43 - -46 cal BP); 3.2%  
107 1957 cal AD (-8 cal BP)), whereas the humin fraction of the upper 3 cm in profile KGI D is  
108 much younger (95.4% 1954 - 1956 cal AD (-5 - -7 cal BP)) (Tab. S1).” (L. 187)

109  
110 With this first rough listing of plants and the degree of coverage we wanted to test whether  
111 different degrees of coverage are reflected in changes of soil properties. We discussed the  
112 influence of increasing/decreasing vegetation coverage on microbial communities in lines  
113 304ff. Much to our regret, we are not able give a more detailed description of the vegetation,  
114 because we had no botanist in our group. We are aware that a detailed description of the  
115 vegetation would help to better understand the mechanisms of soil processes associated with  
116 specific plants (e.g. different moss species). However, this was not the aim of this study and  
117 we do not discuss this issue. To answer this question would have required a different sampling  
118 approach (1 cm depth increments in the upper 10 cm), which would have been plant species  
119 specific. This can be a next step and should be part of the goals of our next field trip. Again,  
120 many thanks for this comment.

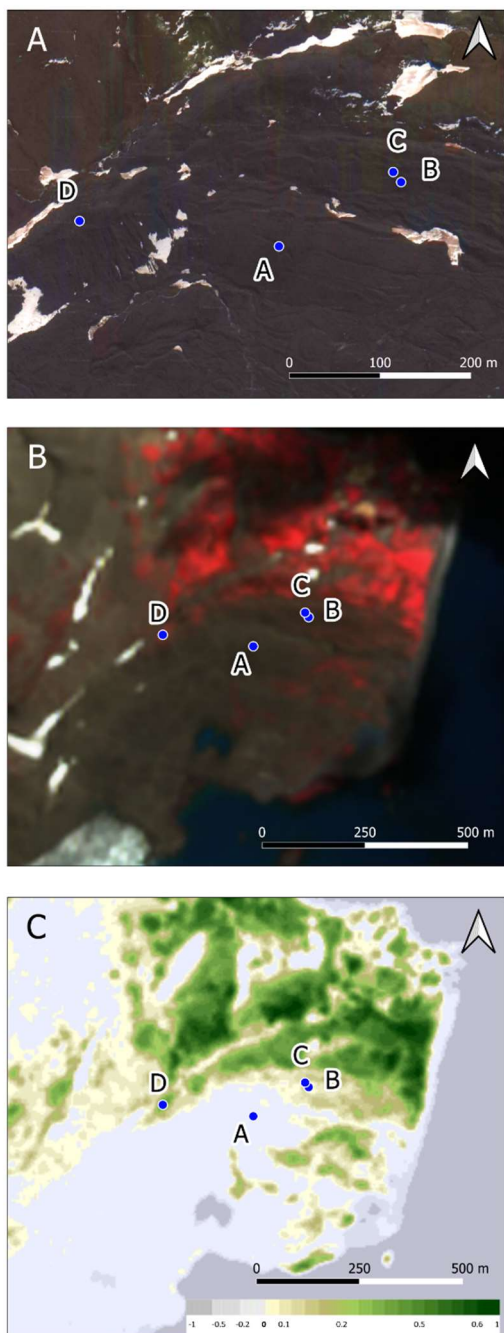
121  
122 **Additionally, it is highly unlikely, that only one species of lichens occurs, which**  
123 **suggests erratic identification. The age dating of the sites is the crucial issue for the**  
124 **study. The appearance of both higher plants native to Maritime Antarctica already on**  
125 **the second youngest soils is directly leading to the question if the dating was done**  
126 **correctly, since this appearance is either limited to highly developed soils or**  
127 **ornithogenic soils. As the soil parameters suggest a young soil, not having enough N**  
128 **for maintaining populations of higher plants on the long run, it is very likely, that these**  
129 **encounters are due to the frequently seen bird dropping effect (excrements containing**  
130 **seed of *Deschampsia* or *Colobanthus* stage the appearance of these plants for a season**  
131 **or two, until the N from the excrement is used up). A coverage of 10% of higher plants**  
132 **on such young soils suggest a high frequency of bird visits, which was excluded by the**  
133 **authors, as this would largely question the results of a real soil development situation**  
134 **of the microbial community, as higher plants alien to the respective community would**  
135 **have been introduced.**

136 Bird droppings cannot be excluded, but we suspect the influence to be small: The P contents  
137 in the profiles KGI A, B, and C are low and very similar for all depths. A high frequency of bird  
138 visits including the bird droppings, as you suggest, would also lead to an increase of the P  
139 content at least in the upper cm of these soils. We included a table with RFA data to the  
140 supplement (Tab. S2). Please also note, *Deschampsia antarctica* and *Colobanthus*  
141 *quitensis* use mycorrhizal fungi (ascomycete fungi), to "...facilitate the acquisition of organic  
142 nitrogen as early protein breakdown products" (Hill et al. 2019). This means that they do not  
143 need N from bird droppings to colonize new areas. Thus, the pedogenic data (particularly pH  
144 and SOC) reflect a "real soil development" as we already have discussed in the manuscript.

145  
146 **With the given data all this stays mere speculation, of course. To solve this issue, at**  
147 **least a much better map of the sites, ideally a satellite map showing the features of the**  
148 **sites as well as their surroundings, along with a concise description of the found**  
149 **vegetation should be offered by the authors, please.**

150 We added a new figure (Figure 2) containing three satellite maps: One with very high  
151 resolution, one with false colours to visualize the vegetation and one with the ndvi to indicate  
152 the presence of chlorophyll. All remaining figures move one number further with their  
153 numbering.

154



155

156

157 We added the caption of Figure 2:

158 "(A) High resolution satellite image from 06.11.2016 (Map data © 2016 Google). (B) False  
159 colour Sentinel-2 image from 19.01.2020 (red = band 8, green = band 4, blue = band 3) for  
160 enhanced visualisation of the vegetation. (C) Normalized Difference Vegetation Index (NDVI),

161 dimensionless with positive values indicating healthy vegetation and negative values indicating  
162 the presence of open water bodies. Contains modified Copernicus data.” (L. 725 - 728)

163 Additionally, we added some information in the Materials and Methods section:

164 “Figure 2B, which shows increasing plant-coverage with an increasing red coloration, shows  
165 no vegetation at KGI A, little vegetation at KGI B and C and a higher vegetation coverage at  
166 KGI D. Conversely, image 2C shows highest values of chlorophyll at KGI D, substantially  
167 less chlorophyll at KGI B and C and no chlorophyll at KGI A.”

168 Regarding the description of the vegetation; please see our answer to your comment above.

169  
170

171 **If possible some proof of the assumed age, for instance by a combination by 14C and**  
172 **15N analyses of the soils, showing the age of organic carbon and the source of N (fixed**  
173 **from air or carried in by birds?) would help explain this very rare combination of**  
174 **observed features reported here. These aspects should be discussed in detail, too.**

175 Thank you for the suggestions.

176 Please note our answer to the first comments regarding age determination.

177 As explained in the answer to an above comment, due to similar P contents at all depths of  
178 profiles A, B, C it is assumed that bird droppings are not of major importance at these sites  
179 and therefore no <sup>15</sup>N analyses were carried out.

180

181 **Regarding the DNA-based identification of bacteria it stays largely unclear to the reader**  
182 **where the usually pretty high number of unidentified OTUs (so to say the bacteria not**  
183 **yet known to science) in soils of Maritime Antarctica has gone. The abundance graph**  
184 **reads as if the authors could attribute every single OTU to a phylum.**

185 Originally, completely unassigned OTUs have been identified, but were very low in their  
186 abundance and summarized with other low abundant phyla under the term “Others” in Figure  
187 3.

188 As suggested by Reviewer 1, the sequencing data was reanalysed by using the amplicon  
189 sequence variant (ASV) approach. Similar to the OTU analysis, this approach resulted in low  
190 abundances of completely unassigned sequences (minimum: 0.01 % in KGI A 10 – 20 cm;  
191 maximum: 0.28% in KGI A 0 – 1 cm). Low abundances of unassigned reads are common in  
192 recent literature (0.28 % of the total data set of Meier et al., 2019; 1.1 % of the total data set in  
193 Kim et al., 2019). The very low abundance of unassigned sequences does not mean that an  
194 identification to genus/species level for the remaining sequences was possible, though. Many  
195 of the OTUs/ASVs shown in Fig. 5, which represent the most abundant reads in the data set,  
196 were just classified to the order or family level.

197 Since both reviews wondered about this very topic, two tables were added to the supplement  
198 as suggested by Reviewer 1: Table S4 shows the “fate” of the raw data, and Table S5 shows  
199 the resulting ASV table. This time, both tables also include the positive as well as the negative  
200 control. We hope the increased transparency is helpful for the evaluation of our sequencing  
201 data.

202

203 **In the discussion, as already indicated by the missing hypotheses, the reader misses a**  
204 **true discussion of the “identification of processes” promised in the objectives. Not even**  
205 **the “influences of microorganisms on soil formation” promised by the title were**  
206 **discussed in detail, what anyway would have been impossible by a study leaving the**  
207 **fungi completely aside.**

208 Thank you. Please note our previous comment on the hypothesis.

209 To account for the missing fungi, we propose to change the title to “Influence of prokaryotic  
210 microorganisms on initial soil formation along a glacier forefield on King George Island,  
211 maritime Antarctica”.

212

213 **After what is discussed in the paper, this study is more investigating the influence of**  
214 **vegetation on the bacterial community of young soils in Maritime Antarctica. A big step**  
215 **towards the originally intended process elucidation of soil formation would be for**  
216 **instance a detailed discussion on the functional traits and abundances of bacteria**  
217 **involved in e.g. C and N accumulation or weathering processes. In the current form, the**  
218 **study is a family list of bacteria of four Antarctic soils, trying to mimic process**  
219 **identification by naming rather weak coincidences. There is surely more to it.**

220

221 Thank you for your suggestions.

222 Since we have not included metagenomics or metatranscriptomics in our study, only limited  
223 conclusions can be drawn about the occurrence or frequency of different functional  
224 characteristics. On the other hand, we discuss the functions and potential effects on the soil  
225 environment based on the taxonomy of certain observed taxa, e.g. microorganisms potentially  
226 linked to weathering processes (L. 394 – 397) or the degradation of organic compounds (L.  
227 265 – 269 ; L. 410 – 414). However, we agree that such aspects can be discussed in more  
228 detail, especially after reanalysis of our sequencing data using the amplicon sequence variant  
229 approach. Therefore, we broadened the discussion and included more information on the  
230 potential involvement of different prokaryotic groups in certain aspects of soil formation and  
231 rephrased the general statement on the observed composition of the community on a phylum  
232 level:

233 “In accordance with observations in other Antarctic habitats (e.g. Ganzert et al., 2011, Yan et  
234 al., 2017; Meier et al, 2019), the investigated soils were characterized by highly diverse  
235 microbial communities including Acidobacteriota, Bacteroidota, Verrucomicrobiota and  
236 especially high abundances of Actinobacteriota and Proteobacteria, which are known to thrive  
237 in recently deglaciated soils and facilitate a multitude of different phototrophic,  
238 photoheterotrophic and chemolithotrophic processes (Rime et al., 2016; Wei et al., 2016;  
239 Garrido-Benavent et al., 2020).

240

241 Additionally, we modified the part discussing the missing indication of “obvious” prokaryotic  
242 organisms associated with phototrophic carbon fixation:

243 “However, our dataset showed only low abundances of prokaryotic organisms most probably  
244 associated with phototrophic carbon fixation, such as the Cyanobacteria-related Tychonema.  
245 Low abundances of Cyanobacteria in recently deglaciated areas are not an uncommon  
246 observation in Antarctic soil environments (Ji et al., 2016; Garrido-Benavent et al., 2020).In  
247 addition to the low abundances of Cyanobacteria, other bacterial groups might be involved in

248 phototrophic carbon fixation, such as Chloroflexi (Lacap et al., 2011). Moreover, based on the  
249 on the amount of chloroplast-related sequences in our dataset, this process is at least partly  
250 facilitated by eukaryotic organisms such as algae in the early stage of soil development.” (L.  
251 288ff)

252

253 We also added additional information on the influence of microorganisms on weathering  
254 processes:

255 “By performing enzymatically catalysed reactions, processes reducing the pH and the  
256 production of complexing agents (Mavris et al., 2010; Styriakova et al., 2012; Ahmed and  
257 Holmström, 2015) microorganisms are able to substantially promote weathering processes  
258 (Schulz et al., 2013).” (L. 391)

259

260 As mentioned above, microorganisms can facilitate processes that lower the local soil pH,  
261 thereby promoting weathering processes. In the absence of vegetation, we observed a  
262 decreased soil pH in the upper centimetres of the KGI A soil profile, which can therefore be  
263 linked to microbial metabolism (L. 412 – 414). This pH reduction pH should increase as soon  
264 as the soils are colonized by plants, since microorganisms are able to degrade organic  
265 compounds and organic acids could be released into the surrounding soil (L. 267 – 269). This  
266 effect can be seen in the pH reduction in relation to increasing plant coverage from KGI A (pH  
267 6.8), KGI B (pH 6.6) to KGI C with a pH of 5.4 in within the first centimetre.

268

269

270 **Table S1: Radiocarbon dating results obtained on the humin fraction of soil organic matter from two sites at the**  
 271 **Ecology Glacier, King George Island.**

Profile	Depth [cm]	Horizon	Material	pMC [%]	δ13C (‰)	cal BP (1σ)	cal CE (1σ)	Lab-Nr.
KGI C	0 – 1		soil organic matter, humin fraction (alkali soluble organics)	112.55 ± 0.42	-26.1	-44 to -45 cal BP	1993 - 1994	Beta- 570459
KGI D	0 - 3	Ah	soil organic matter, humin fraction (alkali soluble organics)	100.62 ± 0.38	-27.9	-5 to -6 cal BP	1954 - 1955	Beta- 570458

272

273

274 **Table S2: Major elements by XRF of four soil profiles from King Georges Island, Antarctica. All data given in weight**  
 275 **percent. LOI (loss on ignition) determined at 1000°C. For location of the profiles, see table 1.**

Profile	Depth [cm]	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	MnO	MgO	CaO	Na <sub>2</sub> O	K <sub>2</sub> O	TiO <sub>2</sub>	P <sub>2</sub> O <sub>5</sub>	LOI	Sum
		%											
KGI A	0-1	49,43	0,93	19,39	8,53	0,20	3,38	5,85	3,13	1,16	0,24	7,46	99,85
	1-10	48,98	0,94	19,30	8,48	0,20	3,15	5,72	3,00	1,18	0,25	8,39	99,73
	10-20	49,25	0,91	19,26	8,26	0,19	3,03	5,95	3,04	1,19	0,25	8,30	99,78
	20-40	49,73	0,93	19,12	8,54	0,20	3,11	6,26	3,41	1,16	0,24	7,12	99,96
KGI B	0-1	49,80	0,95	18,77	8,72	0,21	3,26	6,31	3,74	1,14	0,27	6,28	99,59
	1-10	49,72	0,94	18,95	8,71	0,21	3,29	6,29	3,65	1,12	0,26	6,87	100,14
	10-20	49,92	0,93	18,85	8,69	0,21	3,18	6,34	3,76	1,15	0,27	6,36	99,81
	20-80	49,66	0,94	18,83	8,73	0,20	3,25	6,51	3,71	1,14	0,26	6,43	99,83
KGI C	0-1	48,43	0,85	18,65	8,30	0,20	3,47	6,23	3,62	1,05	0,27	8,73	99,94
	1-10	49,45	0,94	18,91	8,76	0,20	3,22	6,24	3,63	1,09	0,28	6,88	99,75
	10-20	49,66	0,95	18,91	8,89	0,21	3,09	6,29	3,69	1,13	0,29	6,68	99,94
	20-40	49,73	0,94	18,90	8,67	0,19	3,10	6,30	3,73	1,17	0,29	6,35	99,52
KGI D	0-3	44,39	0,79	17,47	7,75	0,17	3,40	5,88	2,98	1,12	1,04	14,6	99,76
	3-15	48,84	0,82	19,22	8,06	0,18	3,54	6,30	3,53	1,19	0,35	7,84	100,01
	15-27	49,85	0,80	19,28	7,81	0,19	3,50	6,80	3,79	1,15	0,21	6,54	100,05
	27-60	49,24	0,82	19,30	8,04	0,20	3,56	7,01	3,71	1,08	0,19	6,49	99,80

276

277 **Table S4: Number of sequencing reads after each processing step.**

sample	input	filtered	denoised	merged	Non-chimera	0.01% cutoff
	number of reads					
KGI_A_0_1_a	823555	760184	744842	728869	722825	706168
KGI_A_0_1_b	416652	386897	378089	368092	365389	359124
KGI_A_0_1_c	923881	848435	824280	799372	786254	772324
KGI_A_1_10_a	473836	436670	427179	416676	413400	410050
KGI_A_1_10_b	234950	218204	212241	204825	203788	201502
KGI_A_1_10_c	352840	330275	323230	316343	314148	311452
KGI_A_10-20_a	36923	33865	29718	26130	25958	25922
KGI_A_10-20_b	18966	17275	14209	12268	12259	12252
KGI_A_10-20_c	25875	23729	19871	17313	17308	17300
KGI_A_20_40_a	905510	839764	828694	814373	808267	790148
KGI_A_20_40_b	846168	790425	779458	764252	758482	737591



KGI_A_20_40_c	893543	830439	821643	808578	801514	778446
KGI_B_0_1_a	802491	738909	726606	711994	705038	683957
KGI_B_0_1_b	767042	707072	685728	660207	650385	634947
KGI_B_0_1_c	776140	717207	691876	662511	644483	630913
KGI_B_1-10_a	870220	801229	775510	742168	726536	700009
KGI_B_1-10_b	763113	708362	698110	683584	679585	655095
KGI_B_1-10_c	984339	904070	867753	821445	790478	763807
KGI_B_10_20_a	902645	835484	829409	818332	809608	796113
KGI_B_10_20_b	50853	47043	41987	37904	37729	37613
KGI_B_10_20_c	45144	41598	36756	32746	32618	32557
KGI_B_20_80_a	797077	739614	734477	727607	713996	706767
KGI_B_20_80_b	666167	617867	613646	605338	594001	587915
KGI_B_20_80_c	59859	55236	50726	47120	46730	46649
KGI_C_0_1_a	748549	692351	673800	648601	635472	605915
KGI_C_0_1_b	896152	828214	784842	728938	687678	661043
KGI_C_0_1_c	780292	724237	701902	672607	650189	619970
KGI_C_1_10_a	1072129	979738	941569	893583	866371	830430
KGI_C_1_10_b	796453	734715	714128	686413	675197	651021
KGI_C_1_10_c	945868	879402	852158	818052	799307	765149
KGI_C_10_20_a	914463	847130	837834	821943	814309	788037
KGI_C_10_20_b	724047	663724	657595	646501	642849	625122
KGI_C_10_20_c	379777	348578	342785	334296	331763	325740
KGI_C_20_40_a	805325	743983	737314	727505	720592	705527
KGI_C_20_40_b	883724	818626	808175	789997	782377	762057
KGI_C_20_40_c	651443	603352	595920	583664	578353	564023
KGI_D_0_3_a	779880	715854	698512	675201	664504	626027
KGI_D_0_3_b	787246	723529	685098	643359	617163	586001
KGI_D_0_3_c	898392	826444	770393	701924	643856	619137
KGI_D_3_15_a	815335	754410	739659	718535	709893	677501
KGI_D_3_15_b	950935	871121	850341	820666	806893	766920
KGI_D_3_15_c	930601	858951	806053	732072	650658	627047
KGI_D_15_27_a	913147	851119	839448	822669	812036	784157
KGI_D_15_27_b	1074041	989909	980950	964946	953467	917368
KGI_D_15_27_c	869976	799507	791364	777160	770119	749250
KGI_D_27_60_a	740686	687100	681491	671687	666209	645648
KGI_D_27_60_b	768476	714698	707202	695134	689840	671215
KGI_D_27_60_c	852927	790621	782334	769492	763600	737767
Negative control	11045	9999	9955	9899	9899	9819
Positive control	651120	590140	589778	585268	585268	585250

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