

A live bacteria enzyme assay for identification of human disease mutations and drug screening

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SUPPLEMENTARY NOTES

Supplementary Note 1. Comparing effects of AG1 on hu-G6PD-Canton and in vitro experiment.

According to the previous experiment, AG1 maximally activated recombinant G6PD-Canton by 1.7-fold¹. Using LEICA, we reverse-estimated the activity of G6PD-Canton from the measured growth rate of humanized *E. coli* for G6PD-Canton ([Fig. 1f](#)) when activated by AG1. Fitted linear regression function of log-enzyme activity vs. growth rate is as follows ([Fig. 1f](#)).

$$GR = 0.0551A + 0.0459 \text{ (Pearson's } R^2 = 0.840)$$

$$\Leftrightarrow A = 15.235GR - 0.6117,$$

where GR is the growth rate and A is activity of G6PD.

Without AG1 treatment, the growth rate of humanized *E. coli* for G6PD-Canton was 0.0855 h⁻¹ (±0.0047, s.d.; n=10). When maximally activated (with 0.3 μM AG1), the growth rate of the same strain was 0.1028 h⁻¹ (±0.0026; s.d.; n=10) (data available in [Source Data](#)). Relative activities are calculated to be 0.6914±0.0714 (0 μM AG1) and 0.9538±0.0403 (0.3 μM AG1). This gives a 1.38-fold increase, consistent with the previous observation (1.7-fold improvement).

Supplementary Note 2. G6PD inhibitors screened.

1. G6PDi-1

A small molecule inhibitor of G6PDi-1 was recently discovered by high-throughput screening². Reported half-maximal inhibitory concentration (IC₅₀) in *in vitro* settings is 0.07 μ M. It exhibited inhibitory effect in cell-based experiments: suppresses T cell (mouse CD8+) cytokine production (at concentration of 10-50 μ M) and neutrophil (bone-marrow-derived macrophages) oxidative burst at 50 μ M².

2. Dehydroepiandrosterone (DHEA)

DHEA is a steroid hormone precursor and its G6PD inhibitory activity has been reported previously. recombinant enzymes³. However, cell-based assay indicated a limited bioavailability² and G6PD inhibition by indirect mechanisms⁴.

3. RRx-001

RRx-001 is one of the interesting classes of energetic compounds. Recently, it has been highlighted as a potential radiosensitizer⁵. Although the mechanisms of chemo/radiosensitizing effect have not fully been understood, its inhibitory activity on G6PD may originate primarily through nitric oxide production⁶.

4 and 5. Brimonidine (BMN) and proparacaine (PPC)

These two are widely used ophthalmologic drugs. Brimonidine (BMN) is a selective α_2 -adrenergic receptor agonist, mainly used to lower intraocular pressure in the clinical treatment of patients with glaucoma^{7,8}. Proparacaine (PPC) is a local anesthetic for surface anesthesia in ophthalmology. In the previous study, BMN has an inhibitory effect of G6PD with IC₅₀ of 29.93 μ M, while PPC has none^{6,9}.

6. Metamizol (MMZ; dipyrone)

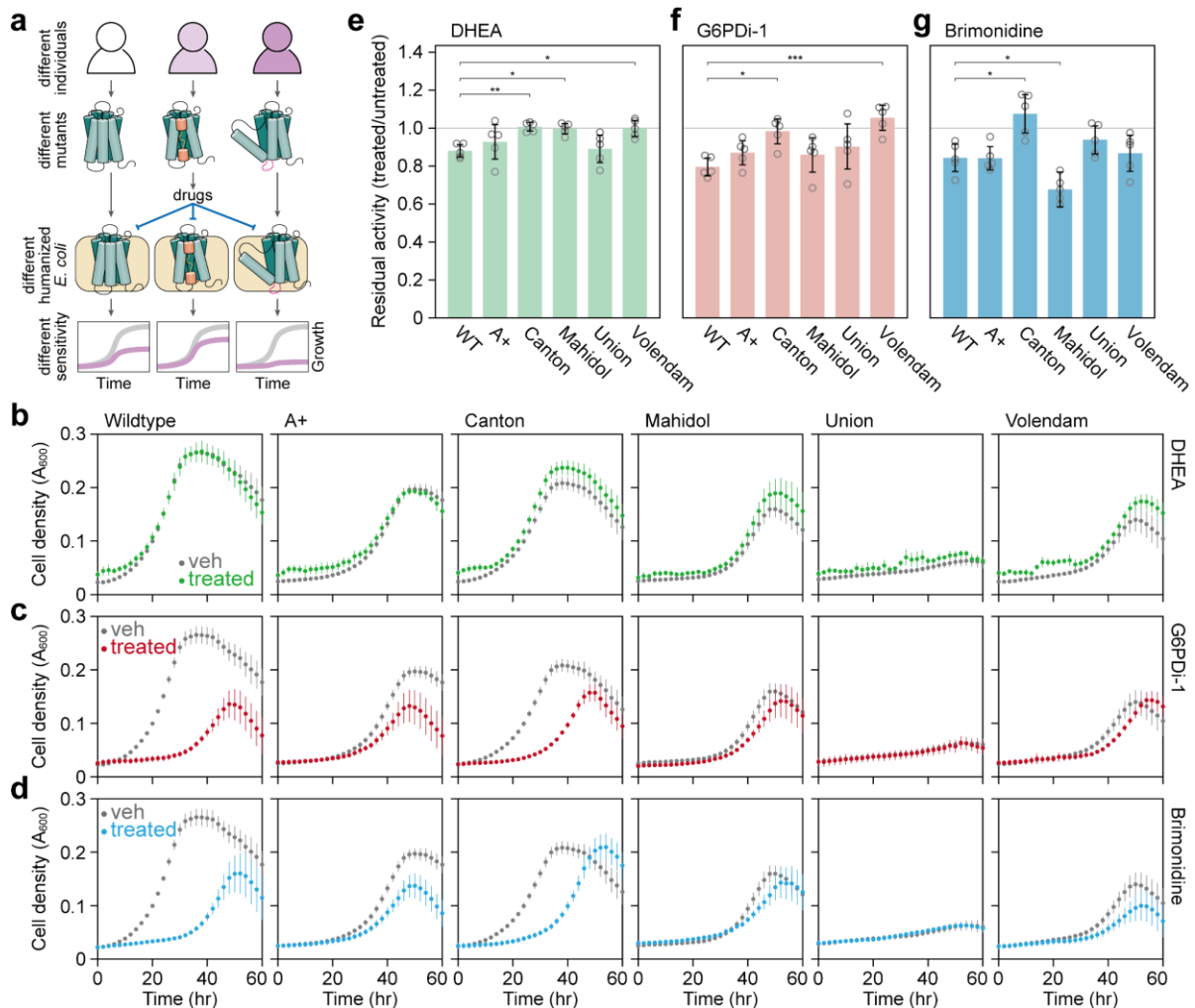
Metamizol is a pain reliever that belongs to pyrazolones. Pyrazolones have been reported to potentially induce severe hemolytic anemia¹⁰. Effect of metamizole on G6PD was addressed previously, showing an inhibitory effect both *in vitro* and *in vivo*, although IC₅₀ was relatively high (17 mM *in vitro*)¹¹.

7. 6-aminonicotinamide (6AN)

We used 6AN as a positive control of assay. It is a non-selective inhibitor of NADP-dependent enzymes exhibiting antiproliferative effects on prostate cancer cells¹².

Supplementary Note 3. Potential applications of LEICA in personalized medicine research.

As genome sequencing becomes increasingly accessible, precision medicine emerges as an innovative approach to treat patients by accounting for characteristics of individuals. A fundamental aspect of personalized medicine involves understanding a person's genetic makeup to identify medical interventions that are more likely to succeed while minimizing potential side effects.



Supplementary Figure 1. Different sensitivities of individual mutants against different compounds. **a**, LEICA of human G6PD variants screens for different compound sensitivities. **b-d**, Growth profiles of humanized *E. coli* in LEICA with different variants under dehydroepiandrosterone (DHEA) (**b**), inhibitor of G6PD 1 (G6PD-1) (**c**), and brimonidine (BMN) (**d**) treatments. All compounds were treated with the final concentration of 100 μ M. Data are presented as mean values $\pm$ SD. Error bars show SD of five replicates. **e-g**, The growth rates of humanized *E. coli* with treatments compared to the untreated controls represent residual G6PD activities. Variants have different levels of inhibition to DHEA (**e**), G6PD-1 (**f**), and BMN (**g**). Data are presented as mean values $\pm$ SD. Error bars show SD of five replicates. Dots are individual data points ($n=5$). * p -value < 0.05. ** p -value < 0.005. *** p -value < 0.001 (two-sided Welch's t -test of log-transformed ratios with Bonferroni correction, the residual activities were inferred by comparing treated samples with the untreated samples).

Our proposed assay method has demonstrated the ability to measure the activities of genetic variants and their responses to drug effects, enabling an exploration of each variant's sensitivity to drugs. We subjected DHEA, G6PDi-1, and BMN, which exerted inhibitory effects on the WT G6PD, to all constructed variants to assess differences in sensitivities ([Supplementary Fig. 1a](#)).

In this assay, variants exhibited different sensitivities to the same treatments ([Supplementary Fig. 1b-d](#)). Upon DHEA treatment, G6PD-Canton, Mahidol, and Volendam variants showed activities comparable to untreated controls, indicating insensitivities to DHEA ([Supplementary Fig. 1e](#)). Similarly, G6PD-Canton and Volendam were insensitive to G6PDi-1, while G6PD-A⁺, Mahidol, and Union exhibited similar levels of inhibition to the WT ([Supplementary Fig. 1f](#)). Interestingly, G6PD-Mahidol was more sensitive to BMN than WT G6PD, exhibiting 67.9% residual activity compared to the untreated control, where WT G6PD retained 84.4% activity ([Supplementary Fig. 1g](#)). In contrast, the G6PD-Canton variant appeared resistant to all three compounds tested ([Supplementary Fig. 1e-g](#)). These differential drug sensitivities of variants may explain why different G6PD-deficient patients with various G6PD variations exhibit varying levels of hemolytic events after drug administration, such as antimalarials¹³. However, a detailed structural understanding of these drug bindings on G6PD is lacking, necessitating comprehensive molecular investigation into the drug-enzyme interactions.

Supplementary Note 4. Nucleotide sequence of synthesized G6PD.

The following double-stranded DNA fragment was synthesized chemically (Integrated DNA Technologies, USA).

Human G6PD cDNA (1548 bp)

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ATGGCAGAGCAGGTGGCCCTGAGCCGGACCCAGGTGTGCGGGATCCTGCGGGAAGAG
CTTTTCCAGGGCGATGCCTTCCATCAGTCGGATACACACATATTCATCATCATGGGTGCA
TCGGGTGACCTGGCCAAGAAGAAGATCTACCCACCATCTGGTGGCTGTTCCGGGATG
GCCTTCTGCCCCGAAAACACCTTCATCGTGGGCTATGCCCGTTCCCGCCTCACAGTGGC
TGACATCCGCAAACAGAGTGAGCCCTTCTTCAAGGCCACCCAGAGGAGAAGCTCAAG
CTGGAGGACTTCTTTGCCCGCAACTCCTATGTGGCTGGCCAGTACGATGATGCAGCCTC
CTACCAGCGCCTCAACAGCCACATGAATGCCCTCCACCTGGGGTCACAGGCCAACCGC
CTCTTCTACCTGGCCTTGCCCCCGACCGTCTACGAGGCCGTACCAAGAACATTCACGA
GTCCTGCATGAGCCAGATAGGCTGGAACCGCATCATCGTGGAGAAGCCCTTCGGGAGG
GACCTGCAGAGCTCTGACCGGCTGTCCAACCACATCTCCTCCCTGTTCCGTGAGGACC
AGATCTACCGCATCGACCACTACCTGGGCAAGGAGATGGTGCAGAACCTCATGGTGCT
GAGATTTGCCAACAGGATCTTCGGCCCCATCTGGAACCGGGACAACATCGCCTGCGTTA
TCCTCACCTTCAAGGAGCCCTTTGGCACTGAGGGTCGCGGGGGGCTATTTTCGATGAATTT
GGGATCATCCGGGACGTGATGCAGAACCACCTACTGCAGATGCTGTGTCTGGTGGCCA
TGGAGAAGCCCGCCTCCACCAACTCAGATGACGTCCGTGATGAGAAGGTCAAGGTGTT
GAAATGCATCTCAGAGGTGCAGGCCAACAAATGTGGTCCTGGGCCAGTACGTGGGGAAC
CCCGATGGAGAGGGCGAGGCCACCAAAGGGTACCTGGACGACCCACGGTGCCCCGC
GGGTCCACCACCGCCACTTTTGCAGCCGTCGTCTCTATGTGGAGAATGAGAGGTGGG
ATGGGGTGCCCTTCATCCTGCGCTGCGGCAAGGCCCTGAACGAGCGCAAGGCCGAGG
TGAGGCTGCAGTTCCATGATGTGGCCGGCGACATCTTCCACCAGCAGTGCAAGCGCAA
CGAGCTGGTGATCCGCGTGCAGCCCAACGAGGCCGTGTACACCAAGATGATGACCAAG
AAGCCGGGCATGTTCTTCAACCCCGAGGAGTCGGAGCTGGACCTGACCTACGGCAACA
GATACAAGAACGTGAAGCTCCCTGACGCCTATGAGCGCCTCATCCTGGACGTCTTCTGC
GGGAGCCAGATGCACTTCGTGCGCAGCGACGAGCTCCGTGAGGCCTGGCGTATTTTCA
CCCCACTGCTGCACCAGATTGAGCTGGAGAAGCCCAAGCCCATCCCCTATATTTATGGC
AGCCGAGGCCCCACGGAGGCAGACGAGCTGATGAAGAGAGTGGGTTTCCAGTATGAG
GGCACCTACAAGTGGGTGAACCCCAACAAGCTCTAA
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SUPPLEMENTARY TABLES

Supplementary Table 1. Human genetic variations in glucose-6-phosphate isomerase (*GPI*) and their clinical manifestations. SNV, single nucleotide variation. P, pathogenic. LP, likely pathogenic. B, benign. LB, likely benign. U, uncertain significance. HA, hemolytic anemia. RBS, red blood cells. ND, not detected. *in vitro* activities of variants were previously reported¹⁴.

Type	Interpretation (#n of evidence in ClinVar; P:LP:B:LB:U)	Nucleotide	AA	Disease association	Historic Name	<i>in vitro</i> activity (IU/mg)	Allele frequency (gnomeAD v4.1.0)
-	Normal	-	-	-	Wild-type	400±39.2	-
SNV	benign (0:0:3:3:0)	T623C	I208T			NA	0.020571
SNV	likely benign (0:0:0:1:1)	G317A	R106Q			NA	0.000229
SNV	pathogenic (2:0:0:0:0)	G1615A	D539N	HA	Fukuoka	314±47.1	0.000005
SNV	pathogenic (2:2:0:0:2)	C671T	T224M	HA	Iwate	353±45.2	0.000011
SNV	pathogenic (1:0:0:0:0)	C14T	T5I	HA	Matsumoto	289±31.7	0.000003
SNV	pathogenic (4:0:0:0:0)	G1040A	R347H	HA		NA	0.000017

Supplementary Table 2. Human genetic variations in glucose 6-phosphate dehydrogenase (G6PD) and their clinical manifestations. P, pathogenic. LP, likely pathogenic. B, benign. LB, likely benign. U, uncertain significance. HA, hemolytic anemia. AHA, acute hemolytic anemia. CNSHA, chronic non-spherocytic hemolytic anemia. F, favism NA, not available. ND, not detected. Source data are provided as a Source Data file.

Type	Interpretation (#n of evidence in ClinVar; P:LP:B:LB:U)	Nucleotide	AA	Disease association	Historic Name	WHO classification	<i>in vitro</i> activity, relative to normal		Allele frequency (gnomeAD v4.1.0)
							k _{cat} (s ⁻¹)	specific activity (IU/mg)	
SNV	conflicting (9:1:1:3:5)	A376G	N126D	mild HA	A+	Class III	0.492±0.003		0.017080
SNV	pathogenic (11:0:0:0:0)	G1376T	R459L	AHA	Canton	Class II	0.541±0.010		0.000133
SNV	pathogenic (12:1:0:0:0)	G487A	G163S	AHA	Mahidol	Class III	0.818±0.075		0.000038
SNV	pathogenic (15:0:0:0:0)	C1360T	R454C	AHA	Union	Class II	0.104±0.005		0.000068
SNV	likely pathogenic (0:1:0:0:0)	C514T	P172S	CNSHA	Volendam	Class I	NA	0.171±0.014	ND
SNV	likely pathogenic (0:1:0:0:1)	T196A	F66I	HA	Songklanagarind	Class II	0.932±0.028		ND
SNV	pathogenic (1:0:0:0:0)	A713G	K238R	CNSHA	Durham	Class I	0.305		ND
SNV	likely pathogenic (0:1:0:0:0)	G488A	G163D	CNSHA	Plymouth	Class I	0.992±0.026		ND
SNV	pathogenic (2:2:0:0:0)	T208C	Y70H	HA	Namoru	Class II	0.160±0.013		0.000005
SNV	pathogenic (2:3:0:0:0)	A209G	Y70C	HA, F	Murcia	Class III	0.903±0.031		0.000006
SNV	pathogenic (1:1:0:0:0)	G593C	R198P	CNSHA	Santiago	Class I	0.012±0.001		ND
SNV	likely pathogenic (0:1:0:0:0)	G921C	Q307H	CNSHA	Omiya	Class I	0.555±0.013		ND
SNV	pathogenic (2:0:0:0:0)	G1192A	E398K	CNSHA	Puerto Limon	Class I	0.652±0.018		ND
SNV	uncertain (0:0:0:0:2)	C1278G	N426K						0.000014
SNV	pathogenic (1:0:0:0:0)	T1292G	V431G	HA	Sumare				ND
SNV	benign (0:0:1:2:0)	G311A	R104H						0.000610
SNV	likely benign (0:0:0:4:3)	G1048C	D350H	HA	Mira d'Aire				0.000007

Supplementary Table 3. Human genetic variations in argininosuccinate lyase (ASL) and their clinical manifestations. SNV, single nucleotide variation. P, pathogenic. LP, likely pathogenic. B, benign. LB, likely benign. U, uncertain significance. HA, hemolytic anemia. RBS, red blood cells. ND, not detected. a: reference. b: gnomeAD. *in vitro* activities were previously reported^{15,16}.

Type	Interpretation (#n of evidence in ClinVar; P:LP:B:LB:U)	Nucleotide	AA	Disease association	<i>in vitro</i> activity (k_{cat} ; s^{-1})	Allele frequency (gnomeAD v4.1.0)
SNV	Normal	-	-	-	34.5±3.0	-
SNV	pathogenic (18:6:0:0:0)	G35A	R12Q	ASLD	2.1±0.11	0.001832
SNV	pathogenic (1:2:0:0:0)	A260G	D87G	ASLD	0.0	0.000001
SNV	pathogenic (12:0:0:0:0)	A857G	Q286R	ASLD	0.6±0.02	0.000091

Supplementary Table 4. Primers used in this study. Overlap extension (OE) PCR was performed to assemble two DNA fragments.

Experiment	Name	Sequence	PCR product (bp)	OE PCR product (bp)
GPI knockout	GPI_KO_SC_F	TTCCAAAGTCACAATTCTCAAAATCAGAAGAGTATTGCTACTAAGGTTGACAATTAATCA	2327	-
	GPI_KO_SC_R	CGGCGTGAACGCCTTATCCGGCCTACATATCGACGATGATACGTGCTCAAGCTTCCTAGG		
zwf knockout	zwf_KO_F	GCGCAAGATCATGTTACCGGTAAAATAACCATAAAGGATAAGCGCAGATAGTGTAGGCTGGAGCTGCTTC	1398	-
	zwf_KO_R	CAAGTATACCCTGGCTTAAGTACCGGGTTAGTTAACTTAAGGAGAATGACGGGATCCGTCGACCTGCAGT		
GPI variants introduction	HsaGPI_F	CTCCAACACCGTTACTTGGGC	1904	2499
	Fukuoka_R	TGGTAGAAGCGTTGTGAGAGGTCACTTGAG		
	Fukuoka_F	GTGACCTCTCACAACGCTTCTACCAATGGG	620	
	HsaGPI_R	CCGCAAGCGCAGATATGGC		
	HsaGPI_F	CTCCAACACCGTTACTTGGGC	958	2499
	Iwate_F	ATGCAGAGATGGCGAAGGAGTGGTTTCTCC		
	Iwate_R	CTCCTTCGCCATCTCTGCATTCGTGATGGT	1561	
	HsaGPI_R	CCGCAAGCGCAGATATGGC		
	HsaGPI_F	CTCCAACACCGTTACTTGGGC	300	2499
	Matsumoto_F	CCGCTCTCATCCGGGACCCCAAGTTCCAGA		
	Matsumoto_R	GGGTCCCGGATGAGAGCGGCCATTAGCAAT	2218	
	HsaGPI_R	CCGCAAGCGCAGATATGGC		
	HsaGPI_F	CTCCAACACCGTTACTTGGGC	603	2499
	R106Q_F	TGCGGAACCAGTCAAACACACCCATCCTGG		
	R106Q_R	GTGTTTGACTGGTTCCGCAGAGCCACGTGC	1915	
	HsaGPI_R	CCGCAAGCGCAGATATGGC		
	HsaGPI_F	CTCCAACACCGTTACTTGGGC	910	2499
	I208T_F	CTGTTCATCACTGCCTCCAAGACCTTTACT		
	I208T_R	CTTGGAGGCAGTGATGAACAGGGAGGACTC	1610	
	HsaGPI_R	CCGCAAGCGCAGATATGGC		
	HsaGPI_F	CTCCAACACCGTTACTTGGGC	1327	2499
	R347H_F	TACCTGCACCACTTTGCTGCGTACTTCCAG		
	R347H_R	CGCAGCAAAGTGGTGCAGGTACTGGTCATA	1193	
	HsaGPI_R	CCGCAAGCGCAGATATGGC		
HsaG6PD and variants cloning	pTrc_bb_F	GTTTAAACGGTCTCCAGCTT	4282	-
	pTrc_bb_R	GGTTTATTCTCTTATTTAATCG		
	HsaG6PD_F	TAAATAAGGAGGAATAAACCATGGCAGAGCAGGTGGCCCT	1591	-
	HsaG6PD_R	AAGCTGGAGACCGTTTAACTTATTAGAGCTTGTGGGGGTTCA		
	HsaG6PD_F	TAAATAAGGAGGAATAAACCATGGCAGAGCAGGTGGCCCT	406	1591
	Aplus_R	TGGAGGGCATCCATGTGGCTGTTGAGGCGC		
	Aplus_F	AGCCACATGGATGCCCTCCACCTGGGGTCA	1205	
HsaG6PD_R	AAGCTGGAGACCGTTTAACTTATTAGAGCTTGTGGGGGTTCA			

HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT	1405	1591
Canton_R	CAGGCCTCAAGGAGCTCGTCGCTGCGCACG		
Canton_F	ACGAGCTCCTTGAGGCCTGGCGTATTTCA	205	
HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA		
HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT	516	1591
Mahidol_R	GGTTCCAGCTTATCTGGCTCATGCAGGACT		
Mahidol_F	GAGCCAGATAAGCTGGAACCGCATCATCGT	1095	
HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA		
HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT	1389	1591
Union_R	CGTCGCTGCACACGAAGTGCATCTGGCTCC		
Union_F	CACTTCGTGTGCAGCGACGAGCTCCGTGAG	221	
HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA		
HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT	543	1591
Volendam_R	TCCCGAAGGACTTCTCCACGATGATGCGGT		
Volendam_F	CGTGGAGAAGTCCTTCGGGAGGGACCTGCA	1068	
HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA		
HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT	225	1591
Songklanagarind_R	CCACGATGATGGTGTTTTCGGGCAGAAGGC		
Songklanagarind_F	GAAAAACACCATCATCGTGGGCTATGCCCGT	1385	
HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA		
HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT	740	1591
Durham_R	GGGCTCCCTGAAGGTGAGGATAACGCAGGC		
Durham_F	CTCACCTTCAGGGAGCCCTTTGGCACTGAGG	869	
HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA		
HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT	516	1591
Plymouth_R	GGTTCCAGTCTATCTGGCTCATGCAGGACTCG		
Plymouth_F	GCCAGATAGACTGGAACCGCATCATCGTGG	1093	
HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA		
HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT	236	1591
Namoru_R	ACGGGCATGGCCCACGATGAAGGTGTTTTC		
Namoru_F	CATCGTGGGCCATGCCCGTTCCCGCCTCAC	1374	
HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA		
HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT	236	1591
Murcia_R	ACGGGCACAGCCCACGATGAAGGTGTTTTC		
Murcia_F	ATCGTGGGCTGTGCCCGTTCCCGCCTCACA	1373	
HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA		
HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT	621	1591
Santiago_R	GGTCGATGGGGTAGATCTGGTCCCTACGGAAC		
Santiago_F	AGATCTACCCCATCGACCACTACCTGGGCA	988	
HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA		
HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT	948	1591

	Omiya_R	CCACGTAGTGGCCCAGGACCACATTGTTGG	661	
	Omiya_F	TCCTGGGCCACTACGTGGGGAACCCCGATG		
	HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA	1222	1591
	HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT		
	Puerto Limon_R	TACACGGCCTTGTTGGGCTGCACGCGGATC	389	
	Puerto Limon_F	CAGCCCAACAAGGCCGTGTACACCAAGATG		
	HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA	340	1591
	HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT		
	R104H_R	TAGGAGTTGTGGGCAAAGAAGTCCTCCAGC	1270	
	R104H_F	TCTTTGCCCACTCCTATGTGGCTGGCC		
	HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA	1077	1591
	HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT		
	Mira d'Aire_R	GCACCCCATGCCACCTCTCATTCTCCACATAGAGG	533	
	Mira d'Aire_F	GAGAGGTGGCATGGGGTGGCCTTCATCCTG		
	HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA	1307	1591
	HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT		
	N426K_R	CTTGTATCTCTTGCCGTAGGTCAGGTCCAG	303	
	N426K_F	CTACGGCAAGAGATACAAGAACGTGAAGCTCCC		
	HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA	1321	1591
	HsaG6PD_F	TAAATAAGGAGGAATAAAACCATGGCAGAGCAGGTGGCCCT		
	Sumare_R	GGGAGCTTCCCGTTCTTGTATCTGTTGCCGT	289	
	Sumare_F	ACAAGAACGGGAAGCTCCCTGACGCCTATG		
	HsaG6PD_R	AAGCTGGAGACCGTTTAAACTTATTAGAGCTTGTGGGGGTTCA		
<i>argH</i> knockout	argH_KO_F	CCGGAGGCGCAGCTTTCGGGCATTGAATTTCAAATAAGGAAACAGAGTTGTGTAGGCTGGAGCTGCTTC	1394	-
	argH_KO_R	TAAAAAAGCCCGGCGATAAGCCAGGCTCAAATTTATACATATAAATGTTCTCCGTCGACCTGCAGTTCGA		
<i>HsaASL</i> and variants cloning	HsaASL_F	TAAATAAGGAGGAATAAAACCATGGCCTCGGAGAGTGGA	1438	-
	HsaASL_R	AAGCTGGAGACCGTTTAAACTTATTAGGCCTGCTGTGCCTGCAGTA		
	R12Q_F	GGGAAGCTTTGGGGTGGCCAGTTTGTGGGTGCAGTGGACC	1403	
	HsaASL_R	AAGCTGGAGACCGTTTAAACTTATTAGGCCTGCTGTGCCTGCAGTA		
	R12Q_F_primer extension	TAAATAAGGAGGAATAAAACCATGGCCTCGGAGAGTGGAAGCTTTGGGGTGGCCA	1438	-
	HsaASL_R	AAGCTGGAGACCGTTTAAACTTATTAGGCCTGCTGTGCCTGCAGTA		
	HsaASL_F	TAAATAAGGAGGAATAAAACCATGGCCTCGGAGAGTGGA	289	1438
	D87G_R	GTGTGGATGCCCTCATCATTGGAGTTTCAGTTTG		
	D87G_F	ATGATGAGGGCATCCACACAGCCAATGAGC	1168	
	HsaASL_R	AAGCTGGAGACCGTTTAAACTTATTAGGCCTGCTGTGCCTGCAGTA		
	HsaASL_F	TAAATAAGGAGGAATAAAACCATGGCCTCGGAGAGTGGA	887	1438
	Q286R_R	GTTTTCTTCCGGGGCATCAGGCTGCTTCC		
	Q286R_F	TGATGCCCCGGAAGAAAAACCCCGACAGTTTGG	571	
	HsaASL_R	AAGCTGGAGACCGTTTAAACTTATTAGGCCTGCTGTGCCTGCAGTA		

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