Supplementary Data

1. Genome resequencing of ancestral strain ABWT and comparison with ADP1 strain

The genome of ABWT was resequenced and showed significant differences with Acinetobacter baylyi ADP1. These diffencies are summarized in table S1. A sequence of about 40 kb was present in ABWT but absent in ADP1. ORF were searched using ORF Finder on line tool and ORFs were blasted. The results are summarized in the table S2.

Table S1. Mutational event in ABWT when compared to A. baylyi ADP1

Index ADP1	Event	Comment
178468	G → –	74 bp upstream start codon of ACIAD0178 atpI ATP synthase protein I
289081	insertion	Insertion of IS1236 between ACIAD 0290 and ACIAD 0291
321573-322809	deletion	Deletion of IS1236_1
364108	G → A	L → F; ACIAD0371 pH adaptation potassium efflux system protein G
943621-944857	deletion	Deletion of IS1236_3
968073	T → C	500 bp upstream start codon of ACIAD0980 vanA (vanillate O-demethylase oxygenase subunit)
1139725	– → A	75 bp upstream start codon of ACIAD1147 putative APC family, S-methylmethionine transporter
1179108	C → T	D → N; ACIAD1182 phrB deoxyribodipyrimidine photolyase (photoreactivation), FAD-binding
1277087	T → –	ACIAD1276 putative amino acid transporter; the frameshift results in a stop codon about 100bp downstream the deletion
1278182	T → –	ACIAD1277; the frameshift results in a stop codon about 100bp downstream the deletion
1444653	T → G	62bp upstream start codon of ACIAD1446 catB (muconate cycloisomerase I)
1468567	C → T	S → L; ACIAD1471 putative transmembrane sensor protein (iron transport)
1648116	C → T	157 bp upstream codon start ACIAD1649 putP (SSS family, major sodium/proline symporter)
1794961-1795034		Putative duplication and putative divergence of the copy
1803858-1803939	Duplication	ACIAD1795
2045478	A → C	69 bp upstream start codon of ACIAD2059 gltJ glutamate/aspartate transport protein (membrane)
2420279	– → C	56 bp upstream start codon of ACIAD2457 putative membrane protein
2542652	G → A	Silent mutation in ACIAD2584 lepA GTP binding protein
2737671-2737674		Putative duplication and putative divergence of the copy
2745958-2749970		Putative duplication and putative divergence of the copy
2765010	A → C	S → R; ACIAD2827 putative periplasmic binding protein of transport/transglycosylase
2803531-2805196		Putative duplication and putative divergence of the copy
2876467	C → T	S → L; ACIAD2944 putative resistance-nodulation-cell division (RND) family (AdeB-like)
3357842	G → A	237 bp upstream start codon ACIAD3437
3392500	C → T	A → V; ACIAD3465 putative 2-component sensor

Table S2. Annotation of a sequence of ABWT absent in ADP1.

index	frame	size	product	organism
222-1523	+3	1301	Phage integrase	*Acinetobacter radioresistens SK82*
315-1544	-3	1229	Mutator family transposase	*Acinetobacter johnsonii*
3127-3933	-1	806	AraC family transcriptional regulator	*Pantoea sp. SL1-M5*
				Klebsiella pneumoniae 342
6405-7790	+3	1385	Citrate trasporter	*Acinetobacter junii SH205*
				Acinetobacter baylyi ADP1
6612-7598	-3	986	NmrA family protein	*Klebsiella oxytoca*
7854-8795	+3	941	GntR family transcriptional regulator	*Pseudomonas syringae Cit7*
9022-10089	-1	1067	Luciferase family protein	*Sphingobacterium spiritivorum*
9120-10520	+3	1400	amidohydrolase	*Pantoea stewartii*
				Klebsiella pneumoniae
10123-10974	-1	851	Hypothetical protein	*Leeuwenhoekiella blandensis*
11025-11975	+3	950	SMP30/ gluconolactonase/LRE family	*Panteoa sp.*
				Klebsiella pneumoniae
13381-14685	-1	304	CaiB/BaiF family class III CoA transferase	*Alcaligenes faecalis*
14686-15594	-1	908	Enoyl-CoA hydratase family	*Alcaligenes faecalis*
16004-17734	-2	1730	SSS sodium solute transporter superfamily	*Acidovorax delafieldii 2AN*
17532-18314	+3	782	IclR family transcriptional regulator	*Acinetobacter sp. P8-3-8*
18410-19591	-2	1181	Iron-containing alcohol dehydrogenase	*Acinetobacter johnsonii*
19663-21198	-1	1535	Methylmalonate-semialdehyde dehydrogenase	*Acinetobacter johnsonii SH046*
22280-22942	-2	662	Regulatory protein, TetR	*Acinetobacter radioresistens SK82*
23012-24001	-2	989	Putative dehydrogenase	*Acinetobacter baumanii AYE*
25238-26212	-2	974	Oxidoreductase	*Klebsiella pneumoniae 342*
27212-28432	+2	1220	Dimethyl sulfoniopropionate lyase	*Alcaligenes faecalis*
30925-31845	+1	920	Transcriptional regulator	*Acinetobacter calcoaceticus*
33303-35948	-3	2645	Aconitate hydratase	*Pseudomonas syringae Cit7*
34143-34754	+3	611	Modulator of drug activity B	*Shewanella sp. HN-41*
34980-35621	+3	641	TetR family transcriptional regulator	*Serratia odorifera*
				Klebsiella pneumoniae
35942-37114	-2	1172	AcnD accessory protein PrpF	*Pseudomonas syringae*
36415-37725	+1	1310	Transporter (MFS family)	*Klebsiella pneumoniae 342*
37115-37870	-2	755	Extracellular solute-binding protein family 3	*Pseudomonas syringae Cit7*

2. Identification of mutational events and building of the tree

All mutations were identified by analysis of the genome resequencings with standard tools (cited in text) except the duplication (ACIAD1848 – ACIAD1861), and the insertion loci for branches B3, B4 and B5.

Duplication was identified by the difference in coverage for this region; as a result the duplication is present in all genome resequencing (endpoint clones, and population sampled at 1900, 2300 and 2800th generation) but absent in ABwt.

The insertion loci for branches B3, B4 and B5 were identified as now described: in the fasta files provided by resequencing of sampled populations, we looked for fragments carrying the beginning sequence and the end sequence of IS1236. We identified all joint sequences, allowing to discover new ones. We blasted them on *Acinetobacter baylyi* ADP1 to find out the insertion loci corresponding to B3, B4 and B5. The insertion of another IS was located in a region different from ADP1 strain (see 1). To estimate the ratio of sub populations, we compared for each locus, the

number of fragments founded in fasta files matching with IS insertion with the number of fragments matching with no insertion (wild type profile). For the five branches (B1, B2, B3, B4 and B5), the sum of the ratios is about 100% for the three sequencing of population, justifying that there are no other relevant insertion loci, and that each bacteria underwent only one of such five insertion events.

How we obtained the data in table S3:

From frozen samples of population, some bacteria were plated and allowed to grow overnight; single colonies were piked and allowed to grow overnight in LB, snap-frozen and stored at -70°C. To extract genomic DNA, bacteria issued from a single colony were grown overnight in LB, centrifuged and resuspended in small volume of Tris EDTA. The samples were then heated at 100°C during 10 minutes; lysed cells were centrifuged, the supernatant recovered, aliquoted and stored at -20°C.

To check the presence of point mutations and small indels (glnK, Ins 9bp, ACIAD1029, Δ8bp, C1903816T, ACIAD1931, ACIAD2054, and qseB in table S3), primers were designed to amplify fragments of about 800 bp (point mutation or indels in the middle of the fragment). The PCR products were purified on electrophoresis gel and sequenced by Eurofin-MWG.

The presence of IS1236 (1236 bp) at two loci (branches B1 and B2) were checked by amplifying each region and PCR product sizes were checked on electrophoresis gel.

To check the large deletion Δ60kb two different PCR were performed per genome: one PCR with sense primer upstream the deleted sequence and antisense primer within the deleted sequence, and one PCR with antisense primer downstream the deleted region. Only one PCR gives a product which could be checked on electrophoresis gel.

To check the large deletion ΔgltI, one PCR were performed per genome with sense primer upstream the deleted sequence and antisense primer downstream the deleted region. PCR product sizes were checked on electrophoresis gel.

To check the inversion, two different PCRs were performed per genome: the same sense primer were used for both PCRs, and two different antisense primers hybridizing in the inverted sequence were used, one giving product only in the case of an inverted sequence and the other giving product only in the case of a no-inverted sequence. The products were checked on electrophoresis gel.

Table S3. Detection of mutations in several isolated clones[1].

1 In ABWT, an IS1236 copy is present in ACIAD0290. The transposition of this IS (event “ACIAD0290 Ins 9bp”) results in an insertion of 9 bp in ACIAD0290 when compared to ADP1.

	glnK	ACIAD0290	citA	ACIAD1029	IS–B1	IS–B2	Δ8bp	C1903816T	ACIAD1931	Inversion	ACIAD2054	ΔgltI	Δ60kb	qseB
	244223	289081	389458	1020409	1230681	1231207	1249430	1903816	1920605	2041592	2042174	2046132	2664637	2891803
	C → T	Ins 9bp	T → C	T → C					Ins A		T → C			G → A
AB85a	no		no				yes	no		no			yes	no
AB85b							yes							
AB85c							yes							
AB85d							yes							
AB85e													yes	
AB85f													yes	
AB215a	no		no		no		yes	no		yes			yes	yes
AB215b					no					yes				
AB215c					no					yes				
AB215d					no					yes				
AB360d	no		no					no		yes			yes	yes
AB360f	no		no					no		yes			yes	yes
AB590a		no			no			no			no			
AB590b					no			no						
AB590c		no			no		yes	yes			no	no		
AB590d					no			no						
AB590e					no			no						
AB590f					no			no						
AB590g					no			no						
AB590h					no			no						
AB590i					no			no						
AB590k					no			no						
AB845a	no		no		no		yes	G1903844A		yes			yes	yes
AB845b	no		no		no			G1903797A						
AB845c					no			G1903797A						
AB845d					no			G1903844A						
AB845e					no			G1903844A						
AB845f					no			G1903797A						
AB845g					no			G1903844A						
AB845h					no			G1903844A						
AB845i					no			G1903844A						
AB845j					no			G1903844A						
AB845k					no			A1903876T						
AB845l					no			A1903876T						
AB845m					no			A1903876T						
AB845n					no			A1903876T						
AB845p					no			A1903876T						
AB845q					no			A1903876T						
AB845r					no			no						
AB845s					no			A1903876T						
AB980a		no			no			G1903844A			no			
AB980b		yes			yes			yes			no			
AB980c		no			no			G1903844A						
AB980d		yes			yes			yes			no			
AB980e					yes			yes			no			
AB980f					yes			yes			no			
AB980g					yes			yes			no			
AB980h		yes		no	no	yes		yes			no	no		yes
AB980i					yes			yes			no			
AB980k					yes			yes			no			
AB1100a		yes			no	no		yes	no		no			yes
AB1100b		yes			no	no		yes	no		no			yes
AB1100c		yes			yes			yes			no			
AB1100d		yes			no	no		yes	no		no			yes
AB1100e		yes			no	?		yes	no					yes
AB1100f		yes			yes			yes			no			
AB1100g		yes			no	no		yes	no		no			yes
AB1100h		yes		no	no	yes		yes			no	no		
AB1100i		yes			no	no		yes	no		no			yes
AB1100k		yes			yes			yes			no			
AB1900a	no	yes	no		yes		yes	yes			no			
AB1900b	no				yes			yes			no			
AB1900c	no	yes			no	no	yes	yes	no		no			yes
AB1900d	no	yes			no	no		yes	no		no			yes
AB1900e	no				yes			yes			no			
AB1900f	no				yes			yes			no			
AB1900g	no				yes			yes			yes			
AB1900h	no				yes			yes			no			
AB1900i	no				yes			yes			yes			
AB1900j	no				yes			yes			yes			
AB2300a	no	yes	no		no	no			no		yes			yes
AB2300b	no	yes	no		no	no			no		no			yes
AB2300c	yes	yes			no	no			no		yes			yes
AB2300d	no	yes			no	no			no		no			yes
AB2300e	no				yes						yes			
AB2300f	no	yes			no	no			no		no			yes
AB2300g	yes	yes			no	no			no		no			yes
AB2300h	yes				yes						no			
AB2300i	no				yes						yes			
AB2300k	no	yes			no	no			no		yes			yes
AB2800a	yes	yes	yes		yes	no	yes	yes		yes		no	yes	yes
AB2800b	no		no	yes	no	yes		yes		yes		yes	yes	yes
AB2800c	no	yes	no	yes	no	yes					yes	yes		yes
AB2800d	yes		yes		yes						yes			
AB2800e	yes		no		yes						no			

3. Transformation rates measured within and out reactors

A genomic DNA containing a resistance to kanamycin (gDNAkan^R) was used in competence assays. gDNAkan^R was obtained by transforming wild type bacteria with a cassette containing the resistance to kanamycin (see below). Genomic DNA was extracted from resistant cells by ethanol precipitation after lysis and separation with chloroform.

Competence was tested with a 20 days evolved bacteria during a control experiment. Four different conditions were tested: within the chemostat (air bubbles aeration + glass wall), in 50ml Falcon with air input tube (air bubbles aeration + plastic wall), in glass tube in shaker (aeration by shaking + glass wall) and in 2ml eppendorf microtubes (aeration by shaking + plastic wall).
For the three last conditions, the bacteria subjected to transformation were sampled from the chemostat. In each case, transformation assay was performed in selection medium supplemented with gDNAkan^R 1 µg/ml and 20 mM KNO3 pulse enhancing tranformation rate. Bacteria were incubated at 30°C for 4.5 h. Samples were plated at different dilutions on LB supplemented with 50 µg/ml kanamycin, and incubated at 30°C overnight. The OD measured before plating allowed to estimate the number of plated cells. The rate of transformation was estimated by the ratio between the number of resistant colonies and the number of plated cells.

In chemostat and in Falcon supplied with air by an input tube, the competence was not detectable (below 10^{-9}). Transformation only took place in condition of aeration by shaking (glass tube and eppendorf microtube), suggesting that air bubbles limits the transformation process.

Protocols used to obtain bacteria resistant to kanamycin: A cassette containing kanamycin resistance was first produced as follows: the gene conferring resistance to kanamycin (kan) was amplified by PCR from the plasmid pUC4K (GE Healthcare), and two fragments (named here H1 and H2) overlapping the extremities of the mutS gene were PCR-amplified with the *Acinetobacter baylyi* genome as template. Primers were designed to introduce sequences allowing subsequent hybridization of the fragments for the later cassette assembly (the sequences added for hybridization are underlined): tttttgatgatgcacgaaaagcggcaaaac (left primer H1), cgcgggcggccgttcttaagtcacgtggtc (right primer H1), gcgggcccggccttgaaattggctacaatc (left primer H2), ttttgtgcttccttgatcacgcttgcaggt (right primer H2), cggccgcccgcgttgtcgggaagatgcgtga (left primer kan), ggccgggcccgcttgggaaagccacgttgt (right primer kan). Each fragment were about 1 kb long. One extremity of each fragment (H1, H2), and the two extremities of the kan fragment were partially digested on the 3' end via the partial exonuclease action of the T4 polymerase in the presence of a limited set of dNTP's: the fragments were each treated via 0.5 µl T4 DNA polymerase, DTT 2.5 mM, dATP 2.5 mM, 10 µl PCR product in 20 µl buffer T4 DNA polymerase during 30 min at 22°C. In such condition, the T4 polymerases chews away the 3' ends unless it encounters an A. The polymerase was then inactivated by a 20 min incubation at 75°C. The fragments with neo-formed cohesive protruding single-stranded ends were then hybridized by incubating 6µl H1, 6 µl H2 and 2 µl kan during 3 hours at 45°C, and ligated overnight at 4°C (0.5 µl T4 DNA ligase, 14 µl hybridized mix in 20 µl ligase buffer). The ligase was inactivated by a 10 min incubation at 65°C. The ligation of the three fragments was confirmed by gel electrophoresis. The cassette was then amplified by PCR with the pfu DNA polymerase (Fermentas).
ABwt was transformed as follows: 20 µl saturated culture of bacteria were incubated with 300 µl LB 2.5 h at 30°C with shaking; 95 µl PCR products were added and incubated with shaking 2 h at 30°C; cells were selected on LB plate supplemented with 50 µg/ml kanamycin. A few clones were isolated and the presence of the cassette in the genome was verified by PCR.

4. Calibration and measurements of nitrate concentration in chemostat

In order to estimate the concentration of nitrate within the reactor during the experiment, samples

were collected from the output of the reactor, centrifuged to remove bacteria and snap-frozen. For measurements, samples were thawed and the nitrate was reduced to nitrite; samples underwent then a Griess test. In detail, the Griess Reaction Nitrate Kit from World Precision Instrument was used: the reduction of nitrate to nitrite is obtained by a reaction using metallic particles of Cadmium; a Griess test follows, which results in a red pink color (540 nm) which level is related to the nitrite concentration. Spectral measurements were done at 600 nm using a photometer (Biophotometer , Eppendorf). Phosphate was found to inhibit the reduction of nitrate. We circumvented this effect by first precipitating Phosphate via the addition of 15% $CaCl_2$ 1 M, and with 10% NaOH 1 M to samples. Samples were then centrifuged one minute in micro-centrifuge at 13000 RPM, the supernatant was recovered and underwent a reduction by cadmium as described in the Nitralyzer kit protocol (World Precision Instrument).

Standards containing minimal medium with different concentration of nitrate were used to calibrate measurements (figure S1). The data were well fitted by polynomial function. Each sample was duplicated and the polynomial fit was used to estimate nitrate concentration in samples. The minimal level of detectable nitrate was estimated to be ~2 µM nitrate.

In an effort to accurately measure a saturation constant, we started another chemostat experiment under the same conditions of the above evolution experiments but with the clone of AB85a for initial seeding. The reactor flow-out was sampled for different dilution rates, the bacteria where eliminated by centrifugation and the sample were snap-frozen and stored for subsequent analysis. This experiment was led during less than 25 generations to limit the evolution of the strain. At four dilution rates (0.19, 0.50, 0.54 and 0.60 h^{-1}), no nitrate was detectable with our experimental procedure. For D=0.70 h^{-1} a value of 82 ± 2 µM nitrate was measured.

The same type of experiment has been made with the clone of AB215b, during about 55 generations. For D=0.60 h^{-1} and D=0.78 h^{-1}, no nitrate was detectable in the flow-out of the reactor, and for D=0.88 h^{-1}, we measured a concentration of 82 ± 1 µM. However, the chemostat was possibly out of equilibrium for this latter value of D because the draining threshold has been estimated to be about 0.85 in this period. It can be deduced from the very low nitrate (<2µM) in the reactor in a wide range of dilution rate that >99.8% of this nitrate present in the fresh medium (1mM) is captured by the bacteria.

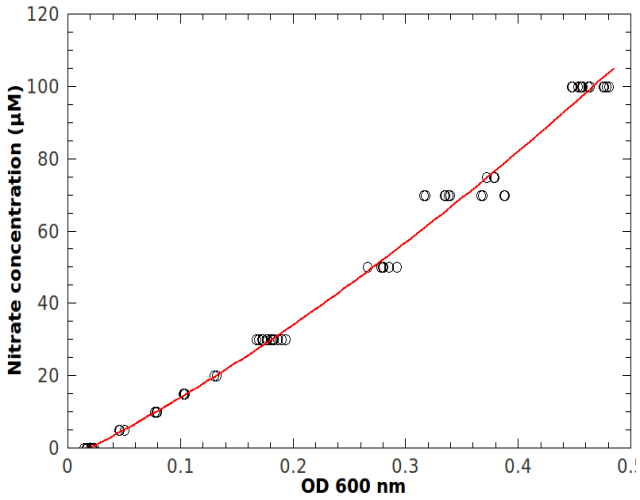


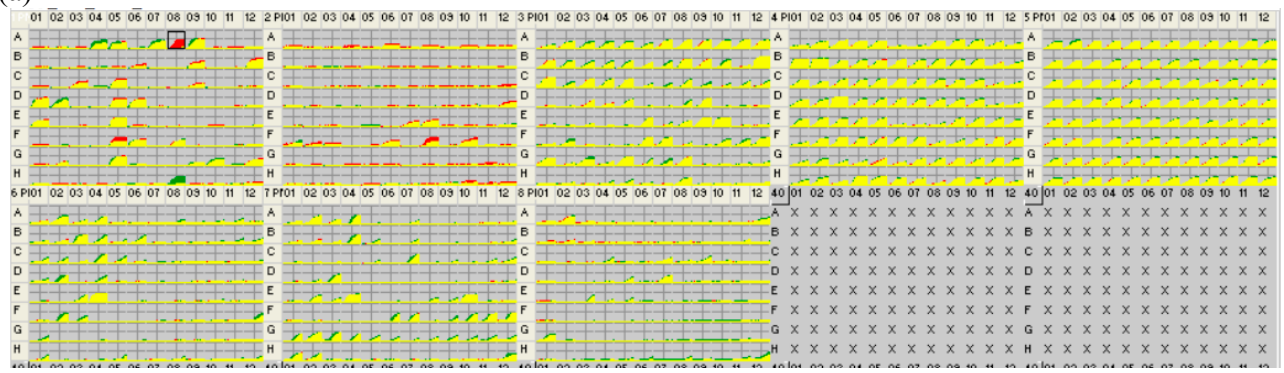
Figure S1: calibration; each point corresponds to a standard sample undergoing the Griess protocol and measured by spectroscopy at 600 nm.

5. Biolog assay

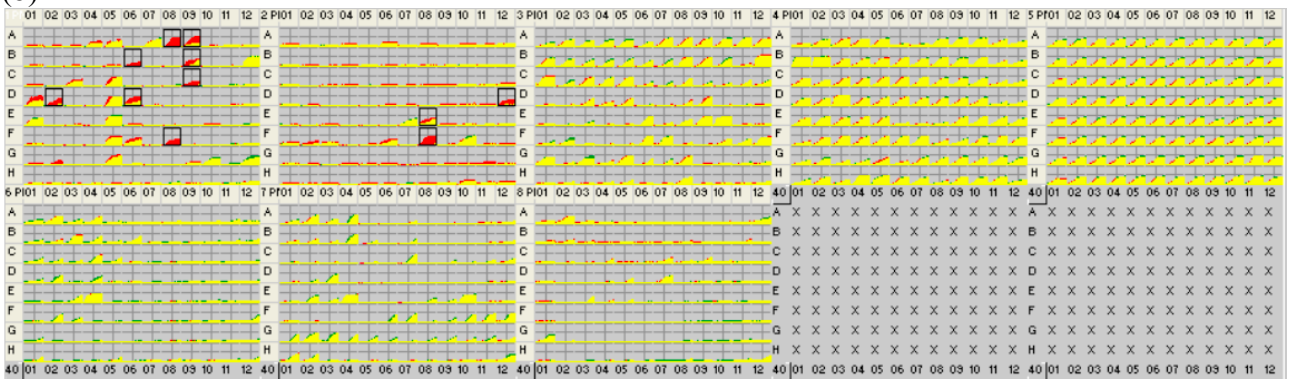
The comparative catabolic activities of the starting strain ABWT and of both endpoint clones AB2800a and AB2800b were determined by the Phenotype MicroArray Services of Biolog, Hayward, CA. The strains were tested on different carbon sources, nitrogen sources, phosphorus

and sulphur sources, nutrient supplements, and peptides nitrogen sources. We also compared the catabolic activities of clones AB1100h and AB2800b

(a)



(b)



(c)

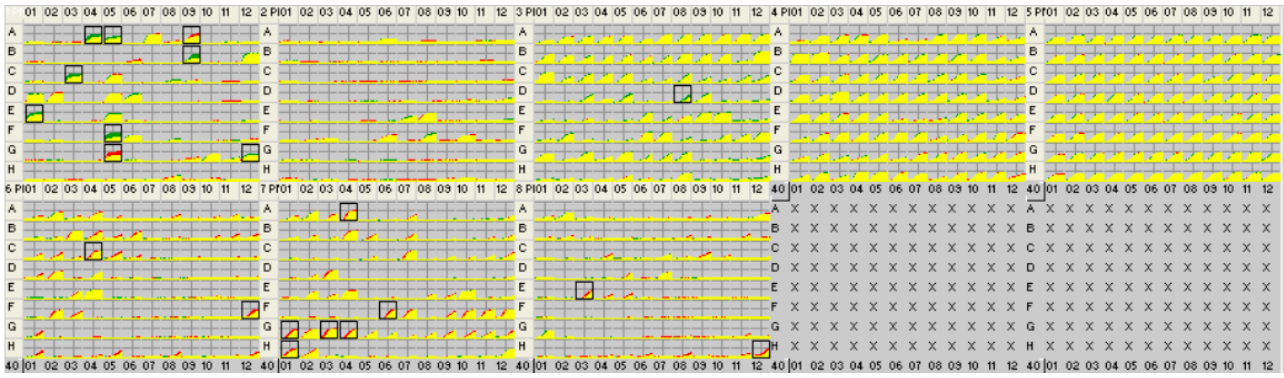


Figure S2: Each plate corresponds to a type of nutrient source tested; each square on a plate corresponds to a different nutrient. Top, from left to right: the two first plates test Carbon sources, the following plates test Nitrogen sources, Phosphorus and Sulfur sources, Nutrients supplements. Bottom: the three plates test Peptide Nitrogen sources.
(a) AB2800b was compared to ABwt. When there are similar phenotypic responses in both cell lines the kinetic curve is yellow, different phenotypic responses are expressed respectively in red for ABwt and green for AB2800b. The relevant differences are black squared by Biolog. For more details, please visit the web site of Biolog (http://www.biolog.com/index.shtml).
(b) AB2800a (green) was compared to ABwt (red)
(c) AB2800b (green) was compared to AB1100h (red)