TECHNICAL MANUAL

horizon™
INSPIRED CELL SOLUTIONS

Yeast Genomic Tiling Collection

Cat #YSC4613

Product description

The Yeast Genomic Tiling Collection consists of over 1,500 glycerol stock cultures each containing a unique clone of a segment of the yeast *S. cerevisiae* genome in an *E. coli*-yeast shuttle vector, designed by Dr. Greg Prelich of Albert Einstein College of Medicine. These plasmid clones represent a unique and virtually complete overlapping clone collection of the entire *S. cervisiae* genome. This clone collection constitutes a "minimal functional pathway" through the yeast genome and represents ~ 97% of the length of the yeast genome at the physical level, and ~ 95% of the genome at the functional level. The genomic location represented by each insert was identified in collaboration with the Sanger Institute by sequencing the insert-vector junctions and comparing the obtained sequences to the complete yeast genome sequence in the public database. Analysis revealed only ~ 284 kb of the genome missing in 92 gaps (for a 3 kb average gap size). The genome coordinates (such as the known yeast genes) and well position on the microtiter plate of each clone is precisely known and supplied to the customer. This collection was designed specifically for systematic overexpression screens.

The library was constructed by partially digesting prototrophic yeast genomic DNA with *Mbo*I and subcloning into the *BamH*I sites of the *E. coli*-yeast shuttle vector, pGP564. Since the library was not made by PCR, it contains no PCR-induced mutations. The proteins are untagged and expressed from their endogenous wild-type promoter. The inserts are flanked by attL sites, allowing transfer of inserts to appropriate destination vectors with attR sites by the Gateway reaction.

The pGP564 shuttle vector contains the *LEU2* selectable marker and 2-micron plasmid sequences necessary for maintenance of a high copy number in yeast. The average insert size in this library is approximately 10 kb, with each insert containing an average of 4-5 genes.

The collection is provided as bacterial cultures of *E. coli* (DH10B) in LB broth with an inert growth indicator + 8% glycerol + kanamycin at a concentration of 50 µg/mL.

Open labs products

We provide clone resources developed by leading academic laboratories. Many of these resources address the needs of specialized research communities not served by other vendors. In order to provide these as a public resource, we depend on the contributing laboratories for quality control. Therefore, these are distributed in the format provided by the contributing institution with no additional product validation or guarantee. Additional information can be found in the product manual as well as in associated published articles (if available).

Shipping and storage

Plates are shipped on dry ice and should be stored at -80 ºC.

Source of yeast genomic DNA

The parental yeast strain for isolation of genomic DNA was FY4, a MAT*a* prototroph, originated from Dr. Fred Winston's lab (Harvard Medical School Department of Genetics).

Plate replication protocol

To allow any CO_2 that may have dissolved into the medium from the dry ice in shipping to dissipate, store plates at –80 °C for at least 48 hours before thawing.

Procedure

Replication of plates

1. Dispense ~ 160 µL of sterile (low salt) LB medium into 96-well microtiter plates. The LB should be supplemented with 8% glycerol and the appropriate antibiotic.

Prepare source plates

1. Remove the foil seals from the source plates. Removing the seals while the source plates are frozen will minimize cross-contamination.
2. Thaw the source plates with the lids on. Wipe away any condensation under the lid.

Replicate

1. Gently place a disposable replicator into the thawed source plate and lightly move the replicator around inside the well to mix the culture. Make sure to scrape the bottom of the plate of the well.
2. Carefully remove the replicator from the source plate and gently place the replicator into the target plate. Lightly move the replicator back and forth in the target plate to transfer cells.
3. Discard the replicator.
4. Place the lids back on the source plates and target plates.
5. Seal the source plates, being mindful to avoid cross contamination.
6. Repeat this process until all plates have been replicated.
7. Return the source plates to the –80 °C freezer.
8. Place the inoculated target plates in a 37 °C incubator. Incubate the plates for 12-24 hours.

Obtaining clone information

Individual clone information and plate locations can be found on the USB drive that accompanies the collection. Alternatively, the datasheet can be downloaded from our website. (dharmacon.horizondiscovery.com)

Reference

Jones, G. M., J. Stalker, *et al.* (2008). A systematic library for comprehensive overexpression screens in *Saccharomyces cerevisiae*. *Nat Methods* **5**(3): 239-41.

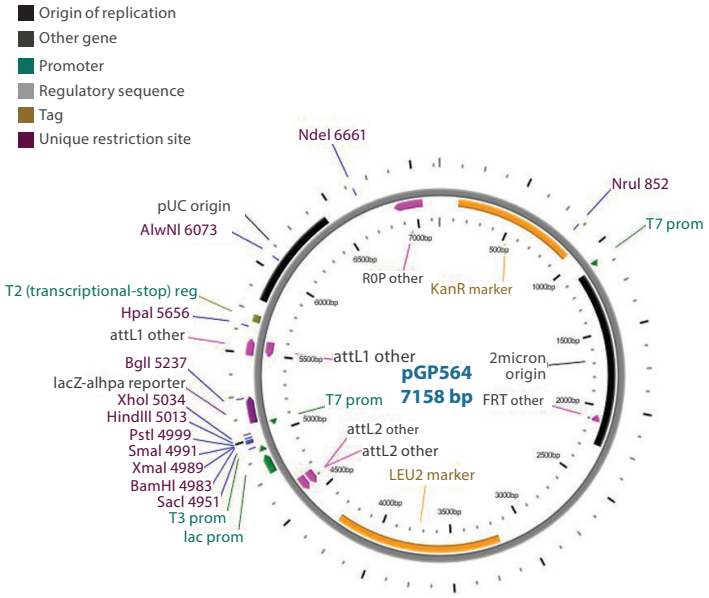

Figure 1. pGP564 Map. The vector is pBR322-based (not pUC-based), so the DNA yield may be a bit lower than a pUC-based vector yield.

Appendix I

Vector sequence pGP564

LOCUS	7158 bp
FEATURES	*Location/Qualifiers*
Marker	125..934
	/gene="KanR marker"
ORF	125..934
	/sequence="ORF_2 rf(6)"
misc_binding	849..854
	/dbxref="REBASE:NruI"
Promoter	1068..1087
	/gene="T7 prom"
Rep_Origin	1102..2262
	/gene="2micron origin"
Other Gene	2065..2112
	/gene="FRT other"
Marker	3175..4281
	/gene="LEU2 marker"
ORF	3175..4281
	/sequence="ORF_1 rf(4)"
Other Gene	4559..4658
	/gene="attL2 other"
Promoter	4774..4889
	/gene="lac prom"
Promoter	4915..4934
	/gene="T3 prom"
misc_binding	4946..4951
	/dbxref="REBASE:SacI"
misc_binding	4982..4987
	/dbxref="REBASE:BamHI"
misc_binding	4988..4993
	/dbxref="REBASE:SmaI"
misc_binding	4988..4993
	/dbxref="REBASE:XmaI"
misc_binding	4994..4999
	/dbxref="REBASE:PstI"
misc_binding	5012..5017
	/dbxref="REBASE:HindIII"
misc_binding	5033..5038
	/dbxref="REBASE:XhoI"
Promoter	5061..5080
	/gene="T7 prom"
Reporter	5088..5246
	/gene="lacZ-alhpa reporter"
misc_binding	5230..5240
	/dbxref="REBASE:BglI"
Other Gene	5489..5588
	/gene="attL1 other"
misc_binding	5653..5658
	/dbxref="REBASE:HpaI"
Regulatory_Seq	5687..5730
	/gene="T2(transcriptional-stop) reg"
Rep_Origin	5821..6440
	/gene="pUC origin"
misc_binding	6067..6075
	/dbxref="REBASE:AlwNI"
misc_binding	6659..6664
	/dbxref="REBASE:NdeI"
Other Gene	6853..7044
	/gene="ROP other"

BASE COUNT

	1888 a	1655 c	1613 g	2002 t	0 others	
ORIGIN						
1	agatctctga	cgctcagtgg	aacgacgcgt	aactcacgtt	aagggatttt	ggtcatgagc
61	ttgcgccgtc	ccgtcaagtc	agcgtaatgc	tctgccagtg	ttacaaccaa	ttaaccaatt
121	ctgattagaa	aaactcatcg	agcatcaaat	gaaactgcaa	tttattcata	tcaggattat
181	caataccata	ttttttgaaaa	agccgtttct	gtaatgaagg	agaaaactca	ccgaggcagt
241	tccataggat	ggcaagatcc	tggtatcggt	ctgcgattcc	gactcgtcca	acatcaatac
301	aacctattaa	tttcccctcg	tcaaaaataa	ggttatcaag	tgagaaatca	ccatgagtga
361	cgactgaatc	cggtgagaat	ggcaaaagtt	tatgcatttc	tttccagact	tgttcaacag
421	gccagccatt	acgctcgtca	tcaaaatcac	tcgcatcaac	caaaccgtta	ttcattcgtg
481	attgcgcctg	agcgagacga	aatacgcgat	cgctgttaaa	aggacaatta	caaacaggaa
541	tcgaatgcaa	ccggcgcagg	aacactgcca	gcgcatcaac	aatattttca	cctgaatcag
601	gatattcttc	taatacctgg	aatgctgttt	ttccggggat	cgcagtggtg	agtaaccatg
661	catcatcagg	agtacggata	aaatgcttga	tggtcggaag	aggcataaat	tccgtcagcc
721	agtttagtct	gaccatctca	tctgtaacat	cattggcaac	gctacctttg	ccatgtttca
781	gaaacaactc	tggcgcatcg	ggcttcccat	acaagcgata	gattgtcgca	cctgattgcc
841	cgacattatc	gcgagcccat	ttatacccat	ataaatcagc	atccatgttg	gaatttaatc
901	gcggcctcga	cgtttcccgt	tgaatatggc	tcataacacc	ccttgtatta	ctgtttatgt
961	aagcagacag	ttttattgtt	catgatgata	tatttttatc	ttgtgcaatg	taacatcaga
1021	gattttgaga	cacgggccag	agctgccagg	aaacagctat	gaccatgtaa	tacgactcac
1081	tataggggat	cgatcctcct	gaacgaagca	tctgtgcttc	attttgtaga	acaaaaatgc
1141	aacgcgagag	cgctaatttt	tcaaacaaag	aatctgagct	gcatttttac	agaacagaaa
1201	tgcaacgcga	aagcgctatt	ttaccaacga	agaatctgtg	cttcattttt	gtaaaacaaa
1261	aatgcaacgc	gagagcgcta	atttttcaaa	caaagaatct	gagctgcatt	tttacagaac
1321	agaaatgcaa	cgcgagagcg	ctattttacc	aacaaagaat	ctatacttct	tttttgttct
1381	acaaaaatgc	atcccgagag	cgctatttttt	ctaacaaagc	atcttagatt	actttttttc
1441	tcctttgtgc	gctctataat	gcagtctctt	gataacttttt	tgcactgtag	gtccgttaag
1501	gttagaagaa	ggctactttg	gtgtctattt	tctcttccat	aaaaaaagcc	tgactccact
1561	tcccgcgttt	actgattact	agcgaagctg	cgggtgcatt	ttttcaagat	aaaggcatcc
1621	ccgattatat	tctataccga	tgtggattgc	gcatactttg	tgaacagaaa	gtgatagcgt
1681	tgatgattct	tcattggtca	gaaaattatg	aacggtttct	tctattttgt	ctctatatac
1741	tacgtatagg	aaatgtttac	attttcgtat	tgttttcgat	tcactctatg	aatagttctt
1801	actacaattt	ttttgtctaa	agagtaatac	tagagataaa	cataaaaaat	gtagaggtcg
1861	agtttagatg	caagttcaag	gagcgaaagg	tggatgggta	ggttatatag	ggatatagca
1921	cagagatata	tagcaaagag	atacttttga	gcaatgtttg	tggaagcggt	attcgcaata
1981	ttttagtagc	tcgttacagt	ccggtgcgtt	tttggttttt	tgaaagtgcg	tcttcagagc
2041	gcttttggtt	ttcaaaagcg	ctctgaagtt	cctatacttt	ctagagaata	ggaacttcgg
2101	aataggaact	tcaaagcgtt	tccgaaaacg	agcgcttccg	aaaatgcaac	gcgagctgcg
2161	cacatacagc	tcactgttca	cgtcgcacct	atatctgcgt	gttgcctgta	tatatatata
2221	catgagaaga	acggcatagt	gcgtgtttat	gcttaaatgc	gtacttatat	gcgtctattt
2281	atgtaggatg	aaaggtagtc	tagtacctcc	tgtgatatta	tcccattcca	tgcggggtat
2341	cgtatgcttc	cttcagcact	acccttttagc	tgttctatat	gctgccactc	ctcaattgga
2401	ttagtctcat	ccttcaatgc	tatcatttcc	tttgatattg	gatcatacta	agaaaccatt
2461	attatcatga	cattaaccta	taaaaatagg	cgtatcacga	ggccctttcg	tctcgcgcgt
2521	ttcggtgatg	acggtgaaaa	cctctgacac	atgcagctcc	cggagacggt	cacagcttgt
2581	ctgtaagcgg	atgccgggag	cagacaagcc	cgtcagggcg	cgtcagcggg	tgttggcggg
2641	tgtcggggct	ggcttaacta	tgcggcatca	gagcagattg	tactgagagt	gcaccatatc
2701	gactacgtcg	taaggccgtt	tctgacagag	taaaattctt	gagggaactt	tcaccattat
2761	gggaaatgct	tcaagaaggt	attgacttaa	actccatcaa	atggtcaggt	cattgagtgt
2821	tttttatttg	ttgtatttttt	ttttttttag	agaaaatcct	ccaatatcaa	attaggaatc
2881	gtagtttcat	gattttctgt	tacacctaac	tttttgtgtg	gtgccctcct	ccttgtcaat
2941	attaatgtta	aagtgcaatt	ctttttccttt	atcacgttga	gccattagta	tcaatttgct

3001 tacctgtatt cctttactat cctccttttt ctccttcttg ataaatgtat gtagattgcg
3061 tatatagttt cgtctaccct atgaacatat tccattttgt aatttcgtgt cgtttctatt
3121 atgaatttca tttataaagt ttatgtacaa atatcataaa aaaagagaat ctttttaagc
3181 aaggattttc ttaacttctt cggcgacagc atcaccgact tcggtggtac tgttggaacc
3241 acctaaatca ccagttctga tacctgcatc caaaaccttt ttaactgcat cttcaatggc
3301 cttaccttct tcaggcaagt tcaatgacaa tttcaacatc attgcagcag acaagatagt
3361 ggcgataggg tcaaccttat tctttggcaa atctggagca gaaccgtggc atggttcgta
3421 caaaccaaat gcggtgttct tgtctggcaa agaggccaag gacgcagatg gcaacaaacc
3481 caaggaacct gggataacgg aggcttcatc ggagatgata tcaccaaaca tgttgctggt
3541 gattataata ccatttaggt gggttgggtt cttaactagg atcatggcgg cagaatcaat
3541 gattataata ccatttaggt gggttgggtt cttaactagg atcatggcgg cagaatcaat
3601 caattgatgt tgaaccttca atgtagggaa ttcgttcttg atggtttcct ccacagtttt
3661 tctccataat cttgaagagg ccaaaagatt agctttatcc aaggaccaaa taggcaatgg
3721 tggctcatgt tgtagggcca tgaaagcggc cattcttgtg attctttgca cttctggaac
3781 ggtgtattgt tcactatccc aagcgacacc atcaccatcg tcttcctttc tcttaccaaa
3841 gtaaatacct cccactaatt ctctgacaac aacgaagtca gtacctttag caaattgtgg
3901 cttgattgga gataagtcta aaagagagtc ggatgcaaag ttacatggtc ttaagttggc
3961 gtacaattga agttctttac ggatttttag taaaccttgt tcaggtctaa cactaccggt
4021 accccattta ggaccagcca cagcacctaa caaaacggca tcaaccttct tggaggcttc
4081 cagcgcctca tctggaagtg ggacacctgt agcatcgata gcagcaccac caattaaatg
4141 attttcgaaa tcgaacttga cattggaacg aacatcagaa atagctttaa gaaccttaat
4201 ggcttcggct gtgatttctt gaccaacgtg gtcacctggc aaaacgacga tcttcttagg
4261 ggcagacata ggggcagaca ttagaatggt atatccttga aatatatata tatattgctg
4321 aaatgtaaaa ggtaagaaaa gttagaaagt aagacgattg ctaaccacct attggaaaaa
4381 acaataggtc cttaaataat attgtcaact tcaagtattg tgatgcaagc atttagtcat
4441 gaacgcttct ctattctata tgaaaagccg gttccggcct ctcacctttc ctttttctcc
4501 caatttttca gttgaaaaag gtatatgcgt caggcgacct ctaggatcag ctggatggca
4561 aataatgatt ttattttgac tgatagtgac ctgttcgttg caacaaattg ataagcaatg
4621 ctttcttata atgccaactt tgtacaagaa agctgggtcg gcgcgcccac ccttgcgccc
4681 aatacgcaaa ccgcctctcc ccgcgcgttg gccgattcat taatgcagct ggcacgacag
4741 gtttcccgac tggaaagcgg gcagtgagcg caacgcaatt aatgtgagtt acctcactca
4801 ttaggcaccc caggctttac actttatgct tccggctcct atgttgtgtg gaattgtgag
4861 cggataacaa tttcacacag gaaacagcta tgaccatgat tacgccaagc gcgcaattaa
4921 ccctcactaa agggaacaaa agctggagct ccaccgcggt ggcggccgct ctagaactag
4981 tggatccccc gggctgcagg aattcgatat caagcttatc gataccgtcg acctcgaggg
5041 ggggcccggt acccaattcg ccctatagtg agtcgtatta cgcgcgctca ctggccgtcg
5101 ttttacaacg tcgtgactgg gaaaaccctg gcgttaccca acttaatcgc cttgcagcac
5161 atccccctttt cgccagctgg cgtaatagcg aagaggcccg caccgatcgc ccttcccaac
5221 agttgcgcag cctgaatggc gaatggcgcg acgcgccctg tagcggcgca ttaagcgcgg
5281 cgggtgtggt ggttacgcgc agcgtgaccg ctacacttgc cagcgcccta gcgcccgctc
5341 ctttcgcttt cttcccttcc tttctcgcca cgttcgccgg ctttccccgt caagctctaa
5401 atcgggggct ccctttaggg ttccgattta gtgctttacg gcacctcgac cccaaaaaac
5461 ttgattaggg tgaagggggc ggccgcggag cctgcttttt tgtacaaagt tggcattata
5521 aaaaagcatt gctcatcaat ttgttgcaac gaacaggtca ctatcagtca aaataaaatc
5581 attatttggg gcccgagctt aagactggcc gtcgttttac aacgtcgtga ctgggaaaac
5641 atccatgcta gcgttaacgc gagagtaggg aactgccagg catcaaataa aacgaaaggc
5701 tcagtcggaa gactgggcct ttcgttttat ctgttgtttg tcggtgaacg ctctcctgag
5761 taggacaaat ccgccgggag cggatttgaa cgttgtgaag caagatctac cccgtagaaa
5821 agatcaaagg atcttcttga gatccttttt ttctgcgcgt aatctgctgc ttgcaaacaa
5881 aaaaaccacc gctaccagcg gtggtttgtt tgccggatca agagctacca actcttttte
5941 cgaaggtaac tggcttcagc agagcgcaga taccaaatac tgtccttcta gtgtagccgt
6001 agttaggcca ccacttcaag aactctgtag caccgcctac atacctcgct ctgctaatcc
6061 tgttaccagt ggctgctgcc agtggcgata agtcgtgtct taccgggttg gactcaagac

6121	gatagttacc	ggataaggcg	cagcggtcgg	gctgaacggg	gggttcgtgc	acacagccca
6181	gcttggagcg	aacgacctac	accgaactga	gatacctaca	gcgtgagcta	tgagaaagcg
6241	ccacgcttcc	cgaagggaga	aaggcggaca	ggtatccggt	aagcggcagg	gtcggaacag
6301	gagagcgcac	gagggagctt	ccaggggaa	acgcctggta	tctttatagt	cctgtcgggt
6361	ttcgccacct	ctgacttgag	cgtcgatttt	tgtgatgctc	gtcagggggg	cggagcctat
6421	ggaaaaacgc	cagcaacgcg	gccttttac	ggttcctggc	cttttgctgg	cctttgctc
6481	acatgttctt	tcctgcgtta	tcccctgatt	ctgtggataa	ccgtattacc	gcctttgagt
6541	gagctgatac	cgctcgccgc	agccgaacga	ccgagcgcag	cgagtcagtg	agcgaggaag
6601	cggaagagcg	cctgatgcgg	tattttctcc	ttacgcatct	gtgcggtatt	tcacaccgca
6661	tatggtgcac	tctcagtaca	atctgctctg	atgccgcata	gttaagccag	tatacactcc
6721	gctatcgcta	cgtgactggg	tcatggctgc	gccccgacac	ccgccaacac	ccgctgacgc
6781	gccctgacgg	gcttgtctgc	tcccggcatc	cgcttacaga	caagctgtga	ccgtctccgg
6841	gagctgcatg	tgtcagaggt	tttcaccgtc	atcaccgaaa	cgcgcgaggc	agctgcggta
6901	aagctcatca	gcgtggtcgt	gaagcgattc	acagatgtct	gcctgttcat	ccgcgtccag
6961	ctcgttgagt	ttctccagaa	gcgttaatgt	ctggcttctg	ataaagcggg	ccatgttaag
7021	ggcggttttt	tcctgtttgg	tcactgatgc	ctccgtgtaa	gggggatttc	tgttcatggg
7081	ggtaatgata	ccgatgaaac	gagagaggat	gctcacgata	cgggttactg	atgatgaaca
7141	tgcccggtta	ctggaacg				

//

FAQS/troubleshooting

We provide certain clone resources developed by leading academic laboratories. Many of these resources address the needs of specialized research communities not served by other commercial entities. In order to provide these as a public resource, we depend on the contributing academic laboratories for quality control.

Therefore, these are distributed in the format provided by the contributing institution "as is" with no additional product validation or guarantee. We are not responsible for any errors or performance issues. Additional information can be found in the product manual as well as in associated published articles (if available). Alternatively, the source academic institution can be contacted directly for troubleshooting.

Label licenses

The gene editing Products, use and applications, are covered by pending and issued patents. Certain Label licenses govern the use of the products, these can be found at dharmacon.horizondiscovery.com/uploadedFiles/dharmacon-licensing-statements.pdf. It is each Buyer's responsibility to determine which intellectual property rights held by third parties may restrict the use of Products for a particular application.

If you have any questions, contact

t +44 (0) 1223 976 000 (UK) **or** +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us **or** dharmacon.horizondiscovery.com/service-and-support
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

horizon™
INSPIRED CELL SOLUTIONS

V2-0119