



TargetRich™ UMI/Index Adapters & Sequencing User Manual for PGX

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To download this and other TargetRich User Manuals, visit:
<http://KailosGenetics.com/targetrich-pgxcomplete>



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TargetRich™ reagents from Kailos Genetics® are based upon PatchPCR™, a technology that facilitates the enrichment of genomic regions of interest for paired-end sequencing on an Illumina® next-generation sequencer (MiSeq®).

Kit Components

Component	Volumes (µl)	Storage
TargetRich UMI/Sample Adapter Plate 48 or 96-Sample plate	10 per well	-20° C
50 µM TargetRich Read 1 Primer Mix	75	-20° C
50 µM TargetRich Index 1 Primer	75	-20° C
50 µM TargetRich Read 2 Primer Mix	75	-20° C

TargetRich UMI/Index Adapter Plate is available in 48-sample (half-plate) or 96-sample (full-plate) format.

The pre-mixed TargetRich UMI/Index Adapter Plate is required for sample barcoding, multiplex sequencing, and tagging of individual captured DNA molecules. Adapter 1 (i7) and Adapter 2 (i5) contain sequences required for clustering and paired-end sequencing. Adapter 1 incorporates an 8-base index for sample identification in multiplexed sequencing. Adapter 2 contains a random 12-base Unique Molecule Identifier (UMI) sequence for tagging of each captured DNA molecule. Adapter mixes provided in the plate contain both adapters and are identified based on the index sequence of Adapter 1. See Appendix A for Adapter 1 (i7) sequences.

TargetRich UMI/Index Adapters are added to each sample during library preparation as indicated in the TargetRich PGx Panel Panel User Manual (II. Patch Ligation).

96-Sample TargetRich UMI/Index Adapter Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	UMI K001	UMI K002	UMI K003	UMI K004	UMI K005	UMI K006	UMI K007	UMI K008	UMI K009	UMI K010	UMI K011	UMI K012
B	UMI K013	UMI K014	UMI K015	UMI K016	UMI K017	UMI K018	UMI K019	UMI K020	UMI K021	UMI K022	UMI K023	UMI K024
C	UMI K025	UMI K026	UMI K027	UMI K028	UMI K029	UMI K030	UMI K031	UMI K032	UMI K033	UMI K034	UMI K035	UMI K036
D	UMI K037	UMI K038	UMI K039	UMI K040	UMI K041	UMI K042	UMI K043	UMI K044	UMI K045	UMI K046	UMI K047	UMI K048
E	UMI K049	UMI K050	UMI K051	UMI K052	UMI K053	UMI K054	UMI K055	UMI K056	UMI K057	UMI K058	UMI K059	UMI K060
F	UMI K061	UMI K062	UMI K063	UMI K064	UMI K065	UMI K066	UMI K067	UMI K068	UMI K069	UMI K070	UMI K071	UMI K072
G	UMI K073	UMI K074	UMI K075	UMI K076	UMI K077	UMI K078	UMI K079	UMI K080	UMI K081	UMI K082	UMI K083	UMI K084
H	UMI K085	UMI K086	UMI K087	UMI K088	UMI K089	UMI K090	UMI K091	UMI K092	UMI K093	UMI K094	UMI K095	UMI K096

Note: 48-Sample plate contains adapters in rows A-D only

Required Illumina Sequencing Kit

- TargetRich PGx Complete: 12 - 48 samples
 - Illumina MiSeq Reagent Kit v3 (150 cycles)
 - Catalog #MS-102-3001

Follow Illumina's instructions for thawing and preparing the sequencing cartridge.

Sequencing TargetRich Libraries

Pool the TargetRich libraries and normalize to 10nM as described in the TargetRich PGx Panel User Manual.

Below is the recommended procedure for preparing, denaturing, and diluting a TargetRich library:

NOTE: 10% or greater PhiX spike is necessary to ensure sufficient nucleotide diversity at each cycle during the sequencing run.

1. Prepare a 4 nM, 10% PhiX library by combining the following volumes of library, PhiX and Buffer EB:
 - o 5.4 μ l, 10 nM pooled library (~1.1 ng/ μ l)
 - o 3.0 μ l, 2 nM PhiX
 - o 6.6 μ l, Buffer EB
2. Denature the 4 nM library as follows:
 - o To 10 μ l of 4 nM library (prepared above), add 10 μ l 0.2N NaOH (prepared fresh), mix quickly by vortex and briefly centrifuge
 - o Incubate for 5 min at RT to 30°C
 - o Immediately add 980 μ l ice cold HT-1 buffer and mix thoroughly
3. Dilute the resulting 40 pM denatured library to 16 pM by adding 600 μ l chilled HT-1 buffer to 400 μ l of 40 pM denatured library.
4. Load 600 μ l of the diluted, denatured 16 pM library into position 17 of the sequencing cartridge.

NOTE: Some optimization of cluster density may be required.
5. Follow the instructions in Illumina's MiSeq System Custom Primers Guide for loading the custom primers into the cartridge:
 - o To dilute Read 1, Index and Read 2 primer mixes to the working concentration of 0.5 μ M, combine 6 μ l of the primer mix with 600 μ l HT-1 buffer.
 - o Load the diluted sequencing primers into the cartridge to the positions indicated in the MiSeq System Custom Primers Guide.
6. Perform paired-end 2 x 78 base reads with 8 cycles for Index1 (i7) and 12 cycles for Index 2 (i5). Index sequences of TargetRich Adapter 1 are provided in Appendix A.
7. An editable Sample Sheet for sequencing TargetRich PGx libraries with Custom Primers can be downloaded at:
<https://www.kailosgenetics.com/files/pgxsamplesheet1>

Appendix A

Index Sequences in TargetRich™ UMI/Index Adapter plate

Row/ Col	Index ID	i7 Index Sequence 5'→3'
A01	K001	ATTACTCG
A02	K002	TCCGGAGA
A03	K003	GAGATTCC
A04	K004	CGCTCATT
A05	K005	ATTCAGAA
A06	K006	GAATTCGT
A07	K007	CTGAAGCT
A08	K008	TAATGCGC
A09	K009	CGGCTATG
A10	K010	TCCGCGAA
A11	K011	TCTCGCGC
A12	K012	AGCGATAG
B01	K013	TACATGCC
B02	K014	TCTGAACG
B03	K015	AAGAAGGC
B04	K016	AACTACGC
B05	K017	AGCATAGC
B06	K018	GTCACAAG
B07	K019	CGTGCTAA
B08	K020	CGTCCTTT
B09	K021	GACGACTA
B10	K022	CCCTTTTC
B11	K023	TTGTACCG
B12	K024	CATCTGCT
C01	K025	CAGGTTAC
C02	K026	CCTAACAC
C03	K027	AAAACGCC
C04	K028	AGCTGTCA
C05	K029	ATGCTCTC
C06	K030	CTCCTGAA
C07	K031	CCCCAATT
C08	K032	CGTAGCTA

Row/ Col	Index ID	i7 Index Sequence 5'→3'
C09	K033	AACCTTCC
C10	K034	TCAGGTIG
C11	K035	TGCAGGTT
C12	K036	GCATGCAT
D01	K037	GTATCTGC
D02	K038	ACGCCAAA
D03	K039	GCGGTIAA
D04	K040	CCGTACAA
D05	K041	TGACATCC
D06	K042	GTTGTCAC
D07	K043	AAGTGGTC
D08	K044	CGGCATAT
D09	K045	CTATCACC
D10	K046	CTAGTCTG
D11	K047	GTGTCCAA
D12	K048	GGCATCAT
E01	K049	TATAGCCC
E02	K050	CACTCTAG
E03	K051	CGGATACT
E04	K052	AGCGAACT
E05	K053	CTAGAGAC
E06	K054	AGTCGTCT
E07	K055	GACAAGAC
E08	K056	CCGAGATT
E09	K057	TACACCAC
E10	K058	TACTGTGG
E11	K059	ACAACACG
E12	K060	TGACACTG
F01	K061	GCCACATT
F02	K062	ATGGGCAA
F03	K063	ATCTTCCC
F04	K064	CCATACCT

Row/ Col	Index ID	i7 Index Sequence 5'→3'
F05	K065	CACGATGT
F06	K066	TCGAAACC
F07	K067	TGGACGTA
F08	K068	CTTGGATC
F09	K069	GCAGTCTT
F10	K070	GAGCATCA
F11	K071	GACAGACA
F12	K072	ATGAGGTG
G01	K073	AAGCTAGG
G02	K074	ACGTATCC
G03	K075	ATGGACTG
G04	K076	CACAAAGC
G05	K077	TACTAGCG
G06	K078	TAGCTCAC
G07	K079	GTAGAACC
G08	K080	TAAGCGCA
G09	K081	GTGAGGAA
G10	K082	GGAATACG
G11	K083	GAACGTCT
G12	K084	CTCAAACG
H01	K085	TCAATGGC
H02	K086	CGCAATAC
H03	K087	GTAGCAGT
H04	K088	AGTATGCG
H05	K089	GTAACGAC
H06	K090	CGATCCAA
H07	K091	TAAGCAGG
H08	K092	CATTTGGC
H09	K093	CCGATAAC
H10	K094	GCTGAGAT
H11	K095	GATGGCAT
H12	K096	TCGTCAAG

Appendix B

Illumina-compatible TargetRich™ adapter sequences

TargetRich™ Index Adapter 2 (i5)

5' -AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNNNN-ACACCGTCTTAGAGAATGAGGAAGGTGGGGAGT[^]*target sequence (top strand)*[^]

UMI_barcode i5 TR_Read1 primer sequence (underlined)

TargetRich™ Index Adapter 1 (i7)

[^]*target sequence (top strand)*[^] AGTGTGGGAGGGTAGTTGGTGTTCAGTCAC-NNNNNNNN-ATCTCGTATGCCGTCTTCTGCTTG-3'

Index Read Primer (underlined) Index_Barcode i7

TargetRich™ Custom Sequencing Primers:

Read1: 5' -ACACCGTCTTAGAGAATGAGGAAGGTGGGGAG*T-3'

Index1: 5' -AGTGTGGGAGGGTAGTTGGTGTTCAGTCA*C-3'

Read2: 5' -GTGACTGGAAACACCAACTACCCTCCCACAC*T-3'

Kailos Genetics' General Terms and Conditions of Sale

1. Contract Terms. These are the contract terms and conditions ("Terms") under which our products and services are provided. These Terms, together with our quotation (if any), create the contract ("Contract") between the parties for the purchase, sale and use of products and/or services. The Contract between us is created when any one of the following is deemed to have occurred: (i) an order is received, (ii) payment for any part of an order is received, (iii) any part of an order is delivered, (iv) the box containing the product is opened, or (v) the product or service is used. If any conditions within the Contract documents conflict with each other, we will give them the following priority: the quotation then these Terms.

2. Delivery, Title and Ordering

2.1 We will try to meet the delivery dates specified in your order, depending on availability and any lead times that may apply. Sometimes orders are delivered in installments. If orders are delivered in installments, a separate invoice will be provided for each delivery.
2.2 Once an order has been placed and accepted, it cannot be cancelled. If delaying the date of delivery would be helpful, please contact Customer Services to see if we can reschedule your delivery.
2.3 All our products are sold FOB our facility. Products are deemed delivered when accepted by any commercial carrier at our facility. At this point you become responsible for risk of loss and damage. If any product is lost or damaged while being transported, we will try to help you in dealing with the carrier. We do not clear products for import into any country. Doing so is solely your responsibility. Title to products will pass to you upon receipt of product by the carrier.

3. Inspection.

3.1 We want you to receive any product in satisfactory condition. Products that are damaged or defective upon delivery may be returned for replacement, if you contact Customer Services within 5 business days from the date of product receipt. When contacting Customer Service, you will be provided instructions for returning the products and replacement. If you do not contact us within this five-day period, the products will be deemed accepted, but you will not lose any warranty rights.
3.2 Custom products made in accordance with your specifications can only be returned if the custom product does not conform to specifications. In any such case, we will, in our sole discretion, either replace the product or issue a refund for an amount not to exceed the price paid for the product.

4. Price. The price for products and services is shown in our quotation to you. If we do not provide you with a quotation, the price will be the list price that applies to your country on the date the order is received. Our prices do not include any taxes (including VAT), duties, levies or other government fees that may apply to your order. If applicable, payment of any taxes, duties, levies or other government fees are your responsibility. If paid by us, the cost will be added to your invoice. You are also responsible for standard delivery and handling charges for each shipment, if any. These charges will be added to your invoice.

5. Payment. Invoices, unless specified otherwise in the quotation, are due within 30 days from the invoice date in the currency specified in our invoice. Each order is a separate transaction, and you may not set-off payments from one order against another. If you are late in making payment, without affecting our other rights, we may suspend delivery or cancel the Contract, reject your future orders, and charge you a late-payment charge, from the due date until paid, at the rate of one percent (1%) per month (12% per year) or, if less, the maximum amount allowed by law. You agree to pay this late charge when demanded. If we appoint a collection agency or an attorney to recover any unpaid amounts, we can charge you and you agree to pay all reasonable costs of collection, including all associated reasonable attorneys' fees.

6. Product Use and Restrictions.

6.1 All products are for RESEARCH USE ONLY, AND NOT FOR HUMAN OR ANIMAL THERAPEUTIC OR DIAGNOSTIC USE. Products are to be used in accordance with our instructions, and you may not purchase products with the intention of reselling them or otherwise act as a distributor of our products. We do not submit our products for regulatory review by any government body or other organization, and we do not validate them for clinical, therapeutic or diagnostic use, for safety and effectiveness, or for any other specific use or application. You are solely responsible for making sure that the way you use our products complies with applicable laws, regulations and governmental policies. You must obtain all necessary approvals, intellectual property rights, licenses and permissions you may need. It is solely your responsibility to make sure the products are suitable for your particular use. If you are looking for commercial use rights to our products (including the right to perform fee-for-services), please contact our outlicensing department at licensing@kailosgenetics.com.

7. Custom Products.

7.1 When you ask us to manufacture a custom product, we may decline the design or manufacture of such custom product, at any stage of the design or manufacture process, if the custom product is unsuitable or commercially impractical to be synthesized in that way. If that is the case, we will notify you as soon as possible and you will not be obligated to pay any fees for any expenses incurred by us in connection with a declined product unless specifically stated in our quotation.
7.2 By submitting an order for a custom product you represent and agree that (a) you have provided us with all information that you are aware of regarding any biological, radiological and chemical hazards associated with the handling, transport, exposure or other usage of the materials you supply to us; and (b) you have the right to cause the sequences that you requested us to manufacture to be manufactured.

8. Intellectual Property

8.1 You acknowledge that all intellectual property rights relating to our products and services, as between you and us, are solely and exclusively owned by us. Our sale of products to you only grants you a limited, non-transferable right, for only you to use the quantity of the products that you have received from us in accordance with the Contract. When we provide products to you, we do not grant you a license to our intellectual property, whether express, implied, by estoppel or otherwise, or grant you the right to make or have made any product or to use the product beyond the scope of this agreement. Nothing in the Contract limits our ability to enforce our intellectual property rights.
8.2 In relation to processes, methods or related synthesis of a custom product, or otherwise in connection with the design or manufacture of a custom product, any inventions (patentable or otherwise), discoveries, improvements, data, know-how or other results that are conceived, developed, discovered, reduced to practice, or generated by or for us, or jointly by us and you, will be and will remain our sole and exclusive intellectual property, and you transfer and assign all of your right, title and interest in and to any such joint intellectual property to us and assist us, at our request and at our expense, in securing and recording our rights in such intellectual property.

9. Intellectual Property Indemnity

9.1 **Our Indemnity of You.** We will defend and indemnify you from and against infringement damages finally awarded in any legal action brought by a third party against you to the extent that the action is based on a claim that our manufacture and sale of a product infringes any patent, copyright, trademark or other intellectual property right of such third party if we had actual knowledge of such intellectual property right and the actual infringement at the time of delivery of the product to you. This indemnity does not apply to products that we made, assembled or labeled in reliance upon your instructions, specifications, or other directions, or to claims based on your use or resale of products, or to modifications made by you or any third party. This indemnity does not apply to products originating from third parties. THIS INDEMNITY IS OUR ONLY LIABILITY TO YOU, AND YOUR ONLY REMEDY, FOR ANY INFRINGEMENT OR CLAIMED INFRINGEMENT OF INTELLECTUAL PROPERTY RIGHTS BY OR IN CONNECTION WITH ANY PRODUCT. As a condition to this indemnity, you must (i) notify us in writing, as soon as you become aware of any claim; (ii) not admit any liability or take any other action in connection with the claim that could affect the defense; (iii) allow us to solely control the defense or settlement of the claim; and (iv) give us your reasonable information, co-operation and assistance.

9.2 **Your Indemnity of Us.** If a third party makes a claim against us for infringement of its intellectual property rights based on our manufacture or sale of a product we make under your instructions, specifications, directions, installation, assembly, or using materials you provide to us, or based on your modification, use or resale of a product, then you will indemnify and hold us harmless from and against any and all claims, losses, damages, liabilities and expenses (including reasonable attorneys' fees and other costs of defending and/or settling any action) that we may have to pay as a result of the claim.

9.3 **Avoidance.** We wish to avoid claims of intellectual property infringement. If we believe a product may be subject to a claim for intellectual property infringement, then you will allow us, at our option and expense, to either: (a) secure for you the right to continue using the product; (b) substitute the product with another suitable product with similar functionality; or (c) require you to return the product to us for a refund of the purchase price you paid.

10. Limitations of Liability.

10.1 TO THE MAXIMUM EXTENT PERMITTED BY APPLICABLE LAW, WE WILL NOT BE LIABLE UNDER ANY LEGAL THEORY (INCLUDING BUT NOT LIMITED TO CONTRACT, NEGLIGENCE, STRICT LIABILITY IN TORT OR WARRANTY OF ANY KIND) FOR ANY INDIRECT, SPECIAL, INCIDENTAL, PUNITIVE, MULTIPLE, EXEMPLARY OR CONSEQUENTIAL DAMAGES (INCLUDING BUT NOT LIMITED TO COSTS OF COVER, LOST PROFITS, LOST DATA, LOSS OF BUSINESS, LOSS OF GOODWILL OR LOSS OF REVENUE) THAT YOU MIGHT INCUR UNDER THE CONTRACT, OR THAT MAY ARISE FROM OR IN CONNECTION WITH OUR PRODUCTS OR SERVICES, EVEN IF WE HAD NOTICE OF THE POSSIBILITY OF SUCH DAMAGES. IN ADDITION, OUR MAXIMUM AGGREGATE LIABILITY ARISING OUT OF OR IN CONNECTION WITH THE CONTRACT, OR ANY PRODUCT OR SERVICE, IS LIMITED TO THE AMOUNT YOU PAID TO US FOR THE PRODUCT OR SERVICE PURCHASED. HOWEVER, THESE PROVISIONS DO NOT LIMIT OUR LIABILITY FOR DEATH OR PERSONAL INJURY CAUSED BY OUR NEGLIGENCE OR FRAUD, FRAUDULENT MISREPRESENTATION OR ANY OTHER LIABILITY THAT CANNOT BE EXCLUDED BY LAW.

10.2 DELIVERY DATES AND TIMES ARE ESTIMATES ONLY AND WE WILL NOT BE LIABLE (IN CONTRACT, DELICT, TORT OR OTHERWISE) FOR ANY LOSSES, EXPENSES, CLAIMS OR DAMAGES CAUSED BY A LATE DELIVERY.



11. Export Control. Products and information that you receive from us are subject to United States, European Union and local export-control laws and regulations. You may not, directly or indirectly, sell, export, re-export, transfer, divert, or otherwise dispose of any such product or information (including products derived from or based on our products or information) to any destination, entity, or person prohibited by United States, European Union or local laws or regulations.

12. Entire Contract.

12.1 The Contract represents the entire agreement between you and us regarding the products and services we provide to you under it, and supersedes and replaces any previous agreements between us (whether written or oral). Any of your additional or different terms and conditions that you may provide to us, are material alternations and we reject them. Our offer to sell products and perform services is expressly limited to the terms of the Contract. If you submit a purchase order, or other document for the purchase of products or services, whether or not in response to a quotation, you are deemed to have accepted and agree to the Contract, to the exclusion of (a) any other terms and conditions appearing in or referenced in your purchase order or other documents you give to us, and (b) any previous course of dealing, course of performance, trade usage or co-existent agreement. The Contract cannot be amended or modified unless you and we agree in writing.

12.2 We reserve the right to change these Terms at any time. Any changes made to these Terms will not apply to the Contract between us for any order we receive before the changes are made. The most recent revision date can be found at the end of these Terms

13. Miscellaneous.

13.1 We will not be responsible or liable for failing to perform our obligations under the Contract to the extent caused by circumstances beyond our reasonable control. In certain situations, we may use our reasonable judgment and apportion products then available for delivery fairly among our customers.

13.2 Our failure to exercise any rights under the Contract is not a waiver of our rights to damages for your breach of contract and is not a waiver of any subsequent breach. If any provision or part of the Contract is found by any court of competent jurisdiction to be invalid or unenforceable, such invalidity or unenforceability will not affect the other provisions of the Contract. No person other than you or us will have any rights under the Contract. Headings are for convenience only and shall not be used in the interpretation of these Terms.

13.3 You agree to keep confidential any non-public technical information, commercial information (including prices, without limitation) or instructions (including any gene sequences, oligo types or sequences) received from us as a result of discussions, negotiations and other communications between us in relation to our products or services.

14. Governing Law. The Contract and performance under it will be governed by the laws of the State of Delaware, USA, without regard to provision on the conflict of laws. The United Nations Convention on Contracts for the International Sale of Goods shall not apply to the Contract.

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visit www.kailossolutions.com

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