



Long-Range PCR for  
CYP2D6 CNV Analysis.  
Supplementary procedure for  
TargetRich™ PGx Assay

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CYP2D6 CNV analysis using this Long-Range PCR and Gel Analysis protocol is provided to determine copy number variation in the CYP2D6 gene and should be conducted in parallel to genotyping using the TargetRich™ PGx Panel workflow. The protocol includes two PCR reactions performed on each sample for detection of CYP2D6 duplication and deletion, respectively.

Optimum DNA input for the Assay is 1-10 ng. DNA preparations obtained from buccal swab extractions usually result in higher concentrations and require dilution. The DNA dilution procedure is included in this protocol.

### Kit Components (for processing of 96 samples)

Component	Volumes (µl)	Cap Color	Storage
2D6_DUP Master Mix	1500	yellow	-20° C
2D6_DEL Master Mix	1500	purple	-20° C
LR DNA Polymerase	50	clear	-20° C
DUP Control DNA (5 ng/µl)	20	orange	-20° C
DEL Control DNA (5 ng/µl)	20	black	-20° C

#### Additional required equipment and materials:

- Nuclease-free water
- Buffer EB (QIAGEN, 10 mM Tris-Cl, pH 8.5)
- HMW DNA ladder (Lamda Biotech, Cat.# M112)
- 1% agarose ethidium bromide gel (Invitrogen, Cat.# G800801)
- Thermal Cycler with ramp speeds of approximately 3°C per second
- *Recommended:* Low retention micropipette tips to prevent reagent loss
- 100 µl PCR tubes or low-profile PCR plates

## Important notes before proceeding

- ❑ Control DNA for CYP2D6 Duplication (2D6\_DUP) and CYP2D6 Deletion (2D6\_DEL) are provided and should be used with every batch of processed samples. For a negative control, use nuclease-free water.
- ❑ To prevent loss of enzymes and master mixes during pipetting, use low retention pipette tips. Additionally, prior to transferring reagents with a multichannel pipette, aliquot reagents into PCR strip tubes.
- ❑ As with all PCR-based protocols, regular cleaning and careful handling should be followed to reduce potential contamination. Avoid cross-contamination by changing pipette tips after every addition to a sample.
- ❑ If performing the library preparation in a PCR plate, seal the plate before every vortex, centrifuge, and thermal cycler step.
- ❑ Thaw all reagents/ samples at room temperature ~15 minutes prior to use.
- ❑ DNA polymerase should be kept at -20°C until ready to use. Always keep the enzyme in a reagent cooler.

## I. Diluting template (for samples with DNA concentration >2 ng/μl)

**NOTE:** Optimum DNA input for the assay is 1-10 ng. If the DNA concentration in the sample is  $\leq 2\text{ ng}/\mu\text{l}$ , then omit steps 1 - 2 and use 10 μl of undiluted DNA sample as an input for CYP2D6 duplication and deletion detection reactions. However, DNA\_DUP and DNA\_DEL Controls still need to be diluted according to this procedure.

**NOTE:** The protocol describes a procedure for processing up to 48 samples at a time, including controls.

**NOTE:** To ensure accuracy of DNA quantification, using a fluorometric assay, such as Qubit or Quant-iT (Life Technologies®) is recommended.

**Tip:** Keeping the receiving plate on a cold block or ice reduces the interference of static electricity with transfers of small volumes from the pipette tip onto the bottom of the well.

1. Transfer 4 μl of sample DNA from the batch into a new PCR plate. Include DNA\_DUP (6 μl), DNA\_DEL (6 μl) and Negative controls (4 μl of water).
  - If following Kailos Blue replace A1 (NTC) with DNA\_DEL and A12 (NTC) with DNA\_DUP controls. At least one water control (NTC) should remain in the batch to control for contaminations.
2. Add 40 μl of PCR-grade water to each well on the batch, including controls, mix by pipetting 3-5 times. This makes a **diluted DNA stock plate**.
3. Transfer 10 μl of DNA from the **diluted DNA stock plate** into two new PCR plates labeled "DUP" and "DEL", respectively.
4. Keep DUP and DEL DNA plates chilled; proceed to adding PCR Master Mixes.

## II. PCR Set Up

### A. CYP2D6 Duplication Detection Reaction

1. Thaw and mix 2D6\_DUP master mix by inverting the tubes or by gentle vortexing. Briefly centrifuge the master mixes to collect the all liquid at the bottom of the tubes and place on ice.
2. Calculate the amount of master mix (14.75 μl/reaction) and the LR DNA

Polymerase (0.25 µl/reaction) required for the batch, including an overage to account for pipetting error.

3. Combine calculated volumes of 2D6\_DUP Master Mix and LR DNA Polymerase in a new chilled tube. Mix by pipetting. Close the tube and mix by inverting the tube 5 times. Centrifuge the tube briefly to collect all liquid at the bottom of the tube. Place the tube on ice.
4. Add 15 µl of pre-mixed 2D6\_DUP Master Mix / LR DNA Polymerase to each sample on "DUP" plate.
5. Mix thoroughly by pipetting, seal the plate and briefly centrifuge to collect all the liquid at the bottom of the wells.
6. Initiate the 2D6\_DUP program on a Thermal Cycler with a heated lid:  
Step 1: 94°C for 2 minutes  
Step 2: 10 cycles of
  - 95°C for 20 seconds
  - 70°C for 4 minutesStep 3: 18 cycles of
  - 95°C for 20 seconds
  - 70°C for 4 minutes + 5 seconds/cycleStep 4: 4°C forever
7. Once the lid has heated, place samples in Thermal Cycler until the program has reached completion
8. **Samples may remain at 4°C overnight or stored at -20°C**

## **B. CYP2D6 Deletion Detection Reaction**

1. Thaw and mix 2D6\_DEL master mix by inverting the tube or by gentle vortexing. Briefly centrifuge the master mix to collect the all liquid at the bottom of the tube and keep on ice.
2. Calculate amount of master mix (14.75 µl/reaction) and the LR DNA Polymerase (0.25 µl/reaction) required for the batch, add overage for pipetting error.
3. Combine calculated volumes of 2D6\_DEL Master Mix and LR DNA Polymerase in a new chilled tube. Mix by pipetting. Close the tube and mix by inverting the

tube 5 times. Centrifuge the tube briefly to collect all liquid at the bottom of the tube. Place the tube on ice.

4. Add 15 µl of pre-mixed 2D6\_DEL Master Mix / LR DNA Polymerase to each sample on “DEL” plate.
5. Mix thoroughly by pipetting, seal the plate and briefly centrifuge to collect all the liquid at the bottom of the wells.
6. Initiate the 2D6\_DEL program on a Thermal Cycler with a heated lid:
  - Step 1: 94°C for 2 minutes
  - Step 2: 10 cycles of
    - 95°C for 20 seconds
    - 68°C for 4 minutes
  - Step 3: 18 cycles of
    - 95°C for 20 seconds
    - 68°C for 4 minutes + 5 seconds/cycle
  - Step 4: 4°C forever
7. Once the lid has heated, place samples in Thermal Cycler until the program has reached completion.
8. **Samples may remain at 4°C overnight or stored at -20°C.**

### III. Gel Analysis of the PCR products

1. Briefly centrifuge the plates with PCR reaction product and keep on ice.
2. Dilute Lambda HMW Ladder 1:10 with EB.
3. To each PCR reaction add 15 ul of EB and mix by pipetting.
4. Load 15 ul of the diluted PCR reaction onto 48-well 1% agarose gel with ethidium bromide (Invitrogen, Cat.# G800801).
  - If working with Kailos Blue use 12 channel pipette to load samples into wells of the gel in the order indicated below.

Gel Well ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
PCR Sample	A1	B1	A2	B2	A3	B3	A4	B4	A5	B5	A6	B6	A7	B7	A8	B8	A9	B9	A10	B10	A11	B11	A12	B12

Gel Well ID	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
PCR Sample	C1	D1	C2	D2	C3	D3	C4	D4	C5	D5	C6	D6	C7	D7	C8	D8	C9	D9	C10	D10	C11	D11	C12	D12

**Note:** 2D6\_DEL and 2D6\_DUP control reactions will be in the top row of the gel, in wells #1 and #23, respectively.

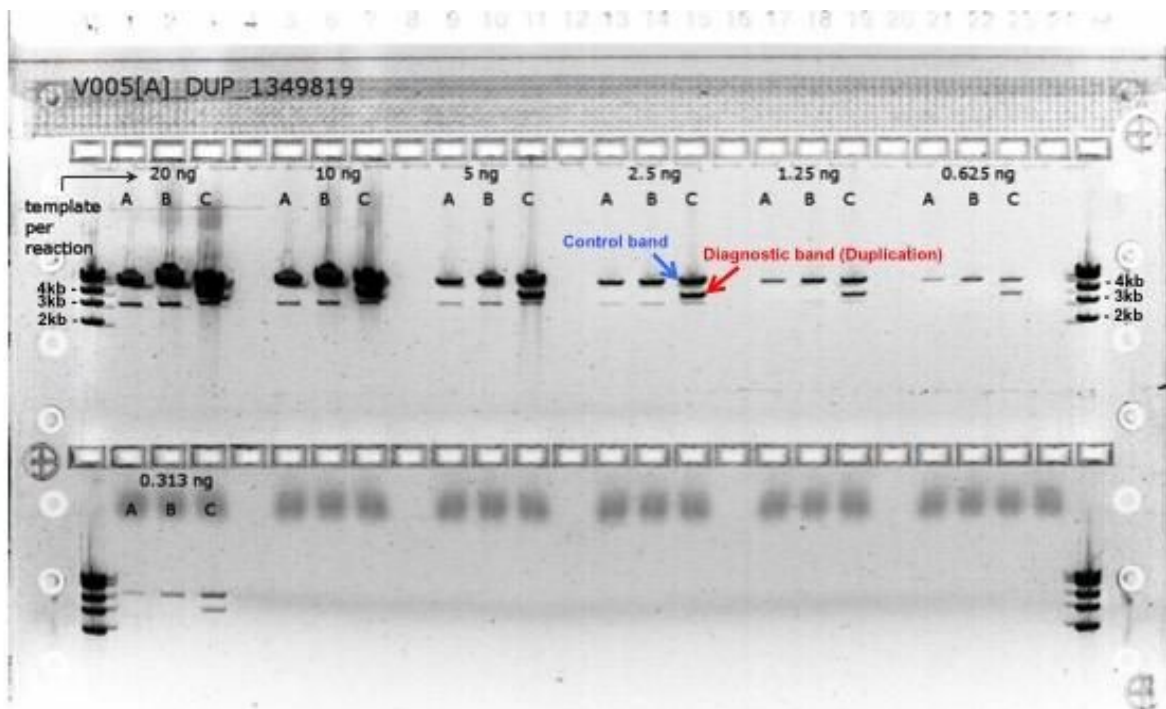
5. Load diluted Lambda HMW Ladder into wells marked "M" on both left and right side of the gel.
6. Run the E-gel on EP program for 15 minutes, or until bromophenol blue (purple dye) in the ladder travels ~1 1/4 inch or more into the gel. Add water to the wells of the E-gel ~ 5-7 minutes after the start of the program to keep the wells from drying out.
7. Visualize DNA bands using UV filter, save images for analysis.

## IV. Reading Electrophoregrams

Normal DNA samples with 2 copies of CYP2D6 will have one control band at 5.1kb. An additional (diagnostic) band 3.5 kb in size in 2D6\_DUP or in 2D6\_DEL reactions indicate presence of duplication or deletion CNV, respectively. If neither the 5.1 kb control nor 3.5 kb diagnostic band are detected in the sample, the reaction must be considered failed.

### CYP2D6 Duplication Detection Gel

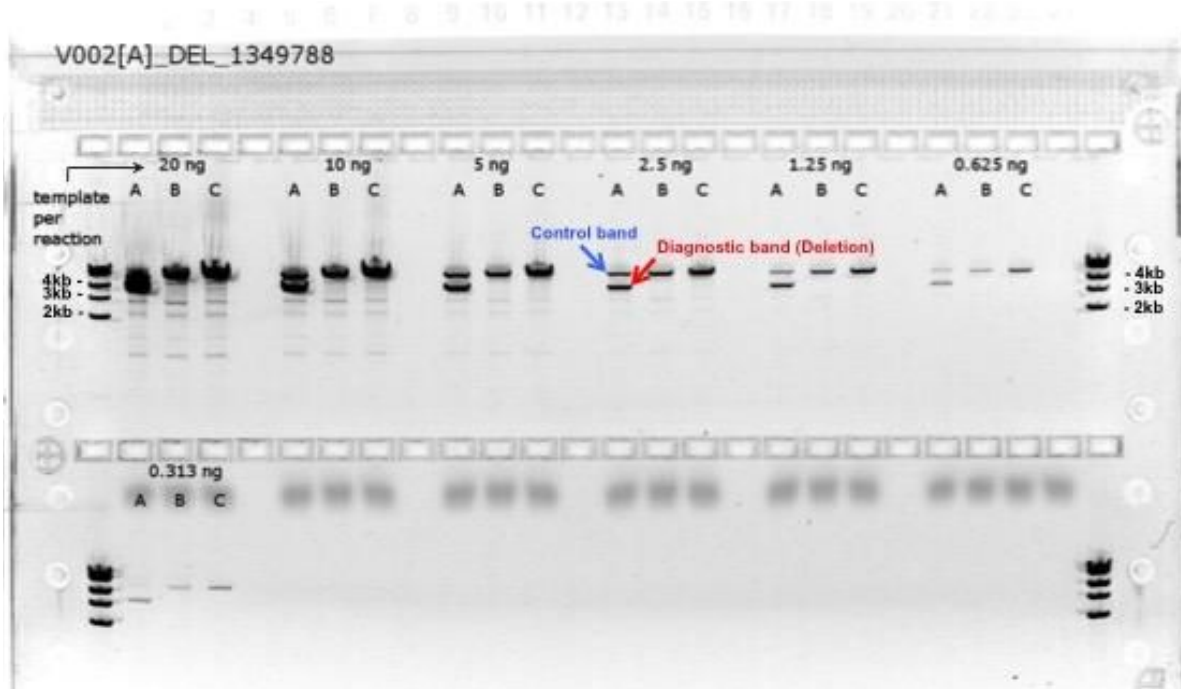
Electrophoregram of the 2D6\_DUP PCR reaction products in Deletion-containing CYP2D6\*1/\*5 (A), Normal CYP2D6\*2/\*2(B) and Duplication-containing CYP2D6\*2/\*2xN (C) samples [1] at a range of genomic DNA inputs.





### CYP2D6 Deletion Detection Gel

Electrophoregram of the 2D6\_DEL PCR reaction products in Deletion-containing CYP2D6\*1/\*5 (A), Normal CYP2D6\*2/\*2(B) and Duplication-containing CYP2D6\*2/\*2xN (C) samples [1] at a range of genomic DNA inputs.



## V. Supplementary Information

Long Range PCR for CYP2D6 duplications and deletions detection was modified from [2].

### Primers for CYP2D6 duplication and deletion detection

Primer	Sequence	Position	Strand
<b>2D6dupl-F</b>	CCTGGGAAGGCCCCATGGAAG	chr22:42,522,003-42,522,023	negative
<b>2D6dupl-R</b>	CAGTTACGGCAGTGGTCAGCT	chr22:42,530,646-42,530,666	positive
<b>5-2D6del</b>	CACCAGGCACCTGTACTCCTC	chr22:42,534,666-42,534,686	negative
<b>3-2D6del</b>	CAGGCATGAGCTAAGGCACCCAGAC	chr22:42,519,025-42,519,049	positive
<b>DPKup</b>	GTTATCCCAGAAGGCTTTCAGGCTTCA	chr22:42,527,113-42,527,140	negative
<b>DPKlow</b>	GCCGACTGAGCCCTGGGAGGTAGGTA	chr22:42,522,040-42,522,065	positive

### Expected test results

Reaction	Primers	PCR product if CYP2D6 CNV status is		
		NORMAL	DELETION	DUPLICATION
2D6_DEL	DPKup/low + 2D6del 5'/3'	5.1 kb	5.1 kb & <b>3.5</b> kb	5.1 kb
2D6_DUP	DPKup/low + 2D6dupl F/R	5.1 kb	5.1 kb	5.1 kb & <b>3.5</b> kb

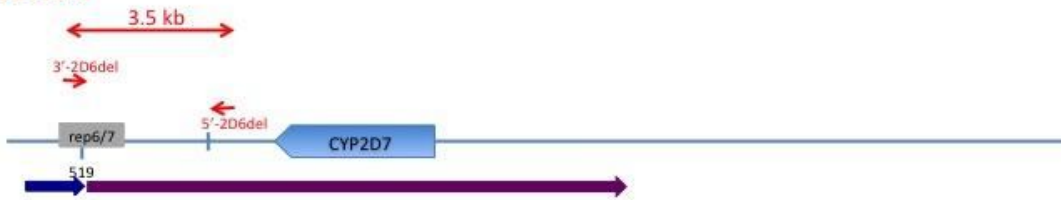
Schematic representation of the regions targeted in CYP2D6\_DEL and CYP2D6\_DUP reactions.

### CYP2D6 deletion detection

#### NORMAL



#### DELETION



### CYP2D6 duplication detection

#### NORMAL



#### DUPLICATION



## VI. References

1. Coriell Cell Repositories <http://www.ccr.coriell.org/>
2. P. Dorado et al., BioTechniques 39: 571-574 (2005): Development of a PCR-based strategy for CYP2D6 genotyping including gene multiplication of worldwide potential use.

## 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](mailto: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 alterations 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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