DNA-PAINT CELLS SLIDE

MASSIVE-Cells 2-PLEX

Additional information

General information about MASSIVE-Cells 2-PLEX slide found in datasheet.

EXCHANGING THE IMAGING MIXTURE AND PROPERLY SEALING THE SLIDE

Exchanging the imaging mixture

- dilute imagers in Massive Photonics Imaging Buffer to create the imaging mix
- remove both black caps from the Slide
- aspirate the complete volume in the channel and fill the channel with 300 µL imaging mix, repeat this procedure 3 times. The duration the channel does not contain liquid should be as short as possible.

Caution: No air bubbles should get stuck in the channel. For optimal filling of the channel lift one side of the slide slightly up and fill the volume in the opposite side (port)

*Optional: aspirate just the volume of the reservoirs (120 µL) and perform exchange by pipetting 120-150 µL volume in one side in and in the opposite side out, this procedure must be performed 6 tim*es for optimal exchange.

Slide can be stored in imaging mix.

Imaging can be performed in a sealed or open channel, but avoid drying out of the channel

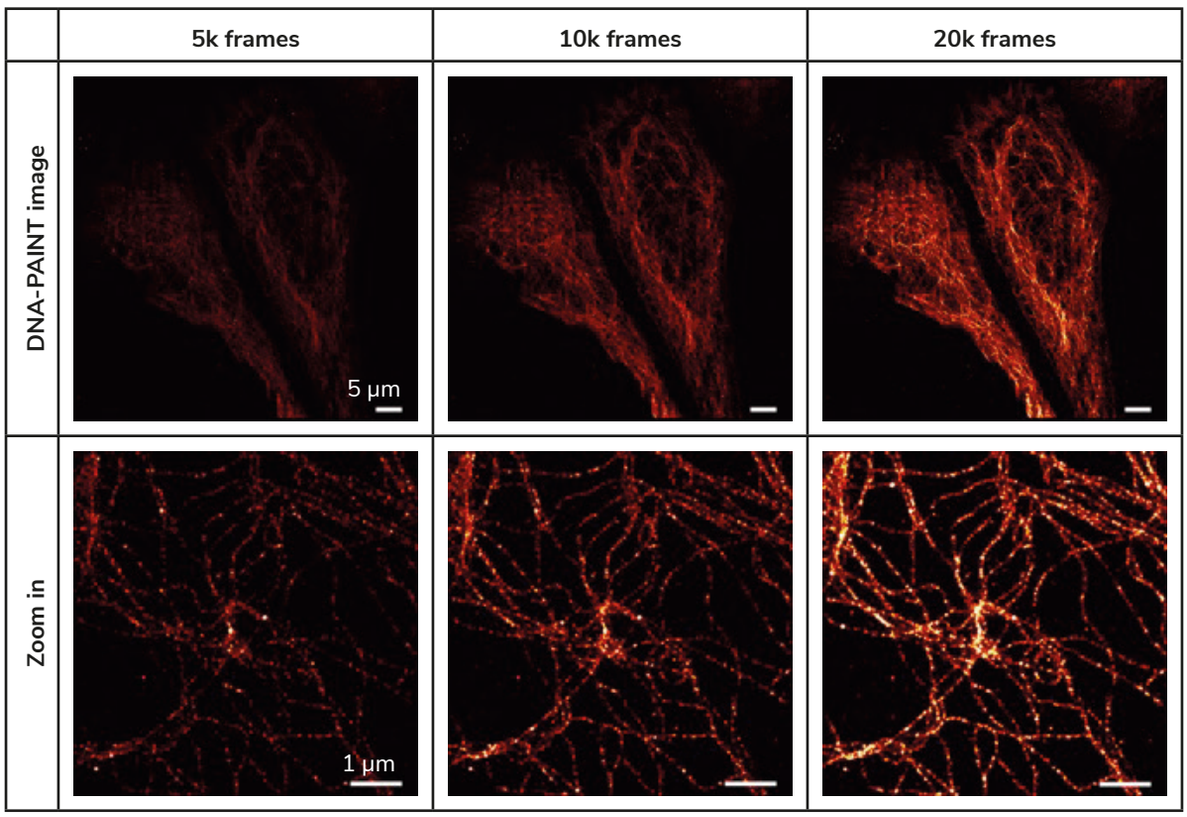
Sealing the Slide

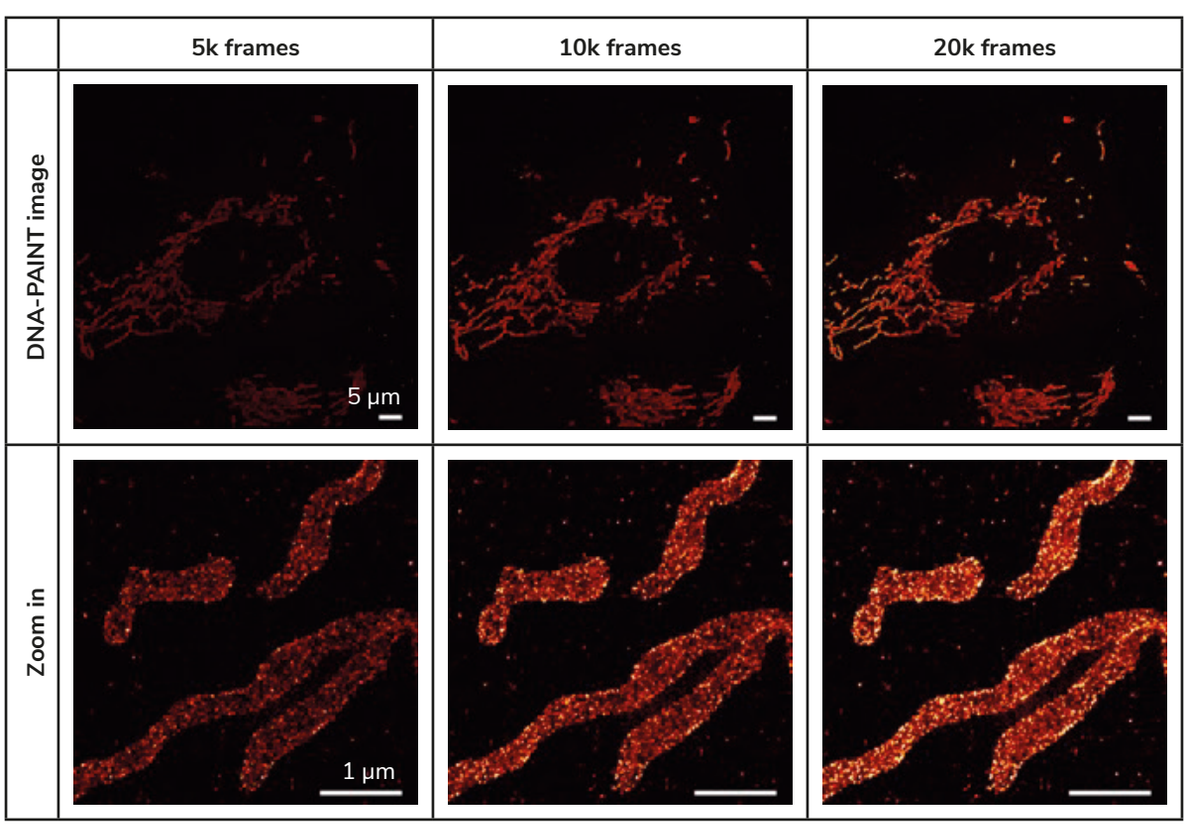
- place slide on a flat surface (e.g. paper towel on lab bench)
- fill single-channel-slide completely (total volume about 450 µL, liquid should form a convex meniscus over both ports)
- fill both black caps (about 70 µL)
- connect 1st black cap
- remove spilled liquid
- squeeze second black cap during connection to second port
- remove spilled liquid
- check whether sample contains air bubbles and both caps are properly connected

Caution: do not push down both sealing caps in parallel, this can cause cracking of the glass slide

SETTING THE NUMBER OF IMAGING FRAMES

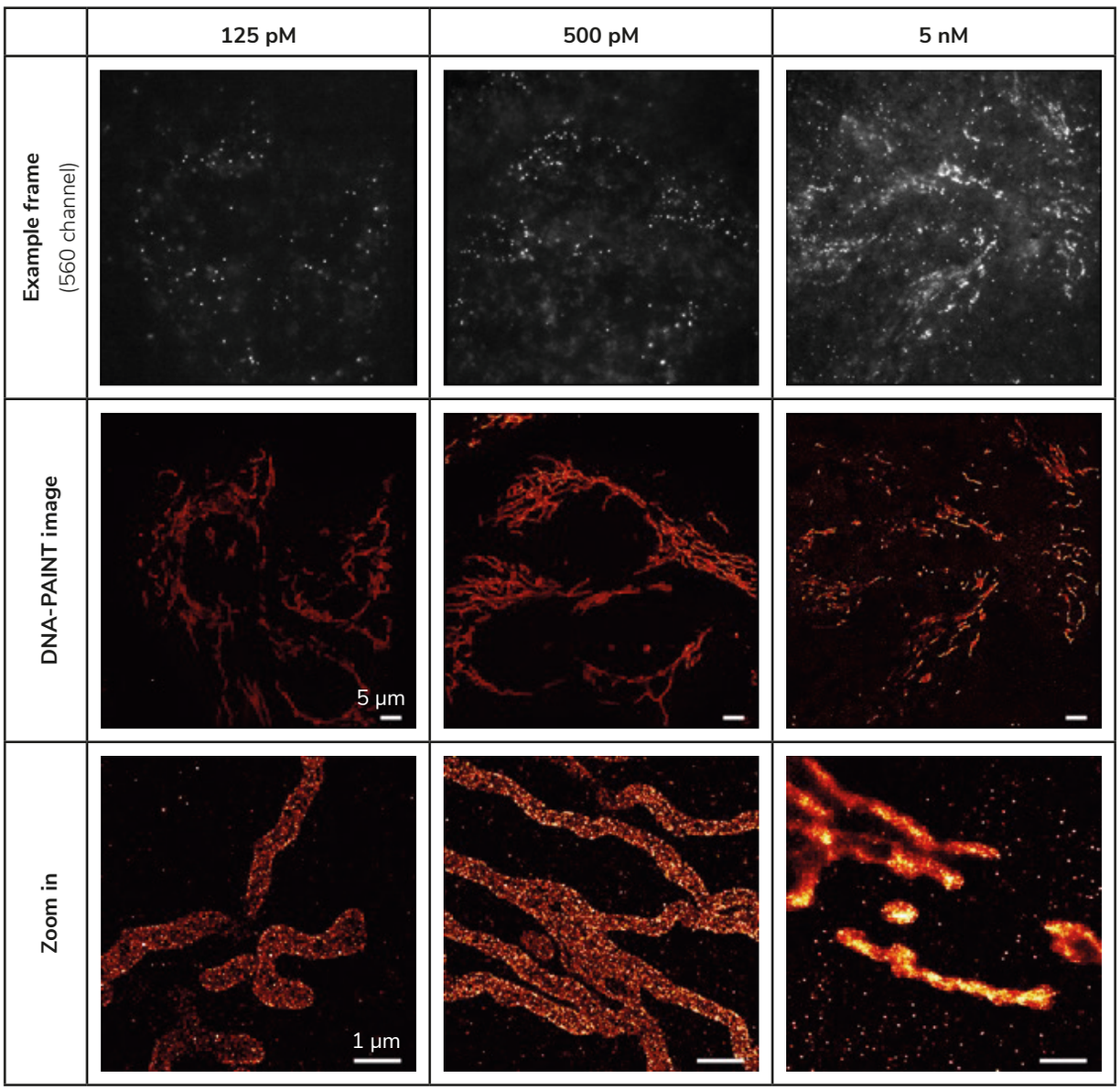
Sampling of the target improves with increased number of frames and/or increased imager concentrations. For a good compromise of resolution and imaging duration, a sweet spot between the number of frames and imager concentration needs to be found.





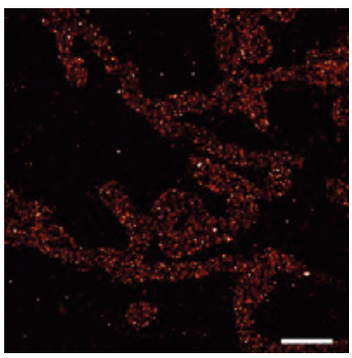
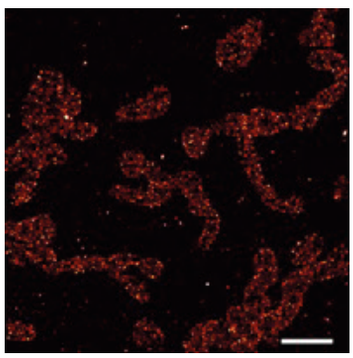
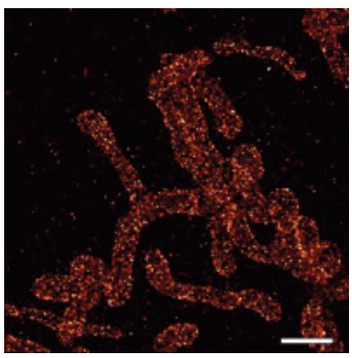
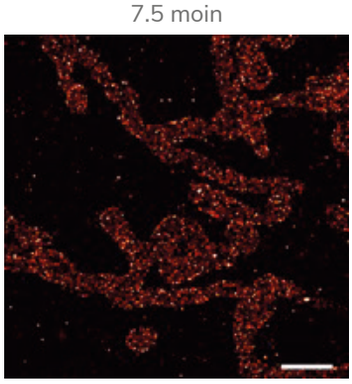
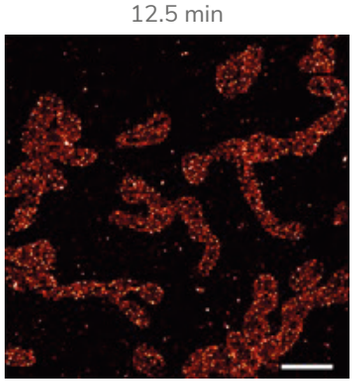
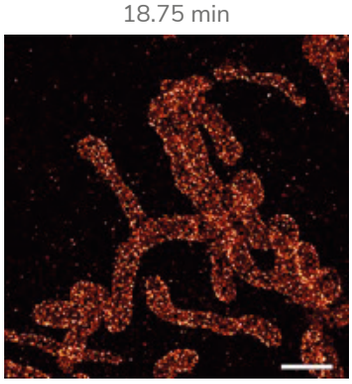
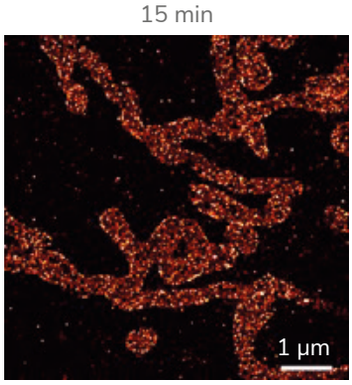
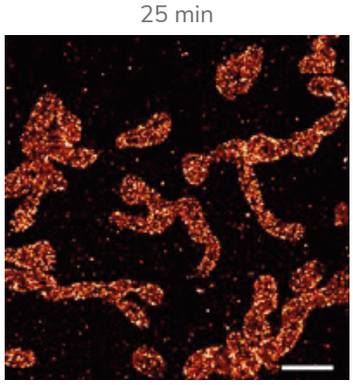
SETTING THE IMAGER CONCENTRATION

Low imager concentrations result in a high resolutions, but for sufficient sampling a higher number of frames needs to be recorded. Too high imager concentrations result in overlapping point spread functions, ultimately resulting in lower resolution.



SETTING EXPOSURE TIME

DNA-PAINT imaging can be performed with a range of camera integration times. The highest localization precisions are achieved with integration time is similar to the amount of time the imager is bound to the docking strand. Using the table below as a guide, camera integration time can be adjusted for your experimental needs.

	30 ms, 60 mW	50 ms, 50 mW	75 ms, 30 mW
Example frame (560 channel)	Measurement takes 5 min 	8.33 min 	12.5 min 
DNA-PAINT image	7.5 moin 	12.5 min 	18.75 min 
Zoom in	15 min 	25 min 	
Median fit precision	8.5 nm	9.0 nm	6.4 nm
NeNA precision	12.3 nm	11.9 nm	5.5 nm

NeNA precision is a localization precision algorithm cited in Literature 2.

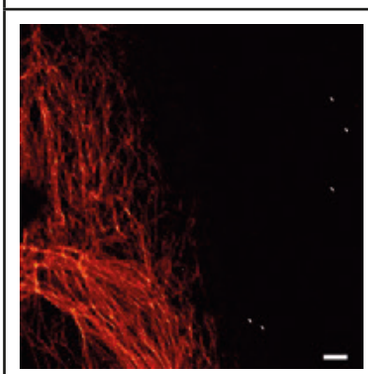
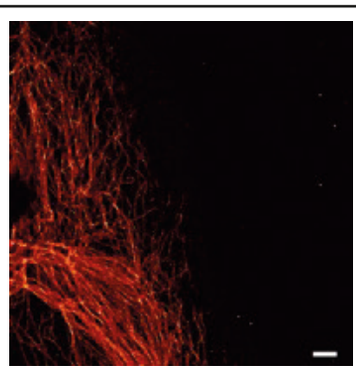
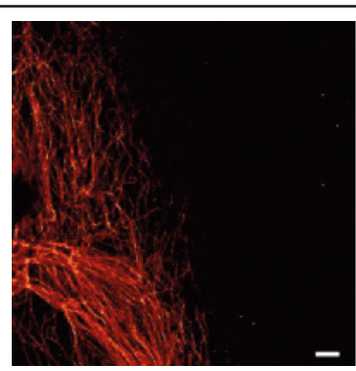
GOLD NANOPARTICLES AS DRIFT MARKERS

An important post-processing step for DNA-PAINT imaging is to compensate for stage drift that occurred during data acquisition. Drift correction can be performed in Picasso using either the localization events themselves or fiducials (in our case gold nanoparticles).
The drift correction based on localization events utilizes a redundant cross-correlation (RCC) algorithm. In this approach, the recorded movie is divided into several temporal segments, and super-resolution images are generated for each segment. Cross-correlating the resulting super-resolution images allows for determining the spatial shifts between the segments, which are then used to interpolate the drift. Another method for drift compensation in localization-based super-resolution microscopy involves using fiducial markers. These luminescent markers are typically observed in the same emission channel as the fluorescent signal. In Picasso, localizations from these fiducial markers can be selected for drift correction, as they can be assumed to originate from a single, point-like source.

The RCC drift correction can be applied in Picasso (https://github.com/jungmannlab/picasso) by navigating to **Render -> Post-processing -> RCC Drif**t Correction

For drift correction using gold nanoparticles, follow these steps:

1. Select a few gold nanoparticles using the **Pick** function, which can be accessed in the **Tools** menu (make sure to adjust the pick diameter in **Tools -> Tool Settings** to fully encircle the drifted gold nanoparticle).
2. Use **Tools -> Pick Similar** to automatically identify and select additional gold nanoparticles.
3. Finally, go to **Postprocess -> Undrift from Picked** to perform the drift correction.

Without drift corre	**RCC drift corrected**	**Drift correction using gold nanoparticles**
		

DILUTION FACTORS FOR VARIOUS IMAGER CONCENTRATIONS

Imager concentration	**Dilution factor** (stock concentration 1 µM)
5 nM	200x
1 nM	1,000x
500 pM	2,000x
250 pM	4,000x
100 pM	10,000x

LITERATURE

1. Schnitzbauer, Joerg, et al. “Super-resolution microscopy with DNA-PAINT.” Nature protocols 12.6 (2017): 1198-1228.
2. Endesfelder, Ulrike, et al. “A simple method to estimate the average localization precision of a single-molecule localization microscopy experiment.” Histochemistry and cell biology 141 (2014): 629-638.
3. Steen, Philipp R., et al. “The DNA-PAINT palette: a comprehensive performance analysis of fluorescent dyes.” Nature Methods (2024): 1-8.

GENERAL TERMS AND CONDITIONS

These Terms and Conditions for Sales of Products constitute a binding legal agreement between Massive Photonics GmbH ("Massive") and the customer or authored distributor ("Purchaser") refers to either the customer and/or authorized distributor unless alternative terms are set forth in a signed written agreement between Massive and the Purchaser.

1. **Payment.** Net amount of invoice is due within 30 days of invoice date unless the invoice states otherwise. Massive may require payment in advance if justified in Massive's opinion by Purchaser's financial condition or payment history.
2. **Shipment.** Unless specified by Purchaser, Massive will select a carrier of its choice. Purchaser will be responsible for costs of transportation, shipping and handling, and applicable importation fees. Shipments are insured at Purchaser's expense and made at Purchaser's risk. Massive may make partial shipments. Shipment dates are estimates only.
3. **Taxes.** All taxes will be added to product price and will be paid by Purchaser, unless Purchaser provides Massive with a valid tax exemption certificate.
4. **Delays.** Massive will not be liable for loss or damage of any kind resulting from delay or failure in fulfilling orders, if such delay or failure is directly or indirectly caused by factors beyond Massive's reasonable control.
5. **Limited Warranties and Remedies.** Massive warrants that products are free from defects in material and manufacture. Should any product prove defective, Purchaser must notify Massive promptly, no later than sixty (60) days from the date of the delivery, stating full particulars in support of Purchaser's claim. Massive will replace defective products or components, or apply an appropriate credit, upon Massive's investigation as may be necessary under the circumstances. The foregoing is Massive's sole obligation, and Purchaser's sole remedy, in the event of a claimed defect. UNDER NO CIRCUMSTANCES SHALL MASSIVE BE LIABLE FOR SPECIAL, INDIRECT, CONSEQUENTIAL OR OTHER DAMAGES, LOSSES, OR EXPENSES IN CONNECTION WITH THE USE OF OR INABILITY TO USE PRODUCTS FOR ANY PURPOSE; NOR SHALL MASSIVE'S LIABILITY IN ANY EVENT EXCEED THE PURCHASE PRICE PAID FOR THE PRODUCTS INVOLVED. The advice of Massive's technical staff may be made available to Purchaser; however, because Massive does not control or supervise use of products after sale, Massive does not warrant or guarantee such advice. NO WARRANTY OR GUARANTEE, EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY AS TO MERCHANTABILITY OR FITNESS FOR ANY PURPOSE, IS MADE EXCEPT AS SET FORTH HEREIN, AND MASSIVE EXPRESSLY DISCLAIMS ALL OTHER WARRANTIES.
6. **Cancellation and Changes.** Except as otherwise expressly provided in Section 4, above, an order once placed and accepted by Massive's confirmation is non-cancellable, unless Massive consents to such cancellation in writing and Purchaser pays any applicable cancellation charges.
7. **Research Use Only.** Purchaser acknowledges that products are for research use only; not for diagnostic use. Products have not been subjected to regulatory review or cleared by the United States Food and Drug Administration ("FDA"). Purchaser agrees not to use any product: (a) in any manner requiring FDA or other regulatory approval or registration, such as uses in diagnostic or therapeutic procedures; (b) for manufacturing; or (c) for quality control related to (a) or (b). The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial services of any kind, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration.
8. **Proper Use.** Purchaser agrees (a) not to use any product for a purpose other than its intended use; (b) to store products according to Massive storage requirements; (c) to use products only as specified in the applicable written instructions provided by Massive; and (d) to use products only for Purchaser's internal research use, and not for resale or commercial distribution. If Purchaser fails to comply with any of these term and conditions, Massive will be released from its performance and liability obligations to Purchaser and Purchaser will pay all costs or damages, including legal fees, incurred by Massive, caused by such failure to comply.
9. **No Reverse Engineering.** Purchaser agrees not to, nor allow any third party to, reverse engineer or otherwise attempt to discover the identity of a composition, nucleic acid sequence, or other component of a product.
10. **Governing Law.** These terms and conditions shall be governed and construed under the laws of the Federal Republic of Germany without reference to principles of conflicts of law. Any action concerning this sale must be filed in the courts where Massive has its place of business and each party submits to the exclusive jurisdiction of these courts.