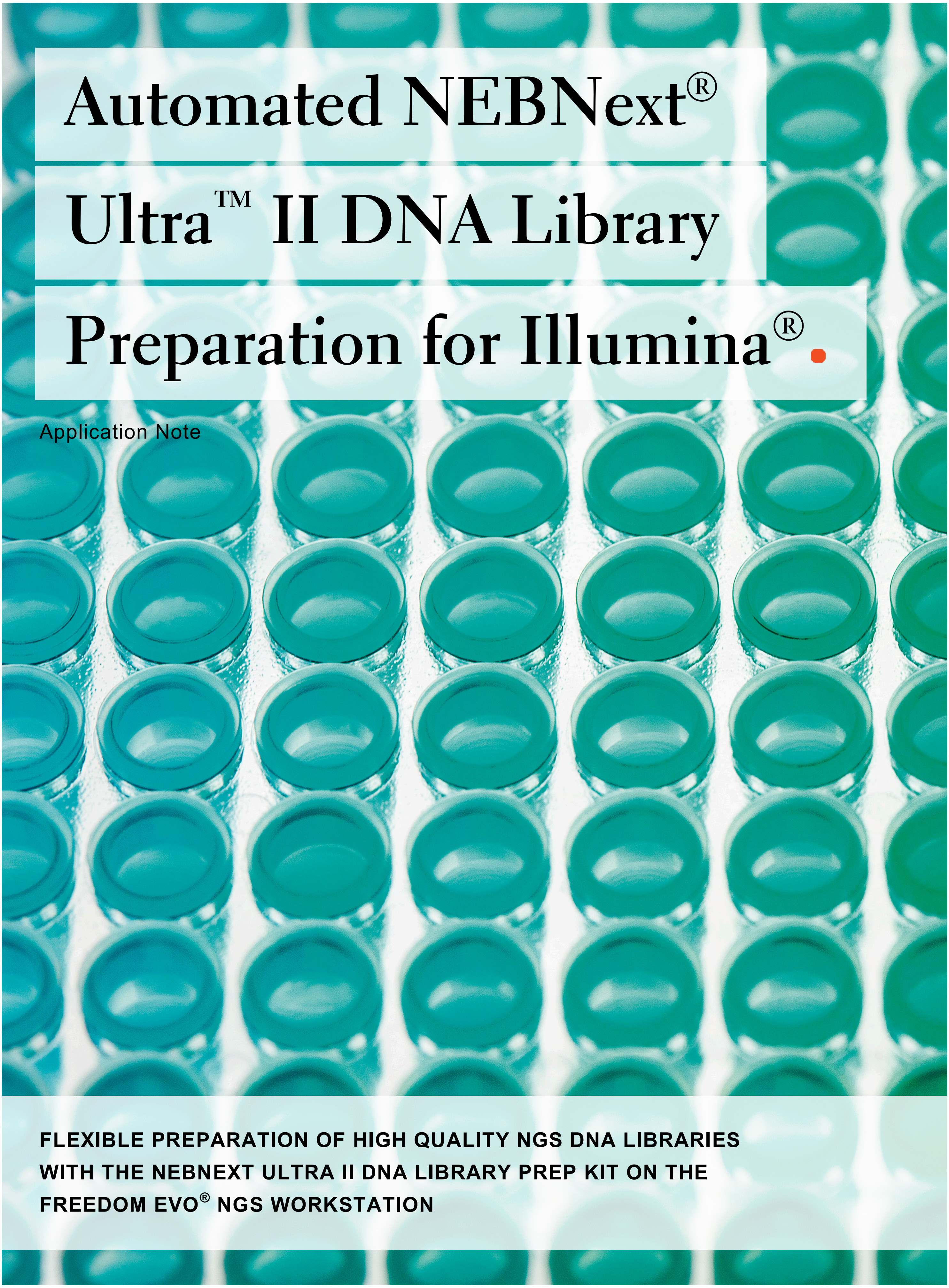
Automated NEBNext® Ultra™ II DNA Library Preparation for Illumina®.



Application Note

FLEXIBLE PREPARATION OF HIGH QUALITY NGS DNA LIBRARIES WITH THE NEBNEXT ULTRA II DNA LIBRARY PREP KIT ON THE FREEDOM EVO® NGS WORKSTATION

TECAN.

INTRODUCTION

Library preparation is a critical part of the next generation sequencing (NGS) workflow, and success requires the creation of high quality and yield libraries. The NEBNext Ultra II DNA library prep workflow combines the end repair and dA-tailing steps with minimal clean-up to reduce sample preparation time. It can accommodate 500 pg to 1 µg of input DNA, sheared by either mechanical or enzyme-based methods. Up to 96 libraries can be prepared per run, and multiplexed using, for example, the NEBNext Multiplex Oligos for Illumina.

Here we describe a flexible, automated NEBNext Ultra II DNA library preparation protocol on the Freedom EVO NGS workstation (Figure 1). This protocol allows highly reproducible library preparation from a wide range of input DNA concentrations, as well as FFPE samples, offering flexible processing of 1 to 96 samples with minimal user intervention. A user-friendly TouchTools™ graphical interface guides users through option selection and workable set-up, reducing training needs and operator-to-operator variability.

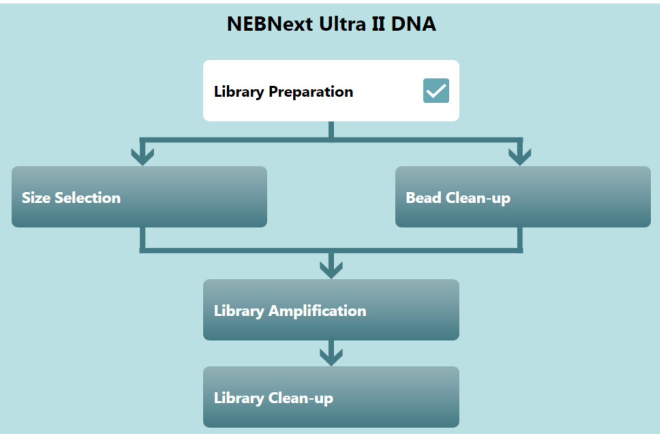


Figure 1: Simple selection of next protocol step with TouchTools user interface.

MATERIALS AND METHODS

A Freedom EVO NGS workstation (deck layout shown in Figure 3) performs automated liquid handling for end repair and adenylation, adapter ligation, bead clean-up or size selection, PCR set-up, normalization and pooling. PCR amplification and long-term incubation steps are performed in an offline thermocycler to optimize the workflow.

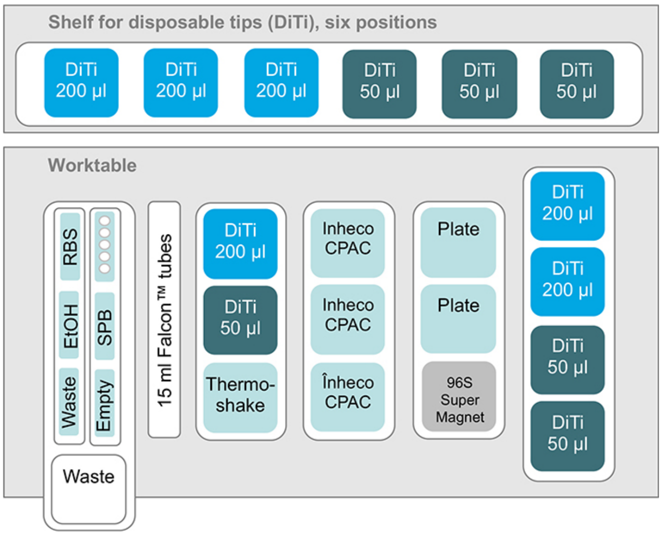


Figure 3: Deck layout of the Freedom EVO NGS workstation set up for NEBNext Ultra II DNA library preparation.

The platform uses advanced air displacement pipetting technology, enabling precise eight-channel pipetting from 1,000 µl down to just 0.5 µl. It also includes three INHECO CPAC thermal devices – allowing reagents to be kept cool and providing optimal conditions for the enzymatic steps – an INHECO Thermoshake heated shaker, a 96-position magnetic plate separator (Alpaqua® 96S Super Magnet) and a Robotic Manipulator Arm. In addition, the compact worktable offers storage space for up to 12 tip boxes, allowing longer unattended runs.

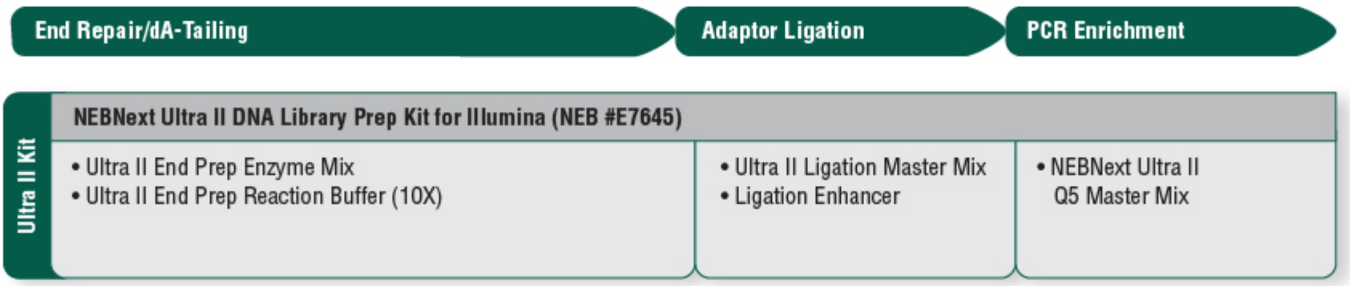


Figure 2: NEBNext Ultra II DNA library prep workflow.

Genomics DNA library preparation was done according to the kit manufacturer's recommendations. Enzymatic reaction set-up, bead clean-up and size selection were all performed on the work deck. End repair, dA-tailing and PCR amplification steps were performed in an offline thermocycler.

High input libraries were prepared from 200 ng of fragmented human genomic DNA and yeast DNA in a single plate. Size-selected library preparation (200 bp insert size) followed the recommended DNA:bead ratios, and amplified with six PCR cycles. Low input libraries were prepared from 2 ng, 1 ng and 500 pg fragmented human genomic DNA in a single plate. Size selection is not recommended for input amounts below 50 ng.

ANALYSIS AND RESULTS

Library QC

Library size distribution and yield was assessed on a Caliper LabChip GX System (software v4.1). As expected, the size-selected (high input) libraries have a narrow size distribution with a mean size distribution of 320-340bp, which corresponds to a 200bp fragment insert size. The size distribution of the non-size selected (low input) libraries correlates to the size distribution of the input DNA (Figure 4A). Automation achieves consistently high library yield (Figure 4B).

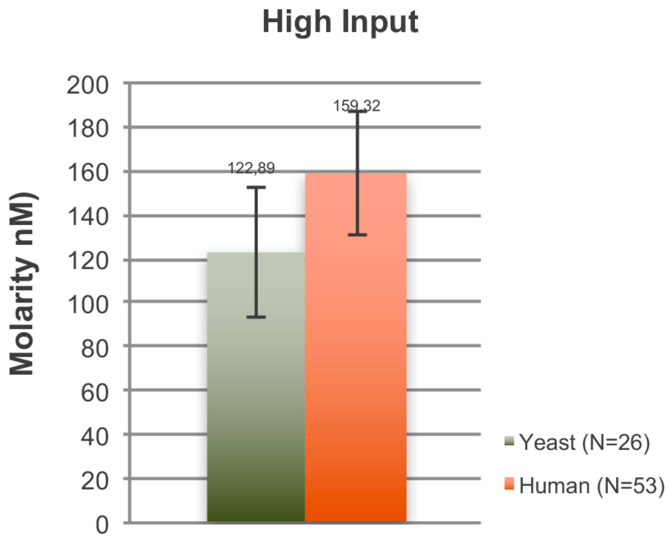


Figure 4b: Consistently high yields of libraries prepared with 200 ng fragmented DNA and only 6 PCR cycles.

Mapping sequencing performance

Three randomly selected high and low input human DNA libraries were sequenced on an Illumina NextSeq® sequencer, generating approximately 400 million (2x75bp) paired-end reads per library, and 700 million (2x75bp) paired-end reads per library, respectively. Reads were mapped to GRCh37 reference using Bowtie 2.2.4 with standard end-to-end settings (Table 1A and Table 1B).

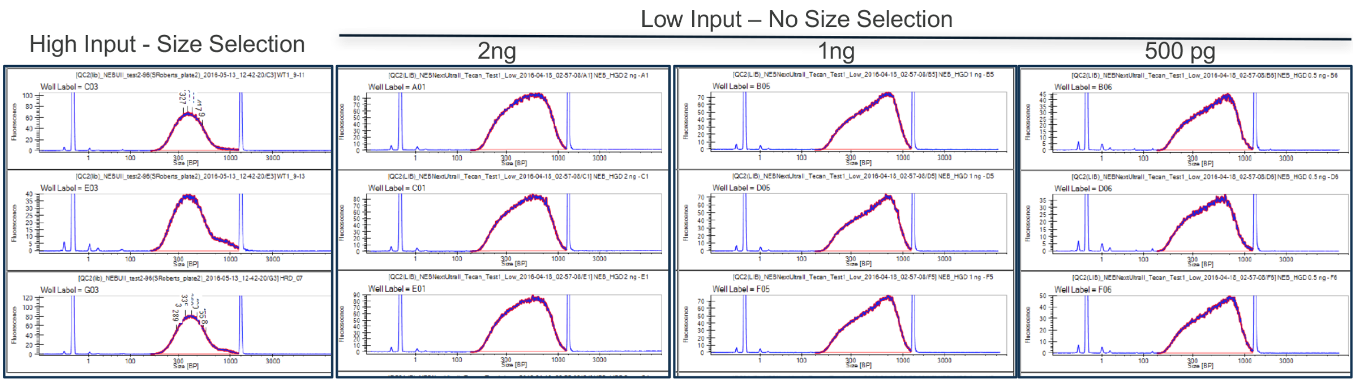


Figure 4a: Library size distribution for high input libraries with size selection and low input libraries with no size selection.

Replicate	Total Reads	% Mapped	% Mapped in Pairs	% Chimeras	% Adapter
Replicate 1	376,701,432	97.93%	99.03%	1.00%	0.001%
Replicate 2	383,870,510	97.74%	99.12%	0.47%	0.001%
Replicate 3	399,541,644	97.73%	99.11%	0.46%	0.001%

Table 1A: Human High Input Libraries

Input	Total Reads	% Mapped	% Mapped in Pairs	% Chimeras	% Adapter
2ng	635,939,854	97.79%	99.06%	0.96%	0.001%
1ng	734,872,382	97.83%	99.11%	0.83%	0.002%
500pg	707,163,276	97.77%	99.12%	0.78%	0.003%

Table 2B: Human Low Input Libraries

Thirteen randomly selected yeast DNA libraries were sequenced on an Illumina HiSeq® 4000 sequencer to generate 13-20 million (2x150bp) paired-end reads per library. Adapter-trimmed sequences were mapped to yeast reference genome, and duplicate reads were removed (Table 1C). The high percentage of aligned reads and low percentage of chimeras and adaptor-mapping reads indicate that the automated protocol enables the generation of high quality sequencing data, even with very low input amounts.

Replicate	Total Reads	% Mapped	% Mapped in Pairs
1	15,654,084	95.75	90
2	13,264,430	95.95	90.64
3	14,011,158	96.65	91.74
4	14,491,384	96.95	92.67
5	15,397,932	95.49	89.73
6	15,295,366	96.44	91.36
7	15,320,706	96.41	91.64
8	14,465,084	94.99	86.48
9	14,559,392	97.11	90.35
10	15,919,990	96.65	90.86
11	15,007,054	97.23	93.25
12	20,869,198	97.5	93.71
13	13,938,170	95.76	88.2

Table 1C: Yeast libraries sequencing performance

Uniformity of coverage

GC coverage was calculated using Picard's CollectGCBiasMetrics (v1.117) (Figure 5A and Figure 5B). The results show that the automated Ultra II DNA libraries have very uniform coverage across the range of GC content.

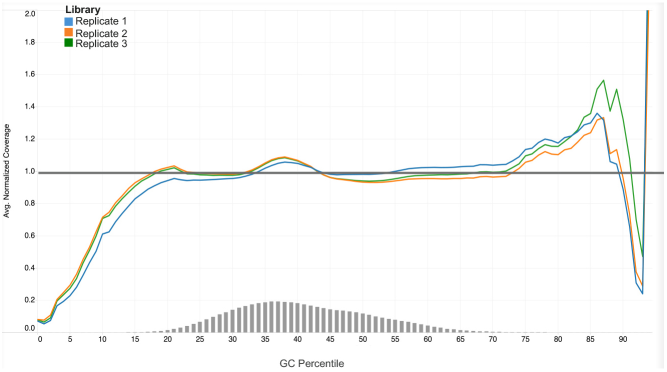


Figure 5A: Very uniform coverage across for the range of GC content for high input libraries.

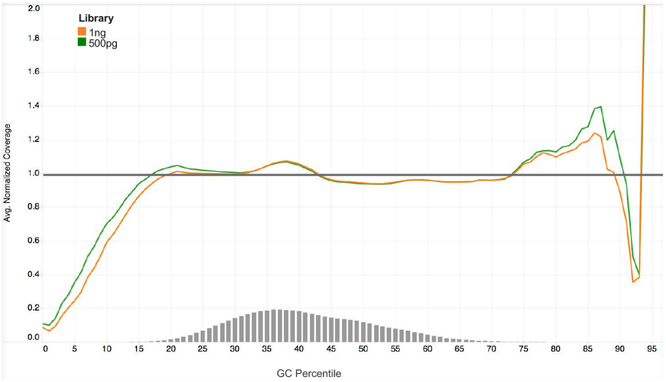


Figure 5B: Uniform coverage across for the range of GC content for low input (500pg & 1ng) libraries.

SUMMARY

The results presented in this application note demonstrate that automation of the NEBNext Ultra II DNA library preparation workflow on the Freedom EVO NGS workstation provides a fast and efficient solution for library preparation. This set-up enables generation of high quality libraries from a broad range of input samples – from 500 pg to 1 µg – while reducing the number of PCR cycles required.
The TouchTools interface ensures a user-friendly experience, reducing training needs, minimizing the risk of manual errors and increasing process reproducibility. Combined with flexible processing of up to 96 samples (with or without size selection) and a number of safe stopping points within the protocol, this set-up provides highly reproducible, sequencing-ready libraries to suit a variety of laboratory workflows.

LEARN MORE

To obtain the automated NEBNext Ultra II DNA library preparation protocols for the Freedom EVO NGS workstation discussed in this application note, contact your Tecan sales representative, visit www.tecan.com/ngs or contact NGSprep@tecan.com.

To learn more about NEBNext II DNA library preparation, visit www.NEBNextUltraII.com.

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Application Note for the NEBNext Ultra II DNA Library Prep kit: https://www.neb.com/products/e7645-nebnext-ultra-ii-dna-library-prep-kit-for-illumina#pd-application-notes

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About the authors

Dr. Daniela Munafo is a field applications scientist at New England BioLabs, Inc. She holds a PhD degree in cellular and molecular biology from university of Buenos Aires, Argentina and a postdoctoral training from the Scripps Research Institute, La Jolla, California.

Dr. Enrique Neumann is an application specialist at Tecan Switzerland. He studied Biology at the University of Santiago de Compostela, Spain. During his PhD at the University of Edinburgh, he focused on the molecular processes in plant cells. He joined Tecan in 2015 and focuses in the development and support of genomic applications for Tecan's liquid handling platforms.

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Australia +61 3 9647 4100 **Austria** +43 62 46 89 33 **Belgium** +32 15 42 13 19 **China** +86 21 28 98 63 33 **Denmark** +45 70 23 44 50 **France** +33 4 72 76 04 80 **Germany** +49 79 51 94 170 **Italy** +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 174 **Singapore** +65 644 41 886 **Spain** +34 93 490 01 74 **Sweden** +46 31 75 44 000 **Switzerland** +41 44 922 89 22 **UK** +44 118 9300 300 **USA** +1 919 361 5200 **Other countries** +41 44 922 8125

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