

## Product Survey: Genotyping Instruments

# Every Single Nucleotide Counts

Genotyping instruments are usually operated by specially trained members of a lab. In these days of scrapie and BSE however, even flock masters and artisan cheese producers must have a basic knowledge of genotyping.

If you are an enthusiastic cheese chomper and have ever travelled through the picturesque and breathtaking landscapes of the Picos de Europa in the mountainous northwestern region of Spain, you should not miss out on a visit to a local market place in one of the small villages. Standing behind small market stalls, the local dairy farmers sell “Queso de Cabrales”, Spain’s most famous blue cheese, named after the Asturian town Cabrales where it has been made since time immemorial. If you’re lucky the cheese will be wrapped in dry chestnut leaves, however, this traditional presentation is against current regulations and has, subsequently, been substituted by profane aluminium foil. Queso de Cabrales is a cheese for “tough guys” since its pungent taste could literally curl your tongue as well as your toes! Although, blue cheese aficionados will undoubtedly relish its delicious aroma of mould and roasted-nuts.

Usually Queso de Cabrales is traditionally made from cows’ milk blended with goat and sheep milk. The latter being the point at which genotyping and genotyping instruments come into play (no don’t worry, you are still reading *Lab Times* and not a gourmet magazine!). Today, even the flock master of a herd of sheep in the most remote Spanish mountain valley will have heard of genotyping. Currently, millions of sheep all over the EU are checked by genotyping for their susceptibility to scrapie, a transmissible spongiform encephalopathy (TSE) affecting the sheep’s central nervous system.

### Genotyping instead of killing

In sheep that have been exposed to the infectious agent of scrapie, the likelihood of progression to disease is very strongly linked to at least three polymorphisms at positions 136 (A or V), 154 (R or H) and 171 (Q, R or H) in the prion protein encoding PrP gene. At codon 136, e.g., valine (V) is linked to scrapie susceptibility, while alanine (A) is linked to resistance. Out of the 12 possible polymorphisms, however, only five occur with any frequency. These five alleles combine to give a total of 15 PrP genotypes that

differ widely in their susceptibility to scrapie. Sheep having the ARR/ARR genotype are almost completely resistant, while animals with the combination VRQ/VRQ (Q stands for glutamine) are extremely susceptible to scrapie. Instead of killing a whole flock when just one of the individuals shows scrapie symptoms, flock masters can separate susceptible from resistant sheep after genotyping to prevent the progression of scrapie. They may also select male ARR-sheep for breeding to increase the flock’s scrapie resistance.

### Plethora of genotyping techniques

Several genotyping techniques are currently applied in order to determine PrP alleles: Classical PCR-restriction fragment length polymorphism (RFLP)-typing, denaturing gradient gel electrophoresis (DGGE), PCR-single-strand conformational polymorphism (PCR-SSCP), Quantitative PCR (Taq-Man), Mass Spectrometry, hybridisation with allele-specific oligos (DNA-Chips) or primer extension to name but a few. The most accurate method, however, is direct sequencing of the region containing the codons at positions 136, 154 and 171.

This is usually done with special genotyping instruments or sequencers that are also widely used in other genotyping studies. According to a market survey published in 2005 by BioInformatics LLC, Applied Biosystems “ABI-PRISM” Sequencers are the most commonly used SNP genotyping instruments. Automatic sequencing with the ABI-PRISM devices is based on Frederick Sanger’s dideoxy sequencing method, coupled with fluorescent dye labelled primers or dideoxynucleotide triphosphates (dye terminators) as signal sources. The sequencers detect the fluorescence coming from the four different dyes used to identify the A, C, G and T extension reactions after separating the DNA fragments in a capillary. In



Engages every one of your taste buds:  
Queso de Cabrales

Applied Biosystems, top models 3730 and 3730xl up to 96 capillaries operate in parallel. GE Healthcare’s MegaBACE Sequencers work with a similar fluorescence-based technique. The major difference to other sequencers using side-illumination lasers is a confocal laser scanning optic, which, according to GE’s fact sheet, provides “a better sensitivity and a consistent signal across all capillaries.”

An alternative method to the classical Sanger-sequencing is pyrosequencing, a technique developed by Pal Nyren at the Royal Institute of Technology (KTH) in Stockholm during the nineties. As Nyren puts it in a *Methods in Molecular Biology* review abstract on the history of pyrosequencing, the basic concept of pyrosequencing is, “to follow the activity of DNA polymerase during nucleotide incorporation into a DNA strand by analysing the pyrophosphate released during the process.” Nyren’s concept sounds simple, however, as is often the case in biochemistry or molecular biology, the road from an idea to a feasible method may be long and stony. It took him 12 years from his initial idea in 1986 to his pyrosequencing patent in 1998. These days, the Swedish company Biotage AB, which emerged from Pyrosequencing AB, founded by Pal Nyren and his KTH colleague Mathias Uhlen in 1997, delivers pyrosequencing systems.

HARALD ZÄHRINGER

## Genotyping Instruments

Company	Type of Instrument	Applications	Time to results	Performance	Miscellaneous Specialities Generally	Price [EUR]
<b>Affymetrix UK</b> Voyager, High Wycombe, UK Contact: Marcus Hausch Marcus_hausch@affymetrix.com	GeneChip Scanner 3000 Targeted Genotyping System	Whole genome association studies, Custom genotyping / fine panning, Copy number analysis, Linkage, Resequencing, CHIP on Chip, Exon analysis and Expression Profiling	3 days	Routinely call rates >99% and accuracy of >99.5% for genotyping products	- Association studies - Copy number - Cytogenetics	-
<b>Applied Biosystems</b> Warrington, UK  http://europe.appliedbiosystems.com  http://www.allsnps.com	Taqman Allelic Discrimination System 7900HT Fast Real Time PCR (Fast 384 and 96 well blocks)	Genotyping, SNP, MNP, In-Del (up to 6 bp) Copy Number Analysis DNA quantitation Instrument also used for RNA analysis: GeneExpression, MicroRNA analysis and Taqman GeneExpression Array Analysis	2 hours, sample to genotype in real time mode 2 minutes, end-point read (requires external thermal-cyclers to complete the reaction)	200.000 GT/ day in end point mode (requires external thermal-cyclers to complete the reaction) 4500 GT/day in real time mode	- 4,5 Million Human TaqMan Pre-Designed SNP Genotyping Assays - 2200 TaqMan Drug Metabolism Genotyping Assays - 10.000 Mouse TaqMan Pre-Designed SNP Genotyping Assays, Custom TaqMan SNP Genotyping Assays for any SNP, any species, Taqman Genotyping MasterMix	0,55-0,15 / GT (Reaction costs at 5 ul reaction volume in 384 well block)
	Taqman Allelic Discrimination System 7300/7500 Real Time PCR (96 well block); 7500 Fast RT PCR (Fast 96 well block)	Genotyping, SNP, MNP, In-Del (up to 6bp) Copy Number Analysis DNA quantitation Instruments also used for RNA analysis: GeneExpression, and MicroRNA analysis	See above	40.000 GT/ day in end point mode (requires external thermal-cyclers to complete the reaction) 1.000 GT/day in real time mode	See above	1,50-0,45 / GT (Reaction costs at 15 ul reaction volume in 96 well block)
	SNaPshot Multiplex Genotyping System 310, 3130, 3130xl, 3730 and 3730xl Genetic Analyzers	Multiplex Genotyping (1-10 plex) BAC fingerprinting Methylation Analysis Instruments also used for sequencing and other fragments analysis applications	8 hours, sample to genotype	48 to 40.000 GT/day (depends on the automation of the laboratory and the number of capillaries of the genetic analyzer)	- SNaPshot Multiplex Kit - SnaPshot Primer Focus Kit	0,15 /GT (Reaction costs, 10 plex, DNA extraction not included)
	SNPlex Genotyping System Applied Biosystems 3130xl, 3730 and 3730xl Genetic Analyzers	Multiplex Genotyping (12-48 plex), SNP, MNP, In-Del (up to 6bp) Instruments also used for sequencing and other fragments analysis applications	14 hours (2 days), sample to genotype	4500 to 400.000 GT/day (see above)	- 96 and 384 well protocols - Manual to different levels of automation supported - SNPlex custom design pipeline (accepts rs ids for up to 20 different species) - SNPlex 4K Linkage Mapping Set	0,05 /GT (Reaction costs, 48 plex, DNA extraction not included)
<b>Biotage AB</b> Uppsala, Sweden www.biotagebio.com Contact: Maria Hjortsmark Phone: +46 18 565900 maria.hjortsmark@eu.biotage.com	PyroMark ID System	SNP association studies. SNP validation. Allele frequency quantification. Complex mutation analysis.	96 samples can be genotyped in 10 minutes	Analyze one assay with up to 96 individual samples or up to 96 different assays in one plate. The system requires 20 µl of PCR product.	- SNP genotyping - Tri/tetra allelic SNP/mutations - InDels - Multiple contiguous mutations	On request
	PyroMark MD System	See above	See above	Analyze one assay with up to 96 individual samples or up to 96 different assays in one plate. High sensitivity light detection system, requires 5-10 µl of PCR product. PyroMark MD Automated version has a built-in robotic mechanism to transfer up to 10 96-well plates for unattended operation.	See above	On request
<b>Eppendorf</b> Hamburg, Germany www.eppendorf.de Contact: Eppendorf Application Hotline Phone +49 1803 666 789 application-hotline@eppendorf.de	epMotion 5070	Real-time-PCR set-up	10 minutes for 96 samples set-up	Precise pipetting down to 1µl Pipetting range from up to 1000µl Processes Microplates and PCR plates up to 384 wells. Eliminates variations between experiments	- Compact control panel & PC version available - Deck control function, fully enclosed system	17.000,- (Starting from)
	epMotion 5070 & 5075	Automated pipetting for SNPlex	Refer to ABI protocol	Fully automated pipetting of all liquid handling steps	See above	As above
	epMotion 5070 & 5075	Automated pipetting for iPLEX	Refer to Sequenom protocol	Fully automated pipetting of all liquid handling steps	See above	As above
	epMotion 5075LH	High throughput real time PCR set up or magnetic bead based nucleic acid purification	10 Minutes for 96 samples set-up Purification depends on the kit used	Precise pipetting down to 1µl Pipetting range from up to 1000µl Processes Microplates and PCR plates up to 384 wells. Eliminates variations between experiments. Purification depends on the kit used	See above	35.000,- (Starting from)

Genotyping Instruments						
Company	Type of Instrument	Applications	Time to results	Performance	Miscellaneous Specialities Generally	Price [EUR]
<b>Eppendorf</b> Contact see page 49	epMotion 5075LH	Vacuum based nucleic acid purification	10 Minutes for 96 samples set-up Purification depends on the kit used, e.g. Sample volume: 200 µl Preparation time: 2.5 hours with QIAamp DNA Blood BioRobot MDx	Precise pipetting down to 1µl Pipetting range from up to 1000µl Processes Microplates and PCR plates up to 384 wells. Eliminates variations between experiments. Purification depends on the kit used Typical yield: 3 -6 µg / 200µl with QIAamp DNA Blood BioRobot MDx	- Compact control panel & PC version available - Deck control function, fully enclosed system - Fully integrated vacuum	35.000,- (Starting from)
<b>Febit Biotech</b> Heidelberg, Germany www.febit.de or www.geniom.com Contact: Russell Golson Phone: +49 6221 6510-330 russell.golson@febit.de	Geniom One Technology Platform Flexible Microarray synthesizer & analyzer	microRNA Chip-CHIP Methylation profiling Resequencing SNP-Typing Bacteria/Virus Typing	Array production within 10 hours / 8 arrays Array processing to final results 6 - 18 hours	6-10.000 features per array 2 colour Reproducibility 0.96-0.99 Pearson	- Ready to use array designs - Custom array designs - Completely automated - Hybridization based - Enzyme enhanced (allele specific primer extension, mini sequencing)	220.000,-
<b>GE Healthcare Europe</b> München, Germany www.gelifesciences.com Contact: Carsten Schlüter Phone +49 514584672 carsten.schluter@ge.com	MegaBace 750/1500 CPO	Genotyping Sequencing SNP-analysis	30 - 100 min	Up to 1000+ Bp	- Capillary electrophoresis instrument - Deeper multiplexing - Many applications possible - High accuracy, 1000+ bp possible - Complete software package	55.000,- to 80.000,-
<b>Genetix</b> Queensway, UK www.genetix.com Contact: Steve Hawkins Phone +44 1425 624 609	QArray Mini	DNA Microarrays, Protein arrays, Oligo saccharide arrays, CGH arrays, Microplate arrays, Reverse transfection, RNAi analysis, SAR libraries	-	Array accuracy and High precision	- Genetix proven, world leading reputation in microarray systems	57.000,-
<b>Hamilton Robotics</b> Martinsried, Germany www.hamiltonrobotics.com Contact: Jörg Katzenberger Phone +49 89 552 649 0 infoservice@hamiltonrobotics.com	Automated liquid handling platform Microlab STAR line	Extremely wide range of applications for Genotyping: Automated DNA purification from bacteria, blood and tissue, (Real Time) PCR and sequencing reaction set-up, Affymetrix; Agilent and Illumina sample preparation on the same platform.	Depending on application and kit	Depending on configuration: up to 16 independent pipetting channels can be combined with 96, 384 or nanopipetting modules on one or two arms	- Nano- and Microliter pipetting on one compact Workstation - "Monitored Air Displacement" pipetting technology - Flexible Software - Asymmetrically spreadable pipetting channels - STARlet workstation can be converted on site to a STARplus by means of an extension module	Depending on configuration
<b>KBiosciences</b> Hoddesdon, UK www.kbiosciences.co.uk Contact: Andy Higgs Phone: +44 7736 065239 andy.higgs@kbioscience.co.uk	Thermal Cycler DT-24 Mini Duncan	SBS format, 96, 384, 1536 wells, water bath based technology	Less than 2 hours for 24 plates Up to 37,864 simultaneous reactions	0.5ul to 1ml volumes Full data tracking LIMS compatible; Temp stability of +/- 0.1°C; Unique very high well-to-well uniformity across the plate	- One-step batch basket loading	75.000,-
	Thermal Cycler DT-108 Super Duncan	SBS format, 96, 384, 1536 wellswater bath based technology	Less than 2 hours for 108 plates. Up to 165,888 simultaneous reactions	-	See above - KBiosciences also offer reagents for SNP Genotyping as well as a service, and also a LIMS system	115.500,-
<b>LTF Labortechnik</b> Wasserburg Germany Contact: Rudolf Walsler Phone +49 8382-98520 info@labortechnik.com www.labortechnik.com	Rotor-Gene 6000	SNP Genotyping Mutation-screening Mutation scanning Haplotyping DNA fingerprinting Gene expression methylation analysis	60 min	In tube real time detection, High speed runs with low probe concentration Alternatively (new): High speed runs with HRM (high resolution melt) Analysis	- Ultimate temperature uniformity +/- 0,01°C - High precision temperature control (0,1°C ramping, Resolution +/-0,02°C) - Up to 100 samples flexible Volume (5µl - 70 µl) - Single copy detection - 6-plex capability - High speed data acquisition	from 29.500,-
<b>Roche Diagnostics</b> Mannheim, Germany www.roche-applied-science.com Contact: Phone +49 621-759-8568 mannheim.biocheminfo@roche.com	LightCycler480	6 channel Real-Time PCR platform 96- or 384 well format	-	-	- Fast PCR with online detection: 40 min for 384-well format; 1 h for 96-well format - High flexibility in choice of detection formats - Accurate data capture across the entire plate - Bar Code Reader	On request
	LightCycler 2.0	See above 32 capillaries	-	-	- Fast PCR with online detection: 20 min - High flexibility in choice of detection formats - 20µl or 100µl capillaries - Kits for RT-PCR and PCR available	On request

## Genotyping Instruments

Company	Type of Instrument	Applications	Time to results	Performance	Miscellaneous Specialities Generally	Price [EUR]
<b>Roche Diagnostics</b> Contact see page 50	LightCycler 1.5	3 channel Real-time PCR system 32 capillaries	-	-	- 20µl capillaries - Fast PCR with online detection: 20 min - Kits for RT-PCR and PCR available	On request
<b>Stratagene</b> www.stratagene.com Contact: Cedric Lemarie Phone +31 203125606 cedric.lemarie@stratagene.com	Mx3000P QPCR System	Genotyping and SNP analysis Allelic discrimination Gene Duplication and other DNA quantification	Standard protocol: about 1H30	96 well format. Multiplexing capabilities (up to 4 dyes), Flexible Set up and Analysis Software, Melting curve analysis with automatic melting temperature (Tm) calculation.	- User selectable filter choices - Open platform supports all standard QPCR chemistries	27.995,- (incl. PC)
	Mx3005P QPCR System	See above	See above	96 well format. Multiplexing capabilities (up to 5 dyes), Flexible Set up and Analysis Software, Melting curve analysis with automatic melting temperature (Tm) calculation.	- User selectable filter choices - Independent filter control - Open platform supports standard and newly emerging QPCR chemistries	29.995,- (incl. PC)
<b>Tecan</b> Crailsheim, Germany www.tecan.de Contact: Jürgen Fetzer Phone +49 7951 9417618 info.de@tecan.com	Microarray Laser ScannerLS Reloaded	Microarray and microfluidic chips using fluorescence for SNP and copy number detection	Depending on resolution, area size, number of dyes: 2-30 min	45,8 Mio pixel per 22x75 mm image size, 6 µm resolution, 16 bit digitalizing, 2 x 90 Mb/6 min	- Confocal /Non-confocal scanning modes with autofocus - Slides, Microplate formats and MiniGels - Autoloader for high throughput - QC tool kit LaserCheck to check the complete system - Scattered light scanning after spotting QC	73.000,- up to 170.000,- (depending on configuration and options)
	Hybridisation Station HS 400 / 4800 Pro	Automated Hybridisation of genotyping slide biochips	Dependent on defined protocol: 20 min to 24 hrs	Temperature control from 4 - 99°C; Temperature change >6°C/min; Sample volume 110 - 130 µl (single Chamber) 20 x 51 mm - 21 x 63.5 mm; or 60µl (dual chamber) 20 x 27.5 mm - 21 x 28.3 mm, Up to 4 and up to 12 slide units, extension up to 48 slides per run	- ABS (Active bubble Suppression) - Active mixing of the sample during hybridisation - Active cooling and heating - Wash process implemented - OSND (on-board slide nitrogen drying)	from 32.400,-