V. DISCUSIÓN GENERAL

En esta tesis se ha abordado el estudio de la presentación de péptidos por moléculas de MHC de clase II utilizando diversas estrategias proteómicas. En primer lugar, el trabajo se centró en la caracterización de los repertorios peptídicos asociados a una APC y a células epiteliales que expresaban moléculas de MHCII, secuenciándose más de 300 ligandos peptídicos, incluyendo diversos péptidos presentados *ex vivo* en tiroides afectados por una patología autoinmune. La mayor parte de los trabajos publicados hasta el momento describían los repertorios presentados en líneas celulares linfoblastoides (Lippolis 2002; Rammensee 1995) por lo que el análisis de los ligandos asociados a células epiteliales es algo novedoso, que aporta información relevante sobre las diferencias que existen entre APC profesionales y células epiteliales. Además, el elevado número de proteínas identificadas a partir de las secuencias caracterizadas ha proporcionado información muy valiosa acerca del proceso de presentación. En este trabajo se han identificado más de 100 proteínas diferentes, caracterizándose 130 epítopos de unión a MHCII. La técnica de nanospray acoplado a la espectrometría de masas en tándem en un instrumento de trampa iónica ha permitido identificar no solo las proteínas mayoritarias en estos extractos, que son probablemente las que generan el mayor número de señales secuenciables en los espectros, sino también otras menos abundantes que con métodos menos sensibles no hubieran sido detectadas. Así, entre las técnicas utilizadas en esta tesis, el nanospray ha mostrado ser el método más idóneo para la caracterización exhaustiva de estos repertorios debido a su sensibilidad y su capacidad para la realización de espectrometría de masas en tándem múltiple. En este sentido debe indicarse que la identificación de los ligandos asociados a DR a partir de tejido tiroideo *ex vivo* no hubiera podido realizarse mediante cualquiera de las otras técnicas utilizadas. En definitiva, las limitaciones tecnológicas determinan en buena parte nuestra capacidad para el análisis de estos repertorios especialmente en muestras muy complejas como es el caso de los tejidos autoinmunes. Afortunadamente, las prestaciones de la espectrometría de masas han aumentado de forma continuada desde su primera aplicación a la secuenciación de péptidos en 1968 (Vilkas 1968) por lo que es de esperar que estos repertorios podrán ser estudiados de forma mucho más exhaustiva en un próximo futuro. En este sentido técnicas recientemente aparecidas, como las fuentes de electrospray basadas en chips de vidrio o silicio, que permiten el análisis automatizado de decenas de muestras en analizadores extremadamente sensibles, como el de resonancia ciclotrónica de iones (FT-ICR), ofrecen ya una capacidad de análisis muy superior a la disponible en estos estudios. Actualmente, es también posible la deposición continuada del eluido cromatográfico directamente en placas para MALDI de modo que pueden evitarse los problemas inherentes a la etapa de fraccionamiento por HPLC de la muestra y los repertorios pueden ser analizados directamente mediante MALDI-TOF/TOF (Zhen 2004). A pesar de la menor eficiencia de esta técnica en la obtención de secuencias comparado con el nESI-ITMS/MS, la velocidad del análisis, el alto número de espectros obtenidos y la automatización que permite esta estrategia puede hacerla ventajosa en muchas aplicaciones.

En una segunda parte del trabajo se ha estudiado el efecto de la transfección de las moléculas HLA-DR, HLA-DM y Ii sobre el proteoma de las células epiteliales y se han identificado diversas proteínas implicadas en este proceso. Se ha mostrado que la transfección de estas moléculas parece estar relacionada con un aumento de la actividad metabólica de estas células, aunque sin producir cambios drásticos en su proteoma global y se ha sugerido que estos cambios podrían implicar a proteínas que, como la oligopeptidasa *Thimet*, podrían intervenir directa o indirectamente en la presentación y ser responsables de parte de las diferencias observadas en los repertorios. Asimismo, se han identificado 30 proteínas que se encuentran asociadas directamente o a través de otras moléculas con MHCII en la célula. Los análisis de expresión diferencial mostraron que la cantidad de estas proteínas unidas a la molécula de MHCII dependía de la expresión de la chaperonas Ii y DM.

A continuación se comentan las principales conclusiones de estos estudios:

1.- Los repertorios peptídicos asociados a moléculas de MHCII HLA-DR4 en células epiteliales endocrinas son diferentes a los generados en células presentadoras profesionales que presentan el mismo alelo y derivan de proteínas distintas. Esto puede estar relacionado con las características propias de la célula epitelial y con su metabolismo especializado.

Mientras que en las células B-LCL el 80% de las proteínas origen identificadas pertenecían a proteínas exógenas, situadas en la membrana celular o residentes en vesículas endocíticas, todas ellas de fácil acceso a la vía endocítica, en las células epiteliales este valor disminuyó hasta el 57% (DR4IiDM), incrementándose el número de péptidos que provenían de proteínas situadas en el citosol y en vesículas secretoras. Estas diferencias cuantitativas en la presentación de ligandos individuales dan información sobre el estado de las células presentadoras y muestran claramente las diferencias funcionales de ambos tipos celulares. Este dato adquiere especial relevancia en el caso de las enfermedades autoinmunes ya que muchos de los autoantígenos descritos en estas patologías son proteínas específicas de las células afectadas, como el caso de la TPO o de la tiroglobulina en la enfermedad de Graves-Basedow (Rapoport 2002). Precisamente un péptido de esta última proteína ha sido detectado por primera vez en este trabajo en tiroides afectado por esta enfermedad.

La especificidad asociada a cada alelo de DR queda patente cuando se compara el repertorio identificado en las células B-LCL con los reportados en la literatura (Chicz 1993; Lippolis 2002; Rammensee 1995). Existe un alto grado de similitud entre los distintos datos y, a pesar de que la líneas celulares y las condiciones de cultivo son diferentes, muchos de los péptidos identificados son los mismos. Sin embargo, la semejanza entre los repertorios de las líneas B-LCL y RIN-

DR4IiDM, ambas homocigotas para el mismo alelo de DR4, es muy baja (solo 3 péptidos idénticos entre más de 100) no detectándose además péptidos comunes entre los repertorios de las B-LCL y las otras transfectantes. Estos datos indican que, a pesar de tener el mismo alelo, los diferentes proteomas y vías de procesamiento determinan los repertorios finalmente presentados. Por otro lado, estas importantes diferencias muestran las limitaciones de las células B-LCL como modelo para el estudio de la presentación en relación a la autoinmunidad órgano-específica u otras patologías que afectan a células no linfoides.

El bajo número de péptidos secuenciados en tejido tiroideo no permite realizar comparaciones similares a las anteriores, aunque parece que los tejidos con el mismo alelo de DR podrían mostrar repertorios muy similares incluso en muestras de distintos pacientes. Además, aunque se identificó una proteína que era común a tejidos con diferente haplotipo, ésta era presentada a través de distintos epítopos.

2. La expresión o no de las chaperonas HLA-DM y Ii afecta al repertorio de péptidos asociados moléculas de MHCII. HLA-DM tiene una función imprescindible en el procesado y presentación de los péptidos, permitiendo la generación de un repertorio peptídico estable incluso en ausencia de la cadena invariante. La cadena invariante, sin embargo, ejerce un papel más relacionado con la eficiencia de la vía biosintética que con el contenido del repertorio.

Tal como se ha descrito en otros modelos celulares y en ratones transgénicos, en ausencia de la chaperona HLA-DM los complejos MHCII-péptido presentan mayoritariamente CLIP (Koonce 2003; Martin 1996; Riberdy 1992; Sette 1992). Además de CLIP, en este trabajo se han identificado otros ligandos peptídicos en cantidades traza. Estos péptidos podrían ser capaces de desplazar a CLIP en ausencia de DM (Avva 1994; Kropshofer 1999; Vogt 1996) o bien podrían ser derivados de otros mecanismos de presentación.

Sin embargo, cuando se expresa HLA-DM pero no la cadena invariante los repertorios peptídicos son muy similares, lo que confirma que la expresión de HLA-DM es suficiente para una edición selectiva del repertorio y que, además, la cadena invariante no es imprescindible para la presentación. Estos resultados están de acuerdo con los datos disponibles sobre la estabilidad de los complejos DR-péptido y la localización celular de estas moléculas (IV.3) y sugieren la existencia de otras proteínas alternativas a Ii que participen en el transporte de MHCII fuera del RE (incluyendo entre ellas HLA-DM que podría jugar un papel tanto en la edición de péptidos como en el proceso de transporte en estas vías). Estos mismos resultados se han reportado recientemente por el grupo de Rudensky que demuestra, mediante el uso de ratones transgénicos, que la interacción CLIP-MHCII no es un factor importante en la

contribución al desarrollo de la autoinmunidad, mientras que la chaperona DM tiene una función crítica en la presentación (Honey 2004).

Independientemente de la presencia de Ii, la expresión de HLA-DM también influye en el motivo de unión de los péptidos presentados, detectándose que en las células DM$^+$ los aminoácidos que predominan son los grandes e hidrófobos, mientras que en las DM$^-$ los que predominan son los pequeños e hidrófobos. Este dato adquiere una especial importancia debido a que en muchos casos de autoinmunidad la expresión de HLA-DM es muy baja con respecto a la de HLA-DR, condicionando de esta forma el tipo de péptidos que se podrán unir a partir de una determinada proteína.

3. En ausencia de las chaperonas cadena invariante y HLA-DM, las moléculas de HLA-DR4 expresadas en células epiteliales presentan péptidos. Estos péptidos son atípicos ya que muchos de ellos no cumplen el motivo de unión y la mayoría son epítopos C- o N-terminales de proteínas citoplasmáticas. Estos datos sugieren la existencia de mecanismos de carga de péptidos alternativos que pueden involucrar la actuación de otras chaperonas distintas a Ii y DM.

La presentación de péptidos en la línea celular DM$^-$/Ii$^-$ ha indicado que éstos pueden unirse a las moléculas de MHCII y presentarse en membrana por mecanismos independientes de las chaperonas HLA-DM y Ii, y que estos procesos implican la unión de péptidos atípicos correspondientes a extremos N- y C- terminales de proteínas citoplasmáticas. La ausencia de las chaperonas Ii y HLA-DM afectaría tanto a los procesos de estabilización y transporte de DR hacía la membrana, como al de edición de los péptidos.

En este sentido, se han identificado diversas proteínas asociadas a la molécula de HLA-DR en estas células epiteliales, que podrían jugar un papel como transportadores y estabilizadores de la moléculas de MHCII, dirigiéndola desde el RE hacía la superficie. La falta de Ii, además podría favorecer la unión de péptidos de baja afinidad en el RE que contribuirían a la estabilización de la molécula de HLA-DR. Se conoce que dos de las proteínas identificadas en complejos con MHCII, las HSP-70 y -90, pueden unir polipéptidos en el citoplasma y posteriormente llegar a los endosomas por endocitosis mediada por receptor. La interacción de las HSPs con HLA-DR podría estar relacionada con la transferencia de estos péptidos a las moléculas de MHCII en los endosomas lo que explicaría el origen citoplasmático de la mayoría de los péptidos presentados por las células I$^-$/DM$^-$. La capacidad de unión de estas proteínas a péptidos y la posible "*cesión*" de estos péptidos a las moléculas de MHCII ya había sido sugería por el grupo de Srivastava (Srivastava 2002).

En el caso de que no haya expresión de Ii pero sí de HLA-DM, esta molécula contribuiría al reemplazo de los péptidos de baja estabilidad en los complejos MHCII-péptido originales por otros más estables, lo que explicaría la semejanza observada con el repertorio de DR4IiDM. Aunque estos repertorios tienen muchas secuencias idénticas, la abundancia de estos péptidos en las células I⁻/DM⁺ es inferior lo que, de acuerdo también con el menor número de moléculas HLA-DR medido en las líneas I⁻, sería probablemente debido a una menor eficiencia de los mecanismos de transporte de DR no asociados a Ii.

4. Las células epiteliales en tiroides afectados por la enfermedad de Graves-Basedow expresan moléculas de MHCII asociadas a un repertorio heterogeneo de péptidos. Las proteínas que generan estos ligandos son intracelulares y extracelulares, incluyendo la tiroglobulina, proteína reconocida como autoantígeno de esta enfermedad.

A partir de tejido tiroideo humano afectado por la enfermedad de Graves-Basedow, se han identificado 28 péptidos asociados a moléculas de MHCII que provenían de 16 proteínas distintas. Los repertorios asociados a DR en este tejido mostraban la características esperada para la presentación de clase II y todas las secuencias identificadas seguían el patrón del motivo de unión a esta molécula. La presencia de diferentes epítopos para una misma proteína en el caso de tejidos con diferente haplotipo y la identidad de los péptidos presentados en el caso de presentar los mismo alelos, incluso con muestras de individuos diferentes, es un reflejo de la especificidad asociada al alelo de DR. Puede ser también relevante el hecho de que los distintos epítopos encontrados para una misma proteína en diferentes alelos se situaban muy cercanos en la secuencia de la proteína origen, lo que podría indicar la degradación de las proteínas a polipéptidos de tamaño grande con una disponibilidad preferente para su unión con MHCII, bien porque se formen en cantidades mayores o bien porque estos polipéptidos tengan ventajas en cuanto a su transporte a través de la vía. Estas secuencias polipeptídicas grandes podrían unirse directamente a la molécula de MHCII, siendo recortadas una vez situadas en el surco de unión para generar los péptidos presentados o bien degradarse a trozos más pequeños conteniendo los epítopos correspondientes.

Más de la mitad de los péptidos identificados derivaban de proteínas de origen exógeno, principalmente de la matriz extracelular, del coloide y de la sangre. El resto derivaba de proteínas de membrana y citoplasmáticas. La mayor parte de los péptidos presentados son de proteínas específicas del tejido tiroideo. El más relevante de todos ellos es probablemente una secuencia derivada de la tiroglobulina, uno de los principales autoantígenos conocidos de esta enfermedad. Curiosamente, el individuo del que provenía este tejido no presentaba anticuerpos anti-tiroglobulina y de hecho la secuencia peptídica identificada no se encuentra en las zonas de esta proteína que se han descrito como inmunorreactivas (Saboori 1999; Thrasyvoulides 2001).

5. Diversas proteínas relacionadas con procesos de transporte de proteínas asociado a membrana y/o la formación de los complejos MHCII-péptido se encuentran formando complejos en la célula con HLA-DR4. Algunas de estas proteínas podrían intervenir en el proceso de presentación observado en las líneas deficientes en las chaperonas cadena invariante y DM [1].

Se han identificado 30 proteínas que interaccionan directa o indirectamente con la molécula HLA-DR4 en estas células epiteliales, la mayor parte no descritas previamente como proteínas asociadas a las moléculas de MHCII. Tal como ya se ha explicado, varias de ellas podrían estar relacionadas con la formación y transporte de los complejos MHCII-péptido hacia la superficie celular. Se han identificado diversas chaperonas residentes en el RE responsables del control de calidad en dicho compartimento, tanto relacionadas con el sistema de control calnexina/calreticulina, como con el sistema basado en la proteína BiP. También se han identificado diversos miembros de la familia de las PDI, enzimas implicados tanto en el plegamiento de las proteínas como en la degradación de éstas. Los complejos derivados de estas proteínas, especialmente los de HSC70 y BiP, eran más abundantes en las células que no expresaban Ii (En el caso de BiP esta observación coincide con datos reportados en los que se describe que en ausencia de Ii las moléculas de MHCII agregan con BiP (Bonerot 1994).

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[1] Los datos obtenidos no permiten emitir conclusiones sobre el papel exacto de estas proteínas. Esperamos que diversos experimentos dirigidos al análisis de la colocalización de estas proteínas con la molécula de MHCII y la cuantificación más exacta de las diferencias de expresión existentes entre las diferentes líneas celulares mediante inmunodetección de cada proteína, aporten más información en este aspecto en un próximo futuro.

VI. CONCLUSIONES

Mediante la utilización de técnicas espectrométricas ha sido posible identificar cerca de 300 péptidos asociados al alelo DR4 de MHC de clase II en líneas celulares en cultivo y se han descrito además por primera vez 28 péptidos presentados en tejido tiroideo autoinmune. Paralelamente se han caracterizado 30 proteínas asociadas directa o indirectamente con las moléculas de MHCII en líneas celulares. La información obtenida en estos estudios nos permiten concluir que:

1. Los repertorios peptídicos asociados a moléculas de MHCII HLA-DR4 en células epiteliales endocrinas son diferentes a los generados en células presentadoras profesionales que presentan el mismo alelo y derivan de proteínas distintas. Esto puede estar relacionado con las características propias de la célula epitelial y con su metabolismo especializado.

2. La expresión o no de las chaperonas HLA-DM y Ii afecta al repertorio de péptidos asociados a moléculas de MHCII. HLA-DM tiene una función imprescindible en el procesado y presentación de los péptidos, permitiendo la generación de un repertorio peptídico estable incluso en ausencia de la cadena invariante. La cadena invariante, sin embargo, ejerce un papel más relacionado con la eficiencia de la vía biosintética que con el contenido del repertorio.

3. En ausencia de las chaperonas cadena invariante y HLA-DM, las moléculas de HLA-DR4 expresadas en células epiteliales presentan péptidos. Estos péptidos son atípicos ya que muchos de ellos no cumplen el motivo de unión y la mayoría son epítopos C- o N-terminales de proteínas citoplasmáticas. Estos datos sugieren la existencia de mecanismos de carga de péptidos alternativos que pueden involucrar la actuación de otras chaperonas distintas a Ii y DM.

4. Las células epiteliales en tiroides afectados por la enfermedad de Graves-Basedow expresan moléculas de MHCII asociadas a un repertorio heterogeneo de péptidos. Las proteínas que generan estos ligandos son intracelulares y extracelulares, incluyendo la tiroglobulina, proteína reconocida como autoantígeno de esta enfermedad.

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VIII. ANEXOS

ANEXO I

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10

Capillary Separations

Montserrat Carrascal and Joaquin Abian

1. Introduction

Capillary liquid chromatography (capLC) utilizes columns with inner diameters below 0.5 mm and flow rates from some few µL/min down to the µL/min range. Miniaturization of chromatographic procedures using capLC offers substantial advantages over conventional LC separation methods. Capillary columns show increased separation efficiency, minimal solvent consumption, higher peak concentration at the detector, and higher peptide recovery. Several reviews on the state of the art can be found in the literature **refs.** *1–4*.

CapLC is probably to LC the same as some decades ago open tubular capillary chromatography was to gas chromatography. Horvarth first reported capLC in 1967 *(5)*. The preparation, analytical characteristics, and some applications of capLC columns were already described in the late 1970s by Scott and Kucera *(6)*, Novotny et al. *(7)*, and Ishii et al. *(8,9)*. Two main classes of capLC columns were developed: wall coated open tubular (OTLC) columns and packed columns. So far, several practical problems (column preparation, very low capacity and injection volumes) relegated OTLC to a more restricted use than packed columns. The latter consists of metal or fused silica capillaries filled with chromatographic supports with particle sizes ranging from 1.0 to 50 µm. CapLC could be divided into micro (0.8–0.15 mm id) LC and nano (20–100 µm id) LC, depending on the inner diameter of the column, although other classifications can be found in **refs.** *2*,*3,* and *10*.

Capillary columns usually show overall performance and efficiency comparable or better than conventional columns. Jorgenson et al. reported 40,000 theoretical plates for resorcinol (k′ = 2,7) on a 25-µm id column 33 cm of length filled with 5-µm particles. These authors observed that column efficiency improved when the column diameters diminished from 50 to 12 µm *(11,12)*.

This improvement was explained as flow dispersion and resistance to mass transfer decreased with smaller column diameters. Wall effects make a higher contribution in capLC, than in conventional LC columns. This leads to relatively higher band broadening at high flow rates. Band broadening can be diminished using electrodriven flows, as performed in capillary electrochromatography (CEC), instead of pressure forces. CEC columns can reach efficiencies over 300,000 theoretical plates/m *(13)*, greatly exceeding the efficiency of the equivalent pressure-driven capLC. Because electrodriven flows are less influenced by particle size than pressure-driven flows, smaller particles and longer columns can be used thus increasing the efficiency that can be obtained. The separation and detection of peptide mixtures at the attomole level by CEC were described recently *(14)*. Lubman reported the analysis of protein digests by the so-called pseudoelectrochromatography, a pressurized CEC mode *(15)*. This technique is an intermediate between capLC and CEC. The preparation of CEC columns and the applications of this technique were reviewed *(4,16-18)*.

In this chapter we will focus on the preparation and handling of packed capLC columns and the corresponding instrumentation for pressure-driven separations. Nowadays, capLC is a widespread technique in laboratories involved in the analysis of peptides and proteins, especially in the proteomics area. The low flow rates employed in capLC allow both the use of expensive solvents (i.e., deuterated) and coupling with detectors that show their best performance at the nanoliter-per-minute range, such as mass spectrometers (MS) with electrospray (ESI) interfaces (ESI-MS). Several companies distribute miniaturized chromatographic systems, including pumps, automatic microinjectors, and microcolumns, as well as suitable cells or interfaces for photometric or MS detection (*see* **Note 1**). These systems can also be built using conventional laboratory equipment with minimal effort at low cost *(19,20)*.

Before Takeuchi and Ishii demonstrated the utility of fused silica capillaries *(21)*, first attempts to prepare packed capLC columns used metal or PTFE tubing *(5,8)*. Although Teflon *(22)* or PEEK lines *(23)* are still used to prepare capillary columns, fused silica is the material most authors choose. Fused silica capillary columns with internal diameters from 500 to about 50 μm can be easily prepared by slurry packing methods *(1,3,17,19,24,25)* (*see* **Subheading 3.1.**). Colon reviewed several other methods for column packing *(16)*.

The first step to column packing is to build a frit at the exit end of the fused silica capillary column. The frit can be prepared sinterizing a plug of silica particles with a flame *(1)* or it can be made pushing the capillary column through a porous membrane sheet two or three times, resulting in a stable plug at the outlet of the column. Membrane frits can also be prepared using Teflon *(24)*, PVDF *(19)*, and glass fiber sheets *(3)*. Alternatively, Lubman used a Valco microbore column fitting with a very small amount of glass wool as a frit. The

thickness was 0.1 mm and it proved to be more stable than glass frits *(15)*. A zero dead volume Valco union can also be used to hold a stainless mesh frit *(17)*.

The sintered glass method is probably the most commonly employed. In our experience, sintered glass frits are stable when using capillaries with outer diameters bigger than 300 μm. Columns with smaller outer diameters (180 μm) are more vulnerable to the heating process that makes the capillary too fragile to handle.

After the frit is ready, the capillary column is coupled to a reservoir filled with the slurry of the packing material. The slurry is pushed under pressure into the column inlet by a flowing stream. The frit allows the solvent through the column and the solid phase fills it in a similar process to filtration. Liquid solvents *(1,12,17,26,27)* and supercritical fluids *(25,28,29)* are used for slurry-packing. Likewise, gas streams are used for dry-packing procedures *(30,31)*. The relative advantages of these methods are the difficulty of packing and the stability and efficiency of the columns obtained.

In common practice, 20 μm is the minimum column diameter for a packed column, but most authors use a minimum id of 50–75 μm. Kennedy and Jorgensson reported the preparation by slurry-packing methods of 12 μm id capLC columns filled with 5 μm particles. Filtering the particles through an 8 μm filter was necessary to prevent the column from plugging with the larger particles in the commercial material *(12)*.

Five micron particles are the most commonly used for column filling. Recently, MacNair et al. introduced ultrahigh pressure capLC (UHPLC) where nonporous particles of 1.0–1.5 μm are used to fill 29–100 μm id capLC columns *(32)*. More than 200,000 theoretical plates (k'=1) can be generated using 25–50-cm-long columns. UHPLC can also produce high-speed separations (less than 100 s) with column efficiencies over 20,000 plates *(33)*. Mac Nair demonstrated the application of this technique to analyse peptides and proteins with an ion trap MS detector *(34)*. To take full advantage of the high speed available, Lee et al. coupled UHPLC to a fast scanning time-of-flight (TOF) MS detector *(33)*.

Other special classes of packed columns with immobilized stationary phases *(35)* or a continuous porous bed (monoliths) *(36,37)*, have been developed. The major advantage of these columns is that they do not need the end frit necessary to retain the stationary phase in conventional packed capLC columns.

The optimal flow rate for a 150-μm column is around 1 μL/min. This demands special requirements of the pumping system especially when a gradient elution is needed. Syringe pumps were used to obtain reproducible flow rates in the microlitre-per-minute range. In this sense, Lee et al. described a brilliant procedure to prepare and store the solvent gradient before the analytical step *(38)*. They also apply a "peak parking" procedure by controlling the pres-

sure of the syringe pump. This enables an efficient characterization of peptide mixtures by tandem mass spectrometry *(39,40)*. More simple setups are based on conventional HPLC gradient pumps with flow splitting. Commercial sources supply calibrated flow splitters that give very reproducible flows (*see* **Note 1**). Even so, most authors prepare their own splitters using a T-piece and some kind of restrictor (*see* **Subheading 3.2.1.**).

CapLC can be coupled to a wide range of detectors including UV, fluorescence, and electrochemical detectors. The analysis of peptides from biological sources is often hindered by its very low concentration in the sample. Attomole/femtomole detection limits are usually required and thus high recovery, high sensitivity separation, and detection techniques are needed. The need for an effective coupling of LC with some emerging MS techniques such as continuous flow fast atom bombardment *(41)* and ESI *(42)* has probably been the driving force towards capLC developmnent in the last decade. These MS techniques were specially suited for biopolymer analyses and offered their optimal performance at the microlitre or submicrolitre per minute flow rate. Coupling MS with LC implied either flow splitting procedures with the resulting sample loss or the development of capLC methods. In this respect, capLC/ESI-MS has become a very powerful analytical technique and, currently, most capLC/MS work is performed using ESI interfaces (*see* **Subheading 3.2.4.**). In the early 1990s, Hunt et al. sequenced subpicomole amounts of antigenic peptides bound to major histocompatibility complex (MHC) molecules using capLC columns and commercially available ESI interfaces *(43,44)*. Further downscaling of the LCMS method by coupling nanoLC to dedicated microESI interfaces resulted in detection limits down to the attomole range *(26,45–47)*. Enzymatic digest from proteomic studies can be on-line separated and sequenced using capLC/ESI-MS with automatic scanning procedures in triple quadrupole, ion trap, or hybrid quadrupole TOF mass spectrometers *(48,49)*. Protein sequence coverage higher than 50% can be routinely obtained from well defined Commassie stained spots (about 1–5 pmol total protein). Yates et al. demonstrated 99% coverage on the characterization of mutant globins (15 KDa) analyzing peptide mixtures from several enzymatic digestions of the same protein *(48)*. CapLC/ESI-MS quantitative determinations of nanomolar amounts of peptides can be performed using internal standards *(26)*. The use of gradient elution or on-line preconcentration microcolumns allows the injection of sample volumes up to 100–200 µL to be injected without deleterious effects on the system. These techniques solve the problem of small injection volumes required by capLC, improve the concentration detection limits and simplify overall sample handling. The use of selective on-line preconcentration procedures can also minimize interferences due to the complexity of the biological sample *(26,50)*. Ion exchange precolumns can be used in combination with reversed-phase capLC

columns for this purpose (*see* **Subheading 3.1.**). A similar, more sophisticated approach is used in multidimensional chromatography. Jorgerson et al. demonstrated zeptomol detection limits for the analysis of peptide digest from porcine thyroglobulin using a two-dimensional (2D) anion exchange-reversed phase capLC system and fluorescence detection. The peak capacity of this 2D method was estimated to be over 2000 components per analysis *(51)*.

In this chapter, we will describe straightforward protocols for beginners to produce capLC separations with minimal effort and economical cost. The use of packed capillary columns with membrane frits and fused silica based interfaces for UV and ESI-MS will be described. The reader is referred to the literature and the notes along the chapter for improved techniques when needed.

2. Materials

2.1. Column Preparation

2.1.1. Analytical Column

1. Fused silica capillaries of 250×350, 50×200, 75×200, and 30×150 (id $\times$ od μm) were obtained from Polymicro Technologies (Phoenix, AZ) (*see* **Note 2**).
2. Whatman GF/A glass fibre filters (Whatman International Ltd., Maidstone, UK).
3. Araldit® glue (Ceys, Ciba-Geigy).
4. Empty 100×1 mm HPLC column holder with the metal filters removed.
5. Bulk stationary phases 5 μm Kromasil C18 and C8 (Teknokroma, Barcelona, Spain), and Poros 20 R1 (Perseptive Biosystems, Cambridge, MA).
6. Fused silica cutter (Alltech Associates, Inc., Deerfield, IL).
7. HPLC pump (any spare pump is valid).
8. Teflon tube (300 and 100 μm id, 1/16 iod, Supelco, Bellefonte, PA).
9. Valco Union and connectors (Valco Instruments Co. Inc., Houston, TX).
10. Acetonitrile (ACN) (LiChrosolv, Merck, Darmstadt, Germany).

2.1.2. Preconcentration Columns

1. Strong-anion exchange μ-Precolumns (5×0.8 mm id; LC Packings, The Netherlands).

2.2. Chromatographic Equipment

2.2.1. General

1. HP-1100 system including high pressure binary HPLC pump and a solvent degasifier (Agilent Technologies, Barcelona, Spain).
2. Rheodyne injector mod 7725i with 5, 10, and 200 μL loops (Supelco).

2.2.2. Chromatographic Flow Splitting

1. Zero dead volume (ZDV) Valco Tee (Supelco).
2. HPLC columns 5 mm 150×2.1 mm (250 mm column), 100×1 (75 mm column), 35×3 mm (For Poros).

2.2.3. Preconcentration Switching Systems

1. Two Position Electric Actuator and VALCO valve (Valco Instruments Co. Inc., Houston, TX).
2. Auxiliary M-6000 HPLC pump (Waters, Milford, MA).

2.2.4. UV Cell Preparation (see Note 3)

1. UV detector ABI 769 A (Applied Biosystems, Foster City, CA).
2. Fused silica capillaries 250×350, 50×200, 150×375, and 30×150 (id × od).

2.2.5. ESI Interface Preparation

1. Fused silica capillaries 50×200, 30×150, 15×150 (id × od).
2. Hypodermic stainless steel capillary (A-M System, Inc., Carlsborg, WA).
3. Microflame torch (Microflame, Minneapolis, MN).

2.3. HPLC Gradient Systems

1. Acetonitrile 190 for UV (Romil, Teknokroma, Barcelona) (for UV detection).
2. Acetonitrile ultragradient (Romil) (for MS detection).
3. Trifluoroacetic acid (TFA) (Uvasol, Merck).
4. Acetic acid (AcOH) (Suprapur, Merck).
5. Ammonium acetate (Merck).

3. Methods
3.1. Column Preparation
3.1.1. Analytical Columns

1. The 250 or 180 μm fused silica capillaries used as columns (*see* **FSC**, **Fig. 1**) are cut to the desired length (11 cm) using a porcelain cutter.
2. The outlet line (*see* **EXC**, **Fig. 1**) is also cut from a 50×200 μm (250 μm id column) or a 50×150 (180 μm id column) capillary long enough to reach the detector (5–20 cm). The shorter the better.
3. Capillaries are flushed with ACN/water 3/1 using an HPLC pump (*see* **Note 4**).
4. A frit is produced at the end of the tube twisting the capillary end against a Whatman GF/A glass fiber filter (*see* **Note 5**). A piece of filter corresponding to the diameter of the capillary is cut out in this way, and remains inside the bore of the tube. The EXC capillary is introduced into the FSC capillary to serve as the outlet line. The frit is pushed 5 mm into the FSC by pressing with the EXC and both tubes are held together with a drop of glue (*see* **Note 6**)

   The same protocol is used for the 75 μm id × 200 od column. In this case, the od of the FSC and the EXC lines are the same and the connection is done using a 2-cm fused silica sleeve (250 μm id × 350 μm id) (*see* **FSS**, **Fig. 1**). The frit is prepared into the FSS and sandwiched between the FSC and the EXC (*see* **Note 7**).
5. The column is slurry packed with the silica particles at 1500 psi. For this purpose, the column is connected to a metallic reservoir (a 1 mm id column holder) using
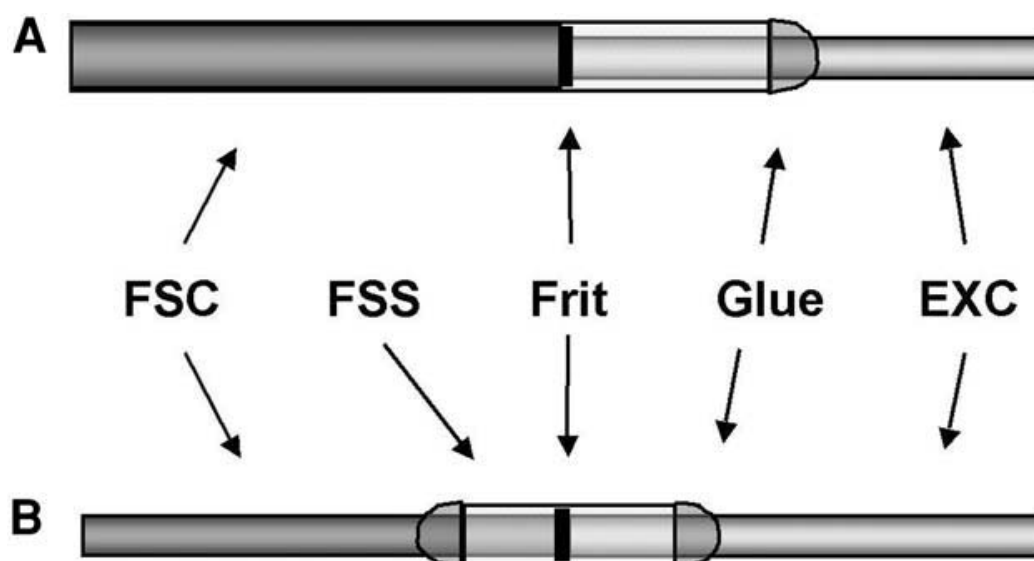
Fig. 1. General scheme for the preparation of fused silica capLC columns with internal diameter **(A)** above and **(B)** below 180 µm. *FSC*, fused silica capillary column. *FSS*, fused silica sleeve. *EXC*, fused silica exit capillary.

1/16 HPLC connectors. A Teflon sleeve is used to adapt the capillary to the ferrule diameter. One mg of stationary phase is dissolved in 4 mL of ACN/water (3/1) and 100 µL of the slurry are introduced into the reservoir with a syringe. The slurry is then pushed into the FSC connecting the reservoir to an HPLC pump (*see* **Note 8**). Flow rate is set at 50–100 µL min for a few minutes to fill the column, and then it is lowered down to approx 20 µL/min in order to compact the stationary phase at a constant pressure. After 20 min, the column can be disconnected ready to be used (*see* **Note 9**).

3.1.2. Preconcentration Columns

Preconcentration columns can be prepared in the same way as analytical columns from 250–530 id FSC tubes (*see* **Note 2** for adequate sizes). Higher column diameters can be used when high volumes have to be preconcentrated in a reasonable time or when complex samples could produce column overloading. Capiello et al. *(23)* described how to produce convenient columns with diameters up to 0.76 mm from PEEK tubing. Conventional ZDV peak or stainless steel connectors are used as column ends with 1/16 nuts and ferrules. The membrane frit at the outlet end is introduced into the ZDV union and sealed in place by pressing the column end against it and then tightening the nut. In the examples given in this chapter, we used a commercial kit consisting of a stainless steel holder and 800 µm id cartridges. The same holder could load cartridges with id from 200 to 800 µm. Cartridges available include C18 and ion exchange materials.
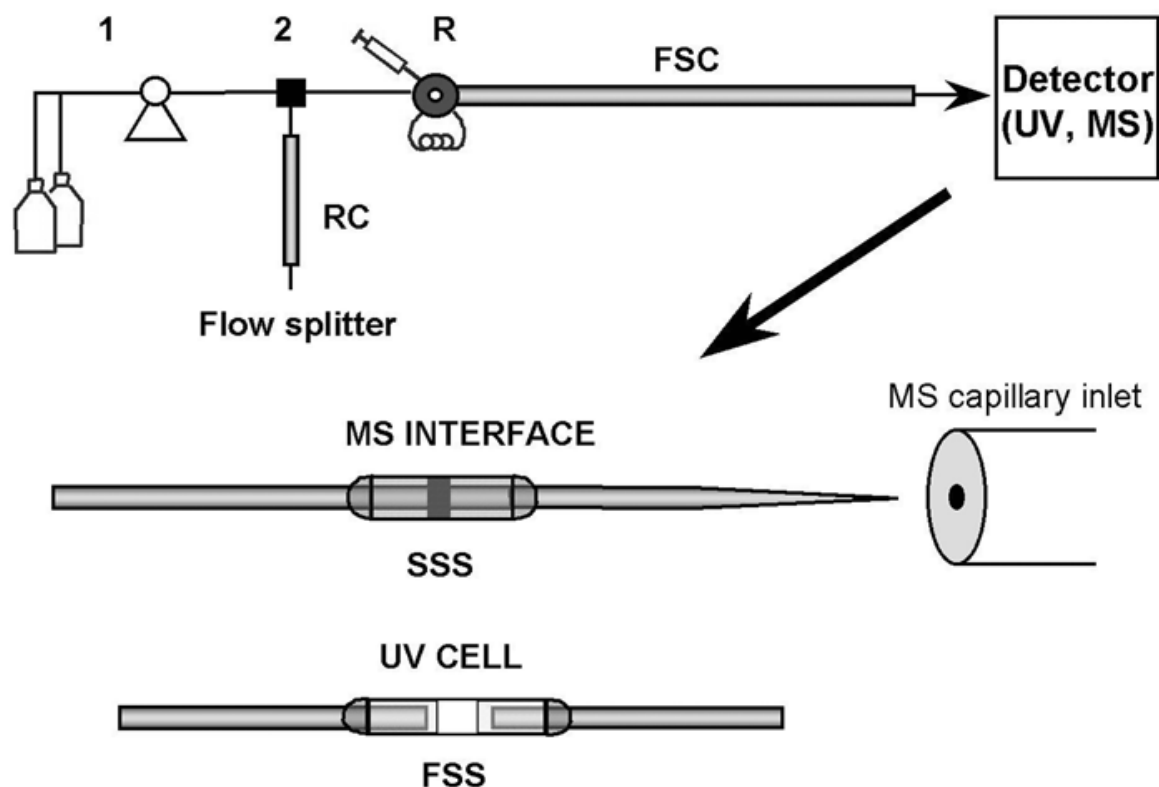
Fig. 2. Scheme of a simple capLC system and the interfaces for MS and UV detection. *1*, Gradient pump and solvents. *2*, ZDV splitting Tee. *R*, injector. *RC*, restriction column. *FSC*, fused silica capillary column. *SSS*, stainless steel sleeve. *FSS*, Fused silica sleeve.

3.2. Chromatographic Equipment

3.2.1. Column Connection

The basic scheme of a capLC system is depicted in **Fig. 2**. The main issue in the setup of capLC is to decrease the dead volumes as much as possible. Some changes can be done in a conventional HPLC pump to optimize its performance at low flow rates. We substituted a 2×0.1 cm precolumn for the mixer of our HPLC-1100 gradient pump and all the connections after the pump were remade with 120 µm id PEEK tube. The tubing connecting the split tee and the injector should be made of 120 µm id tube and as short as possible. The column is connected directly to the injector using a teflon sleeve with an adequate inner diameter (*see* **Fig. 3**). Switching the injector to the "load position" after the sample has been transferred to the analytical column eliminates the delay produced by the injector loop on the solvent gradient.

3.2.2. Chromatographic Flow Splitting

1. The flow splitter is built from a ZDV Valco Tee. The inlet flow from the HPLC is split in a ratio that depends on the relative flow restriction produced by the capillary column and the flow restrictor.
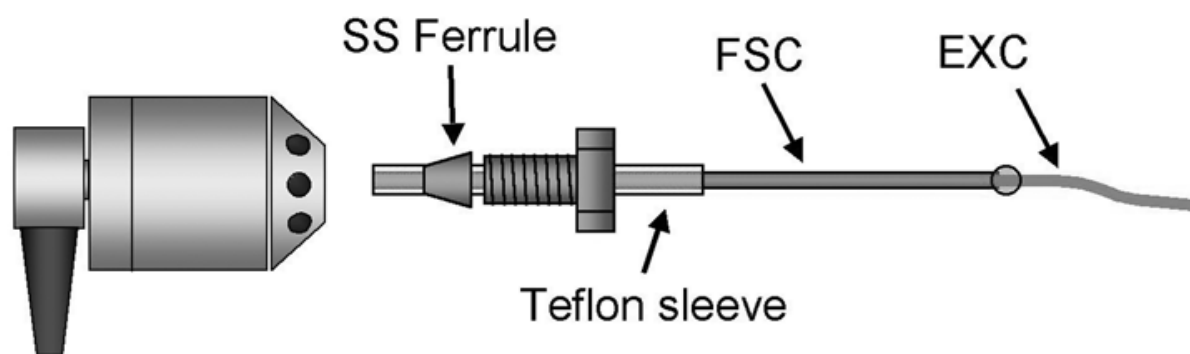
Fig. 3. Connection of the capillary column showing the use of Teflon sleeves for capillary connections. *EXC*, fused silica exit capillary. *FSC*, fused silica capillary column, *SS*, stainless steel.

2. The flow restrictor for a 250 μm id column consists of a conventional 2.1 mm id HPLC column (5 μm Nucleosil, 15 cm long). A 1 mm id column (5 μm Partisil, 10 cm long) is used in the case of 75 mm id columns (*see* **Note 10**).
3. A HPLC flow rate of 100 mL/min is typically used. After flow splitting, about 3–4 μL/min in the 250 μm column or 0.5-1 μL/min in the 75 μm column are obtained.
4. The flow at the exit of the column can be calculated collecting the eluent in a conical vial (500 μL Eppendorf tube) for 10 min and measuring its volume with a 5 or 10 μL Hamilton syringe. Alternatively, the column outlet can be introduced into the bore of the syringe to measure the volume directly from the syringe marks. Final adjustment of the flow rate is done by modifying the flow from the HPLC pump.

3.2.3. Preconcentration Switching Systems

The basic scheme of a preconcentration system is depicted in **Fig. 4**. An auxiliary HPLC pump is used to load the sample into the precolumn and to wash out not retained compounds. The auxiliary pump is permanently working at 50–200 μL min, depending on the precolumn, so that sample loading is typically made in a very fast way even with high volume samples. Sample loops from 20–200 μL are usually employed.

The precolumn is connected to a motor driven Valco switching valve. When the valve is in the "analysis" position, the precolumn is connected in series with the gradient HPLC system and the analytical column. Valve switching can be done manually or automatically using the relay contacts on the HPLC system.

3.2.4. UV Cell Preparation

1. The polyimide coating of a 250 × 350 mm (id × od) fused silica capillary is removed by carefully burning it with a flame. The capillary should be approx 2 cm long and the open window 0.5 cm (*see* **Fig. 2**).
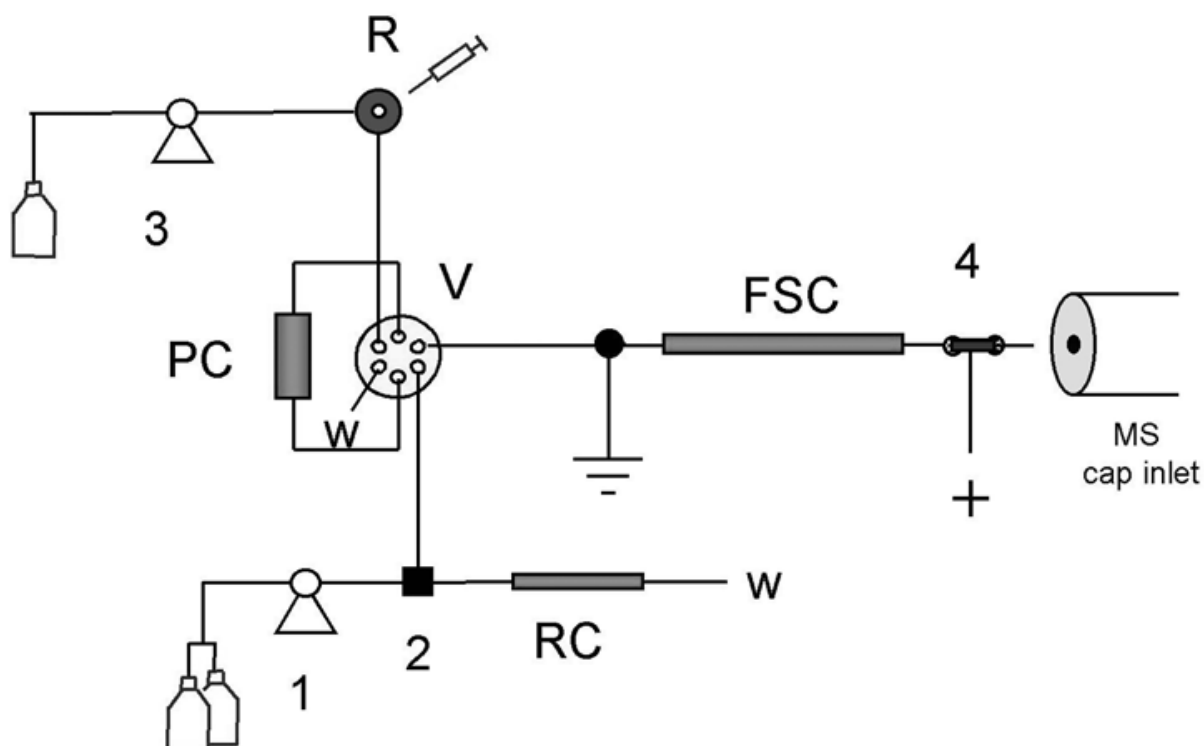
Fig. 4. Scheme of a preconcentration capLC system. *1*, Elution gradient pump and mobile phases. *2*, ZDV splitting Tee. *3*, Preconcentration pump and mobile phase. *4*, microelectrospray interface. *R*, injector. *V*, Valco electrovalve. *W*, waste. *PC*, preconcentration column. *RC*, restriction column. *FSC* fused silica capillary column.

2. Inlet and outlet capillaries are prepared from 50×200 μm (id × od). These capillaries are glued into the cell capillary, leaving a 5-mm space (the window area). The total volume of the flow cell is approximately 250 nL (*see* **Note 11**).
3. The flow cell is placed into the ABI CZE cell holder. The cell is used as a conventional CZE UV cell.

3.2.5. ESI Interface Preparation *(*see **Note 12***)*

1. About 10 cm of a 200×50 μm FS capillary is prepared. A lightweight (10 gr) is fixed to one end of the capillary. The other end is fixed to a support leaving the capillary in a vertical position with the weight hanging free. The flame of a torch is applied to the center of the line allowing the capillary to stretch under gravity. Two capillaries with sharp tips are obtained in this way (*see* **Note 13**). The needle tips are carefully cut to obtain an inner bore of about 5 μm. The bore diameter can be monitored with a stage micrometer (Graticules LTD, Tonbridge, Kent, UK) under a microscope (*see* **Note 14**).
2. The end of a 250-μm ID stainless steel capillary is twisted against a Whatman GF/A glass fiber filter to cut out a piece of filter.
3. The filter is sandwiched between two 200×50 μm capillaries, the electrospray needle prepared as indicated earlier, and the inlet line. These capillaries are glued into the steel capillary that serves as the electrical contact for electrospray (*see* **Fig. 2**).

4. The ESI interface was mounted into an x-y-z-manipulator from a Protana Nanoelectrospray Source Kit.
5. The ESI needle tip was carefully positioned at 3–5 mm from the inlet of a Finnigan LCQ mass spectrometer (ThermoQuest, Barcelona, Spain). For standard analyses, an electrospray voltage between 1.6 and 2.0 kV was applied. For continuous infusion experiments, the inlet capillary of the μESI was connected via an injector to a syringe pump.

3.3. HPLC Gradient Systems

The basic HPLC configurations for the examples listed below are depicted in **Figs. 2** and **4**.

3.3.1. Separation of Peptide Mixtures from Enzymatic Digestions of Proteins

A key point in the analysis of on-gel tryptic digests is the removal of salts and acrylamide contaminants from the sample before peptide separation. In general, desalting can be performed directly on the analytical column provided the peptide concentration is relatively high (clear Commassie spot). Otherwise a column switching system using a preconcentration column would be necessary (*see* **Subheading 3.3.2.**). Proper desalting involves a long washing step with aqueous solvent prior to gradient elution. Special care has to be taken to avoid the loss of very polar peptides during this step. The system described below uses a high volume loop that produces an additional delay on the gradient so that no initial 0% step is required. Examples of capLC separations are shown in **Figs. 5** and **6**.

1. Analytical column: 5 μm Kromasil C8 (250 μm × 10 cm); Injection loop, 10 μL
2. Solvent A : water (1% AcOH for MS detection or 0.05% TFA for UV/MS detection) Solvent B: ACN/water 8/2 v/v (1% AcOH for MS detection or 0.05% TFA for UV/MS detection) (*see* **Note 15**).
3. Gradient: From 0 to 55% solvent B in 60 min; from 55 to 80% B in 5 min; hold 10 min at 80% B. Back to initial conditions in 5 min. Flow rate 3 μL/min.
4. Protein digests are prepared following standard procedures *(52)*. The final extract (about 50–150 μL) is concentrated to 20 mL in order to eliminate the extraction solvent (ACN). A 10-μL aliquot is directly injected into the system.

3.3.2. Separation of Peptides Using Preconcentration Procedures

The quantitative analysis of endothelins in human umbilical vein endothelial cell (HUVEC) supernatants is shown in **Fig. 7**. The use of ion exchange preconcentration prior to on-line reversed phase chromatography provides a very selective method for endothelin analysis. The synthetic endothelin [3,11]Ala-ET-1 (alaET) is used as internal standar for quantitation. Detection limits were found
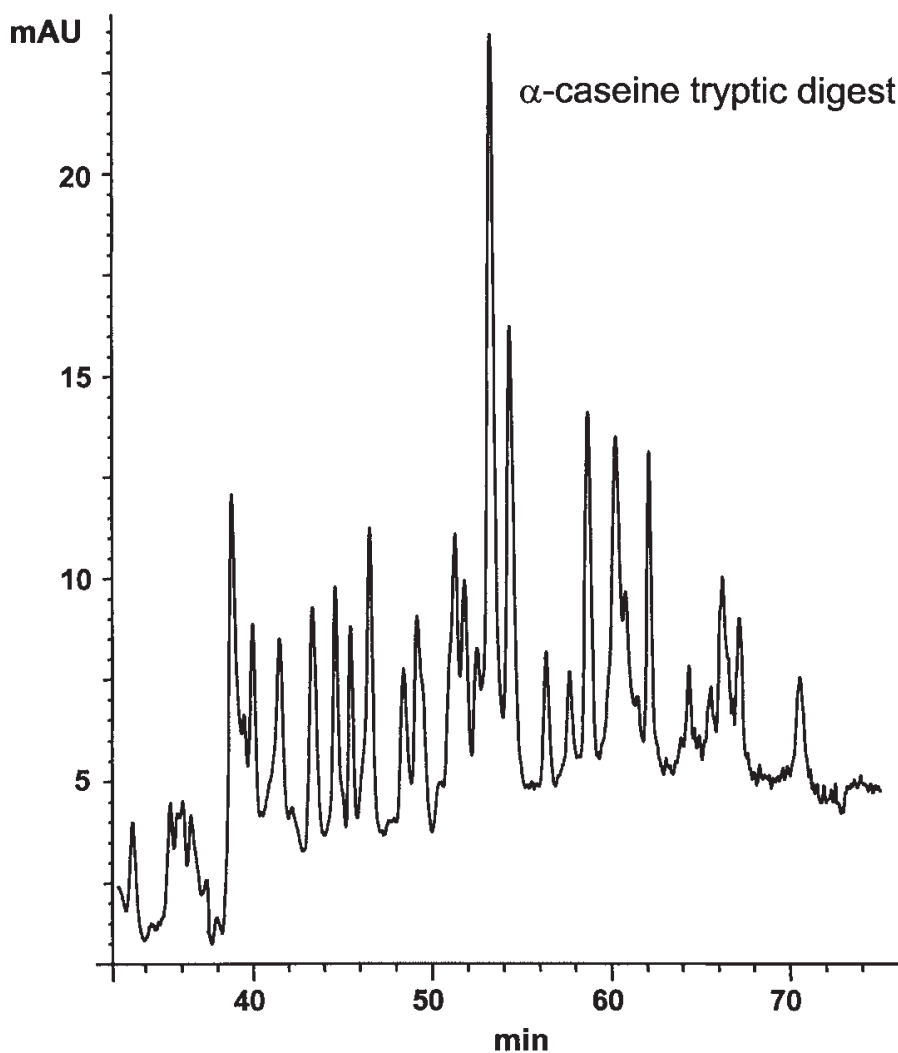
Fig. 5. CapLC-UV analysis of an in-gel tryptic digest of alpha-casein. A blue Commassie spot (1 μg protein) was excised, in-gel reduced, alkylated, and digested following conventional procedures. The peptide mixture was analyzed as indicated in **Subheading 3.3.1.** Peptides were monitored at 214 nm.

Fig. 6. *(see facing page)* Analysis of an in-gel tryptic digest of 90 KDa heat shock protein (HSP90). A protein extract from a lymphoblastoid cell line culture was processed by immunoaffinity chromatography using GST-p21Cip1-Sepharose. The p21Cip1-binding protein fraction was separated by SDS acrilamide gel electrophoresis and stained with Coomassie blue. The selected spots were excised and in-gel reduced, alkylated and digested following conventional procedures. The ThermoQuest LCQ ion trap instrument was programmed for automatic data dependent acquisition. Full MS, Zoom Scan, and Product Ion scans were made consecutively to obtain sequence information over the entire chromatographic elution. As shown in this figure, where spectral data **(right)** was acquired in the elution zone indicated by the asterisk in the chromatogram **(left)** (Retention time 33.03 to 33.14), the sequencing of even very low intensity signals was achieved.
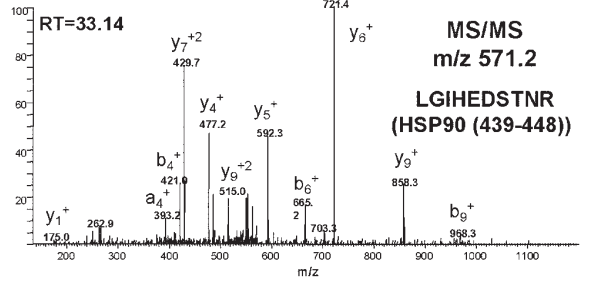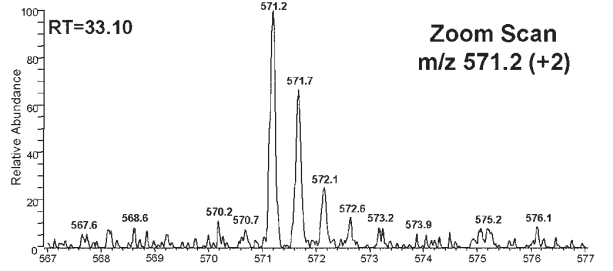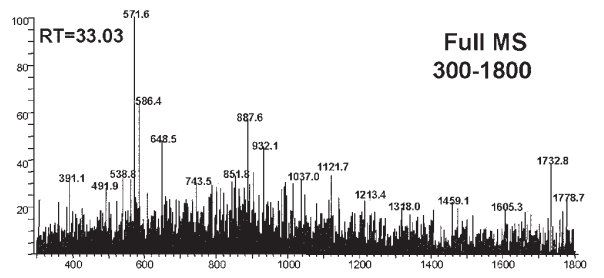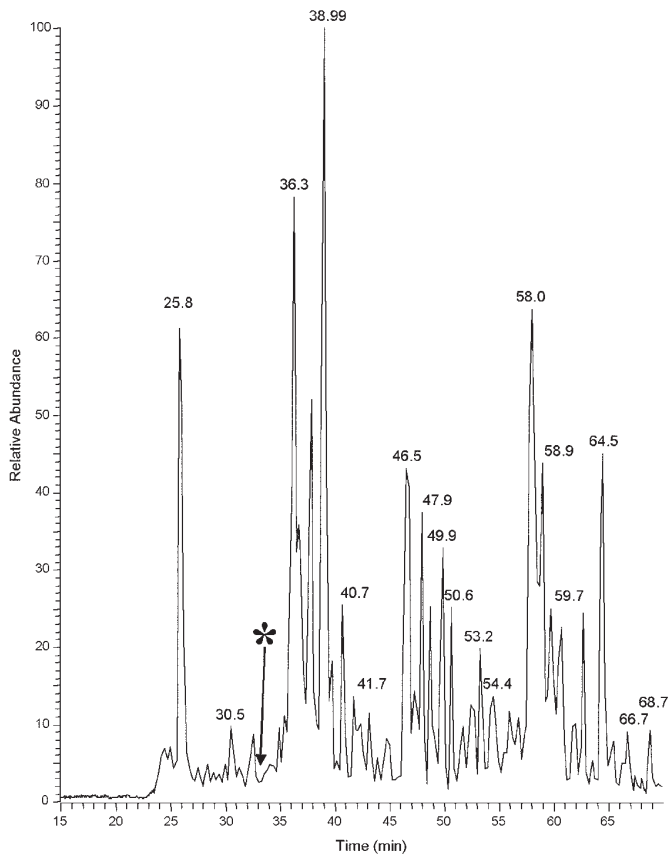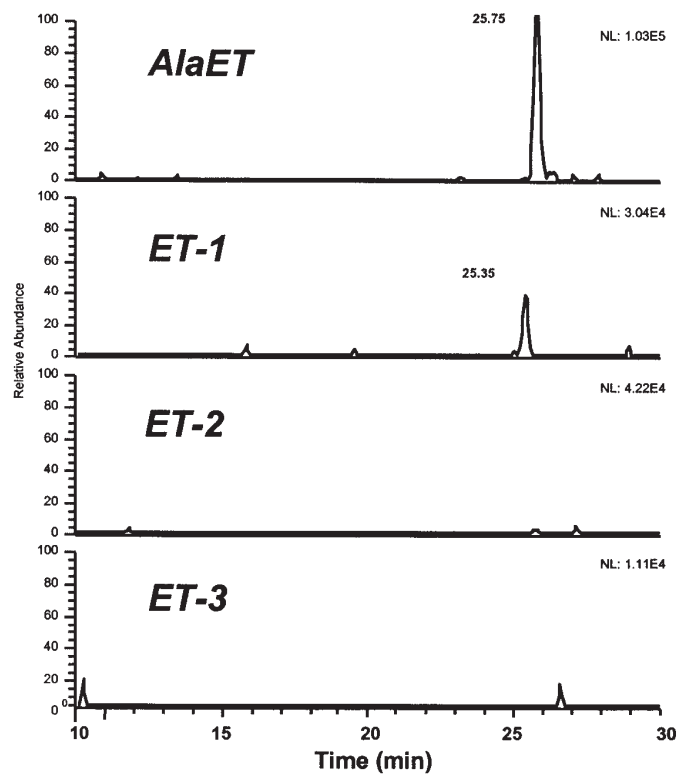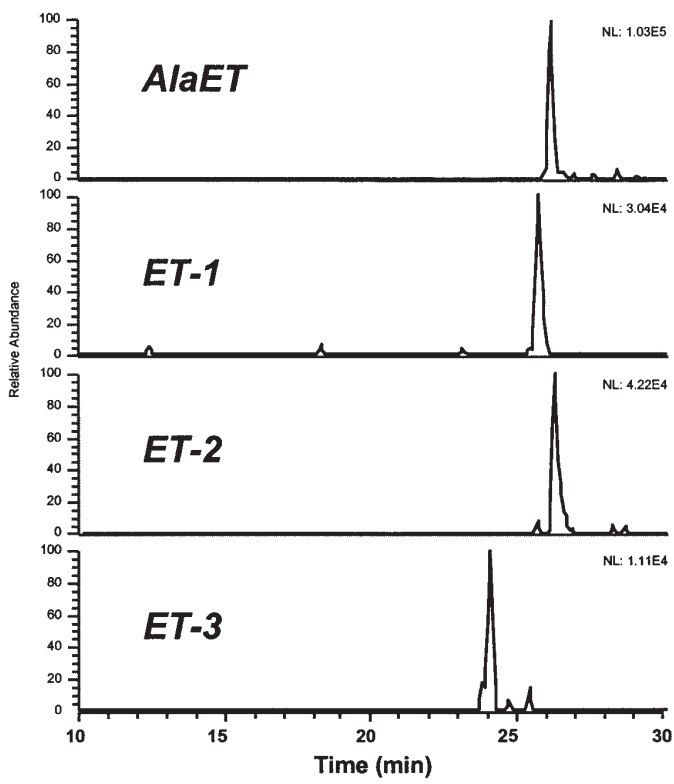
down to 30 pmol/L (1.5 fmol on column) and the method was linear from 50 to 2000 pmol/L *(26)*.

1. Chromatographic Columns:
   Preconcentration precolumn: SAX μ-Precolumn (800 μm × 0.5 cm).
   Analytical column: 5 μm Kromasil C18 (75 μm × 10 cm).
   Injection loop 200 μL.
2. Preconcentration solvent: 10 mmol/L ammonium acetate, pH 7.0 (10:90, v/v).
   Flow rate 200 μL/min.
3. Gradient solvents:
   Solvent A: ACN:water:AcOH:TFA (10:90:1:0.05, v/v).
   Solvent B: ACN:MeOH:water:AcOH:TFA (45:45:10:1:0.05, v/v).
   See **Table 1** for gradient program. Flow rate 550 nL/min.
4. HUVEC supernatants (500 μL) are precipitated with ACN (1 mL). The material is centrifuged and the supernatant evaporated close to dryness. Just before injection, 100 μL of MeOH:water (50:50 v/v) and 1.9 ml of the preconcentration mobile phase are added. A 200-μL aliquot is injected into the analytical system.

4. Notes

1. LC Packings (LC Packings (The Netherlands) BV) were probably the first company to distribute capillary columns and other instrumentation for capLC since the early 1990s. A capillary-perfusion Tool Kit is available from Perseptive Biosystems for the preparation of PEEK capLC columns (ref. 5-2218-00-0000). Low flow pumps and microinjectors for capLC are currently available from major HPLC companies.
2. Other diameters can be selected depending on the application. For example: 530 × 700/150 × 375; 180 × 350/30 × 150 (id × od column / id × od exit line).
3. Any other UV detector provided with cell holders for CZE could be used with homemade fused silica cells. Otherwise, commercial cells can be purchased from LC Packings (*see* **Note 11**). We routinely use a HP1100 UV detector with a LC-Packings U-Z View (CAP) for high sensitivity detection.
4. Capillaries can be deactivated by treatment with *bis*-trimethylsilyl trifluoroacetamide (BSTFA) (Pierce, Rockford, IL). This treatment eliminates the free silanol groups in the silica surface and minimizes peptide adsorption. The capillary is filled with the reagent using a 50-μL glass syringe. After 10–15 min at room temperature, acetonitrile is flushed to remove excess reagent and to clean the capillary. BSTFA is a volatile, strong derivatizing agent that rapidly reacts with moisture. Its handling has to be done in a fume hood. BSTFA is sold in ampoules

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Fig. 7. *(see opposite page)* Analysis of HUVEC supernatants by PC-capLC-ESIMS/MS. **(Left)** HUVEC supernatant spiked with 200 p*M* endothelins and **(right)** HUVEC supernatant. (200 μL injection; internal standard 2 n*M* AlaET). ET, endothelin; AlaET, [3,11 Ala]-ET-1 *(26)*.

Table 1
Time Event of the SAX PC capLC/ESI-MS Method

Time (min)	Concentration of solvent B	Flow rate (mL/min) of LC pump	Valve V position
0	10%	0.4	Preconcentration
10	10%	0.4	
10.1	20%	0.3	
15			Analysis
20	60%	0.3	
20.1	80%	0.3	
25	80%	0.3	
25.1	10%	0.3	

that, after opening, can be stored inside a small flask in the refrigerator for several weeks.

5. Alternatively, frits made of sintered glass can be built as follows: The end of the fused capillary is burned with a flame to remove a section of a few mm of the polyimide coating. Any black residue is carefully wiped out. The capillary is connected to a 500-μL syringe provided with a Teflon connector and flushed with ACN. After ACN removal, the frit is prepared by carefully tapping the tip of the column into a pile of 5 μm silica particles, filling approx 1 mm of the capillary section. This end is then heated with the flame of a microtorch to sinter the silica particles. The resulting frit is usually able to withstand high pressures. However, this procedure requires some practice. The quality of the frit should be tested by flushing some solvent before packing the column. We made stable frits when using capillaries with outer diameters greater than 300 μm. In our experience, columns with smaller outer diameters (180 μm) are more vulnerable to the heating process making the capillary too fragile to handle. Special care needs to be taken to connect the column outlet to the transfer line with the teflon connector (use a preformed connector).

6. Cyanoacrylate glues are too liquid and tended to spread out contaminating the system. Epoxy resins, such as Araldit, are more suitable. The glue must fill the area between the coaxial capillaries, otherwise the connection tends to fail when subjected to pressure. Owing to glue viscosity, this process requires the inner capillary to be pulled 1 mm in and out just after the glue is deposited on the external part of the connection. A gentle warming of the capillary connection is also helpful.

7. This is the general procedure for preparing columns with inner diameters below 150 μm.

8. We commonly use a Vibro-Graver (Burguess Vibro-Crafters, Inc, Graystake, ILL) against the reservoir to prevent the aggregation of particles (some authors immerse the whole system in an ultrasonic bath for this purpose). We do not observe differences in the column quality whether the slurry was previously sonicated or not.

9. This is a very simple procedure that yields reasonable efficiency. Several other solvent mixtures were tested for slurry packing including acetone, methanol, acetonitrile, carbon tetrachloride/paraffin, and n-heptane. In most cases the solvent used for particle suspension was different from the packing (flushing) solvent. Surfactant additives such as sodium lauryl sulphate or Tween were employed to improve particle dispersion. Other packing methods use gases (dry method) or supercritical fluids to pack the particles. A description of available methods can be obtained from **refs. 1**, **12**, **16**, **17**, **19**, **24–31**.

10. When an old LC column is not available, a 50–100 cm section of fused silica capillary can also be used as a restrictor. The split ratio is controlled selecting the appropriate length and internal diameter of the capillary. CapLC columns packed with perfusive chromatography material such as POROS produce a much lower restriction. Thus, the split restrictor must be prepared accordingly. We use a Hypersil C4 0.3 × 3.5 cm column (5 μm, 300 Å particle) as a restrictor for a 250-μm id column (10-cm long) filled with POROS R1.

11. LC Packing offers optimized UV cells for capLC that can be adapted to most commercial detectors. Available cells are prepared to work at three different flow rate ranges: 10–100, 1–10, and 0.1–1 μL/min (Micro, Capillary and NanoLC cells, respectively). Cell volumes are 140 nL (micro), 35 nl (capillary), and 3 nL (nanoLC).

12. The conventional electrospray interface of the Finnigan instruments can be used instead of this dedicated interface when using capLC columns with inner diameter larger than 180 μm at flow rates higher than 3–5 μL/min. When working at flow rates close to or lower than 5 μL/min, sensitivity can be improved by reducing the default distance between the needle tip and the MS entrance (typically 3 cm) to 5 mm.

13. Pulled ESI needles can be prepared with a micropipette puller device. However, some glass pullers do not produce enough energy to fuse the silica. Most authors use laser pullers for this purpose (Sutter Instruments [Phoenix, CA]).

14. Stainless steel, fused silica and glass capillaries have been used as ESI needles. Some changes are usually made to the capillary tip in order to optimize spray formation. Fused silica capillaries with small id are commonly etched with HF *(45)* or the tip is polished with sand paper, resulting in very sharp ESI tips. Electropolishing has also been used with metal capillaries to produce similarly sharp tips *(53)*. ESI tips have been constructed with id values down to 10 μm. Spray tips as small as a few μm can be prepared from glass or fused silica capillaries using a micropipette puller *(54,55)*. Valaskovic et al. *(55)* obtain reproducible tip diameters down to 2 μm by first pulling capillaries and later etching them with HF. These tips can be operated at flow rates from 0.1–20 nL/min, depending on the diameter. New Objective (Cambridge, MA) distribute glass or fused silica tapered ESI tips with different internal diameters.

15. Peptide separations are greatly enhanced by the ion pairing effect of TFA. However, TFA is incompatible with ESI ionization and a higher concentration than 0.2% approx can eliminate the ESI signal completely. For this reason, TFA should

be avoided from capLC/ESI-MS eluents and replaced by formic or acetic acids to maximize sensitivity. In some cases, the use of mixtures containing 1% AcOH and a small amount of TFA (0.02–0.05%) may provide a good balance between chromatographic separation and signal intensity. When on-line UV detection is required, AcOH cannot be used at the concentration indicated earlier because of its high absorbance at 214 nm. In this case, it is advisable to use the minimal amount of TFA (0.05%) to allow both separation and compatibility with MS.

Acknowledgments

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ANEXO II

ANÁLISIS DE LAS PROTEÍNAS RETENIDAS EN LA MEMBRANA DEL CENTRICON DURANTE EL PROCESO DE PURIFICACIÓN DE PÉPTIDOS ASOCIADOS A LAS MOLÉCULAS DE MHC DE CLASE II

El extracto obtenido en el proceso de purificación de los péptidos asociados a MHCII mediante cromatografía de inmunoafinidad debería estar, en principio, compuesto por moléculas de HLA-DR y péptidos de clase II. Adicionalmente se podrían encontrar otras proteínas que se encontraran interaccionando con HLA-DR en la célula, proteínas que interaccionaran de forma inespecífica con el anticuerpo o con el soporte cromatográfico y restos del anticuerpo L243 inmovilizado en la columna que podrían desprenderse durante el proceso de elución. Para aislar los péptidos de clase II, estos extractos se sometieron a una separación por peso molecular utilizando un centricón con una membrana de corte de 10 kDa, de manera que los péptidos atravesaron la membrana y quedaron en el eluido. En la parte superior de la membrana quedaron retenidas todas las moléculas con peso moléculas superior a 10 kDa. Con objeto de caracterizar las proteínas retenidas en esta membrana, se han digerido y analizado los retenidos del centricón obtenidos en la purificación de péptidos de clase II en las células B-LCL y en los tiroides TB270, TB237 y TB448.

Las muestras se digirieron con tripsina previa reducción y alquilación y el extracto peptídico se analizó por MALDI-TOF. En todos los espectros obtenidos se detectaron numerosas señales. Sin embargo, únicamente se logró identificar mediante mapeo peptídico la cadena β de la molécula de MHCII en los extractos procedentes de las células B-LCL (13 péptidos identificados) y del tiroides TB448 (11 péptidos identificados), quedando además muchas señales sin identificar en estos espectros. Estos resultados son los esperados ya que, tal como hemos mostrado en un trabajo previo, el análisis por mapeo peptídico de muestras complejas suele resultar en la identificación preferente de las proteínas mayoritarias (Carrascal, 2002). En este mismo trabajo se demostró que mediante el uso del capLC-μESI-ITMS/MS era posible identificar proteínas minoritarias a partir de este tipo de muestras. En consecuencia, esta última técnica fue la utilizada para el análisis de todos los extractos trípticos. Los cromatogramas obtenidos para las muestras B-LCL y TB 448 se muestran en la figura 1.

A partir del retenido del centricón de las células B-LCL se identificaron un total de 31 péptidos que correspondían con 5 proteínas diferentes. Además de las cadenas α y β de la molécula de MHCII se caracterizaron la actina y piruvato quinasa humanas y la inmunoglobulina de ratón (Tabla II). A partir del retenido obtenido al procesar el tejido autoinmune se identificaron 24, 18 y 28 péptidos en los tiroides TB237, TB270 y TB448 respectivamente (Tablas III, IV y V). A pesar del diferente número de peptidos secuenciados estas secuencias permitireron identificar cinco proteínas en cada caso, algunas de ellas comunes a los diferentes tiroides por lo que el número total de proteínas diferentes identificadas fue de nueve.
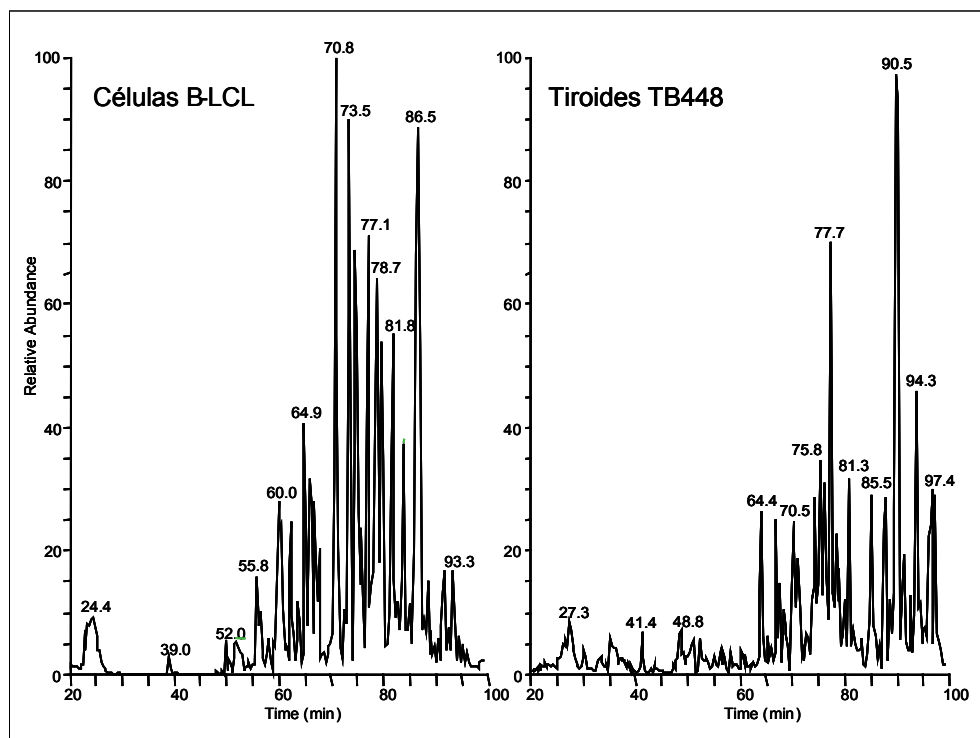
Figura 1: Cromatogramas obtenidos en el análisis por capLC-µESI-ITMS/MS de los extractos trípticos de los retenidos del centricón de células B-LCL y tiroides TB448.

En todos los extractos se han secuenciado péptidos derivados de la molécula de MHC de clase II. En la muestra derivada de las células B-LCL el mayor número de péptidos identificados provienen de las cadenas α y β de esta molécula, mientras que en los extractos procedentes del tiroides el mayor número corresponde a secuencias de diversas inmunoglobulinas de ratón. Estos péptidos provienen del anticuerpo inmovilizado en la columna de inmunoafinidad (L243). La cantidad de anticuerpo inmovilizado es muy alta y una pequeña proporción se desprende de la columna al eluir con 0.1% de TFA. La cantidad de anticuerpo detectado en estos análisis aumenta cuando hay una menor cantidad de otras proteínas en la muestra, aunque esta observación no necesariamente está relacionada con un aumento del sangrado de la columna sino que probablemente está relacionada con la ausencia de otras moléculas que compitan por la ionización. A pesar de que el número de péptidos derivados de inmunoglobulina de ratón es tan elevado, el análisis mediante mapeo peptídico no permitió su identificación ya que estas inmunoglobulinas están presentes en mezclas complejas de diferentes especies de esta familia. En las tablas se han agrupado en inmunoglobulinas humanas y de ratón diferenciándose, en este caso, entre las isoformas kappa, gamma y la cadena pesada..

Debido al elevado grado de homología de secuencia que existe entre los diferentes alelos de las moléculas de MHC, en algunos casos el péptido identificado corresponde a diferentes alelos (ver tablas I, II, III y IV). La identificación de secuencias situadas en zonas polimórficas de la molécula nos ha permitido discernir el alelo correspondiente a cada caso, confirmando, en las muestras de tiroides, el fenotipo determinado por medios convencionales.

El único péptido de clase II identificado en los repertorios peptídicos de estas muestras cuya proteína origen coincide con alguna de las identificadas en los retenidos del centricón es el péptido 62-77 de la molécula de MHCII DR4 (detectado en las células DR4IiDM). Estos resultados sugieren que todos o casi todos los péptidos identificados son ligandos asociados a DR4 y no contaminaciones provenientes de degradaciones ocurridas durante el proceso de purificación.

Se ha identificado actina en 3 de las 4 muestras procesadas. Debido a que la lisis se ha realizado en presencia de una cantidad alta de detergente, la presencia de actina en la muestra podría ser debida a interacciones inespecíficas de esta proteína con el soporte cromatográfico o con el anticuerpo. Sin embargo, se ha reportado que la actina está parcialmente asociada con moléculas de clase II purificadas (Newell, 1988), por lo que esta proteína también podría haber sido copurificada junto con HLA-DR. Barois y colaboradores analizaron el papel del citoesqueleto de actina en la vía de presentación de péptidos de clase II, mostrando que los microfilamentos de actina juegan un papel importante en la degradación de la cadena invariante y en la carga de los péptidos por la molécula de MHCII y que la despolimerización de los microfilamentos de actina inducen la acumulación de complejos recién formados MHCII-Ii en compartimentos no lisosomales. Además, estos microfilamentos están implicados en la endocitosis mediada por receptor y en el control del transporte entre los compartimentos endosomales (Barois, 1998). La importancia de esta proteína en la presentación del antígeno y en la estimulación de las células T ha sido reportada recientemente por los grupos de Rodgers y Fernández (Fernández, 2003; Gordy, 2004).

Tabla I: Péptidos secuenciados y proteínas identificadas en el análisis mediante capLC-μESI-ITMS/MS de los retenidos del centricón de las células B-LCL.

Células B-LCL (5 x 10^8 células)		
Péptidos identificados	**Mr**	**Proteínas identificadas**
AAVDTYCR	954.2	HLA clase II, DRB1-1, -4, -10, -11, DW2.2/DR2.2
FDSDVGEYR	1086.4	HLA clase II, DRB1-1, -4, -7, -8, -9 DRB3-1, -2
RAAVDTYCR	1110.6	HLA clase II, DRB1-1, -4, -10, -11, DW2.2/DR2.2
GHSGLQPTGLLS	1165.4	HLA clase II, DR
GHSGLQPTGFLS	1199.5	HLA clase II, DR
GHSNLQPTGFLS (G4>N)	1256.4	HLA clase II, DR
VYPEVTVYPAK	1264.4	HLA clase II,DRB1-4
RVYPEVTVYPAK	1420.8	HLA clase II,DRB1-4
YFYHQEEYVR	1432.6	HLA clase II,DRB1-4
HNYGVGESFTVQR	1492.8	HLA clase II, DRB1-1,-4, -7, -8, -9, -10, -11, DRB3-1, -2.
AVTELGRPDAEYWNSQK	1963.2	HLA clase II, DRB1-1, -4, -10, -W53, DW2.2/DR2.2
MLSGVGGFVLGLLFLGAGLFIYFR	2546.4	HLA clase II, DRB1-1,-4, -7, -8, -9, -10, -11, DRB3-1, -2.
TQPLQHHNLLVCSVNGFYPGSIEVR	2865.3	HLA clase II, DRB1-4, -10, W53.
TGVVSTGLIQNGDWTFQTLVMLETVPR	2961.4	HLA clase II,DRB1-4
LEEFGR	749.4	
KETVWR	817.6	
ANLEIMTK	934.4	
EPNVLICFIDK	1346.6	
VEHWGLDEPLLK	1434.6	HLA clase II, DRA
FASFEAQGALANIAVDK	1750.8	
NGKPVTTGVSETVFLPR	1801.0	
DGKPVTTGVSETVFFPR	1802.0	
FHYLPFLPSTEDVYDCR	2158.0	
EITALAPSTMK	1176.4	
DSYVGDEAQSK	1197.4	Actina (*H.sapiens*)
QEYDESGPSIVHR	1515.6	
SYELPDGQVITIGNER	1789.8	
IENHEGVR	952.6	Piruvato quinasa (*H.sapiens*)
LDIDSPPITAR	1196.4	
TSTSPIVK	831.4	Inmunoglobulina kappa (*M.musculus*)
HNSYTCEATHK	1303.5	

Tabla II: Péptidos secuenciados y proteínas identificadas en el análisis mediante capLC-µESI-ITMS/MS de los retenidos del centricón del tiroides TB237.

Tiroides TB237 (1.1 g)		
Péptidos identificados	**Mr**	**Proteínas identificadas**
YFYNQEESVR	1333.4	HLA clase II, Dw2.2/DR2.2 cadena
AGFAGDDAPR	975.2	Actina (*H.sapiens*)
AGVETTKPSK	1016.8	
EIQLVQSGGEVK (Q1>E)	1395.4	
EIQLVQSGGEVKK (Q1>E)	1514.6	Inmunoglobulina (*H.sapiens*)
QVGSGVTTDQVQAEAK	1616.8	
VDNALQSGNSQESVTEQDSK	2134.8	
VVSALPIQHQDWMSGK	1795.8	
DLPAPIER	909.2	
APQVYVLPPPEEEMTK	1827.6	
NTEPVLDSDGSYFMYSK	1951.8	
APQVYVLPPPEEEMTKK	1955.1	
QVTLTCMVTDFMPEDIYVEWTNNGK	2990.2	Inmunoglobulina gamma (*M.musculus*)
TISKPK	672.1	
GPTIKPCPPCK	1253.6	
SVVHEGLHNHHTTK	1594.8	
CPAPNLLGGPSVFIFPPK	1910.0	
LLIYGASNR	1005.4	
TSTSPIVK	831.4	
HNSYTCEATHK	1346.6	
QNGVLNSWTDQDSK	1590.6	Inmunoglobulina kappa (*M.musculus*)
FSGSGSGTQYSLK	1317.4	
ASENVVTYVSWYQQKPEQSPK	2468.2	
SLETSASTAYLQINNLK	1851.2	Inmunoglobulina, cadena pesada (*M.musculus*)

Tabla III: Péptidos secuenciados y proteínas identificadas en el análisis mediante capLC-µESI-ITMS/MS de los retenidos del centricón del tiroides TB270.

Tiroides TB270 (3.3 g)		
Péptidos identificados	**Mr**	**Proteínas identificadas**
VHPEVTVYPAK	1238.4	HLA clase II, DRB1-7, -9
FDSDVGEYR	1086.4	HLA clase II, DRB1-1, -4, -7, -8, -9 DRB3-1, -2
FASFEAQGALANIAVDK	1751	HLA clase II, DRA
VAPEEHPVLLTEAPLNPK	1953.4	Actina humana
TELNYK	766.2	
DLPAPIER	909.2	
APQVYVLPPPEEEMTK	1827.4	Inmunoglobulina gamma (*M.musculus*)
VNNKDLPAPIER	909.6	
EIQLVQSGPELK (Q1>E)	1321.6	
LLIYGASNR	1005.4	
TSTSPIVK	831.4	
DSTYSMSSTLTLTK	1533.4	
QNGVLNSWTDQDSK	1590.8	
GQNGVLNSWTDQDSK	1648.6	Inmunoglobulina kappa (*M.musculus*)
NIVMTQSPK	1016.4	
SMSMSVGER	982.2	
SYPYTFGGGTK	1176.2	
YTGVPDR	806.2	

Tabla IV: Péptidos secuenciados y proteínas identificadas en el análisis mediante capLC-µESI-ITMS/MS de los retenidos del centricón del tiroides TB448.

Tiroides TB448 (8 g)		
Péptidos identificados	**Mr**	**Proteínas identificadas**
FASFEAQGALANIAVDK	1751.4	
NGKPVTTGVSETVFLPR	1801.8	HLA clase II, DRA
VEHWGLDEPLLK	1435.2	
YFYHQEEYVR	1432.6	HLA clase II,DRB1-4
SGEVYTCQVEHPSLTSPLTVEWR	2674.2	HLA clase II, DRB1-1, -11, Dw2.2/DR2.2
GHSGLQPTGFLS	1199.6	HLA clase II, DRB1-1,7,4,8,9 / DRB3-1,-2
SGEVYTCQVEHPSVTSPLTVEWR	2661.2	HLA clase II, DRB1-1, -5, Dw2.2/DR2.2
WFLNGQEEK	1149.6	HLA clase II, Dw2.2/DR2.2
YFYNQEEYVR	1333.4	HLA clase II, Dw2.2/DR2.2
YFYNQEEYVR N4>H	1433.2	HLA clase II, Dw2.2/DR2.2
RAEVDTYCR	1168.6	HLA clase II, DR-W53
DIVMTQSPK	1016.4	
APQVYVLPPPEEEMTK	1827.6	
NTMPVLDSDGSYFMYSK, E3>M	1955	
NTEPVLDSDGSYFMYSK	1968.6	
VNNKDLPAPIER	909.8	Inmunoglobulina gamma (*M.musculus*)
GPTIKPCPPCK	1253.6	
NPTIKPCPPCK	1311	
VVSALPIQHQDWMSGK	1795.8	
LLIYGASNR	1005.6	
TSTSPIVK	831.4	
HNSYTCEATHK	1346.6	
QNGVLNSWTDQDSK	1591	
HNSYTCEATHK	1617.3	Inmunoglobulina kappa (*M.musculus*)
SMSMSVGER	982.4	
FSGSGSGTQYSLK	1317.6	
ASENVVTYVSWYQQKPEQSPK	2468	
GLKWMGWINTETGGPTVADDFK	2487.4	Inmunoglobulina, cadena pesada (*M.musculus*)

ANEXO III

LUTIN

INTERFASE DE USUARIO PARA EL PROGRAMA DE SECUENCIACIÓN

DE NOVO LUTEFISK XP

Este programa se ha realizado en el lenguaje de programación XBASIC. XBASIC es un programa de libre distribución, con licencia GPL dentro del proyecto GNU y transportable entre distintos sistemas operativos, incluidos Windows y Linux. La versión de XBASIC utilizada es la vs 6.2.3 para windows y puede obtenerse de http://xbasic.sourceforge.net. El programa actual se ha probado en ordenadores provistos de Windows NT, 2000, win98 y win XP.

Por motivos de espacio algunas líneas del programa (p.e. inicialización de matrices) se han unido en una, si bien es fácil detectar estos puntos para escribirlos de forma correcta. Por la misma razon algunas partes del programa generadas automáticamente por el editor de XBASIC han sido eliminadas.

Este programa provee de una interfase para la utilización sencilla y la automatización del programa de secuenciación *de novo* Lutefisk (R. Johnson y Angem Inc, http:hairyfatguy.com/Lutefisk/) y para la visualización y tratamiento de los archivos generados. El programa Lutefisk es un programa de acceso libre cuya última versión es la LutefiskXP 1.0 de 2003. El programa utilizado se ha compilado para trabajar bajo WIN32.

```
' ####################
' ##### PROLOG #####
' ####################
'
PROGRAM "progname"
VERSION "0.0000"
'
 IMPORT "xst" ' Standard library : required by most programs
 IMPORT "xgr" ' GraphicsDesigner : required by GuiDesigner programs
 IMPORT "xui" ' GuiDesigner : required by GuiDesigner programs
'
INTERNAL FUNCTION Entry ()
INTERNAL FUNCTION InitGui ()
INTERNAL FUNCTION InitProgram ()
INTERNAL FUNCTION CreateWindows ()
INTERNAL FUNCTION InitWindows ()
INTERNAL FUNCTION LUTIN (grid, message, v0, v1, v2, v3, r0, ANY)
INTERNAL FUNCTION LUTINCode (grid, message, v0, v1, v2, v3, kid, ANY)
'
' ####################
' ##### Entry () #####
' ####################
'
FUNCTION Entry ()
 SHARED terminateProgram
 STATIC entry
'
 IF entry THEN RETURN ' enter once
 entry = $$TRUE ' enter occured
'
 InitGui () ' initialize messages
 InitProgram () ' initialize this program
 CreateWindows () ' create main window and others
 InitWindows () ' initialize windows and grids
'
 IF LIBRARY(0) THEN RETURN ' main program executes message loop
'
 XstShowConsole()
 DO ' the message loop
  XgrPeekMessage (@wingrid, @message, @v0, @v1, @v2, @v3, r0, r1)
  IF message = #WindowResized THEN
   XgrGetWindowGrid (wingrid, @grid)
   XuiSendMessage (grid, #Redraw, 0, 0, 0, 0, 0, 0)
  END IF
  XgrProcessMessages (1) ' process one message
 LOOP UNTIL terminateProgram ' and repeat until program is terminated

END FUNCTION
"
' ####################
' ##### InitGui () #####
' ####################
'
FUNCTION InitGui ()
'
 program$ = PROGRAM$(0)
 XstSetProgramName (@program$)

' ***** Funciones de Inicializacion no incluidas *****

END FUNCTION
'
' ####################
' ##### InitProgram () #####
' ####################
'
FUNCTION InitProgram ()
 SHARED counts, PATHESCRITORIO$, PATHFILE$, PATHLUTE$
 SHARED paramf$[], param$[], filetype$[], enzyme$[], cys$[], thres$[], nterm$[],
 cterm$[], inst$[], mode$[]

 DIM paramf$[48], mode$[2], filetype$[4, 1], enzyme$[4]
 DIM cys$[3,1], nterm$[2,1], cterm$[2,1], inst$[2,1], thres$[3,1], param$[52,1,1]

 PATH0$ = "C:" + CHR$(92)
 PATH1$= "WINDOWS"+CHR$(92)+"Escritorio"
 PATHESCRITORIO$= PATH0$+PATH1$
 PATHFILE$ = PATHESCRITORIO$+CHR$(92)+ "LutefiskXP"
 PATHLUTE$ = PATHESCRITORIO$+CHR$(92)+ "LutefiskXP"

filetype$[0,0]="D"        filetype$[1,0]="F"        filetype$[2,0]="L"
filetype$[3,0]="T"        filetype$[4,0]="N"        filetype$[0,1]="dta"
filetype$[1,1]="txt"      filetype$[2,1]="txt"      filetype$[3,1]="txt"
filetype$[4,1]="dat"      mode$[0]="C"              mode$[1]="P"
mode$[2]="A"              enzyme$[0]="T"            enzyme$[1]="K"
enzyme$[2]="E"            enzyme$[3]="D"            enzyme$[4]="N"
cys$[0,0]="103.0100"      cys$[1,0]="160.03065"     cys$[2,0]="161.01466"
cys$[3,0]="208.06703"     cys$[0,1]="Unmod"         cys$[1,1]="carbam"
cys$[2,1]="carboxi"       cys$[3,1]="pyridil"       nterm$[0,0]="1.0078"
nterm$[1,0]="43.0184"     nterm$[2,0]="44.0136"     nterm$[0,1]="Unmod"
nterm$[1,1]="Acetyl"      nterm$[2,1]="Carbamyl"    cterm$[0,0]="17.0027"
cterm$[1,0]="16.0187"     cterm$[2,0]="31.0184"     cterm$[0,1]="Unmod"
cterm$[1,1]="Amide"       cterm$[2,1]="methyl"      thres$[0,0]="0.01"
thres$[1,0]="0.05"        thres$[2,0]="0.1"         thres$[3,0]="0.3"
```

```
thres$[0,1]="1 %"         thres$[1,1]="5 %"         thres$[2,1]="10 %"
thres$[3,1]="30 %"        inst$[0,0]="T"            inst$[1,0]="L"
inst$[2,0]="Q"            inst$[0,1]="TSQ"          inst$[1,1]="LCQ"
inst$[2,1]="QTOF"
counts=0
END FUNCTION
'
' ##### CreateWindows () #####
'
FUNCTION CreateWindows ()
'
IF LIBRARY(0) THEN RETURN
'
 LUTIN (@LUTIN, #CreateWindow, 0, 0, 0, 0, 0, 0)
 XuiSendMessage ( LUTIN, #SetWindowTitle, 0, 0, 0, 0, 0, "De Novo INTERFACE
version 2 JOAQUIN ABIAN 2004")
 XuiSendMessage ( LUTIN, #SetCallback, LUTIN, &LUTINCode(), -1, -1, -1, 0)
 XuiSendMessage ( LUTIN, #Initialize, 0, 0, 0, 0, 0, 0)
 XuiSendMessage ( LUTIN, #DisplayWindow, 0, 0, 0, 0, 0, 0)
 XuiSendMessage ( LUTIN, #SetGridProperties, -1, 0, 0, 0, 0, 0)
END FUNCTION
'
' ####################
' ##### InitWindows () #####
' ####################
'
FUNCTION InitWindows ()
'XstClearConsole()
END FUNCTION
'
' ####################
' ##### LUTIN () #####
' ####################
'
FUNCTION LUTIN (grid, message, v0, v1, v2, v3, r0, (r1, r1$, r1[], r1$[]))
 STATIC designX, designY, designWidth, designHeight
 STATIC SUBADDR sub[]
 STATIC upperMessage
 STATIC LUTIN
'
 $LUTIN = 0 'type = LUTIN          $LDirPath = 1 'type = XuiLabel
 $PathD = 2 'type = XuiTextLine    $PSelFile = 3 'type = XuiPushButton
 $LResult = 4 'type = XuiLabel     $DatName2 = 5 'type = XuiLabel
 $DatName = 6 'type = XuiListBox   $PLoadR = 7 'type = XuiPushButton
 $PSaveR = 8 'type = XuiPushButton  $PNewR = 9 'type = XuiPushButton
 $PPrintR = 10 'type = XuiPushButton  $CZoom = 11 'type = XuiCheckBox
 $CFontR = 12 'type = XuiCheckBox  $LBResultFile = 13 'type =
XuiListButton
 $LPath = 14 'type = XuiLabel      $PathP = 15 'type = XuiTextLine
 $TAreaR = 16 'type = XuiTextArea  $LParFile = 17 'type = XuiLabel
 $ParName = 18 'type = XuiTextLine  $PSavePar = 19 'type = XuiPushButton
 $FileList = 20 'type = XuiFile    $FileType = 21 'type = XuiLabel
 $DFileType = 22 'type = XuiDropButton $LData = 23 'type = XuiLabel
 $Mode = 24 'type = XuiLabel       $DMode = 25 'type = XuiDropButton
 $PLoadD = 26 'type = XuiPushButton  $PSaveD = 27 'type = XuiPushButton
 $PClearD = 28 'type = XuiPushButton  $LMass = 29 'type = XuiLabel
 $Mass = 30 'type = XuiTextLine    $LDataBis = 31 'type = XuiLabel
 $TAreaD = 32 'type = XuiTextArea  $LCharge = 33 'type = XuiLabel
 $DCharge = 34 'type = XuiDropButton  $LMerror = 35 'type = XuiLabel
 $Merror = 36 'type = XuiTextLine  $LFerror = 37 'type = XuiLabel
 $Ferror = 38 'type = XuiTextLine  $LPWithd = 39 'type = XuiLabel
 $TPeakW = 40 'type = XuiTextLine  $Enzyme = 41 'type = XuiLabel
 $DEnzyme = 42 'type = XuiDropButton  $Cys = 43 'type = XuiLabel
 $DCys = 44 'type = XuiDropButton  $Progress = 45 'type = XuiProgress
 $CTerm = 46 'type = XuiLabel      $DCTerm = 47 'type = XuiDropButton
 $NTerm = 48 'type = XuiLabel      $DNTerm = 49 'type = XuiDropButton
 $TAreaZoom = 50 'type = XuiTextArea  $LSeq = 51 'type = XuiLabel
 $TSeq = 52 'type = XuiTextLine    $LSubseq = 53 'type = XuiLabel
 $TSubseq = 54 'type = XuiTextLine  $LMassS = 55 'type = XuiLabel
 $TMassS = 56 'type = XuiTextLine  $LCQual = 57 'type = XuiLabel
 $CQual = 58 'type = XuiCheckBox   $LMaxE = 59 'type = XuiLabel
 $CMaxE = 60 'type = XuiCheckBox   $LTMass = 61 'type = XuiLabel
 $TTMass = 62 'type = XuiTextLine  $LGaps = 63 'type = XuiLabel
 $XuiTextLine931 = 64 'type = XuiTextLine
 $LExt = 65 'type = XuiLabel       $TExt = 66 'type = XuiTextLine
 $LExtT = 67 'type = XuiLabel      $TExtT = 68 'type = XuiTextLine
 $Inst = 69 'type = XuiLabel       $DInst = 70 'type = XuiDropButton
 $LHits = 71 'type = XuiLabel      $THits = 72 'type = XuiTextLine
 $LScoreT = 73 'type = XuiLabel    $TScoreT = 74 'type = XuiTextLine
 $PRun = 75 'type = XuiPushButton  $CBatch = 76 'type = XuiCheckBox
 $Thres = 77 'type = XuiLabel      $DThres = 78 'type = XuiDropButton
 $LIonsW = 79 'type = XuiLabel     $LIonsR = 80 'type = XuiLabel
 $TIonW = 81 'type = XuiTextLine   $TIonR = 82 'type = XuiTextLine
 $LMassO = 83 'type = XuiLabel     $TextMass = 84 'type = XuiTextLine
 $UpperKid = 84 'um
'
 IFZ sub[] THEN GOSUB Initialize
IF XuiProcessMessage (grid, message, @v0, @v1, @v2, @v3, @r0, @r1, LUTIN)
THEN RETURN
 IF (message <= upperMessage) THEN GOSUB @sub[message]
 RETURN
'
' ***** Callback ***** message = Callback : r1 = original message
'
```

```
SUB Callback
 message = r1  callback = message
 IF (message <= upperMessage) THEN GOSUB @sub[message]
END SUB
'
' ***** Create ***** v0123 = xywh : r0 = window : r1 = parent
'
SUB Create
IF (v0 <= 0) THEN v0 = 0
IF (v1 <= 0) THEN v1 = 0
IF (v2 <= 0) THEN v2 = designWidth
IF (v3 <= 0) THEN v3 = designHeight
XuiCreateGrid (@grid, LUTIN, @v0, @v1, @v2, @v3, r0, r1, &LUTIN())
XuiSendMessage ( grid, #SetGridName, 0, 0, 0, 0, 0, @"LUTIN")
XuiSendMessage ( grid, #SetColor, 105, $$Black, $$Black, $$White, 0, 0)
XuiLabel (@g, #Create, 4, 4, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LDirPath, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LDirPath")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetHelpString, 0, 0, 0, 0, 0, @"Directorio file datos y salida")
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"DIR DATA")
XuiTextLine (@g, #Create, 76, 4, 184, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $PathD, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"PathD")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea844")
XuiSendMessage ( g, #SetStyle, 0, 3, 0, 0, 1, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
1, 0)
XuiSendMessage ( g, #SetIndent, 4, 0, 4, 0, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiPushButton  (@g, #Create, 260, 4, 24, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $PSelFile, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"PSelFile")
XuiSendMessage ( g, #SetStyle, 2, 3, 0, 0, 0, 0)
XuiSendMessage ( g, #SetColorExtra, $$BrightGrey, $$LightYellow, $$Black,
$$White, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderFlat1, $$BorderFlat1, $$BorderLower1,
0, 0, 0)
XuiSendMessage ( g, #SetIndent, 18, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetImage, 0, 0, 5, 4, 0, @"C:\\Archivos de
programa\\XBasic\\images\\icon_open.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 16, 16, 0, 0)
XuiLabel (@g, #Create, 288, 4, 364, 24, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LResult, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LResult")
XuiSendMessage ( g, #SetColor, $$BrightGrey, $$LightBlue, $$BrightGrey,
$$BrightGrey, 0, 0)
XuiSendMessage ( g, #SetColorExtra, $$BrightGrey, $$LightYellow, $$BrightGrey,
$$White, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderLoLine1, $$BorderLoLine1,
$$BorderNone, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 240, 600, 700, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"De Novo Result Editor v1.1")
XuiLabel (@g, #Create, 4, 24, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $DatName2, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"DatName2")
XuiSendMessage ( g, #SetStyle, 1, 3, 0, 0, 0, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"DATOS")
XuiListBox  (@g, #Create, 76, 24, 208, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $DatName, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"DatName")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"TextLine")
XuiSendMessage ( g, #SetStyle, 0, 3, 0, 0, 1, 0)
XuiSendMessage ( g, #SetBorder, $$BorderRidge, $$BorderNone, $$BorderNone, 0,
1, 0)
XuiSendMessage ( g, #SetIndent, 1, 0, 1, 0, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 2, @"PressButton")
XuiSendMessage ( g, #SetStyle, 24, 0, 0, 0, 2, 0)
XuiSendMessage ( g, #SetColorExtra, $$Grey, $$LightYellow, $$Black, $$White, 2,
0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleCenter, 0, -1, -1, 2, 0)
XuiSendMessage ( g, #SetBorder, $$BorderRaise1, $$BorderRaise1,
$$BorderRaise1, 0, 2, 0)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 3, @"XuiList882")
XuiSendMessage ( g, #SetStyle, 0, 3, 0, 0, 3, 0)
XuiSendMessage ( g, #SetColorExtra, $$Grey, $$LightYellow, $$Black, $$White, 3,
0)
XuiSendMessage ( g, #SetBorder, $$BorderRidge, $$BorderRidge, $$BorderRidge,
0, 3, 0)
XuiSendMessage ( g, #SetIndent, 0, 0, 0, 0, 3, 0)
XuiPushButton  (@g, #Create, 288, 28, 60, 20, r0, grid)
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XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $PLoadR, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"PLoadR")
XuiSendMessage ( g, #SetStyle, 2, 3, 0, 0, 0, 0)
XuiSendMessage ( g, #SetColorExtra, $$LightRed, $$LightYellow, $$LightRed,
$$LightRed, 0, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderFlat1, $$BorderFlat1, $$BorderLower1,
0, 0, 0)
XuiSendMessage ( g, #SetIndent, 17, 0, 4, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetImage, 0, 0, 3, 3, 0, @"C:\\Archivos de
programa\\XBasic\\images\\icon_open.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 16, 16, 0, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"LOAD")
XuiPushButton  (@g, #Create, 348, 28, 56, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $PSaveR, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"PSaveR")
XuiSendMessage ( g, #SetStyle, 2, 3, 0, 0, 0, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderFlat1, $$BorderFlat1, $$BorderLower1,
0, 0, 0)
XuiSendMessage ( g, #SetIndent, 17, 0, 4, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetImage, 0, 0, 3, 3, 0, @"C:\\Archivos de
programa\\XBasic\\images\\icon_save.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 16, 16, 0, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"SAVE")
XuiPushButton  (@g, #Create, 404, 28, 64, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $PNewR, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"PNewR")
XuiSendMessage ( g, #SetStyle, 2, 3, 0, 0, 0, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderFlat1, $$BorderFlat1, $$BorderLower1,
0, 0, 0)
XuiSendMessage ( g, #SetIndent, 17, 0, 4, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetImage, 0, 0, 3, 3, 0, @"C:\\Archivos de
programa\\XBasic\\images\\icon_new.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 16, 16, 0, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"NEW")
XuiPushButton  (@g, #Create, 468, 28, 64, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $PPrintR, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"PPrintR")
XuiSendMessage ( g, #SetStyle, 2, 3, 0, 0, 0, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderFlat1, $$BorderFlat1, $$BorderLower1,
0, 0, 0)
XuiSendMessage ( g, #SetIndent, 20, 0, 4, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetImage, 0, 0, 7, 2, 0, @"C:\\Archivos de
programa\\XBasic\\images\\printer.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 17, 16, 0, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"PRINT")
XuiCheckBox (@g, #Create, 532, 28, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $CZoom, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"CZoom")
XuiSendMessage ( g, #SetStyle, 2, 3, 0, 0, 0, 0)
XuiSendMessage ( g, #SetImage, 0, 0, 25, 3, 0, @"C:\\Archivos de
programa\\XBasic\\images\\icon_assembly.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 16, 16, 0, 0)
XuiCheckBox (@g, #Create, 592, 28, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $CFontR, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"CFontR")
XuiSendMessage ( g, #SetStyle, 2, 3, 0, 0, 0, 0)
XuiSendMessage ( g, #SetIndent, 17, 0, 4, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"FONT")
XuiListButton (@g, #Create, 288, 48, 364, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LBResultFile, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LBResultFile")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetImage, 0, 0, 300, 5, 0, @"C:\\Archivos de
programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 7, 7, 0, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"hola")
DIM text$[2]
text$[0] = "hola"          text$[1] = "que"          text$[2] = "tal"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"PressButton")
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 1, 0)
XuiSendMessage ( g, #SetImage, 0, 0, 300, 5, 1, @"C:\\Archivos de
programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 7, 7, 1, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 1, @"hola")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 2, @"XuiList871")
DIM text$[2]
text$[0] = "hola"          text$[1] = "que"          text$[2] = "tal"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 2, @text$[])
XuiLabel  (@g, #Create, 4, 44, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LPath, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LPath")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
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XuiSendMessage (g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage (g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage (g, #SetHelpString, 0, 0, 0, 0, 0, @"Directorio archivo de parametros")
XuiSendMessage (g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 0, @"DIR PARAM")
XuiTextLine (@g, #Create, 76, 44, 208, 20, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $PathP, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"PathP")
XuiSendMessage (g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea1069")
XuiSendMessage (g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiTextArea (@g, #Create, 288, 64, 364, 564, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $TAreaR, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"TAreaR")
XuiSendMessage (g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage (g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 1, @"Text")
XuiSendMessage (g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 2, @"ScrollH")
XuiSendMessage (g, #SetStyle, 0, 0, 0, 0, 2, 0)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 3, @"ScrollV")
XuiSendMessage (g, #SetStyle, 0, 0, 0, 0, 3, 0)
XuiLabel (@g, #Create, 4, 64, 72, 20, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $LParFile, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"LParFile")
XuiSendMessage (g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage (g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage (g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage (g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 0, @"PARAMS")
XuiTextLine (@g, #Create, 76, 64, 128, 20, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $ParName, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"ParName")
XuiSendMessage (g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 0, @"default")
DIM text$[0]
text$[0] = "default"
XuiSendMessage (g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea828")
XuiSendMessage (g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiPushButton (@g, #Create, 204, 64, 80, 20, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $PSavePar, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"PSavePar")
XuiSendMessage (g, #SetStyle, 2, 3, 0, 0, 0, 0)
XuiSendMessage (g, #SetAlign, $$AlignMiddleRight, 0, -1, -1, 0, 0)
XuiSendMessage (g, #SetBorder, $$BorderFlat1, $$BorderFlat1, $$BorderLower1, 0, 0, 0)
XuiSendMessage (g, #SetIndent, 4, 0, 7, 0, 0, 0)
XuiSendMessage (g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage (g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage (g, #SetImage, 0, 0, 5, 3, 0, @"C:\\Archivos de programa\\XBasic\\images\\icon_save.bmp")
XuiSendMessage (g, #SetImageCoords, 0, 0, 16, 16, 0, 0)
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 0, @"SAVE PAR")
XuiFile (@g, #Create, 300, 80, 264, 200, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $FileList, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"FileList")
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 0, @"C:\\WINDOWS\\ESCRITORIO\\escrito.txt")
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 1, @"FileNameLabel")
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 2, @"FileNameText")
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 2, @"C:\\WINDOWS\\ESCRITORIO\\escrito.txt")
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 3, @"DirectoryLabel")
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 4, @"FilesLabel")
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 5, @"DirectoryBox")
DIM text$[26]

text$[0] = "A:\\"		text$[1] = "C:\\"	
text$[2] = " WINDOWS"		text$[3] = " ESCRITORIO"	
text$[4] = " "	text$[5] = " "	text$[6] = " "	text$[7] = " "
text$[8] = " "	text$[9] = " "	text$[10] = " "	text$[11] = " "
text$[12] = " "	text$[13] = " "	text$[14] = " "	text$[15] = " "
text$[16] = " "	text$[17] = " "	text$[18] = " "	text$[19] = " "
text$[20] = " "	text$[21] = " "	text$[22] = " "	text$[23] = "D:\\"
	text$[24] = "E:\\"	text$[25] = "F:\\"	text$[26] = "G:\\"

XuiSendMessage (g, #SetTextArray, 0, 0, 0, 0, 5, @text$[])
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 6, @"FileBox")
DIM text$[50]

text$[0] = " "	text$[1] = " "	text$[2] = " "	text$[3] = " "
text$[4] = ""	text$[5] = ""	text$[6] = ""	text$[7] = ""
text$[8] = ""	text$[9] = ""	text$[10] = ""	text$[11] = ""
text$[12] = ""	text$[13] = ""	text$[14] = ""	text$[15] = ""
text$[16] = ""	text$[17] = ""	text$[18] = ""	text$[19] = ""
text$[20] = ""	text$[21] = ""	text$[22] = ""	text$[23] = ""
text$[24] = ""	text$[25] = ""	text$[26] = ""	text$[27] = ""
text$[28] = ""	text$[29] = ""	text$[30] = ""	text$[31] = ""
text$[32] = ""	text$[33] = " "	text$[34] = " "	text$[35] = " "
text$[36] = " "	text$[37] = " "	text$[38] = " "	text$[39] = " "
text$[40] = " "	text$[41] = " "	text$[42] = " "	text$[43] = " "
text$[44] = " "	text$[45] = " "	text$[46] = " "	text$[47] = " "
text$[48] = " "	text$[49] = " "	text$[50] = " "	

XuiSendMessage (g, #SetTextArray, 0, 0, 0, 0, 6, @text$[])
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 7, @"EnterButton")
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 8, @"CancelButton")
XuiLabel (@g, #Create, 4, 84, 72, 16, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $FileType, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"FileType")
XuiSendMessage (g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage (g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage (g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage (g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 0, @"TIPO DAT")
XuiDropButton (@g, #Create, 76, 84, 60, 16, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $DFileType, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"DFileType")
XuiSendMessage (g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage (g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage (g, #SetImage, 0, 0, 50, 2, 0, @"C:\\Archivos de programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage (g, #SetImageCoords, 0, 0, 7, 7, 0, 0)
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 0, @"D")
DIM text$[4]

| text$[0] = ".dta" | text$[1] = ".txt (ICIS)" | text$[2] = ".txt (LCQ)" |
| text$[3] = ".txt (Tab)" | text$[4] = ".dat (SeQ)" | |

XuiSendMessage (g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 1, @"PressButton")
XuiSendMessage (g, #SetColor, $$BrightGrey, $$Green, $$Black, $$White, 1, 0)
XuiSendMessage (g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 1, 0)
XuiSendMessage (g, #SetIndent, 20, 0, 4, 0, 1, 0)
XuiSendMessage (g, #SetTexture, $$TextureNone, 0, 0, 0, 1, 0)
XuiSendMessage (g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
XuiSendMessage (g, #SetImage, 0, 0, 50, 2, 1, @"C:\\Archivos de programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage (g, #SetImageCoords, 0, 0, 7, 7, 1, 0)
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 1, @"D")
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 2, @"XuiPullDown768")
DIM text$[4]

| text$[0] = ".dta" | text$[1] = ".txt (ICIS)" | text$[2] = ".txt (LCQ)" |
| text$[3] = ".txt (Tab)" | text$[4] = ".dat (SeQ)" | |

XuiSendMessage (g, #SetTextArray, 0, 0, 0, 0, 2, @text$[])
XuiLabel (@g, #Create, 140, 88, 144, 20, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $LData, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"LData")
XuiSendMessage (g, #SetColor, $$BrightGrey, $$LightBlue, $$Black, $$White, 0, 0)
XuiSendMessage (g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage (g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage (g, #SetFont, 240, 600, 700, 0, 0, @"Arial")
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 0, @"Archivo Entrada")
XuiLabel (@g, #Create, 4, 100, 72, 16, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $Mode, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"Mode")
XuiSendMessage (g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage (g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage (g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage (g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 0, @"MODE")
XuiDropButton (@g, #Create, 76, 100, 60, 16, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $DMode, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"DMode")
XuiSendMessage (g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage (g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage (g, #SetImage, 0, 0, 50, 2, 0, @"C:\\Archivos de programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage (g, #SetImageCoords, 0, 0, 7, 7, 0, 0)
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 0, @"C")
DIM text$[2]

| text$[0] = "CENTROID" | text$[1] = "PROFILE" | text$[2] = "AUTO" |

XuiSendMessage (g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 1, @"PressButton")
XuiSendMessage (g, #SetColor, $$BrightGrey, $$Green, $$Black, $$White, 1, 0)
XuiSendMessage (g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 1, 0)
XuiSendMessage (g, #SetIndent, 20, 0, 4, 0, 1, 0)
XuiSendMessage (g, #SetTexture, $$TextureNone, 0, 0, 0, 1, 0)
XuiSendMessage (g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
XuiSendMessage (g, #SetImage, 0, 0, 50, 2, 1, @"C:\\Archivos de programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage (g, #SetImageCoords, 0, 0, 7, 7, 1, 0)
XuiSendMessage (g, #SetTextString, 0, 0, 0, 0, 1, @"C")
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 2, @"XuiPullDown772")
DIM text$[2]

| text$[0] = "CENTROID" | text$[1] = "PROFILE" | text$[2] = "AUTO" |

XuiSendMessage (g, #SetTextArray, 0, 0, 0, 0, 2, @text$[])
XuiPushButton (@g, #Create, 140, 108, 48, 32, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $PLoadD, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"PLoadD")
XuiSendMessage (g, #SetStyle, 2, 3, 0, 0, 0, 0)
XuiSendMessage (g, #SetBorder, $$BorderFlat1, $$BorderFlat1, $$BorderLower1, 0, 0, 0)
XuiSendMessage (g, #SetImage, 0, 0, 3, 0, 0, @"C:\\Archivos de programa\\XBasic\\images\\load3.bmp")
XuiSendMessage (g, #SetImageCoords, 0, 0, 42, 42, 0, 0)
XuiPushButton (@g, #Create, 188, 108, 48, 32, r0, grid)
XuiSendMessage (g, #SetCallback, grid, &LUTIN(), -1, -1, $PSaveD, grid)
XuiSendMessage (g, #SetGridName, 0, 0, 0, 0, 0, @"PSaveD")

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XuiSendMessage ( g, #SetStyle, 2, 3, 0, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderFlat1, $$BorderFlat1, $$BorderLower1,
0, 0, 0)
XuiSendMessage ( g, #SetImage, 0, 0, 3, 0, 0, @"C:\\Archivos de
programa\\XBasic\\images\\save3.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 42, 42, 0, 0)
XuiPushButton (@g, #Create, 236, 108, 48, 32, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $PClearD, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"PClearD")
XuiSendMessage ( g, #SetStyle, 2, 3, 0, 0, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderFlat1, $$BorderFlat1, $$BorderLower1,
0, 0, 0)
XuiSendMessage ( g, #SetImage, 0, 0, 5, 0, 0, @"C:\\Archivos de
programa\\XBasic\\images\\new3.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 42, 42, 0, 0)
XuiLabel (@g, #Create, 4, 120, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LMass, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LMass")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetHelpString, 0, 0, 0, 0, 0, @"Help!")
XuiSendMessage ( g, #SetHintString, 0, 0, 0, 0, 0, @"Masa ion precursor")
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"MASS")
XuiTextLine (@g, #Create, 76, 120, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $Mass, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"Mass")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleCenter, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetIndent, 5, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"1234.5")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea785")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleRight, 0, -1, -1, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiLabel (@g, #Create, 140, 140, 144, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LDataBis, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LDataBis")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"mass % ")
XuiTextArea (@g, #Create, 140, 156, 144, 432, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TAreaD, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TAreaD")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
DIM text$[3]
text$[0] = ""  text$[1] = ""  text$[2] = "\t\t  Introduce"
text$[3] = "\t\t\tdatos"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"Text")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 2, @"ScrollH")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 2, 0)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 3, @"ScrollV")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 3, 0)
XuiLabel (@g, #Create, 4, 140, 72, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LCharge, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LCharge")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"CHARGE")
XuiDropButton (@g, #Create, 76, 140, 60, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $DCharge, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"DCharge")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetHelpString, 0, 0, 0, 0, 0, @"Charge of precursor")
XuiSendMessage ( g, #SetHintString, 0, 0, 0, 0, 0, @"Charge of precursor")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"2")
DIM text$[4]
text$[0] = "0"          text$[1] = "1"          text$[2] = "2"
text$[3] = "3"          text$[4] = "4"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"PressButton")
XuiSendMessage ( g, #SetColor, $$BrightGrey, $$Green, $$Black, $$White, 1, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 1, 0)
XuiSendMessage ( g, #SetIndent, 20, 0, 4, 0, 1, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
XuiSendMessage ( g, #SetImage, 0, 0, 50, 2, 1, @"C:\\Archivos de
programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 7, 7, 1, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 1, @"2")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 2, @"XuiPullDown762")
XuiSendMessage ( g, #SetBorder, $$BorderRidge, $$BorderRidge, $$BorderNone, 0,
2, 0)
DIM text$[4]
text$[0] = "0"          text$[1] = "1"          text$[2] = "2"
text$[3] = "3"          text$[4] = "4"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 2, @text$[])
XuiLabel (@g, #Create, 4, 156, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LMerror, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LMerror")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)

XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"MASS error")
XuiTextLine (@g, #Create, 76, 156, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $Merror, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"Merror")
XuiSendMessage ( g, #SetIndent, 15, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"3.2")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea788")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleCenter, 0, -1, -1, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiLabel (@g, #Create, 4, 176, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LFerror, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LFerror")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"FRAG error")
XuiTextLine (@g, #Create, 76, 176, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $Ferror, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"Ferror")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleCenter, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetIndent, 15, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"2.3")
DIM text$[0]
text$[0] = "FE"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea790")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleCenter, 0, -1, -1, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 1, @"2.3")
XuiLabel (@g, #Create, 4, 196, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LPWithd, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LPWithd")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"PEAK Withd")
XuiTextLine (@g, #Create, 76, 196, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TPeakW, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TPeakW")
XuiSendMessage ( g, #SetIndent, 20, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"5")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea911")
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 1, @"Comic Sans MS")
XuiLabel (@g, #Create, 4, 256, 72, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $Enzyme, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"Enzyme")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"ENZIMA")
XuiDropButton (@g, #Create, 76, 256, 60, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $DEnzyme, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"DEnzyme")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetIndent, 0, 0, 4, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 240, 400, 700, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"T")
DIM text$[4]
text$[0] = "Trypsin"       text$[1] = "Lys-C"         text$[2] = "V8"
text$[3] = "AspN"          text$[4] = "None"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"PressButton")
XuiSendMessage ( g, #SetColor, $$BrightGrey, $$Green, $$Black, $$White, 1, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 1, 0)
XuiSendMessage ( g, #SetIndent, 20, 0, 4, 0, 1, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
XuiSendMessage ( g, #SetImage, 0, 0, 50, 2, 1, @"C:\\Archivos de
programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 7, 7, 1, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 1, @"T")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 2, @"XuiPullDown793")
DIM text$[4]
text$[0] = "Trypsin"       text$[1] = "Lys-C"         text$[2] = "V8"
text$[3] = "AspN"          text$[4] = "None"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 2, @text$[])
XuiLabel (@g, #Create, 4, 272, 72, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $Cys, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"Cys")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
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XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"MASS Cys")
XuiDropButton  (@g, #Create, 76, 272, 60, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $DCys, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"DCys")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"Unmod")
DIM text$[3]
text$[0] = "103"        text$[1] = "160"    text$[2] = "161"        text$[3] = "208"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"PressButton")
XuiSendMessage ( g, #SetColor, $$BrightGrey, $$Green, $$Black, $$White, 1, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 1, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
 XuiSendMessage ( g, #SetImage, 0, 0, 50, 2, 1, @"C:\\Archivos de
programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 7, 7, 1, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 1, @"Unmod")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 2, @"XuiPullDown776")
DIM text$[3]
text$[0] = "103"        text$[1] = "160"    text$[2] = "161"    text$[3] = "208"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 2, @text$[])
XuiProgress (@g, #Create, 336, 312, 260, 28, r0, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"Progress")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetColor, $$LightRed, $$Black, $$Black, $$White, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderFrame, $$BorderFrame, $$BorderNone,
0, 0, 0)
XuiLabel  (@g, #Create, 4, 288, 72, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $CTerm, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"CTerm")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
 XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"C Term")
XuiDropButton  (@g, #Create, 76, 288, 60, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $DCTerm, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"DCTerm")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetColor, $$Green, $$Black, $$Black, $$White, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"Unmod")
DIM text$[1]
text$[0] = "None"           text$[1] = "Amida"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"PressButton")
XuiSendMessage ( g, #SetColor, $$BrightGrey, $$Green, $$Black, $$White, 1, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 1, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
 XuiSendMessage ( g, #SetImage, 0, 0, 50, 2, 1, @"C:\\Archivos de
programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 7, 7, 1, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 1, @"Unmod")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 2, @"XuiPullDown814")
DIM text$[1]
text$[0] = "None"           text$[1] = "Amida"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 2, @text$[])
XuiLabel  (@g, #Create, 4, 304, 72, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $NTerm, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"NTerm")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
 XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"N Term")
XuiDropButton  (@g, #Create, 76, 304, 60, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $DNTerm, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"DNTerm")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"Unmod")
DIM text$[2]
text$[0] = "None"           text$[1] = "Acetyl"         text$[2] = "Carbam"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"PressButton")
XuiSendMessage ( g, #SetColor, $$BrightGrey, $$Green, $$Black, $$White, 1, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 1, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
 XuiSendMessage ( g, #SetImage, 0, 0, 50, 2, 1, @"C:\\Archivos de
programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 7, 7, 1, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 1, @"Unmod")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 2, @"XuiPullDown818")
DIM text$[2]
text$[0] = "None"           text$[1] = "Acetyl"         text$[2] = "Carbam"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 2, @text$[])
XuiTextArea (@g, #Create, 288, 360, 364, 268, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TAreaZoom, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TAreaZoom")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 360, 600, 0, 0, 0, @"Courier New")

XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"hola")
DIM text$[5]
text$[0] = "1 hola"         text$[1] = "2 quet"         text$[2] = "3 MS"
text$[3] = "4 VDC"          text$[4] = "5 MNT"          text$[5] = "6 FG"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"Text")
XuiSendMessage ( g, #SetFont, 360, 600, 0, 0, 1, @"Courier New")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 2, @"ScrollH")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 2, 0)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 3, @"ScrollV")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 3, 0)
XuiLabel  (@g, #Create, 4, 324, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LSeq, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LSeq")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
 XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"SEQU max")
XuiTextLine (@g, #Create, 76, 324, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TSeq, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TSeq")
XuiSendMessage ( g, #SetIndent, 5, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"20000")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea916")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiLabel  (@g, #Create, 4, 344, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LSubseq, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LSubseq")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
 XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"SUBSEQ")
XuiTextLine (@g, #Create, 76, 344, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TSubseq, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TSubseq")
XuiSendMessage ( g, #SetIndent, 8, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"5000")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea918")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiLabel  (@g, #Create, 4, 364, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LMassS, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LMassS")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
 XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"MASS Sc")
XuiTextLine (@g, #Create, 76, 364, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TMassS, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TMassS")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetIndent, 14, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"0")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea920")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
XuiLabel  (@g, #Create, 4, 388, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $CQual, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"CQual")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
 XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"Chk Quality")
XuiCheckBox (@g, #Create, 76, 388, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LCQual, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"XuiCheckBox886")
XuiLabel  (@g, #Create, 4, 408, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LMaxE, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LMaxE")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
 XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"MaxEnt3")
XuiCheckBox (@g, #Create, 76, 408, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $CMaxE, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"CMaxE")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiLabel  (@g, #Create, 4, 428, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LTMass, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LTMass")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0,
0, 0)
 XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"MASS prom")
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XuiTextLine (@g, #Create, 76, 428, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TTMass, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TTMass")
XuiSendMessage ( g, #SetIndent, 11, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"5000")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea925")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiLabel (@g, #Create, 4, 448, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LGaps, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LGaps")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"GAPS max")
XuiTextLine (@g, #Create, 76, 448, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $XuiTextLine931, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"XuiTextLine931")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetIndent, 14, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"-1")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea932")
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 1, @"Arial")
XuiLabel (@g, #Create, 4, 468, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LExt, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LExt")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"EXTEN")
XuiTextLine (@g, #Create, 76, 468, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TExt, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TExt")
XuiSendMessage ( g, #SetIndent, 20, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"6")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea927")
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 1, @"Comic Sans MS")
XuiLabel (@g, #Create, 4, 488, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LExtT, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LExtT")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"EXTEN Thres")
XuiTextLine (@g, #Create, 76, 488, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TExtT, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TExtT")
XuiSendMessage ( g, #SetIndent, 12, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"0.15")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea929")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiLabel (@g, #Create, 4, 552, 72, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $Inst, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"Inst")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"MS")
XuiDropButton (@g, #Create, 76, 552, 60, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $DInst, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"DInst")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"TSQ")
DIM text$[2]
text$[0] = "TSQ"          text$[1] = "LCQ"          text$[2] = "QTOF"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"PressButton")
XuiSendMessage ( g, #SetColor, $$BrightGrey, $$Green, $$Black, $$White, 1, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 1, 0)
XuiSendMessage ( g, #SetIndent, 15, 0, 4, 0, 1, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
XuiSendMessage ( g, #SetImage, 0, 0, 50, 2, 1, @"C:\\Archivos de
programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 7, 7, 1, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 2, @"TSQ")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 2, @"XuiPullDown780")
DIM text$[2]
text$[0] = "TSQ"          text$[1] = "LCQ"          text$[2] = "QTOF"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 2, @text$[])
XuiLabel (@g, #Create, 4, 568, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LHits, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LHits")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)

XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"# HITS")
XuiTextLine (@g, #Create, 76, 568, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $THits, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"THits")
XuiSendMessage ( g, #SetIndent, 14, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"5")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea902")
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 1, @"Comic Sans MS")
XuiLabel (@g, #Create, 4, 588, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LScoreT, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LScoreT")
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"SCORE Tresh")
XuiTextLine (@g, #Create, 76, 588, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TScoreT, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TScoreT")
XuiSendMessage ( g, #SetIndent, 14, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"2.3")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea623")
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 1, @"Arial")
XuiPushButton (@g, #Create, 140, 592, 144, 36, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $PRun, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"PRun")
XuiSendMessage ( g, #SetColorExtra, $$Grey, $$LightRed, $$Black, $$White, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 240, 600, 700, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"RUN")
XuiCheckBox (@g, #Create, 4, 608, 132, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $CBatch, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"CBatch")
XuiSendMessage ( g, #SetStyle, 3, 3, 0, 0, 0, 0)
XuiSendMessage ( g, #SetIndent, 33, 0, 4, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"BATCH PROCESS")
XuiLabel (@g, #Create, 4, 240, 72, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $Thres, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"Thres")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 500, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"THRESHOLD")
XuiDropButton (@g, #Create, 76, 240, 60, 16, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $DThres, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"DThres")
XuiSendMessage ( g, #SetStyle, 0, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 700, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"10%")
DIM text$[3]
text$[0] = "1%"          text$[1] = "5%"          text$[2] = "10%"
text$[3] = "30%"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 0, @text$[])
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"PressButton")
XuiSendMessage ( g, #SetColor, $$BrightGrey, $$Green, $$Black, $$White, 1, 0)
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 1, 0)
XuiSendMessage ( g, #SetIndent, 9, 0, 4, 0, 1, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 1, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
XuiSendMessage ( g, #SetImage, 0, 0, 50, 2, 1, @"C:\\Archivos de
programa\\XBasic\\images\\dn7x7.bmp")
XuiSendMessage ( g, #SetImageCoords, 0, 0, 7, 7, 1, 0)
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 1, @"10%")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 2, @"XuiPullDown822")
DIM text$[3]
text$[0] = "1%"          text$[1] = "5%"          text$[2] = "10%"
text$[3] = "30%"
XuiSendMessage ( g, #SetTextArray, 0, 0, 0, 0, 2, @text$[])
XuiLabel (@g, #Create, 4, 508, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LIonsW, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LIonsW")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"IONS wind")
XuiLabel (@g, #Create, 4, 528, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LIonsR, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LIonsR")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"IONS res")
XuiTextLine (@g, #Create, 76, 508, 60, 20, r0, grid)
```

```
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TIonW, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TIonW")
XuiSendMessage ( g, #SetIndent, 14, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"6")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea1496")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
XuiTextLine (@g, #Create, 76, 528, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TIonR, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TIonR")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetIndent, 19, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Comic Sans MS")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"4")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea1498")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Comic Sans MS")
XuiLabel (@g, #Create, 4, 216, 72, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $LMassO, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"LMassO")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, $$JustifyCenter, -1, -1, 0, 0)
XuiSendMessage ( g, #SetBorder, $$BorderNone, $$BorderNone, $$BorderNone, 0, 0, 0)
XuiSendMessage ( g, #SetTexture, $$TextureNone, 0, 0, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"MASS offset")
XuiTextLine (@g, #Create, 76, 216, 60, 20, r0, grid)
XuiSendMessage ( g, #SetCallback, grid, &LUTIN(), -1, -1, $TextMass, grid)
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 0, @"TextMass")
XuiSendMessage ( g, #SetAlign, $$AlignMiddleLeft, 0, -1, -1, 0, 0)
XuiSendMessage ( g, #SetIndent, 13, 0, 1, 0, 0, 0)
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 0, @"Arial")
XuiSendMessage ( g, #SetTextString, 0, 0, 0, 0, 0, @"0.0")
XuiSendMessage ( g, #SetGridName, 0, 0, 0, 0, 1, @"XuiArea1501")
XuiSendMessage ( g, #SetFont, 200, 400, 0, 0, 1, @"Arial")
GOSUB Resize
END SUB
'
' ***** CreateWindow *****   v0123 = xywh : r0 = windowType : r1$ = display$
'
SUB CreateWindow
 IF (v0 == 0) THEN v0 = designX
 IF (v1 == 0) THEN v1 = designY
 IF (v2 <= 0) THEN v2 = designWidth
 IF (v3 <= 0) THEN v3 = designHeight
 XuiWindow (@window, #WindowCreate, v0, v1, v2, v3, r0, @r1$)
 v0 = 0 : v1 = 0 : r0 = window : ATTACH r1$ TO display$
 GOSUB Create
 r1 = 0 : ATTACH display$ TO r1$
 XuiWindow (window, #WindowRegister, grid, -1, v2, v3, @r0, @"LUTIN")
END SUB
'
' ***** GetSmallestSize ***** see "Anatomy of Grid Functions"
SUB GetSmallestSize
END SUB
'
' ***** Redrawn ***** see "Anatomy of Grid Functions"
'
SUB Redrawn
 XuiCallback (grid, #Redrawn, v0, v1, v2, v3, r0, r1)
END SUB
'
' ***** Resize ***** see "Anatomy of Grid Functions"
'
SUB Resize
END SUB
'
' ***** Selection ***** see "Anatomy of Grid Functions"
'
SUB Selection
END SUB
'
' ***** Initialize ***** see "Anatomy of Grid Functions"
'
SUB Initialize
 XuiGetDefaultMessageFuncArray (@func[])
 XgrMessageNameToNumber (@"LastMessage", @upperMessage)
 func[#Callback] = &XuiCallback () ' disable to handle Callback messages internally
 func[#GetSmallestSize] = 0 ' enable to add internal GetSmallestSize routine
 func[#Resize] = 0 ' enable to add internal Resize routine
 DIM sub[upperMessage]
 sub[#Create] = SUBADDRESS (Create) ' must be subroutine in this function
 sub[#CreateWindow] = SUBADDRESS (CreateWindow) ' must be subroutine in
this function
 sub[#GetSmallestSize] = SUBADDRESS (GetSmallestSize) ' enable to add internal
GetSmallestSize routine
 sub[#Redrawn] = SUBADDRESS (Redrawn) ' generate #Redrawn callback if
appropriate
 sub[#Resize] = SUBADDRESS (Resize) ' enable to add internal Resize routine
 sub[#Selection] = SUBADDRESS (Selection) ' routes Selection callbacks to
subroutine
'
 IF sub[0] THEN PRINT "LUTIN() : Initialize : error ::: (undefined message)"
 IF func[0] THEN PRINT "LUTIN() : Initialize : error ::: (undefined message)"
 XuiRegisterGridType (@LUTIN, "LUTIN", &LUTIN(), @func[], @sub[])

 designX = 615            designY = 35
 designWidth = 656        designHeight = 632
```

```
'
 gridType = LUTIN
 XuiSetGridTypeProperty (gridType, @"x", designX)
 XuiSetGridTypeProperty (gridType, @"y", designY)
 XuiSetGridTypeProperty (gridType, @"width", designWidth)
 XuiSetGridTypeProperty (gridType, @"height", designHeight)
 XuiSetGridTypeProperty (gridType, @"maxWidth", designWidth)
 XuiSetGridTypeProperty (gridType, @"maxHeight", designHeight)
 XuiSetGridTypeProperty (gridType, @"minWidth", designWidth)
 XuiSetGridTypeProperty (gridType, @"minHeight", designHeight)
 XuiSetGridTypeProperty (gridType, @"can", $$Focus OR $$Respond OR
$$Callback OR $$InputTextString OR $$TextSelection)
 XuiSetGridTypeProperty (gridType, @"focusKid", $ParName)
 XuiSetGridTypeProperty (gridType, @"inputTextArray", $TAreaD)
 XuiSetGridTypeProperty (gridType, @"inputTextString", $PathD)
 IFZ message THEN RETURN
END SUB
END FUNCTION
'
' #######################
' ##### LUTINCode () #####
' #######################
'
FUNCTION  LUTINCode (grid, message, v0, v1, v2, v3, kid, r1)

 $LUTIN = 0'type = LUTIN              $LDirPath = 1'type = XuiLabel
 $PathD = 2'type = XuiTextLine        $PSelFile = 3'type = XuiPushButton
 $LResult = 4'type = XuiLabel         $DatName2 = 5'type = XuiLabel
 $DatName = 6'type = XuiListBox       $PLoadR = 7'type = XuiPushButton
 $PSaveR = 8'type = XuiPushButton     $PNewR = 9'type = XuiPushButton
 $PPrintR = 10  'type = XuiPushButton   $CZoom = 11  'type = XuiCheckBox
 $CFontR = 12  'type = XuiCheckBox    $LBResultFile = 13  'type =
XuiListButton
 $LPath = 14  'type = XuiLabel        $PathP = 15  'type = XuiTextLine
 $TAreaR = 16  'type = XuiTextArea    $LParFile = 17  'type = XuiLabel
 $ParName = 18  'type = XuiTextLine   $PSavePar = 19  'type =
XuiPushButton
 $FileList = 20  'type = XuiFile      $FileType = 21  'type = XuiLabel
 $DFileType = 22  'type = XuiDropButton  $LData = 23  'type = XuiLabel
 $Mode = 24  'type = XuiLabel         $DMode = 25  'type =
XuiDropButton
 $PLoadD = 26  'type = XuiPushButton  $PSaveD = 27  'type =
XuiPushButton
 $PClearD = 28  'type = XuiPushButton  $LMass = 29  'type = XuiLabel
 $Mass = 30  'type = XuiTextLine      $LDataBis = 31  'type = XuiLabel
 $TAreaD = 32  'type = XuiTextArea    $LCharge = 33  'type = XuiLabel
 $DCharge = 34  'type = XuiDropButton  $LMerror = 35  'type = XuiLabel
 $Merror = 36  'type = XuiTextLine    $LFerror = 37  'type = XuiLabel
 $Ferror = 38  'type = XuiTextLine    $LPWithd = 39  'type = XuiLabel
 $TPeakW = 40  'type = XuiTextLine    $Enzyme = 41  'type = XuiLabel
 $DEnzyme = 42  'type = XuiDropButton  $Cys = 43  'type = XuiLabel
 $DCys = 44  'type = XuiDropButton    $Progress = 45  'type = XuiProgress
 $CTerm = 46  'type = XuiLabel        $DCTerm = 47  'type =
XuiDropButton
 $NTerm = 48  'type = XuiLabel        $DNTerm = 49  'type =
XuiDropButton
 $TAreaZoom = 50  'type = XuiTextArea  $LSeq = 51  'type = XuiLabel
 $TSeq = 52  'type = XuiTextLine      $LSubseq = 53  'type = XuiLabel
 $TSubseq = 54  'type = XuiTextLine   $LMassS = 55  'type = XuiLabel
 $TMassS = 56  'type = XuiTextLine    $LCQual = 57  'type = XuiLabel
 $CQual = 58  'type = XuiCheckBox     $LMaxE = 59  'type = XuiLabel
 $CMaxE = 60  'type = XuiCheckBox     $LTMass = 61  'type = XuiLabel
 $TTMass = 62  'type = XuiTextLine    $LGaps = 63  'type = XuiLabel
 $XuiTextLine931 = 64  'type = XuiTextLine  $LExt = 65  'type = XuiLabel
 $TExt = 66  'type = XuiTextLine      $LExtT = 67  'type = XuiLabel
 $TExtT = 68  'type = XuiTextLine     $Inst = 69  'type = XuiLabel
 $DInst = 70  'type = XuiDropButton   $LHits = 71  'type = XuiLabel
 $THits = 72  'type = XuiTextLine     $LScoreT = 73  'type = XuiLabel
 $TScoreT = 74  'type = XuiTextLine   $PRun = 75  'type = XuiPushButton
 $CBatch = 76  'type = XuiCheckBox    $Thres = 77  'type = XuiLabel
 $DThres = 78  'type = XuiDropButton  $LIonsW = 79  'type = XuiLabel
 $LIonsR = 80  'type = XuiLabel       $TIonW = 81  'type = XuiTextLine
 $TIonR = 82  'type = XuiTextLine     $LMassO = 83  'type = XuiLabel
 $TextMass = 84  'type = XuiTextLine  $UpperKid = 84  ' kidum
'
SHARED counts, manual, bacht, paso, marcador, atime, PATHESCRITORIO$,
PATHFILE$, PATHLUTE$, PATHParam$, busca$, dato$
SHARED ifile$, ifilex$, ifilelong$, ifilelongpath$, lutfilelongpath$, rfilelong$,
nombrefiletype$
SHARED paramf$[], file$[], filed$[], filet$[], param$[], pfile$[], cfile$[], filetype$[],
list$[], enzyme$[]
SHARED cys$[], thres$[], nterm$[], cterm$[], inst$[], mode$[], rfile$[]
'
IF counts=0 THEN
 paso= 1       bacht=0        marcador=1
 DIM vacio$[0]
 XuiSendMessage (grid, #Disable,0,0,0,0, $Progress,0)
 XuiSendMessage(grid,#Disable,0,0,0,0,$FileList,0)
 XuiSendMessage(grid,#Disable,0,0,0,0,$TAreaZoom,0)
 dato$=""      nombrefiletype$ = ""         parfile$ = "Lutefisk"
 counts = 1
 GOSUB LeerParamFile
 GOSUB SetParams
 GOSUB DrawParams
 GOSUB DrawFileName
 GOSUB LoadFile
END IF
```

```
'
    buscoR$= PATHFILE$ + CHR$(92) + "*.txt"
    XstGetFiles (buscoR$,@file$[])
    XuiSendMessage (grid,#SetTextArray,0,0,0,0,$LBResultFile, @file$[])
    IF (message == #Callback) THEN message = r1
'
    SELECT CASE message
    CASE #Selection  : GOSUB Selection  ' most common callback message
    CASE #CloseWindow : QUIT (0)  ' close main window and no cleanup needed
    CASE #CloseWindow : XuiSendMessage (grid, #HideWindow, 0, 0, 0, 0, 0)
    END SELECT
    RETURN

' ***** Selection *****
'
SUB Selection
 SELECT CASE kid
  CASE $LUTIN :
  CASE $PathD :
    XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $PathD, @PATHFILE$)
    GOSUB DrawFileName
  CASE $DatName :
    XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DatName, @ifilex$)
    position = RINSTR(ifilex$, ".")
    extension$ = LCLIP$(ifilex$, position)
    marca=0
    IF extension$ = "dat" THEN marca=1
    IF extension$ = "txt" THEN marca=1
    IF extension$ = "dta" THEN marca=1
    IF marca=1 THEN
     long = LEN(ifilex$)
     ifile$ = LEFT$(ifilex$, position-1)
    ELSE
     ifile$=ifilex$
    END IF
    XuiSendMessage (grid,#SetTextString,0,0,0,0,$DatName, @ifile$)
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DatName,0)
    GOSUB LoadFile
  CASE $TAreaD :
  CASE $TAreaR :
    XuiSendStringMessage (grid, @"GetTextArray", 0, 0, 0, 0, $TAreaR, @rfile$[])
  CASE $DFileType :
    XuiSendStringMessage (grid, @"GetTextArrayLine", v0, 0, 0, 0, 0, @text$)
    letrafiletype$=filetype$[ABS(v0),0]
    nombrefiletype$=filetype$[ABS(v0),1]
    XuiSendMessage (grid,#SetTextString,0,0,0,0,$DFileType, @letrafiletype$)
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DFileType,0)
    GOSUB DrawFileName
  CASE $ParName :
  CASE $Mode :
    XuiSendStringMessage (grid, @"GetTextArrayLine", v0, 0, 0, 0, 0, @text$)
    mode$=mode$[ABS(v0)]
    XuiSendMessage (grid,#SetTextString,0,0,0,0,$DMode, @mode$)
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DMode,0)
  CASE $Mass :
    XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $Mass, @mass$)
  CASE $DCharge :
    XuiSendStringMessage (grid, @"GetTextArrayLine", v0, 0, 0, 0, 0, @text$)
    charge$= STRING(ABS(v0))
    XuiSendMessage (grid,#SetTextString,0,0,0,0,$DCharge, @charge$)
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DCharge,0)
  CASE $Merror :
    XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $Merror, @merror$)
  CASE $Ferror :
    XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $Ferror, @ferror$)
  CASE $DEnzyme :
    XuiSendStringMessage (grid, @"GetTextArrayLine", v0, 0, 0, 0, 0, @text$)
    enzyme$=enzyme$[ABS(v0)]
    XuiSendMessage (grid,#SetTextString,0,0,0,0,$DEnzyme, @enzyme$)
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DEnzyme,0)
  CASE $DCys :
    XuiSendStringMessage (grid, @"GetTextArrayLine", v0, 0, 0, 0, 0, @text$)
    cys0$=cys$[ABS(v0),0]
    cys1$=cys$[ABS(v0),1]
    XuiSendMessage (grid,#SetTextString,0,0,0,0,$DCys, @cys1$)
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DCys,0)
  CASE $DCTerm :
    XuiSendStringMessage (grid, @"GetTextArrayLine", v0, 0, 0, 0, 0, @text$)
    cterm0$= cterm$[ABS(v0),0]
    cterm1$= cterm$[ABS(v0),1]
    XuiSendMessage (grid,#SetTextString,0,0,0,0,$DCTerm, @cterm1$)
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DCTerm,0)
  CASE $DNTerm :
    XuiSendStringMessage (grid, @"GetTextArrayLine", v0, 0, 0, 0, 0, @text$)
    nterm0$= nterm$[ABS(v0),0]
    nterm1$= nterm$[ABS(v0),1]
    XuiSendMessage (grid,#SetTextString,0,0,0,0,$DNTerm, @nterm1$)
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DNTerm,0)
  CASE $DThres :
    XuiSendStringMessage (grid, @"GetTextArrayLine", v0, 0, 0, 0, 0, @text$)
    thres0$=thres$[ABS(v0),0]
    thres1$=thres$[ABS(v0),1]
    XuiSendMessage (grid,#SetTextString,0,0,0,0,$DThres, @thres1$)
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DThres,0)
  CASE $CQual :
  CASE $PLoadD :
    GOSUB LoadFile
  CASE $PSaveR :
    GOSUB SaveResult
  CASE $PClearD :
    XuiSendMessage (grid,#SetTextArray,0,0,0,0,$TAreaD, @vacio$[])
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$TAreaD,0)
  CASE $PRun :
    manual=1
    IF bacht=0 THEN GOSUB RunFile
    IF bacht=1 THEN GOSUB RunBacht
  CASE $PLoadR :
    manual=1
    GOSUB LoadLut
  CASE $PSaveD :
    XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DatName, @ifile$)
    XuiSendStringMessage (grid, @"GetTextArray", 0, 0, 0, 0, $TAreaD, @list$[])
    emptyfile$= PATHFILE$ + CHR$(92)+ "nomequites.txt"
    ofilelongpath$ = PATHFILE$ + CHR$(92)+ "s" + ifile$ + "." + nombrefiletype$
    XstCopyFile (emptyfile$ , ofilelongpath$ )
    linea= UBOUND(list$[])
    FOR k= 0 TO linea-1
     list$[k]=list$[k] + CHR$(13)
    NEXT k
    XstSaveStringArray (ofilelongpath$, @list$[])
  CASE $PPrintR :
  CASE $THits :
    XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $THits, @hit$)
  CASE $TScoreT :
    XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $TScoreT, @score$)
  CASE $TPeakW :
    XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $TPeakW, @peakw$)
  CASE $DInst :
    XuiSendStringMessage (grid, @"GetTextArrayLine", v0, 0, 0, 0, 0, @text$)
    inst0$=inst$[ABS(v0),0]
    inst1$= inst$[ABS(v0),1]
    XuiSendMessage (grid,#SetTextString,0,0,0,0,$DInst, @inst1$)
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DInst,0)
  CASE $PSavePar :
    GOSUB LeerWinParams
    GOSUB LoadArray
    GOSUB GrabarParams
  CASE $PSelFile:
    XuiSendMessage (grid,#Enable,0,0,0,0,$FileList,0)
    XuiSendMessage(grid,#Redraw,0,0,0,0,$FileList,0)
    busca$= "f"
  CASE $FileList :
    XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $FileList,
@ifilelongpath$)
    IF busca$="f" THEN
    XstDecomposePathname(ifilelongpath$, @PATHFILE$, @parent$, @ifilelong$,
@ifile$, @ifiletypex$)
    ifile$=TRIM$(ifile$)
    ifiletype$= LCLIP$(ifiletypex$,1)
    nombrefiletype$= ifiletype$
    GOSUB DrawFileName
    XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $PathD, @PATHFILE$)
    XuiSendMessage (grid, #Redraw, 0, 0, 0, 0, $PathD, 0)
    XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DatName, @ifile$)
    XuiSendMessage (grid, #Redraw, 0, 0, 0, 0, $DatName, 0)
    XuiSendMessage(grid,#Disable,0,0,0,0,$FileList,0)
    XuiSendMessage(grid,#Redraw,0,0,0,0,$TAreaR,0)
    XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $PathD, @PATHFILE$)
    XuiSendMessage (grid, #Redraw, 0, 0, 0, 0, $PathD, 0)
    FOR v=0 TO 4
    IF ifiletype$=filetype$[v, 1] THEN
    letrafiletype$=filetype$[v, 0]
    XuiSendMessage (grid,#SetTextString,0,0,0,0,$DFileType, @letrafiletype$)
    XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DFileType,0)
    GOSUB LoadFile
    END IF
    NEXT v
    END IF
  CASE $LBResultFile :
    manual=1
    lutfilelongpath$= PATHFILE$+ CHR$(92) + file$[v0]
    rfilelong$ = file$[v0]
    GOSUB LoadLut
  CASE $CFontR :
   IF v0=0 THEN
    XuiSendMessage ( grid, #SetFont, 200, 400, 0, 0, $TAreaR, @"Comic Sans MS")
'(old font)
   ELSE
    XuiSendMessage ( grid, #SetFont, 300, 500, 0, 0, $TAreaR, @"Comic Sans MS")
   END IF
    XuiSendMessage(grid,#Redraw,0,0,0,0,$TAreaR,0)
  CASE $PNewR
    XuiSendMessage (grid,#SetTextArray,0,0,0,0,$TAreaR, @vacio$[])
    XuiSendMessage(grid,#Redraw,0,0,0,0,$TAreaR,0)
  CASE $CZoom :
   IF v0=0 THEN
    XuiSendMessage(grid,#Disable,0,0,0,0,$TAreaZoom,0)
    XuiSendMessage (grid, #Resize, 288, 64, 364, 564, $TAreaR,0)
   ELSE
    XuiSendMessage (grid, #Enable,0,0,0,0, $TAreaZoom,0)
    XuiSendMessage (grid, #Resize, 288, 64, 364, 297, $TAreaR,0)
   END IF
    XuiSendMessage (grid, #Redraw,0,0,0,0,$TAreaR,0)
    XuiSendMessage(grid,#Redraw,0,0,0,0,$TAreaZoom,0)
```

```
CASE $CBatch
 bacht= ABS(v0)
END SELECT
END SUB
'
'*******************LeerParamFile*******************
'
SUB LeerParamFile
PATHParam$ = PATHLUTE$ + CHR$(92) + parfile$+ ".params"
XstLoadStringArray (PATHParam$, @paramf$[])
FOR linea = 9 TO 52
  match=0      par$=""       pos=0
  done=0       sit=0
  IF (LEFT$ (paramf$[linea]= "/") THEN DO NEXT
  XstFindArray ( 0, @paramf$[], ":", linea, @pos, @found )
  pos=pos+2
  DO UNTIL done
   string$ = XstNextField$ (paramf$[linea] , @pos , @done )
   IF string$= "|" THEN EXIT DO
   IF string$= " " THEN DO DO
  par$ = par$+string$
  LOOP
param$[linea,0,0] = par$
param$[linea,1,0]= par$   '=param$[linea,0]
NEXT linea
END SUB
'
'******************GrabarParams*********************
'
SUB GrabarParams
FOR line=9 TO 52
IF (param$[line,0,0]=param$[line,1,0]) THEN DO NEXT
XstReplaceArray(0, @paramf$[], param$[line,0,0], param$[line,1,0], line, 12,
@match)
param$[line,0,0]=param$[line,1,0]
NEXT line
XstSaveStringArray(PATHParam$,@paramf$[])
END SUB
'
'*******************DrawParams*********************
'
SUB DrawParams
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $PathP, @PATHLUTE$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $PathD, @PATHFILE$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $ParName, @parfile$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DatName, @ifile$)
'XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $CQual, @cqual$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $Mass, @mass$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DCharge, @charge$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $Merror, @merror$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $Ferror, @ferror$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DFileType, @filetype$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $THits,  @hit$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $TScoreT, @score$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $TPeakW, @peakw$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DMode, @mode$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DThres, @thres1$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DInst, @inst1$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DCys, @cys1$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DEnzyme, @enzyme$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DNTerm, @nterm1$)
XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DCTerm, @cterm1$)
END SUB
'
'*******************LeerWinParams*******************
'
SUB LeerWinParams
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $PathP, @PATHLUTE$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $PathD, @PATHFILE$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $ParName, @parfile$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DatName, @ifile$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $Mass, @mass$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DCharge, @charge$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $Merror, @merror$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $Ferror, @ferror$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DFileType, @filetype$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $THits, @hit$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $TScoreT, @score$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $TPeakW, @peakw$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DMode, @mode$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DEnzyme, @enzyme$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DCys, @cys1$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DNTerm, @nterm1$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DCTerm, @cterm1$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DInst, @inst1$)
XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DThres, @thres1$)
'XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $CQual, @cqual$)
END SUB
'
'*******************SetParams*********************
'
SUB SetParams
 ifilelong$ = param$[9,0,0]
 position = RINSTR(ifilelong$, ".")
 long = LEN(ifilelong$)
 ifile$ = LEFT$(ifilelong$, position-1)
 ifiletype$ = RIGHT$(ifilelong$, long-position)
```

```
cqual$ = param$[10,0,0]            mass$ = param$[11,0,0]
charge$ =param$[ 12,0,0]           merror$ = param$[15,0,0]
ferror$ = param$[16,0,0]           filetype$=param$[23,0,0]
FOR v=0 TO 3
 IF filetype$[v,0]= filetype$ THEN
 nombrefiletype$= filetype$[v,1]
 END IF
 NEXT v
hit$ = param$[50,0,0]              score$ = param$[51,0,0]
peakw$ = param$[25,0,0]            mode$ = param$[24,0,0]
thres0$ = param$[26,0,0]           inst0$ = param$[32,0,0]
cys0$ = param$[37,0,0]
FOR v=0 TO 3
 IF cys$[v,0]=cys0$ THEN
  param$[37,1,1]= cys$[v,1]         cys1$=cys$[v,1]
 END IF
 IF thres$[v,0] =thres0$ THEN
  param$[26,1,1]= thres$[v,1]       thres1$= thres$[v,1]
 END IF
 NEXT v
enzyme$ = param$[38,0,0]           nterm0$ = param$[39,0,0]
cterm0$ = param$[40,0,0]
 FOR v=0 TO 2
 IF nterm$[v,0]= nterm0$ THEN
  param$[39,1,1]= nterm$[v,1]       nterm1$= nterm$[v,1]
 END IF
 IF cterm$[v,0] = cterm0$ THEN
  param$[40,1,1]= cterm$[v,1]       cterm1$= cterm$[v,1]
 END IF
 IF inst$[v,0]=inst0$ THEN
  param$[32,1,1]= inst$[v,1]        inst1$=inst$[v,1]
 END IF
 NEXT v
END SUB
'
'*******************LoadArray*******************
'
SUB LoadArray
 param$[9,1,0]=ifilelong$          param$[10,1,0]=cqual$
 param$[11,1,0]=mass$              param$ [12,1,0]=charge$
 param$[15,1,0]=merror$            param$[16,1,0]=ferror$
 param$[23,1,0]=filetype$          param$[24,1,0]=mode$
 param$[50,1,0]=hit$               param$[51,1,0]=score$
 param$[25,1,0]=peakw$             param$[26,1,1]=thres1$
 param$[32,1,1]=inst1$             param$[37,1,1]=cys1$
 FOR v=0 TO 3
 IF cys$[v,1]=cys1$: param$[37,1,0]= cys$[v,0]
 IF  thres$[v,1]=thres1$: param$[26,1,0]=thres$[v,0]
 NEXT v
 param$[38,1,0]=enzyme$            param$[39,1,1]=nterm1$
 param$[40,1,1]=cterm1$
FOR v=0 TO 2
 IF nterm$[v,1] = nterm1$ : param$[39,1,0] = nterm$[v,0]
 IF cterm$[v,1] = cterm1$ : param$[40,1,0]= cterm$[v,0]
 IF inst$[v,1] = inst1$ : param$[32,1,0]= inst$[v,0]
 NEXT v
END SUB
'
'*****************RUNLute*******************
'
SUB RunLute
 ifilelongpath$ = PATHFILE$ + CHR$(92)+ ifilelong$ ' raw text file
 PATH5$ = PATHLUTE$ + CHR$(92)
 PATH6$= PATH5$+"Lutefisk.exe -q " + ifilelongpath$
 XstSetCurrentDirectory(@PATH5$)
 SHELL(PATH6$)
 lutfilelong$= ifile$ + ".lut"
 lutfilelongpath$= PATHFILE$+ CHR$(92) + lutfilelong$
 rfilelong$ = "r" + ifilelong$
 marcador=0
 GOSUB LoadLut
 XstDeleteFile (lutfilelongpath$)
'
END SUB
'
'*****************LoadFile*******************
'
SUB LoadFile
 XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $PathD, @PATHFILE$)
 XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DatName, @ifile$)
 ifilelong$ = ifile$ + "." + nombrefiletype$
 ifilelongpath$ = PATHFILE$ + CHR$(92)+ ifilelong$ ' raw text file
 XstLoadString (ifilelongpath$, @raw$)
 XstMultiStringToStringArray (@raw$, @list$[])
 FOR k= 0 TO UBOUND(list$[])
 list$[k]=TRIM$(list$[k])
 NEXT k
 XuiSendMessage (grid,#SetTextArray,0,0,0,0,$TAreaD, @list$[])
 XuiSendMessage (grid, #Redraw,0,0,-1,-1,$TAreaD,0)
END SUB
'
'*****************LoadLut*******************
'
SUB LoadLut
IF manual=1 THEN
resultheadselect$= "file = " + rfilelong$
```

```
XuiSendMessage ( grid, #SetTextString, 0, 0, 0, 0, $LBResultFile,        XstDeleteLines (@pfile$[],3, 18)
@resultheadselect$)                                                      XstDeleteLines (@pfile$[],0,1)
XuiSendMessage (grid, #Redraw,0,0,-1,-1,$LBResultFile,0)                  m=n+10
XstLoadStringArray (lutfilelongpath$, @rfile$[])                          REDIM cfile$[m]
END IF                                                                    FOR d=0 TO 9
IF bacht = 1 THEN                                                          cfile$[n+d]= pfile$[d]
XstLoadStringArray (lutfilelongpath$, @rfile$[])                          NEXT d
END IF                                                                    n=m
IF marcador=0 THEN XstDeleteLines(@rfile$[],0,3) 'solo corta si viene de un run   NEXT ix
marcador=1                                                                XuiSendMessage (grid,#SetTextArray,0,0,0,0,$TAreaR, @cfile$[])
IF manual=1 THEN                                                           XuiSendMessage (grid, #Disable, 0,0,0,0, $Progress,0)
XuiSendMessage (grid,#SetTextArray,0,0,0,0,$TAreaR, @rfile$[])            XstSaveStringArray (PATHFILE$ + CHR$(92)+ "c" + ifilex$ + ".txt", @cfile$[])
XuiSendMessage (grid, #Redraw,0,0,-1,-1,$TAreaR,0)                         XuiSendMessage (grid,#Redraw,0,0,0,0,$TAreaR,0)
END IF                                                                    END SUB
XstCopyArray (@rfile$[], @zfile$[])                                       '
XstDeleteLines (@zfile$[],0,21)                                           '*********************SaveResult*****************
REDIM zfile$[8]                                                           '
match=1                                                                   SUB SaveResult
DO WHILE (ABS(match)>0)                                                   XuiSendStringMessage (grid, @"GetTextString", 0, 0, 0, 0, $DatName, @ifile$)
XstReplaceArray (0,@zfile$[],@" ", @" ",@lin,@pos,@match)                 emptyfile$= PATHFILE$ + CHR$(92)+ "nomequites.txt"
head$= "SEQU Rank  Pr(c) PevzScr Qual IntScr X-corr"                      rfilelongpath$ = PATHFILE$ + CHR$(92)+ "r" + ifile$ + ".txt"
zfile$[0]=head$                                                          XstCopyFile (emptyfile$ , rfilelongpath$ )
LOOP                                                                      linea= UBOUND(rfile$[])
XuiSendMessage (grid,#SetTextArray,0,0,0,0,$TAreaZoom, @zfile$[])        FOR k= 0 TO linea-1
XuiSendMessage (grid, #Redraw,0,0,-1,-1,$TAreaZoom,0)                     rfile$[k]=TRIM$(rfile$[k]) + CHR$(13)
END SUB                                                                   NEXT k
'                                                                         IF (bacht = 0) : XstSaveStringArray (rfilelongpath$, @rfile$[])
'****************DrawFileName&FileList*****************                    END SUB
'                                                                         '
SUB DrawFileName                                                          '******************MideTiempo*****************
 buscoD$= PATHFILE$ + CHR$(92) + "*." + nombrefiletype$                   '
 XstGetFiles (buscoD$,@filed$[])                                          SUB MideTiempo
 XuiSendMessage (grid,#SetTextArray,0,0,0,0,$DatName, @filed$[])           XstGetSystemTime (@ztime)
 XuiSendMessage (grid, #SetTextString, 0, 0, 0, 0, $DatName, @ifile$)      atime = ztime-atime
 XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DatName,0)                      END SUB
END SUB                                                                   END FUNCTION
'                                                                         END PROGRAM
'*********************RunFile*****************
'
SUB RunFile
GOSUB LeerWinParams
GOSUB LoadArray
GOSUB GrabarParams
GOSUB RunLute
END SUB
'
'*********************RunBacht*****************
'
SUB RunBacht
XuiSendMessage (grid, #Enable,0,0,0,0, $Progress,0)
XuiSendMessage (grid,#SetValues, 0, UBOUND(filed$[]), 0,0, $Progress,0)
GOSUB LeerWinParams
bacht=1          manual=0       paso=0          n=32
bachtsize=UBOUND(filed$[])+1
FOR ix=0 TO (bachtsize-1)
 XstGetSystemTime (@atime)
 XuiSendMessage (grid,#Redraw,0,0,0,0,$Progress,0)
 ifilelong$=filed$[ix]
 position = RINSTR(ifilelong$, ".")
 extension$ = LCLIP$(ifilelong$, position)
 long = LEN(ifilelong$)
 ifile$ = LEFT$(ifilelong$, position-1)
  ifiletype$ = RIGHT$(ifilelong$, long-position)
 XuiSendMessage (grid,#SetTextString,0,0,0,0,$DatName, @ifile$)
 XuiSendMessage (grid, #Redraw,0,0,-1,-1,$DatName,0)
GOSUB LoadArray
 GOSUB GrabarParams
 GOSUB RunLute
 GOSUB MideTiempo
 IF paso=0 THEN
 XuiSendMessage (grid,#SetValues, 100/bachtsize, 100, 0,0, $Progress,0)
 totalparcial=atime
 totaltime=atime * bachtsize
 END IF
 IF paso=1 THEN
 totalparcial= totalparcial+atime
 totaltime=(totalparcial/(ix+1)) * bachtsize
 XuiSendMessage (grid,#SetValues, (100 * totalparcial)/totaltime, 100, 0,0,
$Progress,0)
 END IF
 XuiSendMessage (grid,#Redraw,0,0,0,0,$Progress,0)
 GOSUB SaveResult
 IF paso=0
 XstCopyArray (@rfile$[], @cfile$[])
 cfile$[18]=cfile$[1] 'problem
 cfile$[19]=cfile$[2]
 cfile$[0]= "CALCULO EN BACHT"
 cfile$[1]= " "
 XstDeleteLines (@cfile$[],2, 1)
 XstDeleteLines (@cfile$[],16, 1)
 XuiSendMessage (grid,#SetTextArray,0,0,0,0,$TAreaR, @cfile$[])
 XuiSendMessage (grid,#Redraw,0,0,0,0,$TAreaR,0)
 paso=1
 DO NEXT
 END IF

 XstCopyArray (@rfile$[], @pfile$[])
```