Microsynth

An internationally recognized
proof of Swiss-Quality

Catalog 2009/2010

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www.microsynth.ch

Philosophy

Dear Valued Customer,
Microsynth's spirit of innovation and flexibility, as well as our reputation for providing quality products are recognized throughout the world. Our primary goal has always been customer satisfaction, which is maintained through close contact with our clients and an openness to new ideas and technology.

All of our divisions make use of state of the art equipment and strive to implement the latest scientific findings. A low error rate and quick delivery of results is possible due to ever increasing automation of procedures and a well integrated quality management system. We guarantee high-quality products and precise analytical results which arrive just in time.

Close contact with our customers and an openness to new ideas form the bases for a partnership that you can rely on. Finally, we believe that open communication is the best way to improve the quality of our services, a strategy which will allow us to maintain high standards for years to come.

This catalog is printed on recycling paper.

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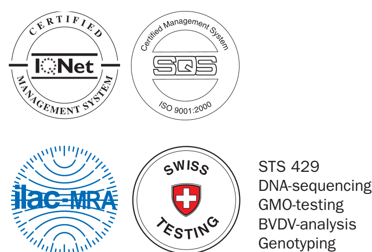
Quality System

In 1989, Microsynth was founded as the first private DNA synthesis laboratory in Switzerland.

In June 2005, Microsynth completed the certification process for all departments according to ISO 9001:2000. Moreover, Microsynth achieved the accreditation of the analysis divisions according to ISO 17025.
Certification and accreditation is our way of showing our commitment to the continuous improvement of all our services that enables us to keep customer satisfaction at it's highest.



Accreditation according to EN ISO 17025

Customer Support

Our customer service can be reached Monday to Friday from 8.00–12.00 and 13.00–16.30.
Please do not hesitate to contact us for support, technical advice and pricing regarding your specific concerns:

DNA Synthesis Service
e-mail: *oligo.support@microsynth.ch*
phone: +41 71 722 83 33 (main line)
phone direct: +41 71 726 12 02

RNA Synthesis Service
e-mail: *fabian.axthelm@microsynth.ch*
phone: +41 71 722 83 33 (main line)
phone direct: +41 71 726 12 07

DNA Sequencing Service
e-mail: *t.gaechter@microsynth.ch*
e-mail: *sequencing@microsynth.ch*
e-mail: *sequencing1@microsynth.ch*
phone direct: +41 71 726 10 05
phone direct: +41 71 726 10 03
phone direct: +41 71 726 10 04

454 DNA Sequencing Service
e-mail: *genome@microsynth.ch*
phone direct: +41 71 726 10 01

Real Time PCR Service
e-mail: *johannes.haugstetter@microsynth.ch*
e-mail: *e.hoerler@microsynth.ch*
phone direct: +41 71 726 12 50
phone direct: +41 71 726 10 02

Genotyping Service
e-mail: *georges.wigger@microsynth.ch*
phone direct: +41 71 726 10 08

Our Terms and Conditions

PRICING

- Prices do not include VAT or other taxes. All extras must be calculated separately.
- Please note that some countries may charge customs taxes or other fees. This is dependent on the rules and regulations of the destination country.
- Payment terms are net 30 days in CHF, EUR or USD.
- Invoices will be issued in Swiss Francs within Switzerland, EUR within European countries and USD in all other countries.
- For your convenience there is no minimum order requirement.
- Catalog and electronical prices are subject to change without notice.

CANCELLING / CHANGING ORDERS

In order to ship your orders as fast as possible your orders are being processed immediately after entry. Therefore, cancelling/changing orders is only possible for a very limited time after submission. **In such a case, it is absolutely required that you contact us by phone during office hours (+41 71 722 83 33). Just writing an e-mail is not sufficient. Note that weekend and holiday orders can not be cancelled.**
Note that if an order is already processed (e.g. an Oligo synthesis has been started) it is not possible to cancel or change the order and the order will be delivered and charged.

SHIPPING TO MICROSYNTH FROM EUROPEAN COUNTRIES

- P.O. Boxes in Austria or Germany facilitate the sending of your samples for sequencing.

SATISFACTION

- Microsynth is committed to offer you the best possible quality. However, if you should once not be satisfied please report the problem to Microsynth within **three months after receipt** of the product. **After 3 months complaints are no longer accepted.**
- It is necessary that you provide the production number (or sequence number) of the product in order to facilitate a quick handling of your complaint.
- Products sent or billed due to an error by Microsynth may be returned for exchange or credit.
- Products sent or billed due to an error by the customer will not be replaced or credited for.
- Note that Microsynth can not be held liable for delivery problems outside our area of control (our production facility). Delivery problems caused by the delivery carrier must be addressed with the carrier directly.

OFFERS

- Please do not hesitate to ask for **special prices** for large orders.
- To facilitate your administrative work we offer **pre-payment** plans which may include **interesting price reductions.**
- Always refer to the offer number in the field labeled «Your Offer No» on the order form.

DNA Synthesis

If you place an order for Oligos at Microsynth, we offer the following advantages:

- >95 % of our desalted Oligonucleotides are shipped out within 24 hours after receiving your order (if they pass the quality control).
- We only need one additional day for HPLC or PAGE purification.

After 20 years of experience in the oligonucleotide synthesis field our aim is to meet customer's highest expectations. In order to guarantee high quality we perform online trityl monitoring of all oligonucleotides to control coupling efficiency at each cycle. Additionally most unmodified and all modified oligos are analyzed by MALDI-TOF MS or PAGE.

DNA Synthesis Scales

Synthesis scale*)	Restrictions	Guaranteed yield for >20mer, unmodified, desalted	Average yield for >20mer, unmodified, desalted
Genomics	11–35 mer, no modification, desalted only	2 OD260	7 OD260
0.04 µmol	11–70 mer	3 OD260	10 OD260
0.2 µmol	2–125 mer**)	13 OD260	20 OD260
1.0 µmol	2–80 mer	50 OD260	100 OD260
15 µmol	2–50 mer	700 OD260	1000 OD260

*) The synthesis scale is the amount of 3'base that we start the synthesis with. It is **NOT** the expected final yield. The yield depends on the length of the Oligonucleotide, the coupling efficiency, and the base composition. The theoretical yield of the full length product can be determined using the formula 0.985^n, wherein 0.985 represents the coupling efficiency and n the length of the Oligo. Hence, the theoretical yield of the full length product of a 50 mer is 47 % at a coupling rate of 0.985. Shorter Oligos can not be eliminated by desalting but instead by HPLC or PAGE. We recommend HPLC or PAGE purification for Oligos longer than 35-mer.

**) Longer oligos on request.

Important Information

- Please inquire for special offers if you plan an order with more than 50 Oligos. Request our convenient customized Excel sheet at *administration@microsynth.ch.*
- When ordering **mixed bases** please use the IUPAC-code (M=A/C, R=A/G, W=A/T, Y=C/T, S=C/G, K=G/T, H=A/C/T, V=A/C/G, D=A/G/T, B=C/G/T, N=A/C/G/T). No additional charges will be applied for internal mixed base positions (wobbles).

Guaranteed Yields

Description	Guaranteed Minimal Yields. Guarantee valid for unmodified Oligos >20mer only.
Desalted Purification Grade*)	
Genomic scale	2 OD260
0.04 µmol scale	3 OD260
0.2 µmol scale	13 OD260
1.0 µmol scale	50 OD260
15 µmol scale	700 OD260
HPLC Purification Grade (restricted for Oligos <51 mer)	
Genomic scale	not available
0.04 µmol scale	1 OD260
0.2 µmol scale	3 OD260
1.0 µmol scale	15 OD260
15 µmol scale	300 OD260
PAGE Purification Grade	
Genomic scale	not available
0.04 µmol scale (from 11–70 mer only)	0.5 OD260
0.2 µmol scale (from 5–125 mer only)	1 OD260
1.0 µmol scale (from 5–80 mer only)	7 OD260
15 µmol scale	not available
NMR Purification Grade (HPLC & Dialysis) (restricted for Oligos 8–50 mer)	
Genomic scale	not available
0.04 µmol scale	not available
0.2 µmol scale	3 OD260
1.0 µmol scale	15 OD260
15 µmol scale	200 OD260

*) Desalting is included in the base price

How Pure is Pure Enough?

In general, the purity level that you require depends on the effect that failed sequences (n-1, n-2, … Oligomers) will have on your experiments.

For some applications, desalted Oligos are satisfactory. For others, only PAGE purification can yield Oligos of sufficient purity. We recommend the use of purified Oligos for high risk projects (for example cloning of Oligos longer than 50 mer) or Oligos which are routinely used for diagnostic purposes.

Please note that yields vary according to the selected purification method.

DESALTED OLIGOS

All Oligos are at least desalted to remove residual by-products from the synthesis, cleavage and deprotection procedures.

Such purification is sufficient for Oligos shorter than 35 and/or Oligos used for non critical applications like PCR, sequencing, probing, mobility shift, or hybridization. They are not recommended for more sophisticated projects like cloning.

HPLC PURIFIED OLIGOS

Oligos 50 bases or shorter can be HPLC purified using a Reverse Phase HPLC system. Residual, truncated DMT-off Oligo molecules from synthesis are therefore removed during the purification process, resulting in 90–95 % purity.

HPLC purified Oligos are recommended for applications such as DNA fingerprinting, RT-PCR, in situ hybridization, microsatellite polymorphisms and mutagenesis. Of course, you can always use HPLC purified Oligos for other applications as well.

PAGE PURIFIED OLIGOS

Oligos 51 bases or longer should be PAGE purified. With the excellent resolution resulting from PAGE, 95–99 % purity can be achieved. In many cases, full length Oligos can be separated from Oligos which are just one base shorter. This purification grade is recommended for all applications including gene synthesis, primer extension, 32P-labeling and cloning of Oligos longer than 50mer.

NMR GRADE OLIGOS (HPLC & DIALYSIS PURIFIED OLIGOS)

If you would like to use antisense Oligos in cell culture, it is important to remove salts within Oligo sample. Our NMR grade Oligos are the perfect choice to avoid problems with salts in your antisense experiments.

Our NMR grade Oligos are precipitated and carefully dialysed after a Reverse Phase HPLC purification.

This method is mainly used for phosphorothioates (PTO), large scale synthesis and NMR grade Oligos. This purification grade is available for any Oligo longer than 7 bases.

5’ and 3’ Modifications

Please note

We provide the following alternative dyes:
Yakima Yellow for VIC™, ATTO550 for NED™, ATTO565 for PET™, Dyomics630 for LIZ™.
Additionally, LI-COR Sequencer compatible dyes as alternatives for **IRDye 700** and **IRDye 800** are available on request.

Some modified Oligos are automatically purified (marked as «purified only»), while others are purified only upon request (marked as «purification on request»).

Modification	Purification	Modification	Purification
5’ AlexaFluor 350/430	Purified only	3’ Digoxigenin	Purified only
3’ AlexaFluor 350/430	Purified only	5’ Dyomics 630/681/781	Purified only
5’ Amino (C6)	Purification on request	3’ Dyomics 630/681/781	Purified only
3’ Amino (C7)	Purification on request	5’ FAM (Fluorescein), HEX, TET	Purified only
5' ATTO550, 565, 620	Purified only	3’ FAM (Fluorescein)	Purified only
3' ATTO550, 565, 620	Purified only	3’ Inosine	Purification on request
3' BHQ-1	Purified only	5’ JOE, TAMRA, ROX	Purified only
3' BHQ-2	Purified only	3’ JOE, TAMRA, ROX	Purified only
5’ Biotin (C6)	Purified only	3’ mixed base	Purification on request
3’ Biotin (TEG)	Purified only	5’ Phosphorylation	Purification on request
3’ Cholesterol	Purification on request	3’ Phosphorylation	Purification on request
5’ Cy3	Purified only	5’ QSY-7	Purified only
5’ Cy5	Purified only	3’ QSY-7	Purified only
5’ Cy5.5	Purified only	5’ QSY-21	Purified only
5’ Cy7	Purified only	3’ QSY-21	Purified only
3' Dabcyl	Purified only	5’ Thiol (C6)	Purified only
3’ deoxy-A, C, G, T	Purification on request	3’ Thiol (C3)	Purified only
5’ Digoxigenin	Purified only	5’ Yakima Yellow	Purified only

For other ATTO-, Dyomics-, and Alexa Fluor-Modifiers, please inquire.

Internal Modifications

Inosine, 5MetdC, dU, internal DIG and Spacer 18 (HEG) are available in 0.2 µmol scale and 1.0 µmol scale.
Please contact us regarding other internal modifications
(5BrdU, 5BrdC, N6MetdA, QSY-21, internal FAM, Amino-dT, Amino-dC, different spacers, etc).

Dual-labelled Fluorescent Probes (TaqMan; Delivery 4 business days)

Please note

We provide the following alternative dyes:
Yakima Yellow for VIC™, $ATTO_{550}$ for NED™, $ATTO_{565}$ for PET™, $Dyomics_{630}$ for LIZ™.

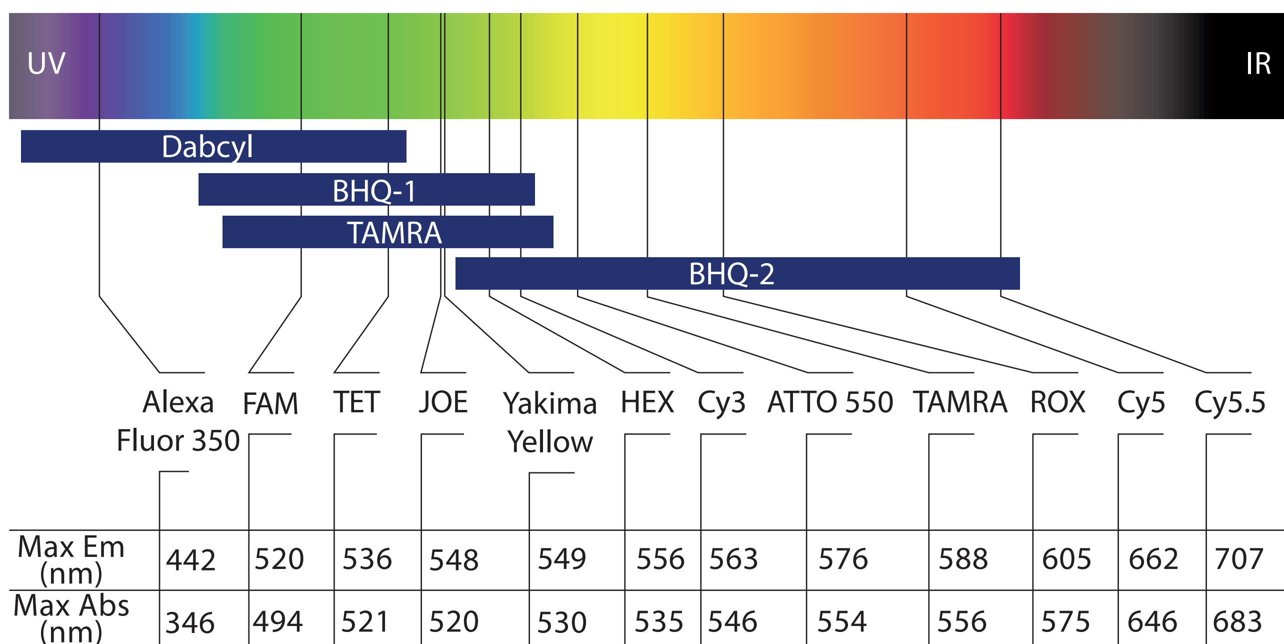
5' Label	3' Label	Synthesis scale	Guaranteed Yield	Average Yield
FAM HEX TET	TAMRA	0.2 µmol	2 OD260	9 OD260
FAM HEX TET	TAMRA	1.0 µmol	10 OD260	25 OD260
FAM HEX TET	QSY-7	0.2 µmol	1.5 OD260	2 OD260
AlexaFluor 350 FAM TET	Dabcyl	0.2 µmol	2 OD260	5 OD260
FAM HEX JOE TET	BHQ-1	0.2 µmol	2 OD260	6 OD260
YAKIMA YELLOW (equivalent to VIC)	BHQ-1	0.2 µmol	2 OD260	5 OD260
ATTO 550 (equivalent to NED) ATTO 620 CY3 CY5 Dyomics 681 ROX TAMRA	BHQ-2	0.2 µmol	1.5 OD260	3 OD260

For other ATTO-, Dyomics-, and Alexa Fluor-Modifier, please inquire.
For 1 µmol synthesis scale, please inquire.

Important Information

- Prices for dual-labelled probes include base addition, dye labeling and two-step purification.
- All probes are MALDI-TOF MS-controlled before shipment.
- Dual-labelled probes are normally aliquoted in 5 tubes to achieve maximal stability and shipped lyophilized. If you wish only one tube please return the order confirmation e-mail with your special request immediately.
- Design of dual-labelled probes and primer pairs on request.
- For more information about our dual-labelled fluorescent probes and design services please visit our homepage www.microsynth.ch.

Fluorophores and Quenchers



	Alexa Fluor 350	FAM	TET	JOE	Yakima Yellow	HEX	Cy3	ATTO 550	TAMRA	ROX	Cy5	Cy5.5
Max Em (nm)	442	520	536	548	549	556	563	576	588	605	662	707
Max Abs (nm)	346	494	521	520	530	535	546	554	556	575	646	683

Schematic of fluorophores and their corresponding quenchers, which can be ordered at Microsynth.

Microsynth offers a wide range of fluorophores and quenchers in various combinations that are suitable for each used method and real-time thermocycler. Traditionally, FAM-TAMRA is one of the most frequently used pairs for TaqMan probes, where FAM acts as the fluorophore and TAMRA as the quencher. Also other quenchers can be used, especially Black Hole Quenchers (BHQ) which capture energy from an excited reporter molecule without subsequent emission of light, i.e. they do not fluorescence.

Probes made by using Black Hole Quenchers tend to be more sensitive in quantitative detection systems, primarily due to lower background fluorescence and a better signal to noise ratio than probes that contain fluorescent quenchers. Moreover, Black Hole Quenchers enable the use of a wider range of reporter dyes, expanding the options available for multiplexed or genotyping assays.

Some fluorophores can be quenched by more than one quencher. In any case the absorption spectrum of the quencher needs to have a good overlap with the emission spectrum of the fluorophore to achieve optimal quenching. Quenchers have a quenching capacity throughout their absorption spectrum, but the performance is best close to the absorption maximum.

For multiplex PCR we recommend the following 5'fluorophore 3'-Black Hole Quencher combination for most qPCR thermo cyclers using TaqMan probes:

Channel 1: FAM-BHQ1
Channel 2: Yakima Yellow*-BHQ1 (*equivalent to VIC)
Channel 3: ATTO550**-BHQ2 (**equivalent to NED)
Channel 4: ROX-BHQ2

Recommended final concentrations for standard experiments:
Primers: 0.9 pmol/µl (0.9 µM)
Probe: 0.2 pmol/µl (0.2 µM)

Recommended RT-PCR reaction (Tm Primer: 59 °C, Tm Probe: 69 °C):
30 sec 95 °C
30 sec 57 °C
30 sec 72 °C, 35 cycles

Design and Functionality Testing of Primer-Probe sets for Real Time PCR

Microsynth offers a functionality testing service for your primer-probe sets (designed by you or Microsynth, synthesized by Microsynth). Your primer-probe-set will be tested in-house on our up-to-date Real Time PCR instruments. You will be sure to get a basically functional assay including **sequences**, oligonucleotides and working conditions (validation certificate). The latter will include PCR-conditions, primer-probe concentrations and an approximate determination of the linear dynamic range of the assay.

What you will get from Microsynth:

- Complete sequence information.
- Universal working conditions.
- Validation results.
- QC-tested oligonucleotides for more than 2000 PCR-reactions at a 25µl end volume.
- Positive control sample.

CONTACT

For further information contact *johannes.haugstetter@microsynth.ch* or *e.hoerler@microsynth.ch*

Phosphorothioates (PTO)/2'-O-methyl-RNA

- Oligos that are used in antisense experiments must be modified to achieve a higher nuclease resistance. There are several types of such modifications. Microsynth offers two possibilities:
  - Modification of the normal phosphodiester backbone (**phosphorothioates**) which is characterized by an increased cell uptake, high nuclease resistance and elicitation of RNAse H activity. We can produce phosphorothioate linkages at any position of your choice.
  - The incorporation of 2'-OMe-nucleotides (**2'-OMe-RNA**) which induces a resistance to a wide variety of ribo- and deoxyribonucleases. This enables the formation of more stable hybrids with complementary RNA strands than equivalent non-modified DNA and RNA sequences.
- If you wish to use antisense Oligos in cell culture, it is important to remove all of the salts within the Oligo sample. We recommend ordering NMR grade purified Oligos. This grade of purification is carried out by reverse phase chromatography and subsequent dialysis.
- 0.2 µmol, 1.0 µmol and 15 µmol scales are available (same guaranteed yields as for unmodified Oligos apply)

Aliquotation

We can supply large numbers of aliquots of sample or sets of samples at economic pricing.

Important Information

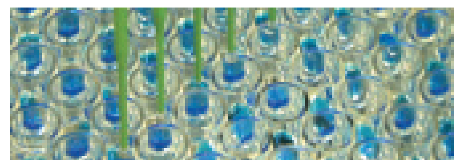
Aliquotation is useful …
… when Oligos must be stored and used over a long period of time without losing activity.
… when Oligos are shared between several groups or laboratories.
… 2 nmol or 5 nmol aliquots are available

High-Throughput DNA Synthesis

In recent years, methods such as DNA microarray analysis, genotyping etc have become more and more important in Molecular Biology and Genomic research. Such methods require large volumes, even thousands of Oligos. Our vast experience with High-Throughput DNA synthesis enables us to offer our customers the following advantages:

- Competitive prices: Please feel free to contact us for a non-binding offer.
- Easy Ordering: Upon ordering please request our convenient customized Excel sheet at *administration@microsynth.ch*.
- High Quality Standards: Certification according to ISO 9001 : 2000.
- Flexibility: Large orders are available in single tubes as well as in microtiter plates, either dry or as a 100 µM solution. Aliquotation is possible in 96 or 384 well plates but will be charged additionally. Please inquire for your specific order.
- Customer-friendly Service: Direct contact with our DNA synthesis team facilitates excellent customer support.
- Short Delivery Time: Our flexibility and large-scale capacity enables easy and fast completion of High-Throughput projects.

Product	Synthesis Scale	
DNA Oligo 96/384 well plate; dry	Genomic 0.04 µmol 0.2 µmol	Prices upon request
DNA Oligo 96/384 well plate; 100 µM	Genomic 0.04 µmol 0.2 µmol	Prices upon request



RNA Synthesis

Our RNAs (siRNAs and single-stranded RNAs) are synthesized using the latest chemistry and synthesis technology. All oligonucleotides are carefully deprotected and purified under RNase free conditions. Similar to DNA synthesis your RNAs may be desalted, HPLC or PAGE purified.

Microsynth offers the fastest synthesis/delivery times (average: 3 days) on the market due to highly automated processes.
RNA oligonucleotides are subjected to the same demanding quality control criteria as our DNA oligonucleotides. Every single RNA is analysed by gel electrophoresis / MS analysis before shipment.

Single-stranded-RNA

We standardly synthesize **RNAs ranging from 5 to 65 bases**. Please inquire for RNAs longer than 65 bases.

	Guaranteed yield for Oligos >20 bases and <40 bases*			
Product	**Desalted**	**HPLC**	**PAGE**	**Average Delivery Time**
Single strand RNA synthesis				
0.04 µmol scale synthesis (10–30 mers)	4 OD260	1.5 OD260	Not available	3 business days
0.2 µmol scale synthesis (5–65 mers)	8 OD260	3 OD260**	1 OD260	3 business days
1 µmol scale synthesis (5–65 mers)	16 OD260	6 OD260	2 OD260	3 business days
15 µmol scale synthesis (15–40 mers)	On request	On request	Not available	On request

*) Guarantees are given for each tube (unmodified oligos only). Average yields are significantly higher.
**) Average yield > 5 OD260.

Single-strand-RNA oligonucleotides are shipped as lyophilized pellets and come together with 1 ml of certified RNase-free water for your convenience.

CHIMERIC OLIGONUCLEOTIDES

Note that we can produce chimeric oligos (containing DNA, RNA, 2'-O-Me-RNA, LNA) nucleotides. Please contact us for all possibilities.

MODIFICATIONS

We offer the following modifications with our RNAs. For an updated list, you may want to check our webpage, as the modification list is constantly growing.

5'	3'
Amino	Amino
Phosphate	Phosphate
Biotin	Biotin
FAM	FAM
TAMRA	TAMRA
Cy3	2'O-Methyl
Cy5	3'O-Methyl
Cy5.5	Cholesterol
Digoxigenin	Digoxigenin
Inverted Thymidine	Inverted Thymidine

Internal modifications: Inosine, Phosphorothioate

Please do not hesitate to contact us for any special modification you might require.

SUPERIOR QUALITY OF OUR RNAs WITH OUR STATE-OF-THE-ART SYNTHESIS CHEMISTRY

RNA synthesis has become a standard as has DNA oligonucleotide synthesis. However, due to degradation-prone characteristics of RNA special precautions have to be taken. Additionally, the free 2'-OH needs to be protected during synthesis.

Microsynth uses modern TOM-chemistry for its RNA synthesis. The use of the TOM 2'-O protective group has several advantages over traditional 2'-O protecting groups such as tBDMS and others. Advantages include the lack of 5'–2' chain elongations, better coupling efficiencies and more. This leads to measurable higher quality of our RNAs compared to our competitor's RNA.

siRNA

siRNAs (small interfering RNAs) are double-stranded RNA molecules used to specifically silence (knockdown) target genes upon delivery into cells. Usually, they are comprised of 21 nucleotides with a 2-base overhang at the 3' end.
Microsynth offers you a broad range of synthesis options for the generation of high-quality siRNA. Synthesized siRNAs undergo the same stringent quality control as every other oligonucleotide produced at Microsynth. Finally, siRNAs are delivered as annealed duplexes (they can also be delivered as two seperate strands upon request).

New to RNAi? Microsynth would like to support the inexperienced scientist in the setup of the experiment and the design of siRNAs free of charge. Contact the RNA-customer service for further information.

GUARANTEED YIELDS

	Guaranteed yield for Oligos > 20 bases and < 40 bases*			
Product	**Desalted**	**HPLC**	**PAGE**	**Average Delivery Time**
siRNA Duplex (2 × 21-mers)				
0.04 µmol scale synthesis	21 nmol	8 nmol	Not available	3 business days
0.2 µmol scale synthesis	35 nmol	16 nmol	5 nmol	3 business days
longer Duplex up to 27-mers per additional RNA Base				
0.04 µmol scale synthesis	21 nmol	8 nmol	Not available	3 business days
0.2 µmol scale synthesis	35 nmol	16 nmol	5 nmol	3 business days

*) Guarantees are given for each tube, average yields are significantly higher (unmodified oligos only).
Modified siRNAs are available – please consider modification table shown for single-strand-RNA.

DESIGN OF siRNAs

Microsynth assists the customer in the design of siRNAs. Many hints can be found on our homepage. Furthermore, a free guaranteed design service is available.

CONTROL siRNAs

Presynthesized control siRNAs (desalted) are available from Microsynth. They are intended to setup RNAi in your lab at an economical price.The sequences are published and show a high degree of knockdown. 4 nmol aliquots are available.

List of available control siRNAs:

Lamin A/C	CUGGACUUCCAGAAGAACAtt
GFP	GCAGCACGACUUCUUCAAGtt

Luciferase	CGUACGCGGAAUACUUCGAtt
Neg. control	AGGUAGUGUAAUCGCCUUGtt

Resuspending and Storage

What is the best way to resuspend Oligos?

- Do a short spin at max. speed in a centrifuge to collect the pellets at the bottom of the tubes.
- Add the appropriate amount of sterile water or buffer.
- Heat 5 mins. at 65 °C.
- Vortex or mix by pipetting vigorously up and down.

What is the best solvent for an Oligo?

For all Oligos (except those with fluorescent dyes) sterile water or buffer (e.g. 10 mM Tris-HCl pH 7.5) is fine. We recommend dissolving fluorescently labelled Oligos in 10 mM Tris pH 7.5 as degradation may occure at low pH (distilled water may have a pH of 6.0!). However Cy-labelled Oligos (Cy3, Cy3.5, Cy5, Cy5.5) degrade slowly at pH > 7.5 and should therefore be dissolved at pH 6.0–7.5.

What are the best storage conditions for Oligos?

Lyophilized (–20 °C)	6 months to several years
Lyophilized (25 °C)	1–2 years
Dissolved (–20 °C)	6 months–2 years
Dissolved (4 °C)	2 months–1 year
Dissolved (25 °C)	1 week–3 months

Important Information

- Please ensure the use of nuclease-free solutions when resuspending Oligos.
- For large experiments Oligos should be aliquoted, lyophilized and stored at –20 °C. This procedure prolongs the shelf-life of our Oligos.
- Please avoid repeated freezing and thawing as the physical forces involved may degrade the Oligos.
- Oligos are more stable at higher concentrations.
- Careful handling is recommended to avoid bacterial contamination.
- Fluorescent Oligos should be stored in the dark, as light can slowly degrade fluorescent moieties.

What is the best way to resuspend siRNAs?

- Do a short spin to collect the pellet at the bottom of the tube.
- Add appropriate amount of provided RNAse-free water.
- Heat 5 minutes at 37 °C and mix.
- Use siRNA (freeze after use).

Alternatively:

to disrupt higher aggregates, which may have formed during lyophilization and to increase the efficiency of your siRNA you may:

- Do a short spin to collect the pellet at the bottom of the tube.
- Add provided RNAse-free water to a final concentration of 40 uM.
- Heat 3 minutes at 90 °C.
- Let siRNA slowly cool down to room temperature.
- Use siRNA (freeze after use).

What is the best way to resuspend single strand RNA Oligos?

- Do a short spin at max. speed in a centrifuge to collect the pellets at the bottom of the tubes.
- Add the appropriate amount of sterile water or buffer (RNase-free!).
- Heat 2 mins. at 65 °C.
- Vortex or mix by pipetting vigorously up and down.

Custom Gene Synthesis

In order to facilitate and speed up your daily bench work, Microsynth can supply you with any gene for which the DNA or amino acid sequence is known.

We provide cloned and fully sequenced products. The genes are cloned into a standard vector. Complete documentation of the sequencing runs, including the sequence chromatograms are delivered with your gene. 100 % sequence identity is guaranteed.

Approximately 3 µg of the vector containing the gene, desicated in a tube are delivered.
The delivery time is 5 weeks or less after your order has been confirmed, this depends on the size of your gene.
As the prices vary based on product length and sequence composition we recommend to ask for a quote. Please include the sequence information.

WHY SYNTHESIZE A GENE?

Your advantages include:

- No need to prepare good cDNA/genomic DNA and clone it yourself. No need for extensive sequence analysis after your cloning.
- No worries about different isoforms/splice variants in different cell lines. You provide the sequence, we deliver as is.
- Optimize its expression by adapting to organism-specific codon tables.

CUSTOM DESIGN AND SYNTHESIS OF LIBRARIES

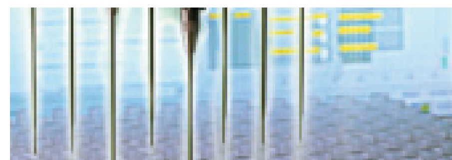
In order to support you in your screening projects we offer you now a design and synthesis service for custom libraries. The validated design and synthesis technology takes a novel approach to enable the realization of highly complex design concepts, while the same time ensuring that libraries generated have the very highest standards of reliability. Please contact us for more information.

Random Primers

Random primers are in stock (available for same-day delivery).

		Amount
Random Hexamer	d(N)6	1 OD260 (17 nmol)
Random Nonamer	d(N)9	1 OD260 (11 nmol)
Random Decamer	d(N)10	1 OD260 (10 nmol)

For ordering Random primers please send an e-mail to: *administration@microsynth.ch*.



DNA Sequencing

Our quality management system is in accordance with ISO 17025, ensuring the highest quality for your results. Our outstanding sequencing service is based on 15 years experience and is appreciated by customers all over the world. As collaborators in diverse genome projects (e. g. *Arabidopsis* genome sequencing project in 1997, *Mycoplasma conjunctivae* sequencing project 2006, diverse cosmid and BAC sequencing projects, exon sequencing projects, etc) we have developed methods which yield extremely low error rates. At Microsynth, we know that high quality standards are of utmost importance for your diagnostic demands.

Why should you send your samples to our sequencing department?

STATE-OF-THE-ART EQUIPMENT AND OPTIMIZED PROCESSES

- Small Sample Volumes: Our robot pipetting systems significantly reduce the amount of DNA required.
- Low Human Error Rate: Automatisation is steadily expanding and increasing our service standards. We are dedicated to improving both reliability and speed.
- Speed: We are committed to completing your orders on time. Single sequencing reactions are completed within one day; double-stranded 5 kb sequences within 1–2 weeks and cosmids or BACs within 1–2 months!
- Capacity: We offer High-Throughput sequencing services for 96-well plates or for 384-well plates.

OUTSOURCE YOUR SEQUENCING TASKS

- Choose from our diverse services to meet your research needs.
- Benefit from our expertise and problem-oriented solutions.
- Save time and money.
- Feel confident that your data will be treated with confidentiality.

A) Single Sequencing Services

OVERVIEW OF THE DIFFERENCES BETWEEN PREMIUM RUN AND ECONOMY RUN/BARCODE ECONOMY RUN

Topic	Premium Run A1)	Economy Run A2) Barcode Economy Run A3)
Sample	Separate, without primer	Premixed with specific primer
Standard primer	Added free of charge	Added free of charge
Specific primer	Must be added at Microsynth	Must be added by the customer
Amount of DNA	Depends on number of reactions	Defined amount per reaction
Support	**Intensive**	Basic
Sequence length	up to 1000 bases	up to 1000 bases
Time schedule	1 day per reaction	1 day per reaction
Editing	Your choice	Not available
Paper print-out of chromatograms	Your choice	Not available
Repetition	**Yes**, failed reactions repeated free of charge	Failed reactions repeated upon request free of charge
Trouble-Shooting	**Yes**, full support including problem-oriented solutions	No
Additional Services	Primer design, Plasmid preparation, Follow-on reaction, Special treatment (C/G-rich, hairpin, …), One-Drop Sequencing – available upon request	Not applicable
Storage of samples	Templates: 3 months, primers: 1 year	No

A1 Premium Run

What we provide

- Sequencing results for plasmids or PCR fragments **within one business day.**
- Standard primers are added free of charge.
- Specific primers are added by Microsynth (may have also been synthesized at Microsynth).
- Reading length: up to 1000 bases.
- Skilled interpretation of your sequencing results.
- Trouble-shooting based on qualified expertise and suggested solutions.
  Common problems include poly stretches, repeats, G/C-rich sequences and hairpin structures.
- Results from either purified DNA or *E. coli* single clones.

DETAILED TROUBLE SHOOTING AND SUPPORT

- All DNA concentrations are measured upon receiving your sample.
  You will be informed if the DNA concentration is too low and provided with options.
- Reading length is up to 1000 bases, depending on template quality or difficult sequences (e.g. poly stretches, high G/C content, hairpin structures, etc).
- Results are provided as textfiles and attached chromatograms by e-mail or secure HTPPS-Download.
- Paper printouts are available upon request.
- Sequences may be edited or non-edited depending on your requirements. Editing includes a manual proofread of your chromatogram by our experienced staff. Mistakes and uncertainties resulting from computer generated base calling (wherein the chromatogram is translated into your sequence) are corrected.
- In case of failed or poor reactions your results will be accompanied by suggestions for improvement.
  However, whether we continue with sequencing or stop your order is ultimately your decision.

TURNAROUND TIME

DNA samples arriving in the morning are processed immediately.
Results are sent to your e-mail account or provided to be downloaded the following business day.

CUSTOMIZED SERVICES (see page 24) are applicable:

- One-Drop Sequencing.
- Special Treatment.
- DNA Preparation from *E. coli.*

Important Information

DNA quality control reaction: We are always curious when a sequencing reaction fails.
Therefore for plasmids our Premium Run Service includes a repeated reaction and a control reaction (to exclude problems due to poor DNA quality). This control reaction is a test system developed here at Microsynth which allows us to identify the type of problem within one day. **NEW:** PCR control system.

Amounts and Concentrations of DNA and Primers

A1) PREMIUM RUN

DNA samples should have a **minimum volume of 20 µl** (sufficient for repetition and control reaction).
Primers should have a **minimum volume of 20 µl**.

Template	Concentration	Amount (in 20 µl)	For each additional reaction
Plasmid	100 ng/µl	2 µg	+ 5 µl
PCR (> 5000 bp)	100 ng/µl	2 µg	+ 5 µl
PCR (< 5000 bp)	40 ng/µl	0.8 µg	+ 5 µl
PCR (< 1000 bp)	20 ng/µl	0.4 µg	+ 5 µl
PCR (< 500 bp)	10 ng/µl	0.2 µg	+ 5 µl
PCR (< 200 bp)	5 ng/µl	0.1 µg	+ 5 µl
Primer	5 pmol / µl = 5 µM	100 pmol	+ 5 µl

A2) ECONOMY RUN A3) BARCODE ECONCOMY RUN

All samples (with or without primer) must have an **exact volume of 10 µl**. One tube is required for each reaction.

Template	Amount
Plasmid	0.8 µg
PCR	15 ng/100 bases
Primer	20 pmol (unless you use Microsynth's standard primers) = 2 µl of 10 µM Primer

A4) HIGH-THROUGHPUT

Please send the DNA in 96-well plates.

Template	Amount
Plasmid	0.3 µg
PCR	6 ng/100 bases
Primer	10 pmol (unless you use Microsynth's standard primers)

B–D) PRIMER WALKING REACTIONS

- **Double-stranded:** 2 µg of plasmid-DNA for a 1 kb insert and another 1 µg for each additional kb.
- **Single-stranded:** 1 µg of plasmid-DNA for a 1 kb insert and another 0.5 µg for each additional kb.

Alternatively, you may send your *E. coli* strain. DNA is isolated free of charge for all Walking Services on plasmids.

A2 Economy Run

What we provide

- Single runs on plasmids or PCR fragments **within one business day**.
- Reading length: up to 1000 bases.
- Cost-effective pricing.
- Sequencing from small pre-defined amounts of DNA.

SEQUENCING SUPPORT

- The sequences are non-edited.
- Results are provided as textfiles and attached chromatograms by e-mail or HTPPS Download.
- All reactions are charged.
- Repetitions are provided upon request free of charge.

TURNAROUND TIME

DNA samples arriving in the morning are processed immediately. Results are sent to your e-mail account or provided to download the following business day.

Important Information

The required amount of DNA must be provided **pre-mixed with the specific primer** in 10 µl volume.
Standard primers selected from our standard primer list will be added in-house free of charge.
Specific primers ordered directly from our oligo department can only be added within our Premium Run Service.
One separate tube is required for each reaction.

A3 Barcode Economy Run

Within our Economy Run Service we perform Barcode Economy Runs using Barcode labels.

Your advantages:

- Prepaid barcode labels without expiry date.
- Further economization related to Barcode labels batch sizes.
- Less administration expense (one invoice for several orders).
- Lost barcodes will be replaced free of charge.
- Comfortable labeling of sample tubes.

Barcode labels can be ordered easily on our online sequencing orderform.
Batch sizes: 50 labels
100 labels
250 labels

What we provide

- Prepaid Labels for Barcode Economy Runs.
- Single runs on plasmids or PCR fragments **within one business day.**
- Reading length: up to 1000 bases.
- Cost-effective pricing.
- Sequencing from small pre-defined amounts of DNA.
- Fast processing of all reactions within one order.

SEQUENCING SUPPORT (see Economy Run Service)

TURNAROUND TIME (see Economy Run Service)

A4 High-Throughput Service

Single sequencing reactions are performed in 96- or 384-well plates.

- DNA samples (and primers) should be sent dried, well covered liquid or frozen on dry ice.
- Standard primers and specific primers will be added in-house.
- Prices are valid for full 96- or 384-well plates only. Price relates on the degree of utilisation.

SAMPLE PREPARATION

Plasmids

We also accept *E. coli* instead of isolated and purified plasmids. Clone picking and plasmid preparation is then performed in-house.

PCR-Products

Please send a gel-image of at least 10 PCR-products (random-check) along with your samples.
PCR-purification can also be done in-house.

HOW TO SET UP YOUR PLATE

The reactions are processed according to their placement on the plates, in **vertical columns top to bottom**, left to right (e. g. for a 96-well plate A1, B1, C1, … H11, H12).
If you are interested in this service, please do not hesitate to contact us. Most of the standard plates with conical wells are accepted. Plates can also be requested free of charge.

B–D) Primer Walking Services

In order to accelerate the analysis of longer sequences (>1600 bases) we offer diverse Primer Walking Services for plasmid-DNA or PCR-products.

Plasmid isolation and internal primer design and synthesis is included in the base price. Occasionally primer walking projects can not be started with standard primers. In this case, specific primers can be ordered or designed in-house.

Important information such as cloning sites, vector names and sequences, standard primer binding sites, etc should be sent by e-mail. Known sequences should be sent as text files. If similar constructs are sequenced with the same primers multiple walking reactions can reduce the cost.

Extra charges may be applied for unforeseen expenses resulting from difficult sequences. You will always be consulted before such steps are taken.

Important Information

Walking primers are synthesized **in-house**! This means High-Speed Results, as quick as one run per day.
For example, a 5 kb sequence can be completed in only 4 business days!

B1 NON-ASSEMBLED, SINGLE-STRANDED

B2 NON-ASSEMBLED, DOUBLE-STRANDED

DNA is sequenced by primer walking reactions. The primers are designed and synthesized in-house; creating single reactions which can be overlapped to produce a reliable assembly. We provide the sequences – you do the assembling.

You receive

- Project data sheet.
- Electronic text files and chromatograms for each single reaction.
- Paper print-outs of the chromatograms.
- Edited sequences.
- Internal primers (only upon request).

C1 ASSEMBLED, SINGLE-STRANDED

One strand of DNA is sequenced by primer walking reactions and the assembly is done in-house. You will receive the single walking reactions as well as the complete assembly as a text file.

You receive

- Electronic and printed versions of the assembled sequence.
- Sequencing strategy.
- Project data sheet.
- Electronic textfiles and chromatograms of single runs.
- Paper print-outs of the chromatograms.
- Edited sequences.
- Internal primers (only upon request).

C2 ASSEMBLED, DOUBLE-STRANDED; PUBLICATION QUALITY (RELIABILITY > 99.99%)

Primer Walking Reactions are performed until double-stranded coverage of the sequence of interest is achieved. Assembly of the single sequences is completed quickly and accuratly in-house.

You receive

- Electronic and printed versions of the alignment of the sequence of interest, double-stranded verified.
- Sequencing strategy.
- Project data sheet.
- Electronic text files and chromatograms of all single runs.
- Paper print-outs of the chromatograms.
- Edited sequences.
- Internal primers (only upon request).
- Delivery time: approx. 5 days for 3 kb.

D1 VERIFICATION, SINGLE-STRANDED

The sequence of interest is verified by primer walking reactions on one strand. Please send by e-mail a text file of the known sequence.

D2 VERIFICATION, DOUBLE-STRANDED; PUBLICATION QUALITY (RELIABILITY > 99.99%)

The sequence of interest is accurately verified by primer walking reactions on both strands. Double-stranded coverage is obtained over the entire sequence, hence a very high level of confidence is achieved. Please send a text file of the known sequence by e-mail.

You receive (in addition to Service C2)

- Description of discrepancies resulting from the **comparison against the original sequence**.
- Delivery time: approx. 3 days for 3 kb.

E) Sequencing on Large Constructs

Sequencing reactions on large constructs (Genomes, BAC, PAC, Fosmids, Cosmids) demand special sequencing conditions and methods. In general, two different strategies are applicable:

- **«Direct Sequencing» of parts** *or*
- **«Entire Sequencing» of a construct or genome.**

S1-projects only can be carried out.

E1) DIRECT SEQUENCING ON BAC, PAC, FOSMID, COSMID

We offer both single runs and primer walking reactions.

Condition of DNA

- **DNA** is provided by the customer.
  Single Sequencing Run: 2 µg per reaction.
  Walking Reactions: 4 µg/1000 bases (single-stranded sequence).
  8 µg/1000 bp (double-stranded sequence).
  We would appreciate receiving *E. coli* single clones of your construct as a back-up system.
- *E. coli* **clone** is provided by the customer.
  DNA isolations are performed by Microsynth (see Service G3). Contrary to plasmid-DNA isolations, isolations of BAC, Fosmid, Cosmid-DNA are not included in the primer walking price per base.

E2) DIRECT SEQUENCING ON GENOMIC DNA

The DNA quality and size of the genome (<4–5 MB) are very important factors when sequencing genomic DNA directly. We therefore use special protocols for our sequencing procedures.

Condition of DNA

- We receive isolated genomic DNA (Microsynth does not offer genomic DNA isolations).
- Amount of genomic DNA: at least 2–3 µg/reaction, 10 µl of 200 ng/µl – 300 ng/µl DNA.

E3) ENTIRE SEQUENCING OF BAC, PAC, FOSMID, COSMID

Entire large constructs are sequenced to x-fold defined coverage or publication-quality. This is accomplished by first creating a shotgun library and then sequencing the resulting fragments of defined sizes.

Different degrees of coverage are achieved depending on the total number of sequencing reactions.
In order to achieve **publication quality** single reactions on shot gun clones are joined together and any remaining gaps are closed. Gap-closure can be accomplished through primer walking reactions spaning the unknown sites or through sequencing of PCR-fragments created from these areas. The entire sequence is provided as text file and all sequencing data can be provided on CD upon request.
Schedule: upon request

Condition of DNA:

- Agar stab culture.
- 1 µg purified DNA for restriction digest control.

E4) ENTIRE SEQUENCING OF WHOLE GENOMES

We will either apply:

- 454 sequencing of the whole genome combined with a 454 paired-end strategy
  or
- 454 sequencing of the whole genome combined with Sanger sequencing of the ends of a fosmid library.
  For the second option, we will construct a fosmid library (33 kb inserts).

Condition of DNA

- We receive isolated genomic DNA (Microsynth does not offer isolations of genomic DNA).
- Amount of genomic DNA: > 30 µg.

Important Information

For your large sequencing projects see also our 454 sequencing service

F) Customized Services (only in combination with Premium Run Service)

F1) ONE-DROP SEQUENCING

Do you need to sequence a plasmid but you have less DNA?

- **Approx. 50 ng** of DNA is needed for a One-Drop sequencing reaction.
- This service is applicable only for plasmid DNA.
- Turnover time is 2 days.

F2) SPECIAL TREATMENTS (HAIRPIN STRUCTURES, G/C-RICH SEQUENCES)

The structural properties of some DNA sequences can severely affect the sequencing results. Special protocols are applied to increase the probability of sequencing difficult structures (like hairpins, G/C rich sequences). This additional service is especially useful for sequencing **siRNA constructs**.
Intensive investigations lead us to obtain tools to enhance success rate continously. Success rate of sequencable difficult structures came up to more than 98 %.

There are two stages available:
Stage 1: most of the demanding constructs are successfully sequenced.
Stage 2: very difficult structures are cracked at a high probability.

G) DNA Preparation From *E. coli*

G1) SINGLE PLASMID PREPARATION

E. coli should be sent as single colonies grown on agar plates or in LB medium. We need to know the antibiotic resistance.
Schedule: Plasmid DNA from *E. coli* arriving in the morning at Microsynth are amplified over night and sequenced the following business day.

G2) HIGH-THROUGHPUT PLASMID PREPARATION

Plasmid preparations are performed in the 96-or 384-well-format. *E. coli* colonies should be sent in 96-or 384-well-plates containing agar or glycerol-stocks (for automated clone picking) or on agar plates (for manual clone picking). We need to know the antibiotic resistance.

G3) BAC, PAC, FOSMID, COSMID DNA PREPARATION

E. coli clones containing your construct should be sent as single colonies on agar plates or as stab cultures. We need to know the antibiotic resistance.

H) DNA Purification

H1) PURIFICATION OF PLASMIDS

Plasmid-DNA is purified.

H2) PURIFICATION OF PCR PRODUCTS

PCR primers, leftover dNTPs and salt-contaminants are removed from your PCR reactions.

H3) PURIFICATION OF PCR PRODUCTS IN 96-WELL FORMAT

PCR-Primers, leftover dNTPs and salts are removed in 96-well plates.

Important Information

This is not a gel-purification of your PCR-products, it will not seperate individual PCR fragments!

I) Exon Sequencing & Mutation Detection

Discovering the correlations between mutations and genetic diseases is of tremendous importance for many areas of research. DNA sequencing analysis is your most accurate tool for genetic research. Sequencing results can be used to identify mutations in genes or exons with great accuracy on a nucleotide level. Here at Microsynth, we use sequencing to compare exons from various samples with the objective of finding point-mutations.

We offer

- **Project management** and consulting.
- Establishing PCR and Sequencing Systems.
- PCR-amplification of exons.
- Double-stranded sequencing of the resulting PCR products.
- Mutation reports of alignment of different samples against reference sequences.

If you are interested or have questions please do not hesitate to contact our DNA Sequencing Service.

Important Information

For your large sequencing projects see also our 454 sequencing service

Practical Notes

SAMPLE PREPARATION

It is essential that DNA samples are sent **without EDTA** (EDTA inhibits the sequencing enzyme). We highly recommend that you resuspend your samples in water or 10 mM Tris/pH 8.5.
We only accept DNA which has been purified using commercial kits which are certified to yield proper DNA for automated fluorescent sequencing. Please consult your purification kit supplier for further advice.

Plasmids

Some plasmids have a low copy number in *E. coli* cells. These low copy plasmids must be purified for sequencing using an additional purification procedure. If you do not purify the sample, please instruct us to purify your sample. We offer plasmid purifications in-house.

E. coli

E. coli must be provided as visible, **single colonies** on agar plates or as single clones in LB-medium. *E. coli* arriving in the morning are amplified overnight and sequenced the next business day.

Agar must be firm enough for proper shipment (liquid agar tends to slide on to the lid during transport). We recommend stable boxes when sending plates in order to arrive undamaged in our lab. Please do not wrap your plates with your paper print-out of the order form.

PCR Products

For reliable results impurities such as dNTPs, PCR primers etc. must be eliminated from the PCR product before sequencing. It is necessary to quantify the purified single-banded products. We have good experiences using fluorimetric measurements.

Gel image of PCR products: If possible, please send a picture of the gel image after the final purification step.

Please make sure that your PCR primers meet the requirements for sequencing primers. Sometimes usage of internal sequencing primers is helpful.

Important Information

A further purification step may improve the quality of your sequencing results.
PCR products < 100 bases are problematic for sequencing. We recommend cloning into vectors.

SAMPLE SHIPMENT

The samples **and the paper print-out** of the **orderform** should be sent to Microsynth.

TUBES

Only 1.5 ml Sarstedt screw cap tubes will be accepted (please contact us for details).
Sarstedt product no. 72.692.005

To order call:
Switzerland: Phone +41 81 750 1880
Germany: Phone +49 2293 305 0
Austria: Phone +43 2236 61682

Please do not wrap tubes in parafilm. These tubes have a tight seal and are quite stable.

LABELING

Label your tubes with a waterproof pen on the tubes themselves (not the lids only)!
Please choose your names carefully. We recommend using numbers optionally followed by a sample name.
(e.g. 1_xy, 2_zz, …).

SHIPPING CONDITIONS

All samples arriving in the morning are sequenced the same day.

Switzerland

DNA samples & primers should be sent in **liquid form** at room temperature. It is important that you mark «**A-Priority**» on the padded envelopes. Send samples to our Swiss address:
Microsynth AG, Postfach 63, 9436 Balgach, Switzerland

*) Swiss P.O. Box is emptied on Saturdays and samples are stored for sequencing on Monday.

Austria

DNA samples & primers should be sent in **liquid form** at room temperature. Send samples to our P. O. box in Austria:
Microsynth AG, Postfach 58, 6961 Wolfurt-Bahnhof, Austria

Germany

DNA samples & primers should be sent in **liquid form** at room temperature. Send samples to our P. O. box in Germany:
Microsynth AG, Postfach 3351, 88115 Lindau, Germany

Other countries

DNA samples & primers should be sent in **liquid form** at room temperature. Most appropriate address is automatically choosen in the online orderforms.

Courier Address for Express Deliveries:

Microsynth AG, Schützenstrasse 15, 9436 Balgach, Switzerland

Sequencing Primers

OPTIONS FOR SEQUENCING PRIMERS

1. You can use one of our standard primers free of charge.
2. You can send the specific primers along with your samples.
3. You can have your primers designed and synthesized at Microsynth. In this case, please provide essential details such as your known sequence or the standard vector sequence.
4. You can design your own primer to be synthesized at Microsynth. You may directly order your primers in the Premium Run order form («Order now»). If you order the primers through our Oligo order form make sure that the primers will be kept for sequencing at Microsynth.

GUIDELINES FOR PRIMER DESIGN

- Primer length approx. 20 bases (+/–2).
- G/C content approx 50 %.
- Avoid hairpins, palindromic sequence and dimers.
- Avoid primers with 90 % assembly with a second binding site or where the last 7 bases from the primer's 3'-end match perfectly with another site.
- Please remember that 30–60 nucleotides get lost between the 3'-end of the primer and the start of sequence. Take this into account when you choose your primer position.

PRIMER STORAGE

All primers used for Premium Runs are stored at our facility for 1 year. During this time these primers or aliquots of them will be sent to you only upon request. After one year they will be disposed of.

454 DNA Sequencing

With the acquisition of the **Roche Genome Sequencer FLX system**, Microsynth offers you the best choice for rapid completion of whole genome or other large DNA sequencing projects. We combine the new technology with the classical Sanger sequencing for a complete solution.
Our long know-how and high quality standards in Sanger sequencing are continued in the «Next Generation Sequencing».

Our strengths

- Project management & flexibility.
- Regular contact and discussions in order to optimise your project.
- Choice of the most appropriate plate size and capacity .
- Best ratio: quality / costs.
- Average reading length: 250 –400 bases per read.
- FLX Titanium (400 Mb, 1'000'000 reads) series & FLX standard series (250 Mb, 400'000 reads).

Microsynth services using the 454 Technology

Important information

DNA Quality control
Microsynth performs for every project a quality control of the input DNA. Depending on the project,
this is done by agarose gel electrophoresis, Agilent bioanalyzer electrophoresis or Sanger sequencing reactions.

I. Whole genome sequencing: De Novo and resequencing

Microsynth proposes shotgun reads and/or 3 kb long-tag paired end reads.

The use of the **shotgun library** preparation produces reads with an average of 250 – 400bases which are assembled with the GS de Novo assembler (or the GS Reference Mapper) in long contigs.
The use of the **3 kb Long-Tag paired end** protocol allows the sequencing of approximately 100 bases from each end of a 3000 base-span. The contigs obtained are more numerous (see table 1) but with this approach a scaffolding of the genome is provided.
Microsynth prepares the DNA for the FLX run according to one of the two options mentioned above and performs the sequencing run on either a full large PTP (picotiterplate), on a ½, ¼, ⅛ or 1⁄16 PTP.
An electronical assembling is made with the software «GS de Novo Assembler» and or «GS Reference Mapper».

You receive

The sequencing data will be delivered on a DVD. The data consist of Raw Reads (FASTA format, Quality Scores, Sff files) and the Assembly data (Fasta formats and Ace files).

TABLE 1: EXAMPLE OF DE NOVO ASSEMBLY RESULTS

	Organism 1 De Novo Shotgun	Organism 1 De Novo Paired end	Organism 2 De Novo Shotgun
Total of bases	153.4 Mb	115.6 Mb	133.7 Mb
Total of reads	607'102	694'566	572'232
Large contigs (>500bp)			
Nb of large contigs	82	305	87
Largest Contig	158.4kb	59 kb	421.4 kb
Avg contig size	33.7 kb	9 kb	57 kb
N50 contig size	60.7kb	17.4 kb	226.2 kb
Nb of bases	2.763 Mb	2.762 Mb	4.971 Mb
All contigs			
Total nb. of contigs	141	963	141
Total Nb of bases	2.777 Mb	2.883Mb	4.984 Mb
Scaffold			
Nb. of scaffolds	-	16	-
Nb. of bases	-	2.765 Mb	-
Largest Scaffold	-	1.555 Mb	-
Avg. Scaffold size	-	172.8 kb	-
N50 Scaffold size	-	1.555 Mb	-

Organism. 1 :*Clostridium chauvoei* – with the courtesy of Prof. J. Frey, Institute of Veterinary Bacteriology, University of Bern

Organism 2 :*Escherichia coli strain* – with the courtesy of Prof. R. Stephan, Institute for food safety and hygiene, University of Zurich

Sequencing was done with GS FLX standard reagents.

Important information

In resequencing or comparative genomic projects, large discrepancies between the sequenced genome and the reference genome can happen and make any alignment of short reads impossible. In opposition to other technologies the long reads (250/400 bases) of the FLX system give a unique advantage. It allows to perform an assembling as a de Novo project by using the «GS de Novo Assembler» and to obtain by this way usable data.

RECOMMENDED COVERAGE FOR WHOLE GENOME SEQUENCING

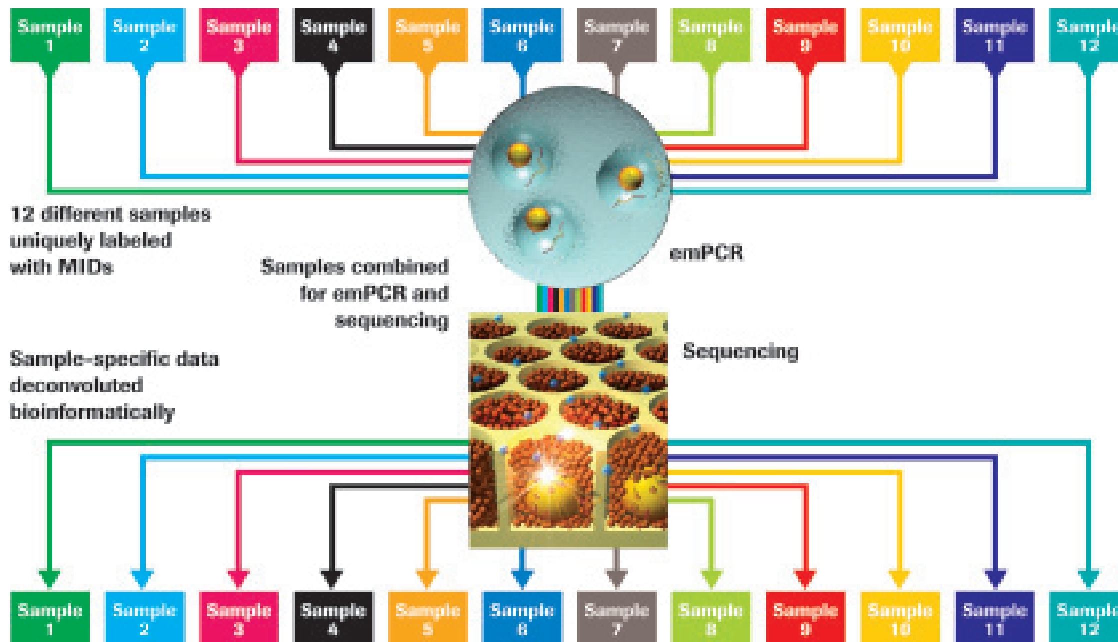
For de Novo sequencing we recommend 20x coverage. For resequencing 15x.

II. BAC sequencing

Depending on their sizes several BACs can be sequenced during one run. Two options can be used:

1. The PTP is divided physically in 2, 4, 8 or 16 regions by the use of a gasket.

2. Multiplex Identifiers (MIDs) containing a unique nucleotide sequence are ligated to the samples during the libray preparation and are used to tag the samples. The samples can then be pooled together.



The preparation of the BAC-DNA libraries for the GS FLX , the sequencing runs, the electronical assembling and the transmission of the data are similar to what is described above in the whole genome sequencing section.

Important information

If the two options are used together, it is possible to process simultaneously up to several hundreds samples per run.

III. cDNA sequencing

cDNA for direct sequencing or RNA for cDNA synthesis (full-length cDNA, small non-coding cDNA) and subsequent sequencing can be sent to Microsynth.

– < 600 bases: the DNA fragments are processed without fragmentation
– >2000 bases: the DNA fragments are fractionated by a shotgun procedure

The preparation of the cDNA for the GS FLX, the sequencing runs and the transmission of the data are similar to what is described above in the whole genome sequencing section.

IV. Amplicon sequencing (Exon Sequencing & Mutation Detection)

The procedure for preparing a DNA sample for Amplicon Sequencing consists of a simple PCR amplification, but requires special Fusion Primers which must be designed by the user according to the specific requirements of the experiment.

The 5'-part of each Fusion Primer, Primer A and Primer B, are always the same, as dictated by the requirements of the FLX Genome Sequencer System. They have the following characteristics:
a. Length: 19 nt
b. Sequence:
I. Primer A: 5' GCCTCCCTCGCGCCATCAG 3'
II. Primer B: 5' GCCTTGCCAGCCCGCTCAG 3'

The 3'-part of each Fusion Primer is specific to each Amplicon and is used by the Amplicon Variant Analyser software to assign reads:
a. Length: typically 20-25 nt (may vary)
b. Sequence: Tag + specific sequence to each side of the desired Amplicon
The amplicons are pooled (equimolar pooling) in 1, 1/2, 1/4, 1/8 or 1/16 run. The number of amplicons that can be pooled together is a compromise between ensuring a high probability of finding a variant and pooling the largest possible number of amplicons.
As an example: if a desired sensitivity for a variant within a sample is 10%, 5% or 2%, and 50 reads per variant are required, the number of amplicons that can be pooled in 1/4 of a PTP plate will be 140, 70 and 28 respectively.
The sequencing runs and the transmission of the data are similar to what is described above in the whole genome sequencing section.
Electronical analysis is made with the software «GS Amplicon Variant Analyser software»

TABLE 2: EXAMPLE OF DEEP SEQUENCING OF PCR PRODUCTS

To pool several samples within one region, the PCR products were barcoded by fusionprimers. Six different fusionprimers with a 6 bp barcode were used to deep sequence totally 36 samples on six regions. The results of six samples on one region are shown below.

Sample	Region	Barcode	Reads per region	Reads per sample
1	1	1	9476	1688
2		2		1866
3		3		1220
4		4		1479
5		5		1293
6		6		1925

Number of reads received per region and sample after equimolar pooling of the PCR products. With the courtesy of Prof I. Roditi, Institute of Cell Biology, University of Bern.

Sequencing was done with the GS FLX standard reagents.

V. Metagenomics

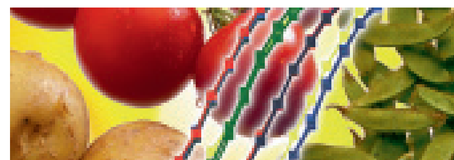
Two approaches are possible for metagenomic projects:

- Analysis of the 16sRNA of a community. This approach is equivalent to an Amplicon project.
- Shotgun sequencing of the whole community DNA.

Contact

For further information and price offers contact *genome@microsynth.ch*

Note: 454 DNA Sequencing related images have been provided by Roche Diagnostics GmbH, Roche Applied Science, 68298 Mannheim, Germany



Real Time PCR Analysis Service

In recent years detection or quantitative determination of specific DNA or RNA sequences through **Real Time PCR** has become an industry standard. The main **benefits** of **state-of-the-art** Real-Time PCR techniques include the elimination of carry-over of PCR products and high accuracy and precision of the results.

For more than a decade we have been successfully testing samples for the presence of genetically modified organisms (GMO). Our services are based on carefully developed PCR techniques combined with sophisticated robotic pipetting systems. With the experience gained from testing numerous samples, we offer routine processing methods that guarantee a smooth-running treatment of your samples.

OUR SERVICES

- Quantitative or qualitative high-throughput GMO testing (raw materials, food, luxury goods)
- Quantitative or qualitative high-throughput testing of samples from various sources for the presence of specific DNA- or RNA-sequences of interest (e.g. pathogen detection, gene expression assays etc.)

OUR ADVANTAGES

- Vast experience in the field of molecular biology.
- Fully equipped for both qualitative and quantitative PCR diagnostics.
- Separate laboratories and a unidirectional work flow minimize the risk of cross contaminations.
- Attractive pricing.
- Accredited according to ISO 17025.

CONTACT

For further details, technical questions and pricing information please contact our Real Time PCR Service:

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Phone direct: +41 71 726 12 50
Dr Emanuel Hoerler
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Phone direct: +41 71 726 10 02

We crack difficult regions in more than 98 %

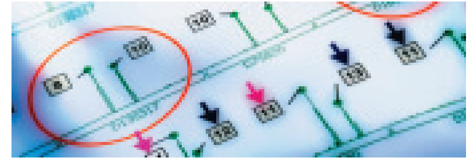
Voucher for
One Special Treatment

(hairpin structure, GC-rich sequence)

Valid in May and June 2009

Enclose it to your sequencing Premium Run order.

Genotyping



We can carry out your fragment length analysis project from A–Z or a single step out of it.
To complete our range of services we use our well funded knowledge in the field of molecular biology to offer a high-quality custom Genotyping Service. A routine application offered by the Microsynth genotyping division is paternity testing services.
With competent service Microsynth helps you to speed up your projects.

Which of the following steps would you like to outsource?
- DNA isolation.
- PCR.
- DNA cleaning steps.
- Electrophoresis.
- Fragment length analysis (STRs).

Important Information

Every project has its own characteristics. For larger projects we should therefore run some test samples first. This gives us the basis for working out an offer.

OUR ADVANTAGES
- Unidirectional working methods to prevent DNA contaminations.
- Our capillary electrophoresis system guarantees a high capacity.
- Work flows according ISO 17025 ensure a sophisticated quality management system.

CONTACT
Please contact Dr Georges Wigger *(georges.wigger@microsynth.ch)* for further information or price offers.

Frequently Asked Questions

CALCULATIONS/CONVERSION FACTORS

What is the definition of OD260?

One OD unit of DNA is the amount of DNA that gives an absorbance reading of 1.0 at 260 nm for a sample dissolved in 1.0 ml total volume of ddH2o which is read in 1 cm quartz cuvette. (OD = «Optical Density»).
For Oligos, 1 OD is approximate 33 µg of DNA.

How is Melting Temperature (Tm) estimated for Oligos?

The melting temperature (Tm value) of an Oligo depends on the length of the sequence, the G+C content of the sequence, and the type and concentration of present cations, particularly sodium ions (Na+).
A variety of formulas have been used for predicting Tm values. These tend to be fairly accurate for long DNA sequences, but are less exact for Oligos.
The following formula is recommended for Oligos ranging in length from 20 to 100 residues, and [Na+] concentrations ranging from 0.01 M to 1.0 M:
Tm (in degree C) = 81.5 + 0.41 (G % + C %) + 16.6 log [Na+] – 500/length
For example, for Oligos with a 55 % G + C content in a 0.1 M Nacl solution:

Tm	= 81.5 + 0.41(55) + 16.6 log [0.1] – 500/length
	= 81.5 + 22.5 – 16.6 – 500/length
	= 87.5 – 500/length
eg	20 mer: 62 °C; 25 mer: 67 °C; 30 mer: 71 °C;
	35 mer: 73 °C

How can I calculate the annealing Temperature for PCR?

Calculate the melting temperature as above and subtract 5 to 10 °C.
If the two Oligos have different melting temperatures, do NOT average the numbers. Use the lower number so that both of the Oligos can anneal.

eg	20 mer: 52–57 °C; 25 mer: 57–62 °C; 30 mer: 60–65 °C; 35 mer: 63–68 °C)

MOLECULAR WEIGHT

How is the molecular Weight of an Oligo calculated?

$$MW = (dA \times 313.1) + (dC \times 289.1) + (dG \times 329.1) + (dT \times 304.2) - 61$$

where dA, dC, dG, dT are numbers of dA's, dC's, dG's and dT's in an Oligo and the 5' and 3' ends contain free hydroxyl groups. Add 78 to the MW for 5'phosphorylated Oligos.

What are the approx. conversion factors?

1 OD260 unit of an Oligo	~ 33 µg
1 OD260 unit of ss RNA	= 40 µg
1 OD260 unit of ds DNA	= 50 µg
1 µg of 1 kb DNA	= 1.62 pmol (3.24 pmoles of ends)
1 µg of pBR322 DNA	= 0.36 pmol DNA

nmoles of Oligo = OD × 100/length of Oligo (rule of thumb).
nmoles of Oligo = OD × 1000/millimolar extinction coefficient.

Molecular Extinction Coefficient

How do I calculate the millimolar extinction coefficient?

$$0.9 \times (15.4 \times nA + 7.3 \times nC + 11.7 \times nG + 8.8 \times nT)$$

SCALE

What is the synthesis scale?

The scale of synthesis is the amount of 3' base that we start the synthesis with. It is NOT the expected final yield. The yield depends on the size of the Oligonucleotide, the coupling efficiency, and the base composition.

What is the theoretical yield?

The theoretical yield of the full length product can be determined using the formula 0.985^n, wherein 0.985 represents the coupling efficiency and n the length of the Oligo. Hence, the theoretical yield of the full length product of a 50 mer is 47 % at a coupling rate of 0.985. Truncated Oligos are not eliminated by desalting but by HPLC or PAGE purification.

How many reactions can I carry out with my Oligo?

In PCR, the Genomic scale yields approx. 700–3500 reactions, the 0.04 µmol scale approx. 1000 to 5000 reactions and the 0.2 µmol scale 4000 to 20000 reactions.

PURITY

What does «Desalting» of an Oligo mean?

All our Oligos are at least desalted. Desalting is necessary to remove protecting groups (which prevent unwanted

side reactions during synthesis) and residual salts which can affect the biological activity of the Oligos. Desalting is especially important for standard sequencing and in vitro mutagenesis.

How do I know which purification grade is sufficient?
The purity required for a specific application depends on the potential problems which would result from the presence of failure sequences (n–1, n–2, … Oligomers).
Please read the recommendations in this catalogue. If you intend to use an Oligo for a high risk project or in routine diagnostic purpose, we recommend purified Oligos.

siRNA

Which overhang is best for my siRNA?
The most determinant factor for effectiveness is the 19 bp core sequence. Any overhang (RNA or DNA) may be chosen. DNA overhangs may be more nuclease resistant, therefore, Microsynth recommends you to use dTdT overhangs. This is also the most economical choice.
Recent studies suggest that siRNA is very stable once introduced to the cells.

My siRNA does not show an effect. What can I do?
A lot of factors influence the effectivnes of your siRNA. As first step we recommend the following:

Titrate the amount of siRNA used in your experiment. *Use a positive control with a simple readout (e.g. GFP).*

Optimize transfection/delivery efficiency. *It should exceed 90 % if you look at a pool of cells.*

Change your readout system – e.g. look at the mRNA instead of the protein. *Your protein migh have a long half-life.*

Re-anneal your siRNA (see how to resuspend siRNAs page 16). *Lyophilization might decrease activity.*

Make sure that your gene of interest is expressed in your cell line of interest. *There may be cell-type specific splice forms.*

Check the sequence information in the database in the context of your cell line. *Same as above.*

Choose a different siRNA. *Not all siRNAs are potent downregulators.*

Mutations in Oligos (see our homepage)

EDITING OF YOUR SEQUENCE

What is the difference between non-edited and edited sequencing reactions?
In automated sequencing, different fluorescent dyes absorb the excitation energy of a laser and allow DNA to be detected in the gel during electrophoresis. A software program then measures the signal strength of the fluorescent bands and creates a chromatogram. Thereafter, automated «base calling» is applied by the instrument which «translates» the chromatogram into a sequence.
Unfortunately, some artefacts cannot be recognized by a «base-caller» program, resulting in the translation of wrong bases or non-defined N-bases. Only experienced «eyes» can detect and correct such uncertainties.

IUB Code for mixed base sites:	
K = G, T	B = G, T, C
M = A, C	D = G, A, T
R = G, A	H = A, T, C
S = G, C	V = G, A, C
W = A, T	N = G, A, T, C
Y = T, C	

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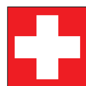

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STS 429
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GMO-testing
BVDV-analysis
Genotyping

Accreditation according to EN ISO 17025