Section F

Molecular Biology Services

Flexible and user-friendly services for gene hunter

- cDNA preparation
- cDNA library construction
- cDNA normalization
- cDNA subtractive hybridization
- bacterial genome subtraction
- eukaryotic genome subtraction
- PCR/RT-PCR cloning
- subcloning
- RACE and full-length cDNA isolation
- genome walking
- site-directed mutagenesis
- gene synthesis

Section content

cDNA preparation and library

Services available:

cDNA preparation

Full-length-enriched double-stranded cDNA is produced using modified SMART technology. The method combines cDNA synthesis and amplification and results in representative cDNA population enriched with full-length sequences even from small amounts of starting materials.

Construction of standard cDNA libraries

Full-length-enriched double-stranded cDNA is prepared from poly(A+) or total RNA. After synthesis, cDNA is size fractionated, cloned (directionally or non-directionally) into an appropriate plasmid vector, and transformed into *E. coli*.

cDNA normalization and construction of normalized cDNA libraries

cDNA normalization results in equalization of the abundance of different transcripts and increase in the number of previously non-detected genes in a full-length-enriched cDNA library. This can noticeably raise the effectiveness of EST sequencing, transcriptome analysis, and rare gene discovery. Normalization is performed using highly effective duplex-specific nuclease (DSN)-based method prior to library cloning.

cDNA depletion and construction of depleted cDNA libraries

The recurring appearance of transcripts that have already been analyzed often makes cDNA analysis an unacceptably time-consuming procedure. The selective depletion of these transcripts from a cDNA library significantly accelerates expression cloning and functional screening. Evrogen performs cDNA depletion of SMART cDNA prior to library construction using a novel DSN-based method.

cDNA subtraction and construction of subtracted cDNA libraries

cDNA subtraction is an optimal means to compare two cDNA populations and to find differentially expressed genes. Two cDNA populations are compared using well-known Suppression subtractive hybridization (SSH) method, cloned into a plasmid vector, and transformed into *E. coli*. Special Mirror orientation selection (MOS) approach that is a part of cDNA subtraction service efficiently eliminates false-positive clones from SSH-generated libraries.

cDNA preparation service

- High-quality full-length-enriched amplified cDNA for various applications

- From limited amounts of total or poly(A+) RNA

Service description

cDNA preparation is performed from customer-supplied poly(A+) or total RNA using SMART technology [1-4].

SMART-amplified cDNA is extremely useful for

- cDNA subtraction
- cDNA normalization or depletion
- Isolation of full-length cDNAs by RACE
- Construction of representative cDNA libraries
- Virtual Northern blot [5]

In addition, PCR amplification of long cDNA fraction can be performed using our long PCR product generation system [6]. This specific approach allows to notably decrease the representation of short transcripts in a cDNA sample.

Service terms and conditions

You provide:

At least 1.0 µg of total RNA or poly(A+) RNA
see "How to send samples to Evrogen" section for details (page G-4)

We perform:

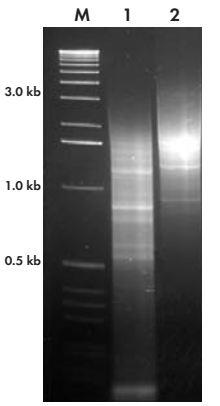
1. First-strand cDNA synthesis
2. cDNA amplification using SMART technology

You will receive:

1. Any leftover starting materials (upon request)
2. First-strand cDNA (with specific adapters on both ends)
3. SMART-amplified double-stranded cDNA (at least 1 µg)
4. PCR primers for cDNA amplification
5. cDNA preparation report

Service	Cat.#	Price
cDNA preparation	CS030	380 €
Extra options		
Amplification of long cDNA fraction	adds	320 €

Minimal order — 1000 €. Prices do not include shipping&handling. Prices are valid from January, 2007 and subject to change without notice. Please verify current prices at Evrogen Web site: www.evrogen.com. We offer DISCOUNTS for MULTIPLE ORDERS!



Regulation of the average length of an amplified cDNA sample for library construction purposes.
M — 1-kb DNA size markers; lane 1— amplified cDNA sample prepared from total RNA of freshwater planarian using SMART approach; lane 2 — biased cDNA sample amplified using Long PCR Product Generation System.

Extra options:

- Amplification of long cDNA fraction

Turnaround time

From 3 to 4 weeks

This time is estimated based on average turnaround time for this type of orders. Actual time may depend on the complexity of starting materials and/or deliverables.

Related services

- Construction of standard cDNA libraries — see page F-5
- cDNA normalization — see page F-6
- cDNA depletion — see page F-8
- cDNA subtraction — see page F-9
- RACE — see page F-18

References

1. Zhu *et al.* (2001) Biotechniques. 30(4): 892-897.
2. Matz M. (2002) Methods Mol Biol. 183: 3-18.
3. Matz *et al.* (1999) Nucleic Acids Res. 27(6): 1558-1560.
4. Matz *et al.* (2003) Methods Mol Biol. 221: 41-49.
5. Franz *et al.* (1999) Nucleic Acids Res. 27: e3.
6. Shagin *et al.* (1999) Nucleic Acids Res. 27: e23.

Confidentiality Statement:
All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Standard cDNA library construction service

- cDNA preparation from limited amounts of total or poly(A+) RNA

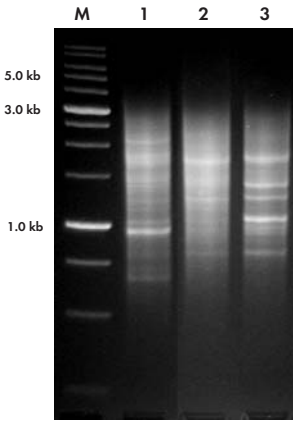
- *E. coli* libraries from high-quality full-length-enriched amplified cDNA

Service description

Full-length-enriched double stranded (ds) cDNA is synthesized using SMART technology [1-4]. After synthesis, ds cDNA is size fractionated, and cloned (directionally or nondirectionally) into a plasmid vector from our collection (or into an appropriate customer-supplied plasmid vector), and transformed into *E. coli*. For directional cloning we use *Sfi*IA — *Sfi*IB, *Srf*I — *Not*I, or *Asc*I — *Not*I restriction sites.

As a quality control measure, a percentage of recombinant clones and average insert size is determined by gel electrophoresis of 33 clones picked at random. cDNA library will contain 750 000 or more (on request) independent clones.

Please inquire about other variants of cDNA preparation and special vector requirements.



ds cDNA prepared on the basis of total RNA from different human tissues.

Lane 1 — embryonic lung; lane 2 — testis; lane 3 — small intestine; M — 1 kb DNA size markers.

Service terms and conditions

You provide:

At least 2.0 µg of total RNA or poly(A+) RNA

see "How to send samples to Evrogen" section for details (page G-4)

We perform:

1. cDNA synthesis from customer-supplied RNA
2. Ligation of the cDNA into an appropriate vector
3. Transformation of the ligate into *E. coli*
4. PCR amplification of the inserts from 33 randomly picked clones to determine a percentage of recombinant clones and average insert size

You will receive:

1. Any leftover starting materials (upon request)
2. SMART-amplified double-stranded cDNA (at least 1 µg)
3. PCR primers for cDNA amplification
4. Amplified and characterized cDNA library (containing at least 750 000 independent clones)
5. Service report

Service	Cat.#	Price
cDNA library construction	CS040	2900 €
Extra options		
Modification of a customer-supplied vector for cloning using restriction endonucleases noted in the service description		agreed

Prices include shipping&handling. Prices are valid from January, 2007 and subject to change without notice. Please verify current prices at the Evrogen Web site: www.evrogen.com. We offer DISCOUNTS for MULTIPLE ORDERS!

Extra options:

- Modifications of a customer-supplied vector for cloning

Turnaround time

From 6 to 8 weeks

This time is estimated based on average turnaround time for this type of orders. Actual time may depend on the complexity of starting materials and/or deliverables.

Related services

- cDNA preparation — see page F-4
- cDNA normalization — see page F-6
- cDNA depletion — see page F-8
- cDNA subtraction — see page F-9

References

1. Zhu *et al.* (2001) Biotechniques. 30(4):892-897.
2. Matz M. (2002) Methods Mol Biol. 183, 3-18.
3. Matz *et al.* (1999) Nucleic Acids Res. 27(6): 1558-1560.
4. Matz *et al.* (2003) Methods Mol Biol. 221: 41-49.

Confidentiality Statement:

All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

cDNA normalization service

- Significant increase of the transcriptome sequencing efficiency
- Equalization of full-length-enriched cDNA population
- Normalization of cDNA prepared from small amounts of total or poly(A+) RNA
- cDNA normalization order processing is set up and monitored by the inventors of the DSN-normalization technology.

Service description

Direct sequencing of clones from standard cDNA libraries is inefficient for discovering rare transcripts because of the repeated occurrence of intermediately and highly abundant cDNA types. cDNA normalization significantly increases the efficiency of sequence analysis of cDNA libraries by decreasing the prevalence of cDNA clones representing abundant transcripts.

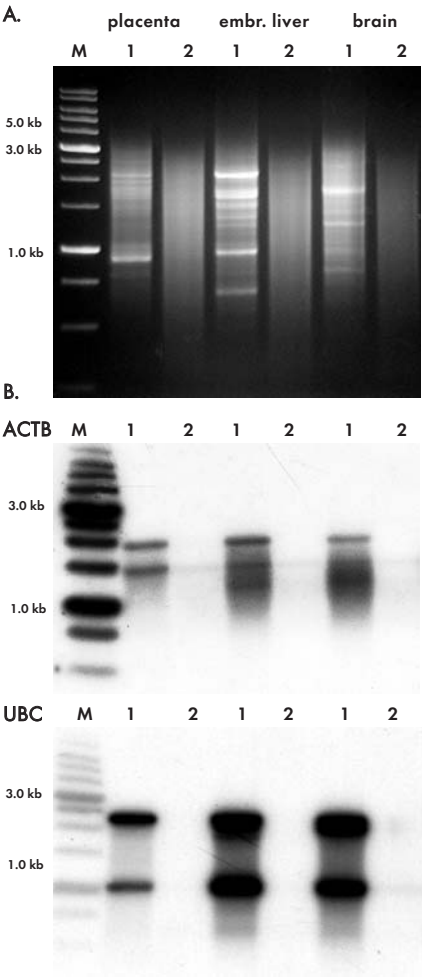
The Evrogen cDNA normalization service is a simple and reliable means to obtain normalized cDNA libraries. We offer cDNA normalization using a proprietary DSN-normalization technology [1,2]. In contrast to other methods, Evrogen technology allows to perform normalization of cDNA enriched with full-length sequences before library cloning.

Our service is adapted for normalization of ds cDNA prepared using SMART [3,4] and Mint approaches. Normalized cDNA can be cloned into a plasmid vector from our collection or an appropriate customer-supplied vector nondirectionally or directionally using *Sfi*IA — *Sfi*IB, *Srf*I — *Not*I, or *Asc*I - *Not*I restriction sites.

The library constructed will contain at least 400 000 independent clones. Preparation of the normalized cDNA library containing more clones should be performed by agreement.

In addition, normalization efficiency analysis can be performed using PCR or Virtual Northern blotting [5] with two marker genes.

Please inquire about other variants of cDNA preparation and special vector requirements.



ds cDNA preparation and normalization result.
(A) — Agarose gel electrophoresis of non-normalized (1) and normalized (2) cDNA from human tissues; (B) — reduction of the concentration of beta-actin (ACTB) and ubiquitin C (UBC) transcripts in normalized cDNA samples revealed by Virtual Northern blot.
M — 1-kb DNA size markers, embr. — embryonic.

Service terms and conditions

You provide:

At least 2.0 µg of total RNA or poly(A+) RNA
see "How to send samples to Evrogen" section for details (page G-4)

We perform:

Level 1

1. cDNA synthesis from customer-supplied RNA
2. cDNA normalization

Depending on your particular needs, normalized cDNA can be flanked by adapter sequences suitable for directional cloning using *Sfi*IA — *Sfi*IB, *Srf*I — *Not*I, or *Asc*I — *Not*I restriction sites.

Level 2 (also includes all services provided in level 1)

3. Ligation of normalized cDNA into an appropriate vector;
4. Transformation of ligate from step 3 into *E. coli* (library will contain at least 400 000 independent clones)

Level 3 (also includes services provided in levels 1 and 2)

5. Plating of the resulting library in one 96-well plate
6. Purification of plasmid DNA from 90 clones
7. Single run sequencing of these 90 clones

You will receive:

Level 1

1. Any leftover starting material (on request)
2. Amplified non-normalized cDNA (at least 1 µg)
3. Amplified normalized cDNA (at least 1 µg)
4. PCR primers for cDNA amplification
5. cDNA normalization report

Additionally for Level 2

6. Amplified and characterized *E. coli* library (at least 400 000 independent clones)

Additionally for Level 3

7. Purified plasmid DNA from 90 clones
8. Insert sequencing data of these 90 clones

Service	Cat.#	Price
cDNA normalization + nondirectional cloning of cDNA library into a vector from Evrogen collection		
Level 1	**CS010-1**	1900 €
Level 2	**CS010-2**	3600 €
Level 3	**CS010-3**	5000 €
cDNA normalization + directional cloning of cDNA library into a vector from Evrogen collection		
Level 1	**CS011-1**	2400 €
Level 2	**CS011-2**	4500 €
Level 3	**CS011-3**	5900 €
Extra options		
Normalization efficiency analysis	adds	550 €
Cloning of the library into a customer-supplied vector or using unusual sites		agreed
Cloning of a library containing more than 400 000 independent clones		agreed

Prices include shipping&handling. Prices are valid from January, 2007 and subject to change without notice. Please verify current prices at the Evrogen Web site: www.evrogen.com.

We offer DISCOUNTS for MULTIPLE ORDERS!

Extra options:

- Normalization efficiency analysis

Virtual Northern blot is performed for samples from human and mouse with ACTB and UBC genes-derived probes (high abundance in most tissues). For samples from other sources, PCR analysis is used, if sequences of two abundant transcripts are provided by a customer; or Virtual Northern blot is used, if two cloned fragments corresponding to abundant transcripts are provided.

- Cloning of the cDNA library into a customer-supplied vector or using unusual restriction sites

- Preparation of the normalized cDNA library containing more than 400 000 independent clones

Turnaround time

Level 1: from 4 to 6 weeks
Level 2: from 5 to 7 weeks
Level 3: from 7 to 9 weeks

This time is estimated based on average turnaround time for this type of orders. Actual time may depend on the complexity of starting materials and/or deliverables.

Related services

- cDNA preparation — see page F-4
- Construction of standard cDNA libraries — see page F-5
- cDNA depletion — see page F-8

References

1. Zhulidov *et al.* (2004) Nucleic Acid Res., 32: e37.
2. Zhulidov *et al.* (2005) Russian Journal of Bioorganic Chemistry 31 (2): 170 177.
3. Zhu *et al.* (2001) Biotechniques. 30(4):892-897.
4. Matz *et al.* (2003) Methods Mol Biol. 221: 41-49.
5. Franz *et al.* (1999) Nucleic Acids Res. 27: e3.

Confidentiality Statement:
All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

cDNA depletion service

- **Specific removal of already analyzed transcripts from cDNA populations**
- **Preparation of depleted full-length-enriched cDNA libraries for functional screenings**

Service description

Evrogen offers specific removal of already analyzed transcripts from cDNA populations and preparation of depleted cDNA libraries. Analysis of the resulting depleted cDNA libraries significantly accelerates discovery of unknown genes. The service is adapted for full-length-enriched ds cDNA. During depletion procedure selected transcripts are effectively eliminated without of importance cDNA size loss. cDNA depletion is accompanied by partial cDNA normalization that results in reduction of the concentration of abundant transcripts.

For library construction, the depleted cDNA is size fractionated, cloned (directionally or nondirectionally) into a plasmid vector from our collection (or into an appropriate customer-supplied plasmid vector), and transformed into *E. coli*.

Service terms and conditions

You provide:

List of the genes to be removed (up to 20 genes, sequences or GenBank IDs are required)

At least 2.0 µg of total RNA or poly(A+) RNA

see "How to send samples to Evrogen" section for details (page G-4)

We perform:

Level 1

1. cDNA synthesis from customer-supplied RNA
2. cDNA depletion
3. PCR confirmation of the depletion efficacy

Depending on your particular needs, depleted cDNA can be flanked by adapter sequences suitable for directional cloning using *Sfi*IA — *Sfi*IB, *Srf*I — *Not*I, or *Asc*I — *Not*I restriction sites.

Level 2 (also includes all services provided in level 1)

4. Ligation of the depleted cDNA into plasmid vector
5. Transformation of the ligate from step 4 into *E. coli*

You will receive:

Level 1

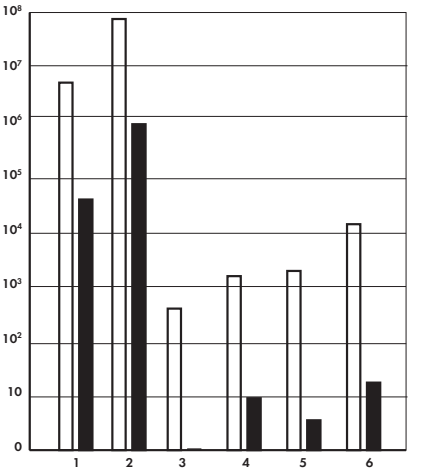
1. Any leftover starting material (on request)
2. Amplified undepleted cDNA (at least 1 µg)
3. Amplified depleted cDNA (at least 2 µg)
4. PCR primers for cDNA amplification
5. cDNA depletion report

Additionally for Level 2

6. Amplified and characterized *E. coli* library

Service	Cat.#	Price
cDNA depletion and library construction		
Level 1	CS012-1	agreed
Level 2	CS012-2	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!



Typical depletion result revealed using quantitative PCR.

1 — Beta-actin; 2 — B2M gene. These two abundant genes were used as a control of normalization process in the sample.
3 — PDE10A; 4 — PDE1A; 5 — PDE1C; 6 — PDE6G. Phosphodiesterase (PDE) catalytic domains of genes 3—6 were removed using Evrogen depleted procedure from the cDNA library intended for functional screening of novel targets having phosphodiesterase activity.
White columns — transcript level in the cDNA sample before depletion; black columns — after depletion.

Extra options:

- Cloning of the cDNA library using unusual restriction sites

Turnaround time

Level 1: from 5 to 7 weeks

Level 2: from 6 to 8 weeks

This time is estimated based on average turnaround time for this type of orders. Actual time may depend on the complexity of starting materials and/or deliverables.

Related services

- cDNA preparation — see page F-4
- Construction of standard cDNA libraries — see page F-5
- cDNA normalization — see page F-8

References

1. Zhu *et al.* (2001) Biotechniques. 30(4):892-897.
2. Matz M. (2002) Methods Mol Biol. 183, 3-18.
3. Matz *et al.* (1999) Nucleic Acids Res. 27(6): 1558-1560.
4. Matz *et al.* (2003) Methods Mol Biol. 221: 41-49.
5. Shagin *et al.* (2002) Genome Res. 12, 1935-1942.

Confidentiality Statement:

All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

cDNA subtraction service

- Identification of differentially expressed genes
- Construction of subtracted cDNA libraries
- Special approach to eliminate false-positive clones from subtracted libraries
- cDNA subtraction order processing is set up and monitored by the inventors of the SSH technology

Service description

To understand molecular regulation of biological processes, differentially expressed genes of interest must be identified, cloned, and studied in details. Suppression subtractive hybridization (SSH) is proved to be a powerful tool in the identification of differentially expressed cDNAs [1-4].

Mirror orientation selection (MOS) service is a part of cDNA subtraction service. MOS eliminates false-positive clones from SSH-generated libraries [5].

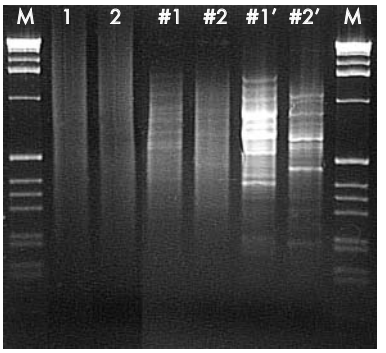
MOS is especially recommended

- for closely related or complicated (e.g. brain or testis RNA) samples;
- if primary SSH PCR requires more than 30 cycles to generate visible PCR products;
- if the percentage of differentially distributed clones in your SSH-subtracted library is very low (i.e. 1—5%);
- if most of the differentially distributed clones found in your SSH-subtracted library are false positives.

Depending on your particular needs, we offer five levels of custom subtraction service. The levels differ in the intensity of work performed and may be finely tuned to exactly suit your research project.

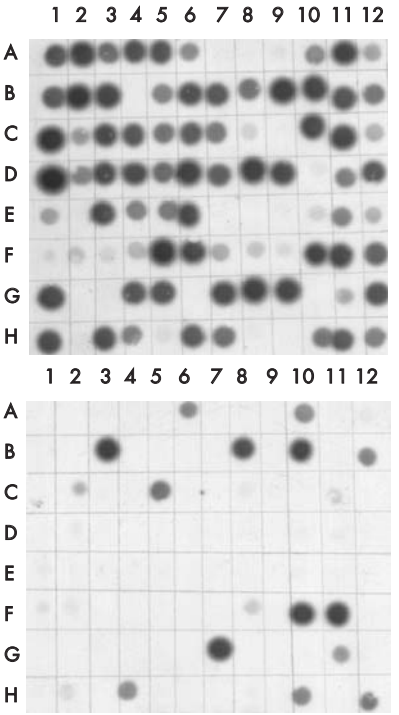
For construction of subtracted cDNA libraries, cDNA populations enriched with differentially expressed genes are nondirectionally cloned into a plasmid vector from our collection (or into an appropriate customer-supplied plasmid vector), and transformed into *E. coli*.

Note: SSH includes the stage of cDNA digestion by restriction endonuclease. Therefore, recombinant clones in the subtracted library contain an average insert size of about 250 bp. To identify full-length sequences of the genes of your interest, use our RACE service (see page F-18).



Typical results of cDNA subtraction and MOS procedure.

M —1-kb DNA size markers; Lanes 1, 2 — cDNA samples 1 and 2; #1 — (sample 1 vs sample 2) subtraction; #2 — (sample 2 vs sample 1) subtraction; #1′ — #1 subtraction after MOS; #2′ — #2 subtraction after MOS. MOS reveals real differential bands and eliminates most of false positives.



Typical differential screening result of a subtracted cDNA library.

Randomly picked clones from #1' subtracted cDNA library were hybridized with radiolabeled #1' (upper filter) and #2' (under filter) subtracted cDNA probes.

Service terms and conditions

You provide:

At least 1.0 µg of total RNA or poly(A+) RNA
see "How to send samples to Evrogen" section for details (page G-4)

We perform:

Level 1

1. cDNA preparation from customer-supplied RNA
2. Subtraction in both directions (forward = A-B; reverse = B-A)
2'. MOS procedure on the subtracted samples (optional, on request)

Level 2 (also includes all services provided in level 1)

3. Ligation of subtracted cDNA into an appropriate vector

Levels 3,4, and 5 (also includes services provided in levels 1 and 2)

4. Transformation of ligates from step 3 into *E. coli*

5. Plating of the two subtracted libraries (in both directions) in 96-well plates:

Level 3: ONE plate of clones per library for both directions

Level 4: FIVE plates of clones for one direction library and ONE plate of clones for the other direction library

Level 5: FIVE plates of clones per library for both directions

6. Differential screening of 96-well plates with two probes to determine the percentage of differentially expressed clones in each subtracted library (probes: forward subtracted cDNA, reverse subtracted cDNA)

7. Purification of plasmid DNA from the differential clones (up to 100 clones from each direction)

8. Virtual Northern hybridization of five clones from each library to confirm differential expression

9. Sequence of differential clones found (up to 10 clones)

You will receive:

Level 1

1. Any leftover starting material (on request)
2. Amplified non-subtracted driver and tracer cDNA
3. Subtracted cDNA samples and all other cDNA samples generated during SSH
4. Primers for amplification of subtracted cDNA and SSH products
5. SSH report

Additionally for level 2

6. Transformation-ready subtracted cDNA ligated into an appropriate vector

Additionally for levels 3-5

7. Subtracted cDNA libraries
8. Differential screening data
9. Purified plasmid DNA from the differential clones
10. Virtual Northern hybridization data
11. Description of the libraries obtained (number of clones and percentage of clones with insertions)
12. Plates with clones used for differential screening

Service		Cat.#	Price
cDNA subtraction			
	Level 1	CS021-1	1700 €
	Level 2	CS021-2	2500 €
	Level 3	CS021-3	5100 €
	Level 4	CS021-4	6500 €
	Level 5	CS021-5	7600 €
MOS		adds	1000 €

Prices include shipping&handling. Prices are valid from January, 2007 and subject to change without notice. Please verify current prices at the Evrogen Web site: www.evrogen.com. We offer DISCOUNTS for MULTIPLE ORDERS!

Turnaround time

Level 1: from 3 to 5 weeks

Level 2: from 4 to 6 weeks

Level 3: from 5 to 7 weeks

Level 4 or 5: from 7 to 11 weeks

If you are taking advantage of our MOS service, please allow an additional week for SSH order turnaround.

This time is estimated based on average turnaround time for this type of orders. Actual time may depend on the complexity of starting materials and/or deliverables.

Related services

- cDNA preparation — see page F-4
- RACE — see page F-18
- Bacterial genome subtraction — see page F-12
- Eukaryotic genome subtraction — see page F-14

References

1. Luk'ianov *et al.* (1994) Bioorg Khim. (Russ), 1994, 20(6):701-704.
2. Diatchenko *et al.* (1996) Proc Natl Acad Sci USA. , 93(12): 6025-6030.
3. Gurskaya *et al.* (1996) Anal Biochem. 240(1): 90-97.
4. Diatchenko *et al.* (1999) Methods Enzymol. 303: 349-80.
5. Rebrikov *et al.* (2000) Nucleic Acids Res. 28(20): e90.

Confidentiality Statement:

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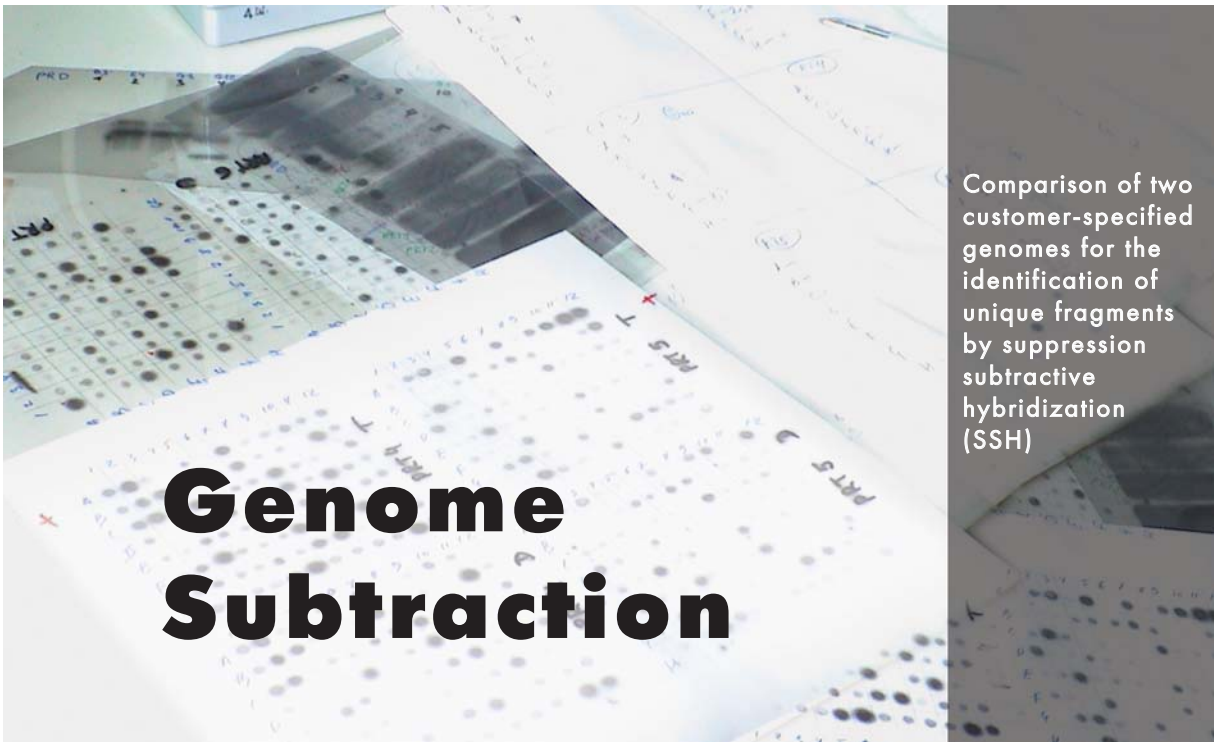


Genome Subtraction

Comparison of two customer-specified genomes for the identification of unique fragments by suppression subtractive hybridization (SSH)

Services available:

Bacterial genome subtraction

Comparison of bacterial genomes using SSH

Eukaryotic genome subtraction

Comparison of eukaryotic genomes using SSH with Mirror orientation selection (MOS)

Bacterial genome subtraction service

- **Identification of differentially distributed sequences in bacterial strains**
- **Subtraction order processing is set up and monitored by the inventors of the SSH technology**

Service description

SSH is the most powerful non-sequencing approach to find genetic difference between bacterial strains. The ability of SSH to identify nearly all major sequence differences between two closely related bacteria has been demonstrated by several independent research groups [1-5].

Depending on your particular needs, we offer five levels of custom bacterial genome subtraction service. The levels differ in the intensity of work performed and may be finely tuned to exactly suit your research needs.

Service terms and conditions

You provide:

Bacterial genomic DNA isolated from the two bacterial strains that you wish to compare
see "How to send samples to Evrogen" section for details (page G-4)

We perform:

Level 1 1. Subtraction in both directions (forward = A-B; reverse = B-A)

Level 2 (also includes all services provided in level 1)

2. Ligation of subtracted DNA into an appropriate vector

Levels 3,4, and 5 (also includes services provided in levels 1 and 2)

3. Transformation of ligates from step 2 into *E. coli*

4. Plating of the two subtracted libraries (in both directions) in 96-well plates:

Level 3: ONE plate of clones per library for both directions

Level 4: FIVE plates of clones for one direction library and ONE plate of clones for the other direction library

Level 5: FIVE plates of clones per library for both directions

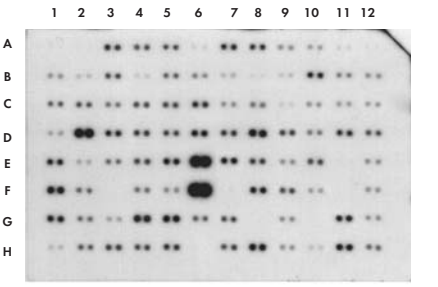
5. Differential screening of 96-well plates with two probes to determine the percentage of differentially distributed clones in each subtracted library (Probes: forward subtracted DNA, reverse subtracted DNA)

6. Purification of plasmid DNA from the differential clones (up to 100 clones from each direction)

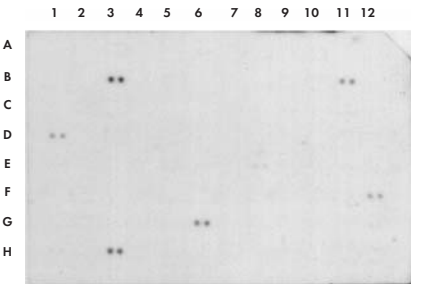
7. Virtual Northern hybridization of five clones from each library to confirm differential distribution

8. Sequence of differential clones found (up to 10 clones)

ZW probe



29213 probe



Typical result of the differential screening for bacterial SSH-generated library.

DNA from the *Staphylococcus aureus* ZW strain was used as a tester and DNA from the S aureus 29213 strain was used as a driver in SSH. Differential screening of ZW-specific SSH generated library with unsubtracted ZW and 29213 strain-specific probes showed that this library contains about 95% of differential clones.

You will receive:

Level 1

1. Any leftover starting material (on request)
2. PCR-amplified subtracted DNA samples and all other DNA samples generated by SSH
3. Primers for amplification of subtracted DNA and SSH products
4. SSH report

Additionally for level 2

5. Transformation-ready subtracted DNA ligated into an appropriate vector

Additionally for levels 3-5

6. Subtracted DNA libraries
7. Differential screening data
8. Purified plasmid DNA from the differential clones
9. Southern hybridization data
10. Description of the libraries obtained (number of clones and the percentage of clones with insertions)
11. 96-well plates with clones used for differential screening

Turnaround time

Level 1: from 3 to 5 weeks

Level 2: from 4 to 6 weeks

Level 3: from 5 to 7 weeks

Level 4 or 5: from 7 to 11 weeks

This time is estimated based on average turnaround time for this type of orders. Actual time may depend on the complexity of starting materials and/or deliverables.

Service	Cat.#	Price
Bacterial genome subtraction		
Level 1	CS022-1	1700 €
Level 2	CS022-2	2500 €
Level 3	CS022-3	5100 €
Level 4	CS022-4	6500 €
Level 5	CS022-5	7600 €

Prices include shipping&handling. Prices are valid from January, 2007 and subject to change without notice. Please verify current prices at the Evrogen Web site: www.evrogen.com.

We offer DISCOUNTS for MULTIPLE ORDERS!

Related services

- cDNA subtraction — see page F-9
- Eukaryotic genome subtraction — see page F-14

References

1. Akopyants *et al.* (1998) Proc Natl Acad Sci USA. 95(22): 13108-13113.
2. Agron *et al.* (2002) FEMS Microbiol Lett. 211(2): 175-182.
3. Winstanley C. (2002) J Med Microbiol. 51(6): 459-467. Review.
4. Rebrikov *et al.* (2002) Annal Periodontol. 7(1): 17-28.
5. Rebrikov *et al.* (2003) In Applications of genomics and proteomics for analysis of bacterial biological warfare agents. V.G. DelVecchio and V. Krcmery (eds.). IOS Press, Amsterdam , Netherlands, 352: 95-105.

Eukaryotic genome subtraction service

- **Identification of differentially distributed sequences in eukaryotic genomes**

Service description

Combination of suppression subtractive hybridization (SSH) with mirror orientation selection (MOS) allows to compare similar eukaryotic genomes to identify genetic difference between them [1,2].

Terms, turnaround time, and prices for eukaryotic genome subtraction service are by agreement.

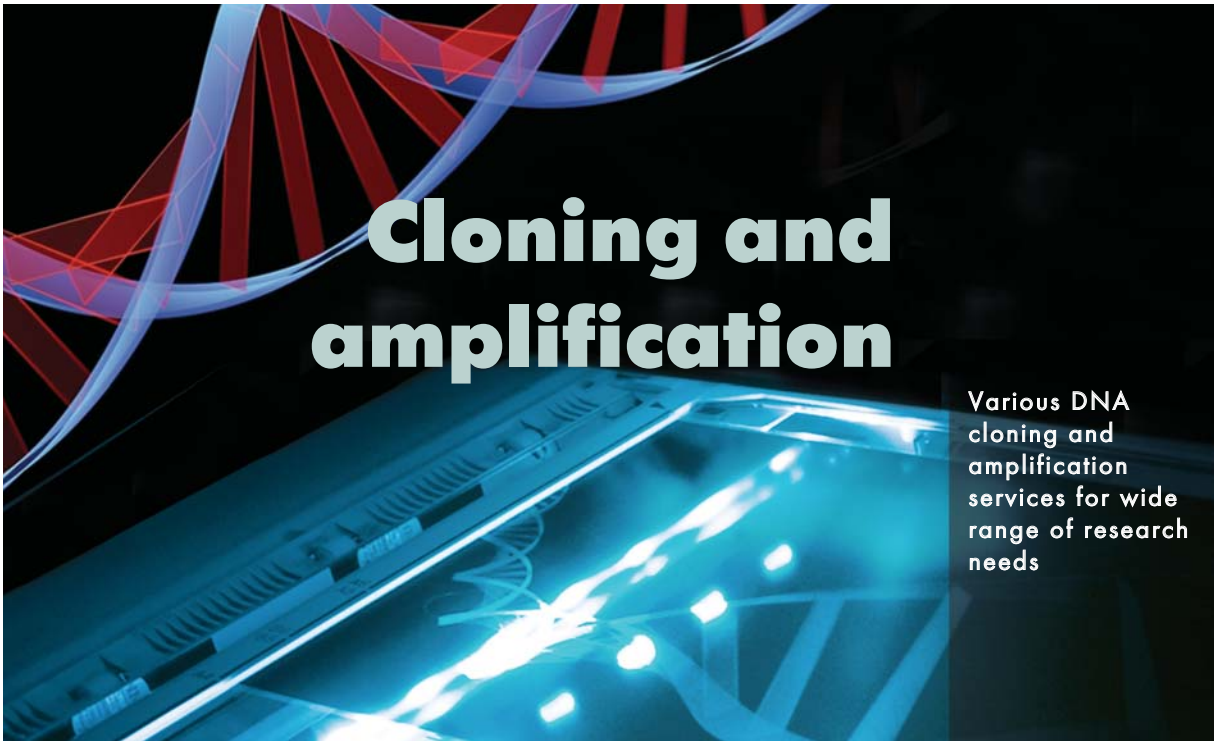
Please contact us via e-mail (evrogen@evrogen.com) to discuss your particular project.

Service	Cat.#	Price
Eukaryotic genome subtraction	CS023	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

Related services
- cDNA subtraction — see page F-9
- Bacterial genome subtraction — see page F-12
- Genome walking — see page F-20

References
1. Rebrikov *et al.* (2002) Mol Biol (Russ). 36(6): 1002-1011.
2. Rebrikov *et al.* (2002) BMC Genomics. 3(1): 15.



Cloning and amplification

Various DNA cloning and amplification services for wide range of research needs

Services available:

PCR and RT-PCR cloning

PCR amplification and cloning of target sequences

Full-length cDNA isolation (RACE)

Isolation of full-length cDNAs when only partial nucleotide or amino acid sequence is known

Subcloning

Cloning of insert of your interest into a bacterial vector of your choice

Genome walking

Rapid cloning of promoters and other upstream regulatory elements of selected genes

PCR and RT-PCR cloning service

- Fast cloning of fragments of interest for various applications: array preparation, Southern blot, Northern blot, etc.

The service includes PCR amplification of target sequences from your DNA/cDNA source, cloning of PCR product(s) into a vector of your choice and purification of plasmid DNA with target inserts. The resulting cloned products are confirmed by direct sequencing.

Service terms and conditions

You provide:

Vector and vector map

Sequence information

Starting material (cDNA, RNA, genomic DNA, plasmid DNA, etc.)

see "How to send samples to Evrogen" section for details (page G-4)

We perform:

1. Synthesis of primers designed for PCR amplification
2. cDNA synthesis and amplification (if required)
3. Amplification of target fragment
4. Cloning of fragment into the standard pUC-based vector
5. Clone confirmation:

Level 1: Clone confirmation by sequencing in one direction. We will select the clone that contains the target insert, but will not verify the perfect coincidence between the sequence of the insert and the customer-provided sequence information.

Level 2: Sequence verification of the perfect coincidence between the sequence of the insert and the customer-provided sequence information.

6. Plasmid purification of a single correct clone (from 5—10 ml culture volume).

You will receive:

1. Any leftover starting material (on request)
2. PCR primers for amplification of a target fragment
3. Purified plasmid comprising the target fragment
4. Sequencing data of the clone selected
5. Cloning report

Service	Fragment length	Cat.#	Price per fragment Level 1	Level 2
PCR and RT-PCR Cloning		CS031		
	0.5 — 1.5 kb		500 €	1100 €
	1.5 — 2.5 kb		650 €	1600 €
	2.5 — 3.5 kb		1050 €	2350 €
	3.5 — 4.5 kb		1800 €	3600 €
	larger than 4.5 kb		agreed	agreed
Extra options			agreed	

Minimal order — 1000 €. Prices do not include shipping&handling. Prices are valid from January, 2007 and subject to change without notice. Please verify current prices at Evrogen Web site: www.evrogen.com. We offer DISCOUNTS for MULTIPLE ORDERS!

Extra options:

- Cloning of the fragment into the bacterial vector of your choice
- Large- or mid-scale plasmid preparation

Turnaround time:

Agreed

Related services

- Subcloning — see page F-17
- RACE — see page F-18
- cDNA preparation — see page F-4
- Genome walking — see page F-20
- Gene synthesis — see page F-22
- Site-directed mutagenesis — see page F-21

Confidentiality Statement:

All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Subcloning service

- Generation of various expression constructs from your initial plasmids
- Modification of a customer-supplied constructs
- Generation of constructs for use in chimeric/fusion protein production
- Flexible service network

Service terms and conditions

You provide: Insert DNA

Vector and vector map.

see "How to send samples to Evrogen" section for details (page G-4)

Alternatively, you can provide a nucleic acid sample* (total RNA, poly(A+) RNA, cDNA, or DNA) and sequence data so that we could obtain the target insert — see PCR and RT-PCR cloning service.

Our subcloning service may involve some or all of the following procedures:

Restriction digests to excise the target DNA fragment and isolation of the obtained fragment from low-melt agarose

Generating inserts by PCR

Custom designed synthetic insert generation

Modification of the DNA fragment: i.e. addition of a linker

Ligation of the fragment into an appropriate vector

Transformation into *E. coli*

Selection of a correct clone

PCR of DNA segment of known sequence

Verification of the size/orientation by restriction analysis

Small-scale plasmid purification of one correct clone (5—10 ml culture volume)

All procedures can be adjusted to meet your research needs!

You will receive:

1. Any leftover starting material (on request)
2. Purified target insert in the selected vector
3. Report on subcloning procedure

Extra options:

Sequence verification of the clone selected

Mid- or large-scale plasmid preparation.

Turnaround time:

From 4 to 6 weeks

This time is estimated based on average turnaround time for this type of orders. Actual time may depend on the complexity of starting materials and/or deliverables.

Service	Cat.#	Price
Subcloning	CS032	agreed

Minimal order — 1000 €. We offer DISCOUNTS for MULTIPLE ORDERS!

Related services

- PCR and RT-PCR cloning — see page F-16
- RACE — see page F-18
- cDNA preparation — see page F-4
- Genome walking — see page F-20
- Gene synthesis — see page F-22
- Site-directed mutagenesis — see page F-21

Confidentiality Statement:

All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Full-length cDNA isolation (RACE) service

- **Isolation of full-length cDNAs when only partial nucleotide or amino acid sequence is known**

Service description

Evrogen offers isolation of full length cDNA(s) correspondent to a known fragment using Step-Out-RACE technology [1,2]. This method allows fast isolation of 3′ and 5′ cDNA ends of the target transcript with no background noise.

The method can be successfully applied to total RNA as well as poly (A+) RNA and is applicable even if only a short nucleotide (20—30 bp) or protein sequence (8—10 aa) is available.

Service terms and conditions

You provide:

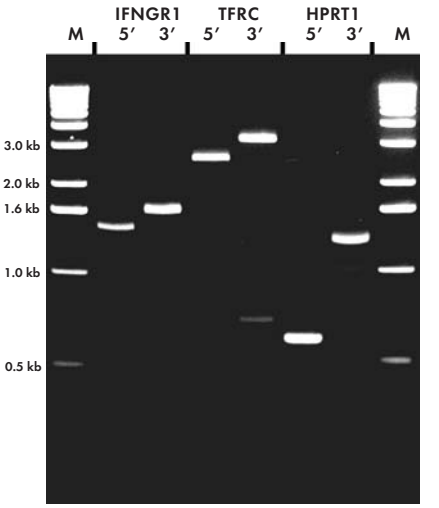
At least 1.0 μg of total RNA or poly(A+) RNA
see "How to send samples to Evrogen" section for details (page G-4)

We perform:

1. First-strand cDNA synthesis from customer-supplied poly(A+) or total RNA samples
2. Construction of oligonucleotide primers (gene-specific or degenerative) based on customer-supplied sequence of interest
3. Amplification of the target cDNA ends (3' and 5' RACE)
4. Cloning of the target cDNA ends into an appropriate vector
5. Partial sequencing of the cloned cDNA fragments
6. Construction of oligonucleotide primers to amplify the target full-length cDNA
7. Amplification of the target full-length cDNA
8. Cloning of the target full-length cDNA into an appropriate vector
9. Screening of the clones obtained to select those containing target full-length cDNA inserts
10. Purification of vector DNA with the target full-length cDNA insert

You will receive:

1. Any leftover starting materials (upon request)
2. First-strand cDNA sample (upon request)
3. SMART-amplified double-stranded cDNA
4. PCR primers for cDNA amplification
5. PCR products generated by RACE (0.5 μg, upon request)
6. Target full-length cDNA (0.5 μg, upon request)
7. Purified vector containing the target full-length cDNA insert
8. All primers designed and synthesized during RACE
9. Report on full-length cDNA isolation



5′- and 3′- Step-Out RACE results.
Amplified cDNA was prepared from human placenta total RNA and used for 5′ and 3′- RACE of several marker genes: IFNGR1 — interferon gamma receptor 1; TFRC — transferrin receptor; HPRT1 — hypoxanthine phosphoribosyltransferase-1. M — 1-kb DNA ladder.

Extra options:

- Complete sequencing of the target full-length cDNA

Turnaround time:

Agreed

Service	Cat.#	Price
Isolation of full-length cDNA	CS033	agreed

Minimal order — 1000 €. We offer DISCOUNTS for MULTIPLE ORDERS!

Related services

- PCR and RT-PCR cloning — see page F-16
- cDNA preparation — see page F-4
- Genome walking — see page F-20

References

1. Matz *et al.* (1999) Nucleic Acids Res. 27(6): 1558-1560.
2. Matz *et al.* (2003) Methods Mol Biol. 221: 41-49.

Confidentiality Statement:

All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Genome walking

- Cloning of genomic sequences flanking known DNA fragment

Service description

Evrogen provides rapid cloning of promoters and other upstream regulatory elements of selected genes using genome walking based on suppression PCR effect [1].

Service terms and conditions

You provide:

Minimum 3 µg of genomic DNA*;

Minimum 30-bp sequence of the gene of interest.

see "How to send samples to Evrogen" section for details (page G-4)

We perform:

1. Construction of oligonucleotide primers based on the supplied sequence
2. Amplification of target gene regions
3. Cloning of target DNA regions into an appropriate vector
4. Partial sequencing of cloned DNA fragments

You will receive:

1. Any leftover starting material (on request)
2. Genome walking-generated PCR products (at least 0.5 µg, upon request)
3. Purified vector DNA with the target insert
4. All primers designed and synthesized during order processing
5. Report on genome walking including all sequence data

Extra options:

Complete sequencing of the target full-length cDNA

Turnaround time:

Agreed

Service	Cat.#	Price
Genome walking	CS035	agreed

Minimal order — 1000 €. We offer DISCOUNTS for MULTIPLE ORDERS!

Related services

- PCR and RT-PCR cloning — see page F-16
- RACE — see page F-18

References

1. Siebert *et al.* (1995) Nucleic Acids Res. 23(6): 1087-1088.

Services available:

Site-directed mutagenesis

Any type of mutations, such as deletion, insertion, or substitution

Gene synthesis

Synthetic gene construction and codon usage optimization

Site-directed mutagenesis

Service description

Any type of mutations, such as deletion, insertion, or substitution, can be introduced into the gene you are working with. All procedures is conducted to the highest standards and confirmed by direct sequencing.

Service terms and conditions

You provide:

Plasmid DNA with the target gene inserted

Plasmid map

Sequence of the target gene with a list of the bases to be changed

see "How to send samples to Evrogen" section for details (page G-4)

We perform:

1. Gene mutagenesis
2. Sequence verification in both directions
3. Plasmid purification of one verified clone (5—10 ml cul-

You will receive:

1. Purified plasmid DNA containing the target sequence with verified mutations
2. Mutagenesis report

Extra options:

- Cloning of the target gene to be mutated
- Large-scale plasmid preparation

Turnaround time

Agreed

Service	Cat.#	Price per mutation
Site directed mutagenesis	CS041	
	1-3 mutations	240 €
	more than 3 mutations	220 €
Extra options		agreed

Minimal order — 480 €. Prices do not include shipping&handling. Prices are subject to change without notice. Please verify current prices at Evrogen Web site: www.evrogen.com. We offer DISCOUNTS for MULTIPLE ORDERS!

Related services

- Gene synthesis — see page F-22

Confidentiality Statement:

All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.

Gene synthesis

- Gene synthesis
- Codon usage optimization

Service description

Evrogen offers synthesis of genes 300—3 000 bp long by assembly of short oligonucleotides followed by cloning of the synthetic gene into an appropriate vector and confirmation of clone integrity by direct sequencing. In particular, Evrogen offers codon usage optimization for gene expression in various heterological systems.

Service terms and conditions

You provide:

The sequence of your interest
Vector of your choice (if required)
see "How to send samples to Evrogen" section for details (page G-4)

We perform:

1. Computer analysis of the sequence and assistance in the gene design (upon request)
2. Oligonucleotide synthesis
3. Assembly of oligonucleotides
4. Cloning of the target gene into an appropriate vector
5. Verification of the sequence by direct sequencing
6. Small-scale plasmid purification on one correct clone (5—10 ml culture volume)

Usually, cloning is performed into a vector from our collection. However, cloning of the target gene into a vector of your choice is also available under the agreement.

You will receive:

1. Purified plasmid DNA containing the target sequence
2. Detailed report

Turnaround time

From 4 to 12 weeks, partially depending on the length of

Service	Cat.#	Price
Synthetic gene construction	CS042	
ds DNA up to 2 kb length subcloned into a plasmid vector from our collection		1.50 € per bp
ds DNA more than 2 kb length		agreed

Minimal order — 500 €. Prices do not include shipping&handling. Prices are valid from September, 2006 and subject to change without notice. Please verify current prices at Evrogen Web site: www.evrogen.com. We offer DISCOUNTS for MULTIPLE ORDERS!

Related services

- Site-directed mutagenesis — see page F-21

Confidentiality Statement:

All data and materials derived from these services are the physical and intellectual property of the purchaser and will be held strictly confidential by Evrogen.