Molecular Biology Services

- 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

Contents

Overview

Evrogen offers various laboratory services to facilitate your gene discovery including

cDNA preparation and library construction services

Full-length-enriched double-stranded cDNA is prepared from poly(A)+ or total RNA. cDNA can be normalized, depleted or subtracted. Depending on your particular needs, you can obtain uncloned or cloned cDNA as well as standard, normalized, depleted or subtracted cDNA libraries.

- cDNA preparation (see page 118)
- Construction of standard cDNA libraries (see page 119)
- cDNA normalization and construction of normalized cDNA libraries (see page 120)
- cDNA depletion and construction of depleted cDNA libraries (see page 122)
- cDNA subtraction and construction of subtracted cDNA libraries (see page 124)

Genome Subtraction services

Comparison of two customer-specified genomes for the identification of unique fragments is performed by suppression subtractive hybridization (SSH). If require SSH is supplemented with mirror orientation selection.

- Bacterial genome subtraction (see page 126)
- Eukaryotic genome subtraction (see page 128)

Amplification and cloning

We provide PCR and RT-PCR cloning of known genes for array preparation and other common applications, subcloning of the target inserts into a vector of your choice, isolation of complete cDNA or genomic sequences by RACE or genome walking approaches.

- PCR cloning (see page 129)
- Subcloning (see page 130)
- RACE and full-length cDNA isolation (see page 131)
- Genome walking (see page 132)

Gene synthesis and modification

Various types of target sequence modification are available including *de-novo* gene synthesis, codon usage optimization, and introduction of any type of mutations, such as deletion, insertion, or substitution.

- Gene synthesis (see page 133)
- Site-directed mutagenesis (see page 134)

Fluorescent protein related services

We offer custom optimization of our vectors expressing fluorescent proteins in accordance with your particular needs and development of live-cell assays utilising Evrogen fluorescent technologies.

- Custom optimization of Evrogen expression vectors (see page 135)
- Custom assay development (see page 136)

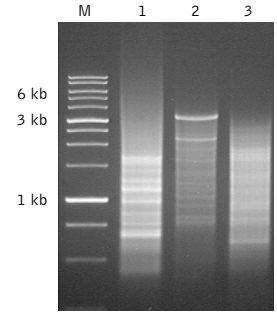
cDNA preparation service

- High-quality full-length-enriched amplified cDNA for various applications
- From limited amount of total or poly(A)+ RNA

Full-length-enriched double-stranded cDNA is produced using modified SMART technology [Matz et al. 1999; Matz 2002; Zhu et al. 2001]. 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. Depending on research purposes, cDNA can be flanked by same (for nondirectional cloning) or different (for directional cloning) adapter sequences.

The cDNA can be used for:

- Construction of representative cDNA libraries (see page 119)
- Subtractive hybridization (see page 124)
- cDNA normalization (see page 120)
- Isolation of full-length cDNAs by RACE (see page 131)
- PCR cloning / array development (see page 129)
- Virtual Northern blot



Typical result of cDNA synthesis.
1 - mosquito grub; 2 - copepod *Pontella sp.*; 3 - tomato *Lycopersicon esculentum*. M - 1 kb DNA size marker, SibEnzyme, Russia.

Service terms and conditions

Cat.#	Service	Turnaround time
CS030	cDNA preparation	3-4 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. At least 1.0 μg of total RNA or poly(A)+ RNA
   See "How to send samples to Evrogen" section for details (page 140)

You will receive:

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

REFERENCES

Matz (2002). Methods Mol Biol, 183: 3–18 / pmid: 12136765

Matz et al. (1999). Nucleic Acids Res, 27 (6): 1558–1560 / pmid: 10037822

Zhu et al. (2001). Biotechniques, 30 (4): 892–897 / pmid: 11314272

Standard cDNA library construction service

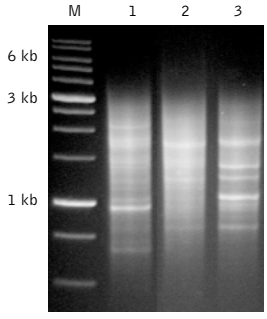
- *E. coli* libraries from high-quality full-length-enriched amplified cDNA
- From limited amount of total or poly(A)+ RNA

Full-length-enriched cDNA is usually produced using SMART technology [Zhu et al. 2001; Matz 2002]. The method combines cDNA synthesis and amplification and results in a representative cDNA population enriched with full-length sequences, even from small amounts of starting materials. After cDNA synthesis, the double stranded cDNA is size fractionated, directionally cloned into a plasmid vector from our collection using SfiIA - SfiIB, SrfI - NotI, or AscI - NotI restriction sites, and transformed into *E. coli*.

Then, the library cDNA is amplified as follows: the resulting library is arrayed in five 25x25 cm plates (approximately 10^5 colonies per plate); clones are eluted by LB/Amp medium.

As a quality control measure, a percentage of recombinant clones and average insert size is determined by gel analysis of 33 clones picked at random.

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



ds cDNA synthetized on the basis of total RNA from different human tissues.
Agarose/EtBr gel-electrophoresis result: M - 1-kb DNA size markers (SibEnzyme); lane 1 - embryonic lung; lane 2 - testis; lane 3 - small intestine.

Service terms and conditions

Cat.#	Service	Turnaround time
CS040	Standard cDNA library construction	4-6 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. At least 2.0 μg of total RNA or poly(A)+ RNA
   See "How to send samples to Evrogen" section for details (page 140)

You will receive:

1. Any leftover starting material (on request)
2. Amplified double-stranded cDNA (at least 1 μg)
3. PCR primers for cDNA amplification
4. Amplified and characterized cDNA library (the library will contain at least 750 000 independent clones)
5. Service report

Extra options:
Modification of the customer-supplied vector for cloning using retriction sites described above.

REFERENCES

Matz (2002). Methods Mol Biol, 183: 3–18 / pmid: 12136765

Zhu et al. (2001). Biotechniques, 30 (4): 892–897 / pmid: 11314272

cDNA normalization service

- Significant increase of the transcriptome sequencing efficacy
- Equalization of cDNA population prior to library cloning
- Normalized cDNA is suitable for 454 sequencing
- Order processing is set up and monitored by the inventors of the technology

A great range in representation of different mRNAs in cells and tissues makes it extremely difficult to analyze rare message in cDNA libraries. Therefore, for rare transcript searching and analysis, creation of equalized (normalized) cDNA is required.

Our proprietary duplex-specific nuclease (DSN)-based normalization is a highly efficient and well-proved approach to equalize transcript concentration in a cDNA population enriched with full-length sequences [Zhulidov et al. 2004; Zhulidov et al. 2005; Bogdanova et al. 2008].

Both total or poly(A)+ RNA is suitable for cDNA synthesis. Full-length-enriched cDNA is usually produced using SMART technology [Zhu et al. 2001; Matz 2002]. After cDNA synthesis, the double-stranded cDNA is normalized using DSN-normalization method.

Depending on your particular needs, normalizad cDNA can be used for 454 sequencing (preparation of normalized cDNA over 2,0 μg is available as an extra option) or construction of normalized full-length-enriched cDNA library. Please use level descriptions at page 121 to choose the service variant you desire.

High-throughput sequencing of the normalized cDNA can be ordered at Macrogen, Korea (http://www.macrogen.com).

Service terms and conditions

You provide:

1. At least 1.0 μg of total RNA or poly(A)+ RNA
   See "How to send samples to Evrogen" section for details (page 140)

We perform:

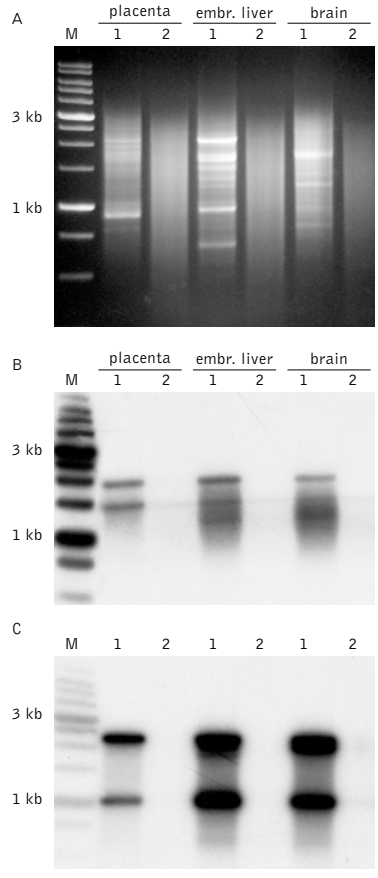
See description of available service levels on page 121

You will receive:

See list of output materials for each service level at page 121

Extra options:

1. Normalization efficiency analysis by PCR or Virtual Northern blot
2. Preparation of additional normalized cDNA up to 5-15 μg



cDNA normalization result.
(A) Agarose/EtBr gel-electrophoresis of non-normalized (1) and normalized (2) human cDNA samples;
(B, C) Concentration of abundant transcripts in these samples revealed by Virtual Northern blot. (B) - β-actin; (C) - ubiquitin C;
M - 1-kb DNA size markers (SibEnzyme); embr. - embryonic.

REFERENCES

Bogdanova et al. (2008). Mol Biosyst, 4 (3): 205–212 / pmid: 18437263

Matz (2002). Methods Mol Biol, 183: 3–18 / pmid: 12136765

Zhu et al. (2001). Biotechniques, 30 (4): 892–897 / pmid: 11314272

Zhulidov et al. (2004). Nucleic Acids Res, 32 (3): e37 / pmid: 14973331

Zhulidov et al. (2005). Bioorg Khim., 31 (2): 186–194 / pmid: 15889793

Level description for cDNA normalization service:

Procedures/Cat.#	Level 1a CS010	Level 1b CS011-1	Level 2a CS011-2a	Level 2b CS011-2b	Level 2c CS011-2c	Level 3a CS011-3a	Level 3b CS011-3b
1a. Synthesis of ds cDNA suitable for further non-directional cloning	+	–	–	–	–	–	–
1b. Synthesis of ds cDNA suitable for further directional cloning	–	+	+	+	+	+	+
2. DSN-normalization of cDNA	+	+	+	+	+	+	+
3. Ligation of normalized cDNA into an appropriate vector*	–	–	+	+	+	+	+
4. PCR quality control of 22 randomly picked clones	–	–	+	+	+	+	+
5a. Preparation of non-amplified normalized cDNA library containing at least 100 000 independent clones	–	–	–	+	–	+	–
5b. Preparation of amplified normalized cDNA library containing at least 400 000 independent clones**	–	–	–	–	+	–	+
6. Purification of plasmid DNA from the 90 clones	–	–	–	–	–	+	+
7. Single run sequencing analysis of these clones	–	–	–	–	–	+	+
Turnaround time, weeks	4-6	4-6	4-6	5-7	5-7	6-8	6-8
Output materials:							
1. Amplified non-normalized cDNA (at least 1 μg)	+	+	+	+	+	+	+
2. Normalized cDNA (at least 2 μg)	+	+	+	+	+	+	+
3. PCR primers for cDNA amplification	+	+	+	+	+	+	+
4. Service report	+	+	+	+	+	+	+
5. Normalized cDNA ligated into a vector	–	–	+	–	–	–	–
6. Non-amplified normalized cDNA library (at least 100 000 independent clones)	–	–	–	+	–	+	–
7. Amplified normalized cDNA library (at least 400 000 independent clones)	–	–	–	–	+	–	+
8. Purified plasmid DNA from the 90 clones and insert sequencing data of these 90 clones	–	–	–	–	–	+	+

We offer DISCOUNTS for MULTIPLE ORDERS!

NOTES:

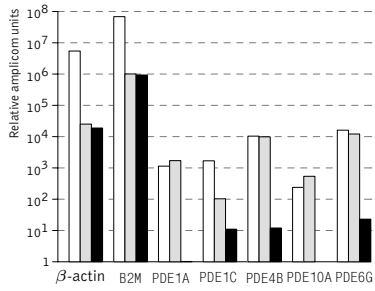
*Library cloning is performed into a vector from our collection using SfiIA - SfiIB, SrfI - NotI, or AscI - NotI restriction sites. Cloning using other sites or into a customer-provided vector can be performed by agreement.

**To prepare an amplified cDNA library, the non-amplified library is arrayed in five 25x25 cm plates (approximately 10^5 colonies per plate); clones are eluted by LB/Amp medium; then the library is supplied with 17% glycerol and stored at -70°C. Preparation of the normalized amplified cDNA library containing more than 400 000 independent clones can be performed by agreement.

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 and library construction service

- Specific removal of already analyzed transcripts from cDNA populations
- Preparation of depleted cDNA libraries for functional screenings
- Order processing is set up and monitored by the inventors of the technology



Evrogen offers a highly efficient technology for 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 by expression cloning.

Full-length-enriched cDNA is usually produced using SMART technology [Zhu et al. 2001; Matz 2002]. 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. After cDNA synthesis, the double stranded cDNA is depleted using a duplex-specific nuclease (DSN)-based method [Bogdanova et al. 2009]. The method allows specific removal of selected transcripts without loss of average cDNA size.

Depletion is accompanied by partial cDNA normalization. The depleted cDNA is size fractionated, directionally cloned into a plasmid vector from our collection (or into an appropriate customer-supplied plasmid vector), and used for preparation of depleted cDNA library.

Service terms and conditions

Cat.#	Option	Turnaround time
CS012-1	Level 1	5-7 weeks
CS012-2	Level 2	6-8 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. At least 1.0 μg of of total RNA or poly(A)+ RNA
2. List of the genes to be removed; up to 40 genes (sequences or GenBank IDs are required)

See "How to send samples to Evrogen" section for details (page 140)

We perform:

Level 1

1. Ds cDNA preparation using SMART approach
2. cDNA depletion
3. PCR confirmation of the depletion

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

4. Ligation of the depleted cDNA into an appropriate vector (cDNA is directionally cloned using SfiIA - SfiIB, SrfI - NotI, or AscI - NotI restriction sites)

REFERENCES

Bogdanova et al. (2009). Mol Biotechnol, 41 (3): 247–253 / pmid: 19127453

Matz (2002). Methods Mol Biol, 183: 3–18 / pmid: 12136765

Zhu et al. (2001). Biotechniques, 30 (4): 892–897 / pmid: 11314272

5. Transformation of the ligate into *E. coli* and preparation of amplified depleted cDNA library. For library amplification, it is arrayed in five 25x25 cm plates (approximately 10^5 colonies per plate); clones are eluted by LB/Amp medium; and the library is supplied with 17% glycerol and stored at -70°C

You will receive:

1. Any leftover starting material (on request)
2. Amplified double-stranded cDNA (at least 1 μg)
3. Depleted cDNA (at least 2 μg)
4. PCR primers for cDNA amplification
5. Amplified and characterized *E.coli* library (only for Level 2)
6. Service report

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 and library construction service

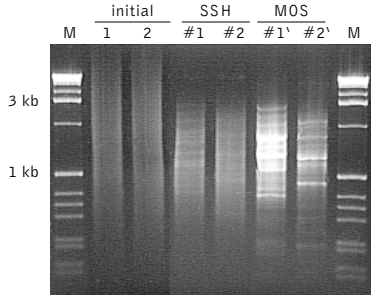
- Isolation of differentially expressed genes
- Special approach to eliminate false-positive clones
- Order processing is set up and monitored by the inventors of the SSH technology

cDNA populations are compared using well-known Supression Subtractive Hybridization (SSH) and Mirror Orientation Selection (MOS) technologies [Lukyanov et al. 1994; Diatchenko et al. 1996; Gurskaya et al. 1996; Diatchenko et al. 1999; Rebrikov et al. 2000].
SSH has been used in hundreds of model systems and is now one of the most popular molecular genetic technologies in the world that appropriate for comparison of RNA populations. MOS substantially improves the results of SSH (especially for cDNA samples of high complexity) by significant decrease in the number of false-positive clones and increase in the number of differential clones in SSH-generated libraries.

MOS is specially recommended:

- for complicated samples (e.g. brain RNA, eukaryotic genomic DNA);
- for closely related 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 service. The levels differ in the intensity of work performed and may be finely tuned to exactly suit your research project. All procedures are set up and monitored by the inventors of the SSH and MOS tecnologies.



Typical results of cDNA subtraction and MOS procedure.
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. M - 1-kb DNA size markers.

Service terms and conditions

Cat.#	Level	Turnaround time
CS021-1	Level 1	3-5 weeks
CS021-2	Level 2	4-6 weeks
CS021-3	Level 3	5-7 weeks
CS021-4	Level 4	5-11 weeks
CS021-5	Level 5	5-11 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. At least 1.0 μg of of total RNA or poly(A)+ RNA
See "How to send samples to Evrogen" section for details (page 140)

We perform:

Level 1

1. cDNA preparation from customer-supplied RNA
2. Subtraction in both directions (forward = A-B; reverse = B-A)

2a. 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

Level 3, 4 and 5 (also includes all services provided in level 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. Description of the libraries obtained (number of clones and percentage of clones with insertions)
9. Differential screening data
10. Plates with clones used for differential screening
11. Purified plasmid DNA from the differential clones
12. Virtual Northern hybridization data

REFERENCES

Diatchenko et al. (1996). Proc Natl Acad Sci U S A, 93 (12): 6025–6030 / pmid: 8650213

Diatchenko et al. (1999). Methods Enzymol, 303: 349–380 / pmid: 10349654

Gurskaya et al. (1996). Anal Biochem, 240 (1): 90–97 / pmid: 8811883

Lukyanov et al. (1994). Bioorg Khim, 20 (6): 701–704 / pmid: 7945464

Rebrikov et al. (2000). Nucleic Acids Res, 28 (20): e90 / pmid: 11024192

Bacterial genome subtraction service

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

Supression subtractive hybridization (SSH) is the most powerful non-sequencing approach to find genetic difference between bacterial strains [Akopyants et al. 1998]. The ability of SSH to identify nearly all major sequence differences between two closely related bacteria has been demonstrated by several independent research groups [Agron et al. 2002; Rebrikov et al. 2002; Rebrikov et al. 2003; Winstanley 2002].
Depending on your particular needs, we offer five levels of custom service. The levels differ in the intensity of work performed and may be finely tuned to exactly suit your research needs. All procedures are set up and monitored by the inventors of the SSH technique.

Service terms and conditions

Cat.#	Level	Turnaround time
CS022-1	Level 1	3-5 weeks
CS022-2	Level 2	4-6 weeks
CS022-3	Level 3	5-7 weeks
CS022-4	Level 4	5-11 weeks
CS022-5	Level 5	5-11 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. 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 140)

We perform:

Level 1

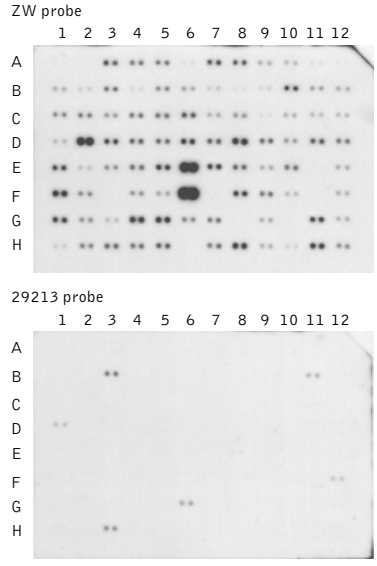
1. Subtraction in both directions (forward = A-B; reverse = B-A)

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

2. Ligation of subtracted DNA into an appropriate vector

Level 3, 4 and 5 (also includes services provided in level 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



Typical result of differential screening for bacterial SSH-generated libraries.
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 (A and B, respectively) showed that this library contains about 95% differential clones.

REFERENCES

Agron et al. (2002). FEMS Microbiol Lett, 211 (2): 175–182 / pmid: 12076809

Akopyants et al. (1998). Proc Natl Acad Sci U S A, 95 (22): 13108–13113 / pmid: 9789049

Rebrikov et al. (2002). Ann Periodontol, 7 (1): 17–28 / pmid: 16013213

Rebrikov et al. (2003). "SSH Subtractive Cloning: Comprehensive Survey Of Genome Differences In Prokaryotes." In: *Applications of Genomics and Proteomics for Analysis of Bacterial Biological Warfare Agents.* Ed. by DelVecchio, V.G. and Krcmery, V. IOS Press, Amsterdam , Netherlands. 352:95–105.

Winstanley (2002). J Med Microbiol, 51 (6): 459–467 / pmid: 12018652

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)

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

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.

Eukaryotic genome subtraction service

- Identification of differentially distributed sequences in eukaryotic genomes

Combination of Suppression Subtractive Hybridization (SSH) with Mirror Orientation Selection (MOS) allows comparison of similar eukaryotic genomes to identify genetic difference between them [Rebrikov et al. 2002].
Terms, turnaround time, and prices for eukaryotic genome subtraction service are by agreement. Please contact us via email service@evrogen.com to discuss your particular project.

Service terms and conditions

Cat.#	Option	Turnaround time
CS023	Eukaryotic genome subtraction	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

REFERENCES

Rebrikov et al. (2002). BMC Genomics, 3 (1): 15 / pmid: 12065025

PCR cloning service

- Fast cloning of DNA fragments for various applications

Evrogen offers large-scale PCR and RT-PCR cloning of known genes for array preparation and other common applications. The service includes PCR amplification of target sequences from your DNA/RNA source, cloning of PCR product(s) into the vector of your choice, and purification of the plasmid DNA with target inserts. The resulting cloned products are then confirmed by direct sequencing.

Service terms and conditions

Cat.#	Option	Turnaround time
CS031-1	Level 1	agreed
CS031-2	Level 2	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. Starting materials (cDNA, RNA, genomic DNA, or plasmid DNA)
2. Vector and vector map
3. Sequence information

See "How to send samples to Evrogen" section for details (page 140)

We perform:

1. Synthesis of primers designed for PCR amplification
2. cDNA preparation (if required)
3. Amplification of the target fragment
4. Cloning of fragment into the standard pUC-based vector from our collection
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 specific to the target fragment
3. Purified plasmid DNA comprising the target fragment
4. Sequencing data of the clone selected
5. Service report

Extra options:

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

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

Evrogen offers the service to produce various expression constructs from your initial plasmid, modify an existing construct, and/or generate constructs for use in chimeric/fusion protein production. All of these procedures can be adjusted to meet your specific research needs. All products are confirmed by direct sequencing.

Service terms and conditions

Cat.#	Option	Turnaround time
CS032	Subcloning	4-6 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. Insert DNA or sequence information
2. Vector and vector map

See "How to send samples to Evrogen" section for details (page 140)

We perform:

1. Isolation of the target DNA fragment: depending on your needs, we can perform (1) restriction digests to excise the target DNA fragment and isolation of the obtained fragment from low-melt agarose; (2) generation of inserts by PCR; or (3) custom DNA synthesis
2. Modification of the target DNA fragment: construction of fusions, introduction of mutations desired, etc.
3. Ligation of the fragment into an appropriate vector
4. Transformation of the ligate from step 3 into *E. coli*
5. Selection of a correct clone and clone confirmation using restriction analysis or direct sequencing
6. Plasmid purification of one correct clone from 5-10 ml culture volume

You will receive:

1. Any leftover starting material (on request)
2. Purified target insert in the selected vector
3. Service report

Extra options:

1. Mid- or large-scale plasmid preparation

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.

RACE and full-length cDNA isolation service

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

Evrogen offers isolation of full length cDNA(s) correspondent to a known fragment using Step-Out-RACE technology [Matz et al. 1999; Matz et al. 2003].
This method allows fast isolation of the 5‘- and 3‘-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 (30-50 bp) or protein sequence (15-30 aa) is available.

Service terms and conditions

Cat.#	Option	Turnaround time
CS033	RACE and full-length cDNA isolation	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. At least 1.0 μg of of total RNA or poly(A)+ RNA
2. Sequence information

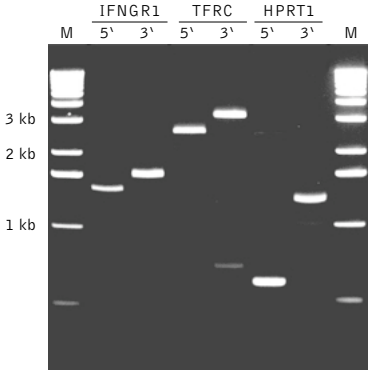
See "How to send samples to Evrogen" (page 140)

We perform:

1. cDNA preparation
2. Construction of oligonucleotide primers on the customer-supplied sequence of interest
3. Amplification of the target cDNA ends (5′- and 3′-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 and amplification of the target full-length cDNA
7. Cloning of the target full-length cDNA into an appropriate vector and screening of the clones to select those containing target full-length cDNA inserts
8. Purification of vector DNA with the target full-length cDNA insert

You will receive:

1. Any leftover starting material (on request)
2. First strand cDNA (on request)
3. Amplified double-stranded cDNA (at least 1 μg)
4. PCR primers for cDNA amplification
5. PCR products generated by RACE (0.5 μg each, upon request)
6. Purified vector containing the target cDNA insert
7. All primers designed and synthesized during RACE
8. Service report



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 size markers.

Extra options:

1. Complete sequencing of the target DNA

REFERENCES

Matz et al. (1999). Nucleic Acids Res, 27 (6): 1558–1560 / pmid: 10037822

Matz et al. (2003). Methods Mol Biol, 221: 41–49 / pmid: 12703732

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 service

- Cloning of genomic sequences flanking known DNA fragment

Evrogen provides rapid cloning of promoters and other upstream regulatory elements of target genes using suppression PCR-based genome walking method.

Service terms and conditions

Cat.#	Option	Turnaround time
CS035	Genome walking	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. Minimum 50-bp sequence of the gene of interest
2. Minimum 3.0 μg of of genomic DNA

See "How to send samples to Evrogen" section for details (page 140)

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 each, on request)
3. Purified vector DNA with the target insert
4. All primers designed and synthesized during order processing
5. Service report

Extra options:

1. Complete sequencing of the target DNA

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 service

- Gene synthesis
- Codon usage optimization

Evrogen offers synthesis of DNA fragments 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

Cat.#	Fragment length	Turnaround time
CS042-1	up to 2 kb	4-7 weeks
CS042-2	more than 2 kb	6-12 weeks

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. Sequence information
2. Vector and vector map (if required)

See "How to send samples to Evrogen" section for details (page 140)

We perform:

1. Oligonucleotide synthesis and assembly of the gene
2. Cloning of the target gene into an appropriate vector from our collection (cloning of the target gene into a vector of your choice is also available under the agreement)
3. Verification of the sequence by direct sequencing
4. Small-scale plasmid purification on one correct clone (5-10 ml culture volume)

You will receive:

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

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.

Site-directed mutagenesis service

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

Service terms and conditions

Cat.#	Option	Turnaround time
CS041-1	1-3 mutations	agreed
CS041-2	more than 3 mutations	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

You provide:

1. Plasmid DNA with the target gene inserted
2. Plasmid map
3. 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 140)

We perform:

1. Gene mutagenesis
2. Sequence verification by sequencing in both directions
3. Plasmid purification of one verified clone from 5-10 ml culture

You will receive:

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

Extra options:

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

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.

Custom optimization of Evrogen expression vectors

Cat.#	Option	Turnaround time
FPS00	Custom optimization of Evrogen expression vectors	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

Optimization of Evrogen vectors expressing fluorescent proteins can be performed in accordance with your particular needs.
Terms, turnaround time, and prices for the service are by agreement.
Please contact us via email service@evrogen.com to discuss your particular project.

We offer:

1. Generation of fusions of proteins, domains or localization signals of your interest to fluorescent proteins using mammalian vectors from our collection
2. Generation of constructs for gene expression analysis using promoterless vectors encoding fluorescent proteins from our collection
3. Codon usage optimization of fluorescent proteins from our collection for expression in heterological system of your interest
4. Cloning of fluorescent protein coding sequences into specific vectors of your interest

Notice to Purchaser:
Custom-modified Evrogen vectors comprising fluorescent proteins (FP) shall be used by purchaser for research purposes only in accordance with Limited Use Label License.
Evrogen retains non-exclusive right to use the Custom-modified Evrogen FP vectors for research and commercial purposes.
Customer information of confidential nature which may be necessary for the service execution will be treated confidential and not used for other purposes by Evrogen without Customer approval.

Custom assay development

Cat.#	Option	Turnaround time
AD001	Custom assay development	agreed

We offer DISCOUNTS for MULTIPLE ORDERS!

Fluorescent proteins (FPs) act as endogenously expressed biolabels for fluorescence microscopy that makes them particularly suitable for use in various HTS/HCS-type, live-cell, drug-discovery screening assays. Red and far-red FPs, which are easily distinguished from cellular autofluorescence and from the typical fluorescence wavelength spectrum of many medicinal chemistry compounds, provide technologically useful possibilities for the design of sophisticated, novel assay formats for drug discovery.
Selection and evaluation of an appropriate FP marker along with optimization of assay parameters is a laborious and time consuming procedure.

We offer the following custom research services to assist with FP selection/assay development:

1. Making an agreed number of different constructs of interest (FP–linker–target protein)
2. Testing the constructs in an number of model cell lines to find the best ones
3. Testing the selected constructs in various cell lines to select the most appropriate cells in terms of fusion localization/redistribution
4. Making stably transfected cell lines based on the testing results above

For customers, who wish to multiplex HCS readout to achieve real multiparameter imaging, we offer the unique option to fuse protein of interest with bright monomeric red (TagRFP) and far-red (mKate2) fluorescent proteins that could be used in combination with dyes/FPs visible in UV, blue and green part of spectrum. Both proteins are perfectly fit for fusions.

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.