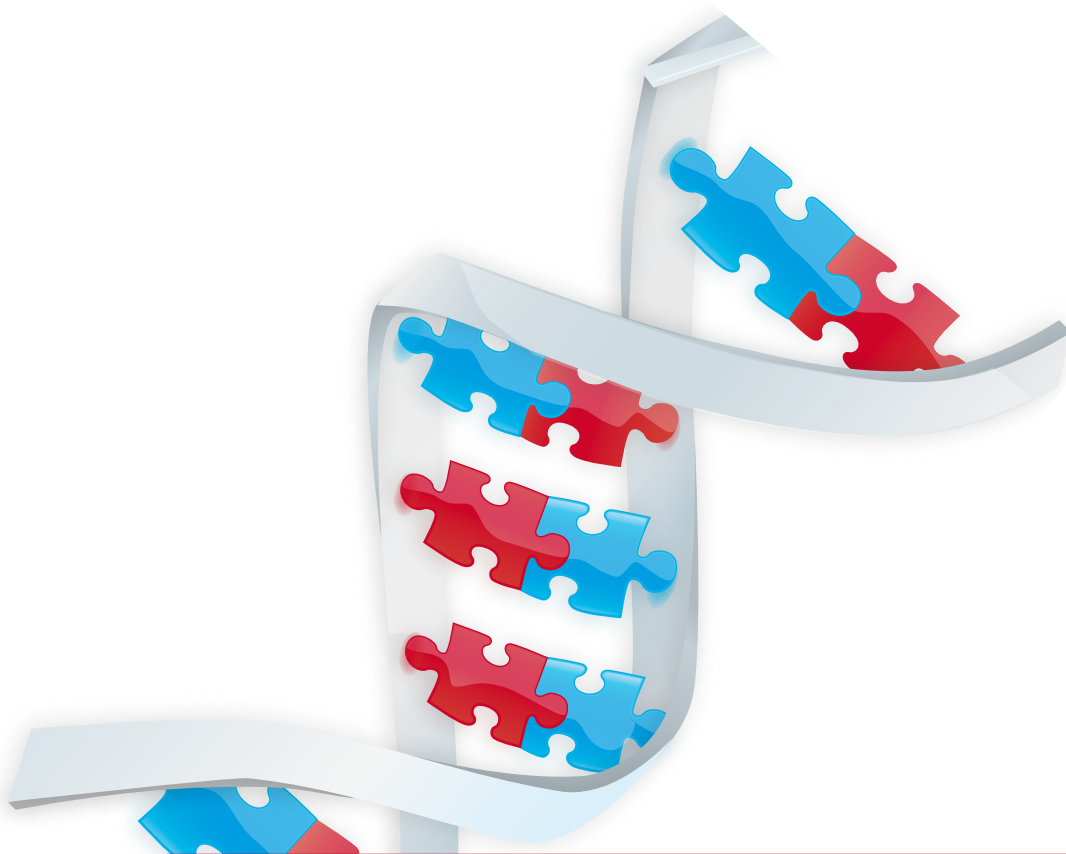


cDNA library construction



We offer you

- Customized cDNA library construction services
- EasyClone cDNA library construction kits
- cDNA normalization
- Collection of cDNA libraries for yeast two-hybrid screening

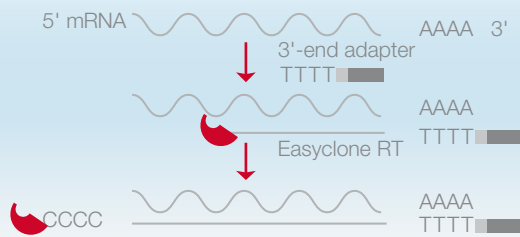
cDNA library construction

Applications

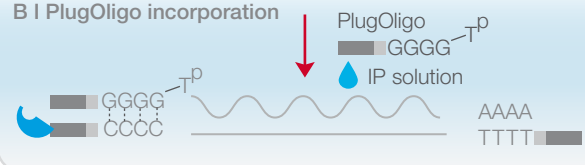
- cDNA libraries for yeast two-hybrid screening
- Custom cDNA library construction in a vector of your choice
- Available as custom services and kits

Schematic outline of the cDNA synthesis workflow

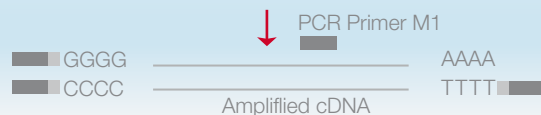
A | First strand cDNA synthesis



B | PlugOligo incorporation



C | ds cDNA preparation by PCR



A | First strand cDNA synthesis

During first strand cDNA synthesis the EasyClone reverse transcriptase (RT) starts synthesis at the Oligo(dT) adapter and ends by adding several non-template nucleotides to the 3' end of the newly synthesized strand.

B | PlugOligo incorporation

The PlugOligo (second adapter) base-pairs with the newly synthesized oligo(dC) stretch, leading to the incorporation of the PlugOligo sequence at the 3' end of the cDNA strand by RT.

C | ds cDNA preparation by PCR

In the final step, EasyClone polymerase and special PCR primers are used to synthesize full length cDNA flanked by PlugOligo and 3'-end adapter sequences.

Normalized cDNA libraries

Benefits

- Normalization removes abundant transcripts
- Decreased rate of false positives in yeast-based screenings
- Increased chance of identifying rare interactors in yeast-based screenings

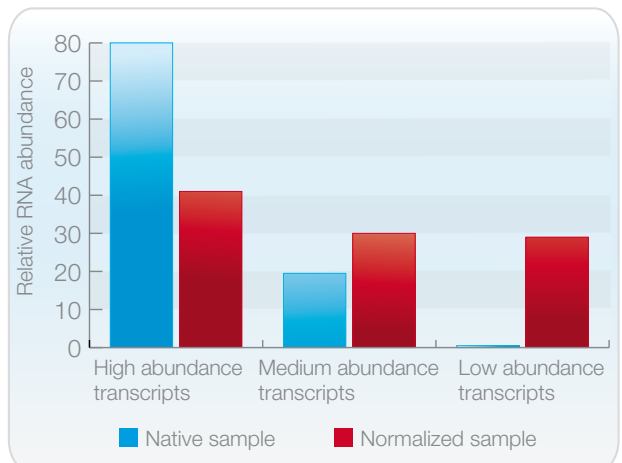
Normalization process

A double stranded cDNA sample is normalized based on differential re-association kinetics after denaturation, since abundant transcripts re-associate faster than rare ones. Transcripts are denatured and then re-annealed under carefully controlled conditions. The reannealing process is stopped after a short time, when the majority of abundant transcripts have reformed into double stranded cDNA, whereas rare transcripts are still single stranded.

Subsequently, double stranded cDNA is removed by digestion, the remaining transcripts are denatured again and annealed to double stranded cDNA. The resulting cDNA population is normalized, i.e. the majority of abundant transcripts has been removed.

A few abundant transcripts dominate RNA populations

Typical eukaryotic mRNA populations consist of three abundance classes:





**Now available:
cDNA normalization service**

■ cDNA library construction services

Benefits

- RNA quality control by Experion chip and *in silico* gel electrophoresis
- High representation of 5' ends
- Guaranteed coverage of tissue complexity
- Large average insert sizes
- Constructed in a vector of your choice
- Normalization of cDNA available

Specifications and deliverables

- Average insert sizes > 1.4 kb
- High complexities (1×10^6 – 5×10^7 independent clones)
- Cloned into DUALhybrid (yeast two-hybrid), DUALmembrane / DUALhunter library vectors or a vector of your choice
- Average turnaround time 2 – 3 months
- You receive primary glycerol stocks and 500 µg of purified plasmid DNA

Requirements

- 5 µg total or polyA+ RNA
- Sufficient tissue for preparation of 5 µg polyA+ RNA
- 5 aliquots with 5×10^7 cells

Ordering information

Order number	Service
S03002	Custom standard cDNA library construction
S03012	Custom normalized cDNA library construction
S03000	Total RNA isolation from cells or tissue
S03001	Poly(A)+ RNA isolation from total RNA
S03004	Vector modification for cDNA library construction

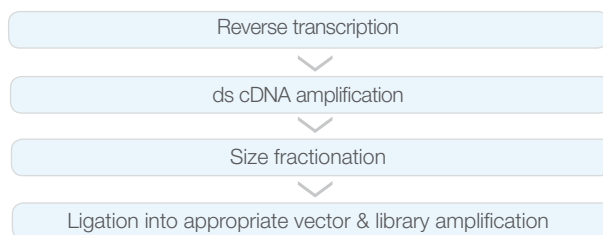


**Kit for construction of
normalized cDNA libraries!**

■ EasyClone cDNA library construction kits

Benefits

- Fast and easy synthesis of highly complex, representational cDNAs
- High representation of full length transcripts
- Directional cloning of cDNAs into a vector of your choice
- Only small starting amounts of RNA required
- Sufficient reagents to construct 10 libraries



EasyClone cDNA library construction kit

EasyClone cDNA synthesis is based on a novel technology to create full length enriched double stranded cDNA from total or polyA+ RNA samples.

No extensive know-how in library construction is needed - the kit supplies all reagents for library construction and the detailed manual covers all steps of library construction.

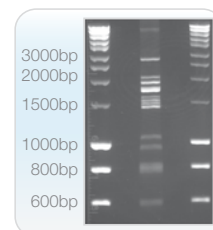
EasyClone normalized cDNA library construction package

- Quick and easy construction of normalized cDNA libraries

Normalization is the process of removing abundant RNAs from the starting material. Normalized cDNA libraries contain approximately equal amounts of all RNAs and therefore increase the occurrence of rare proteins. This significantly increases the quality of yeast two-hybrid screens.

EasyClone PCR kit

The EasyClone PCR kit contains an optimized mixture of proof-reading polymerases to ensure highly accurate, representational PCR's and preferred amplification of large fragments from complex cDNA mixtures.



15 different templates, ranging in size from 0.6 kb to 3 kb, were amplified in one multiplex reaction using the EasyClone PCR kit. 5 µl of the reaction was loaded onto a 1% agarose gel, flanked by size markers.

Ordering information

Order number	Product	Size
P01010	EasyClone cDNA library construction kit	20 reactions
P01011	EasyClone normalized cDNA library construction package	20 reactions
P01012	EasyClone PCR kit	100 reactions

Interested?

Phone +41 44 738 50 00 | www.dualsystems.com/libraries


Our cDNA library collection

We supply a wide range of premade cDNA libraries for use with our yeast-based screening systems. All cDNA libraries are delivered as purified plasmid DNA, ready for screening.

Libraries for the DUALhybrid (yeast two-hybrid) system

cDNAs are cloned into pGAD-HA or pGAD-DS (N-terminal GAL4-fusion)



Order number	Product Size: 200 µg	Order number	Product Size: 200 µg
Mouse tissue			
P02101	11d total embryo	P02401	Normalized adult brain
P02107	Adult heart		
Human tissue			
P02102	Colon	P02402	Normalized fetal brain
Human cell line			
P02104	LNCaP human prostate cancer cells	P02106	Hepatocellular carcinoma cells
P02103	HeLa cell line		

Order number	Product Size: 200 µg	Order number	Product Size: 200 µg
Normalized 			
P02401	Normalized adult mouse brain	P02402	Normalized human fetal brain
Model organisms			
P02105	<i>C. elegans</i> whole adult	P02109	<i>A. thaliana</i>
P02108	<i>S. cerevisiae</i>		

Libraries for the DUALmembrane and the DUALhunter system

cDNAs are cloned into pPR3-N (N-terminal NubG-fusion) or pPR3-C (C-terminal NubG-fusion)

Order number	Product Size: 200 µg	Order number	Product Size: 200 µg
Human tissue NubG-x			
P02213	Embryonal brain	P02226	Adult kidney
P02215	Adult colon	P02230	Adult lung
P02220	Adult liver	P02302	Normalized spleen
P02221	Adult brain	P02304	Normalized fetal brain
Human tissue x-NubG			
P02208	Adult kidney	P02227	Adult brain
Human cell lines NubG-x			
P02205	Jurkat T, unst.	P02228	LNCaP
P02212	HeLa	P02232	Mammary epithelial
Plant NubG-x			
P02236	Medicago nodules	P02210	<i>A. thaliana</i>
Plant x-NubG			
P02235	Medicago nodules		
Rat cells NubG-x			
P02233	Neonatal cardiomyocytes		

Order number	Product Size: 200 µg	Order number	Product Size: 200 µg
Mouse tissue NubG-x			
P02201	Adult whole brain	P02234	Whole embryo, 11d
P02207	Adult heart	P02303	Normalized brain
P02231	Adult kidney		
Mouse tissue x-NubG			
P02206	Adult heart	P02301	Normalized whole embryo, 17d
P02224	Adult spleen		
Model organisms NubG-x			
P02210	<i>A. thaliana</i>	P02237	<i>S. cerevisiae</i>
P02218	<i>C. elegans</i> adult	P02222	<i>C. elegans</i> eggs
P02223	<i>D. melanogaster</i> whole embryo (16h)	P02229	<i>D. melanogaster</i> whole adult
Normalized NubG-x 			
P02302	Human spleen	P02303	Mouse brain
P02304	Human fetal brain		
Normalized NubG-x 			
P02301	Mouse whole embryo, 17d		

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