cDNA Synthesis

VERTIS has developed an innovative portfolio of robust techniques to synthesize cDNA in highest quality from the different RNA species - from coding and non-coding RNA - from eukaryotic and from prokaryotic organisms - from the whole range of tissues, organisms and environmental samples.

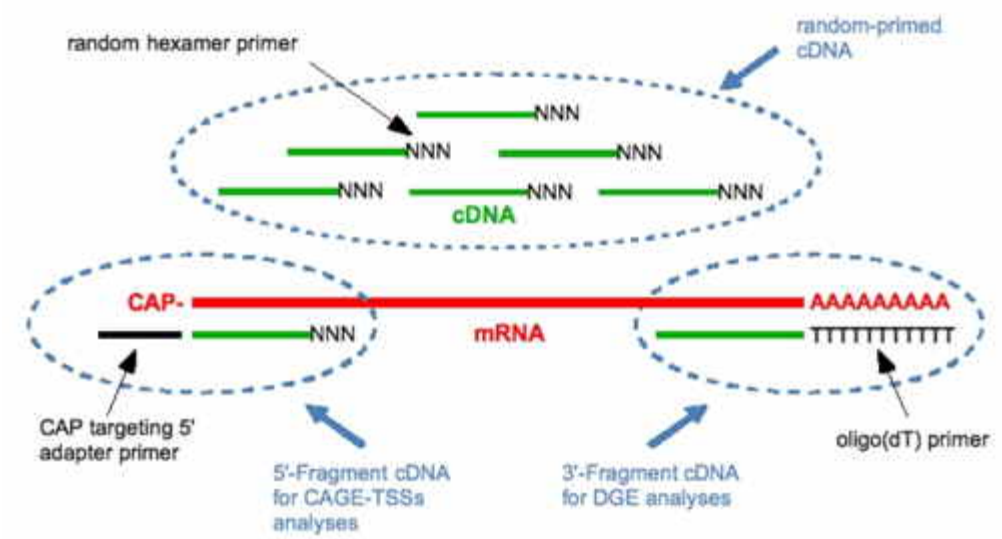
cDNAs ready for 454, SOLiD or Illumina sequencing

The cDNAs carry attached to their 5' and 3' ends the adapter sequences as specified by Roche/454 Life Sciences, ABI or Illumina/Solexa; therefore, the cDNAs delivered are ready for sequencing with the 454, SOLiD or the Illumina protocol. In addition, each cDNA will carry a specific 4 - 6 nt long barcode sequence attached to its 5' end. This way, several cDNA preparations can be mixed and sequenced together in one run. The specific barcodes will allow the assignment of each sequence to the starting material from which it originates. All cDNAs prepared by vertis are strand-specific, allowing transcriptome sequencing and expression profiling in a strand-specific manner.

Depending on your particular needs, you can choose between different techniques to synthesize cDNA:

- **Random-Primed cDNA, enriched for 5' and 3' ends of the transcripts for whole transcriptome sequencing**
- **5'-Fragment cDNA for mapping of transcription start sites**
- **3'-Fragment cDNA for sequencing of the 3'-ends of the transcripts for transcript profiling**

Strategies to synthesize cDNA from eukaryotic mRNA



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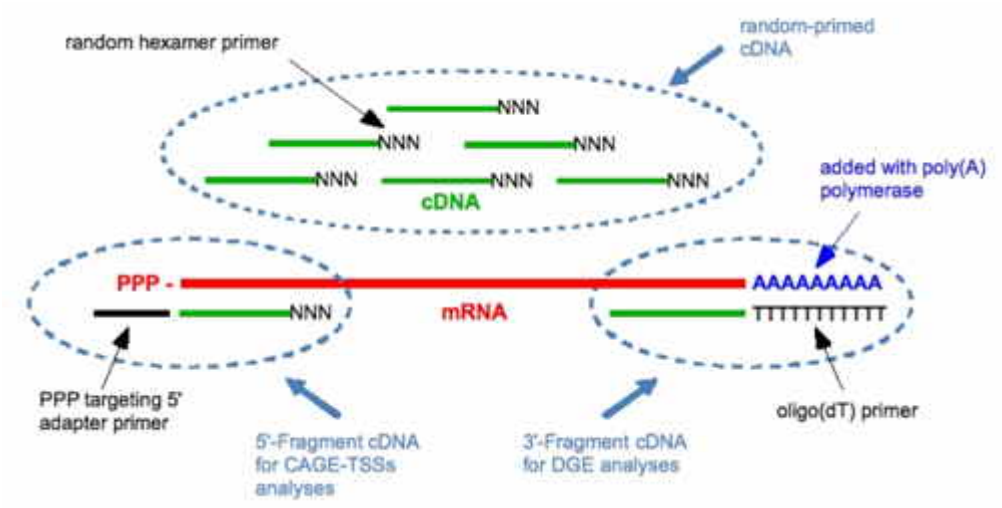
Strategies to synthesize cDNA from prokaryotic Mrna



Oligo(dT) primed cDNA for nebulization or for cloning

A) Full-length enriched cDNA (FLE):

FLE cDNA synthesis is our standard method for the preparation of representative high-quality cDNA libraries also from limited amounts of starting material. The technology assures that 1.-strand cDNA is completely converted into ds-cDNA.

B) True full-length cDNA (TFL) (CAP targeting cDNA synthesis):

TFL cDNA synthesis is the method of choice for the production of cDNAs with complete 5'-ends. It specifically targets intact mRNA carrying a 5'-CAP-structure. With the CAP-targeting method, a selection for full-length cDNA takes place. Routinely > 90% of the clones in such libraries represent full-length clones (the success of the method strongly depends on the quality of the starting material)

Disadvantage of the TFL compared to the FLE technology:

- Less sensitive
- Less representative cDNA population; transcripts with the following properties will be missing or will be underrepresented in the resulting cDNA pool:
    - Transcripts lacking a 5'-CAP
    - Transcripts carrying strong secondary structure, hindering the reverse transcription along the entire RNA molecule into the ligated 5'-adapter
    - Transcripts carrying structures which hinder the ligation of the RNA oligonucleotide to their 5'-end

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Sample requirements

cDNA synthesis method	Minimum amounts
Random-proming	25 µg of total RNA respectively 250 - 500 ng of poly(A)$^+$ RNA, 100 mg of tissue or 10 million cells
5' Fragment	Eukaryotic transcripts: 25 µg of total RNA respectively 250 - 500 ng of poly(A)$^+$ RNA, 100 mg of tissue or 10 million cells Bacterial transcripts: 10 µg of total RNA
3' Fragment	This cDNA synthesis is very sensitive - already a few ng of total RNA are sufficient for cDNA preparation
oligo(dT) primed FLE	FLE cDNA synthesis is very sensitive - already a few ng of total RNA are sufficient for cDNA preparation
oligo(dT) primed TFL	25 µg of total RNA respectively 250 - 500 ng of poly(A)$^+$ RNA, 100 mg of tissue or 10 million cells. The quality of the starting material is a very crucial criterion for the success of the strategy because it strictly depends on a high content of full-length transcripts carrying a 5'-CAP structure; already slightly degraded RNA is converted into TFL cDNA less efficiently.

Quality controls

- Analysis of RNA quality by capillary electrophoresis
- Examination of cDNA samples after each manufacturing step by means of capillary electrophoresis

Documentation

All steps during preparation of the cDNA libraries are documented and are supplied in form of a quality report.

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