Genom Anotasyonu

ATGGGCAATGCAAGGTACGGTGAGCAGGTAAGTGATTAATGCATTTCTCGCAGTGGCTAGACGATGCATAG
M G N A R Y G E Q .... W L D D A *
M G N A R Y G E Q V S D *

5' ▤▤AG GTAAGT▤▤ 3'   Consensus
                         sequence

5' ▤▤AG GTTAGT▤▤ 3' ⎫
5' ▤▤AG GTAACT▤▤ 3' ⎬ Actual
5' ▤▤TT GTAAGT▤▤ 3' ⎬ sequences
5' ▤▤AC GTACGT▤▤ 3' ⎭

(A) The tRNA cloverleaf structure

Acceptor arm
TψC arm
D arm
V loop
Anticodon arm

Figures and Tables are taken from: Terence A. (T.A.) Brown. Genomes 4. Fourth edition. 2017. Garland Science: New York, NY. For educational purpose only.

(A) Gene organization

(B) DNA sequences

--GACAGTTAGCAATCGGAT--

--GATAGTTATCAATCCGAT--   --GACAGCTATCAATCCGAA--

Genome being annotated

Short ORF

Related genomes

Initiation codon

Termination codon

5'   Gene   3'

Transcription

mRNA

5'-untranslated region

3'-untranslated region

Cells

Extract RNA

Denaturing
agarose gel
electrophoresis

rRNA bands

Blotting, northern
hybridization,
autoradiography

DNA probe hybridizes to
a single RNA transcript

Human   Chimpanzee   Cow   Rabbit

DNA markers

Southern hybridization
with human DNA probe

Probe hybridizes to all DNA samples

5' ━━━━━━━━━━━━━━━━ 3' RNA

Anneal a DNA primer

5' ━━━━━━━━━━━━━━━━ 3'
                ┃┃┃┃

cDNA synthesis with reverse transcriptase

5' ━━━━━━━━━━━━━━━━ 3'
    ━━━━━━━━━━━━━━
3'   5'

cDNA

Denature

NNNN ▬
3'

Add As to 3'-end with terminal transferase

AAAAANNNN ▬
3'

Anneal anchor primer

5' NNNNNNTTTTT
         ┃┃┃┃┃
   3' AAAAANNNN ▬

▬▬▬
   ┃┃┃┃
   ━━━━━━━━━━

Second-strand synthesis with Taq polymerase

━━━━━━━━━━━━━━━━
┃┃┃┃┃┃┃┃┃┃┃┃┃┃┃┃
━━━━━━━━━━━━━━━━

Continue as for a standard PCR

Gene

R1   R2

400 bp restriction fragment

Clone restriction fragment in M13

Single-stranded DNA

Anneal RNA

Nuclease S1 degrades all single-stranded regions

R2

┃┃┃┃┃┃

Alkali degrades RNA

R2

Size DNA by electrophoresis, say 200 bp

R1   R2

Start point of transcription

200 bp

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Exon-trap vector

R

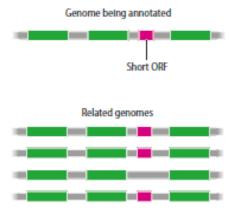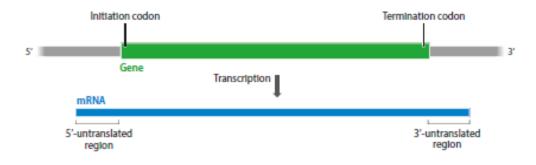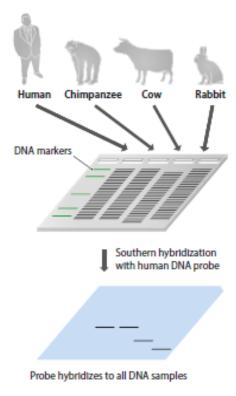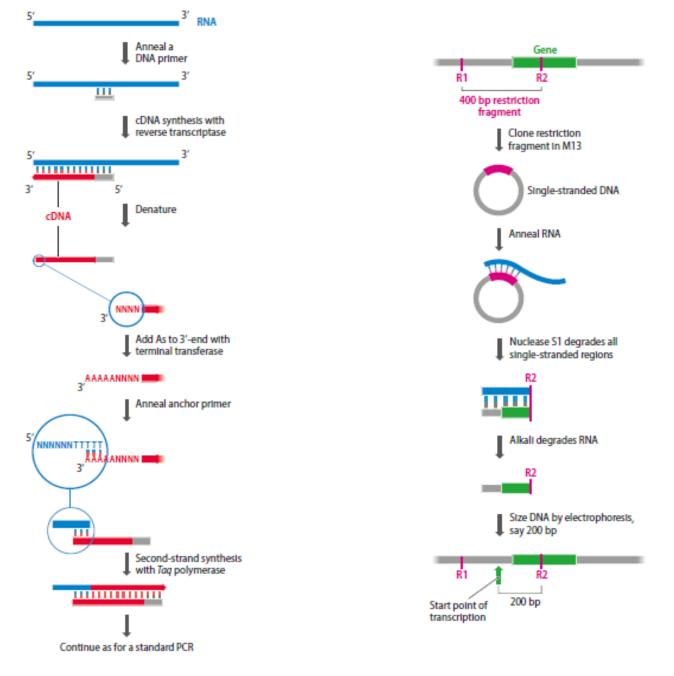Insert new DNA

R   R

Introduce into eukaryotic
host, transcription occurs

RNA transcript

Splicing

Primers for RT-PCR

KEY

Promoter sequences

Exon

R

Restriction site

20 nucleotide overlap

100-mer oligonucleotides

Overlapping tiling array

DNA

Gapped tiling array

20 nucleotide gap

Light-activated synthesis

A A A A

Light

A

T   G
C   C
A   T
C   G
I   I

A   G
T   C
C   T
A   G
C   I
I

Addition only to
activated oligonucleotides

Cell
extract

RNA +
0.5M NaCl

Water

Oligo(dT)–
cellulose

Poly(A)⁻
RNA

Poly(A)⁺
RNA

(A) Map RNA-seq reads directly onto the genome

RNA-seq reads

Genome sequence

Transcribed regions

(B) Construct contigs and map the contigs onto the genome

Contigs

Genome sequence