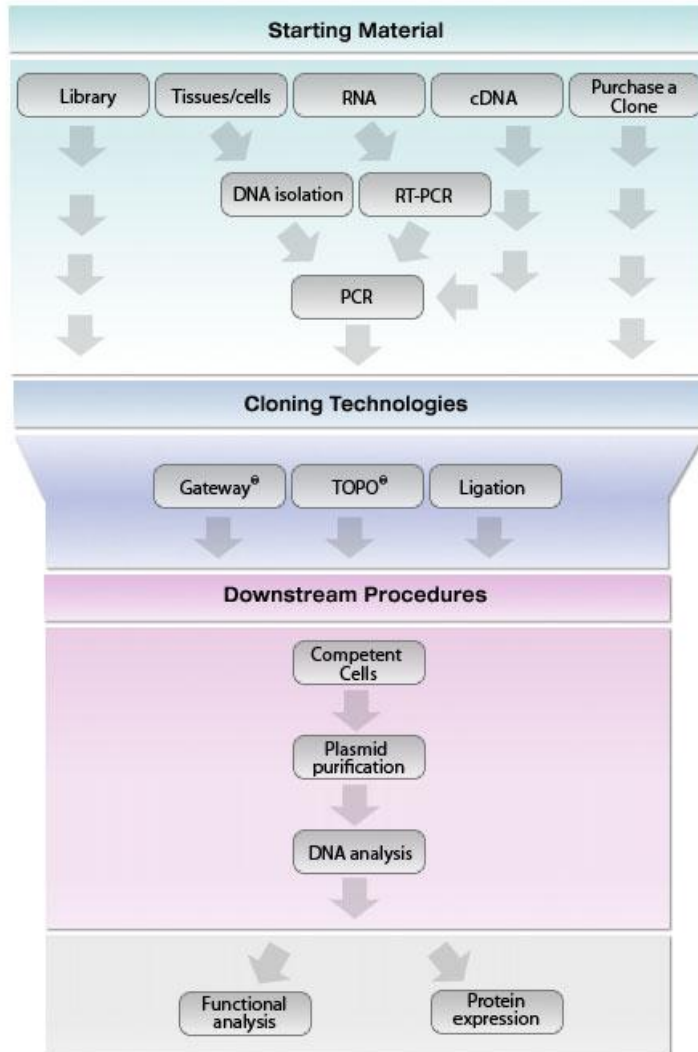


# ***Klonování a expresní systémy II.***

Heidi Onderková

# Základní postup experimentu



1. Příprava materiálu
2. Klonování
3. Postklonovací kroky
4. Aplikační analýzy

# 1. Příprava materiálu

## CloneMiner™ cDNA Library Construction Kit



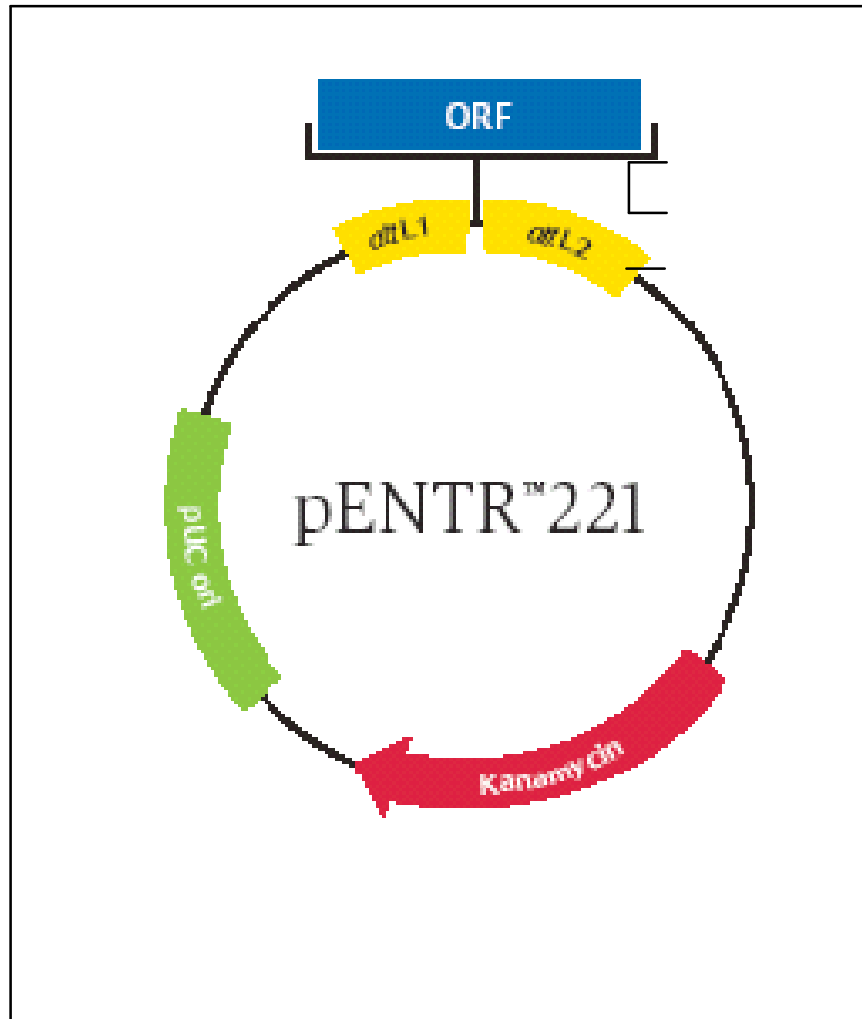
- Construct cDNA libraries without restriction enzymes and ligases-get clones never before available
- fully intact clones from high-quality cDNA libraries.
- enables you to construct highly representative cDNA libraries without the risk of restriction enzymes cutting your precious gene

# ***1. Příprava materiálu***

Nucleic acid isolation:

- Genomic DNA
- Plasmid DNA
- Plasmid DNA Purification Services
- RNA Purification
- Viral RNA/DNA Purification
- PCR Product Clean-up Kits
- Agarose Gel Extraction Kits
- Automated Nucleic Acid Purification

# 1. Příprava materiálu

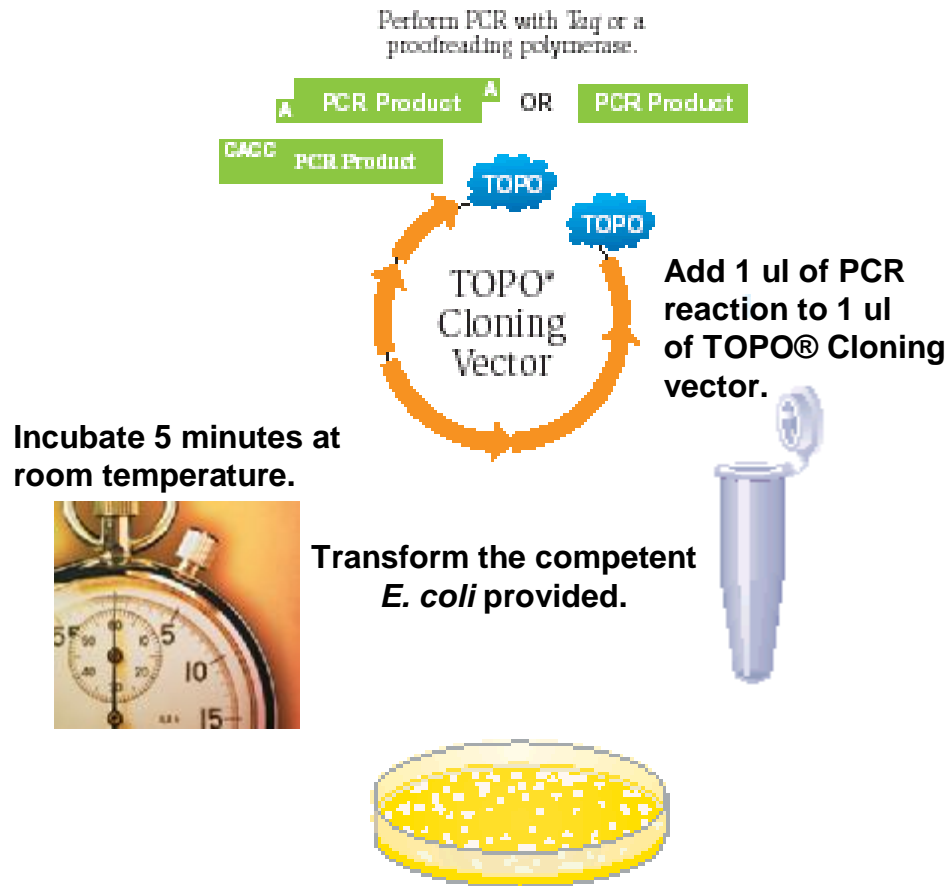


## Ultimate™ ORF clones

- ready to use Human or Mouse genes, in the pENTR221 Gateway® entry vector.
- almost 15,000 Human and over 2,600 Mouse clones available.
- inserts are double strand sequenced, and their amino acid sequence is 100% guaranteed to match the public GenBank database sequences and does not have 5' or 3' untranslated region (UTR) sequence.

-

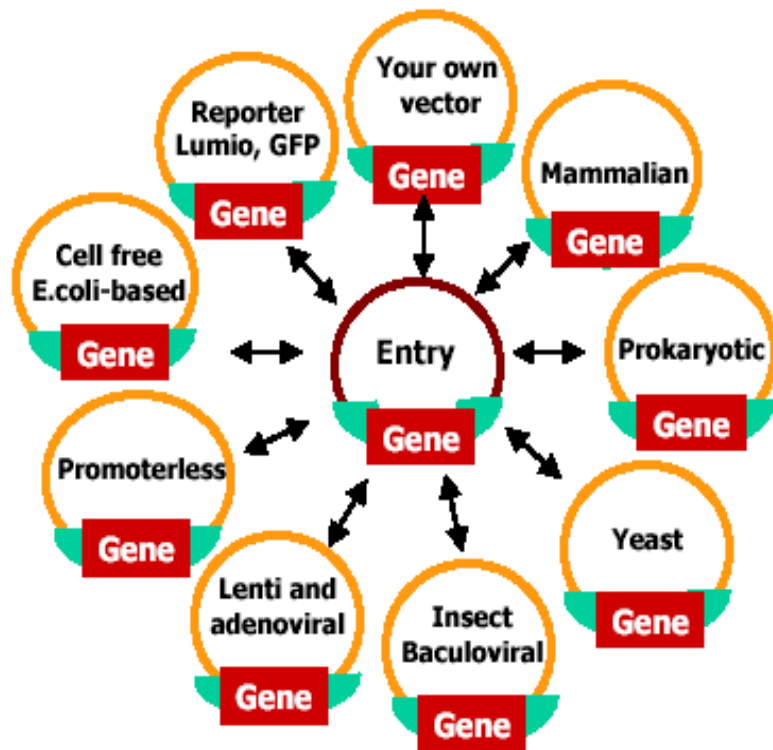
## 2. Klonování



### TOPO TA Cloning

- provides simple steps and fast reactions for cloning of PCR fragments, and has a superior (>99%) efficiency for cloning of a regular sized PCR fragment (<3kb).
- Works with PCR products generated by any polymerase(s)
- Tailor made kits work for many downstream applications
- Compatible with most competent cells

## 2. Klonování

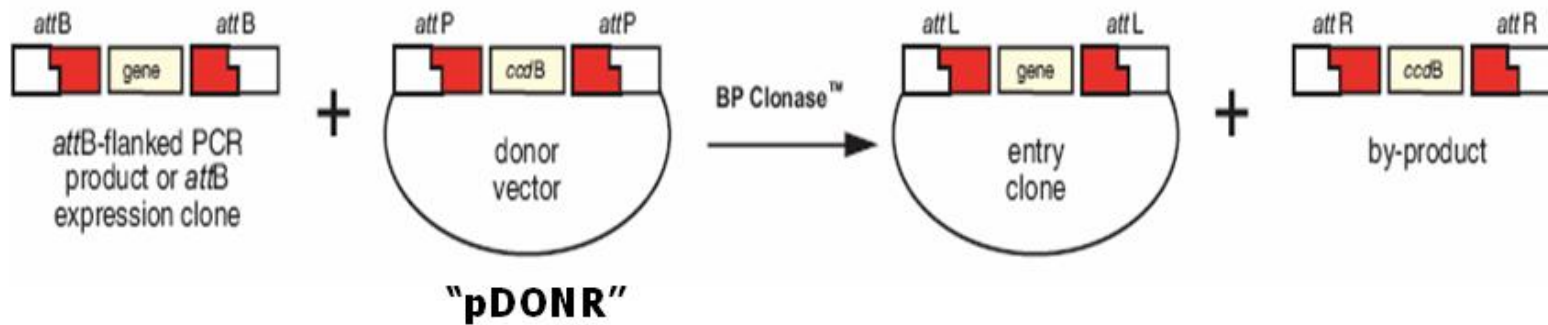


### Gateway® Technology

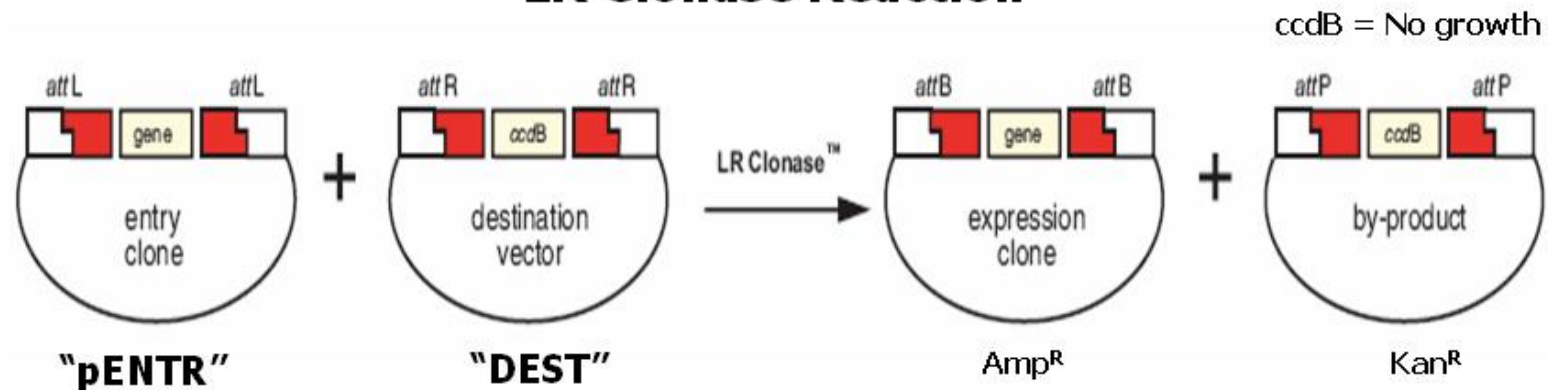
- powerful system designed to simplify and provide a rapid and highly efficient route to multiple expression and functional analysis options.
- transfer DNA fragments across multiple systems and into multiple vectors without restriction enzyme cloning
- eliminates time-consuming cloning and subcloning steps
- any entry clone, containing the gene of interest, can be recombined with various expression vectors that fit the needs of the experimental design

# Gateway® Technology: 2 steps **Reversible**

## BP Clonase Reaction

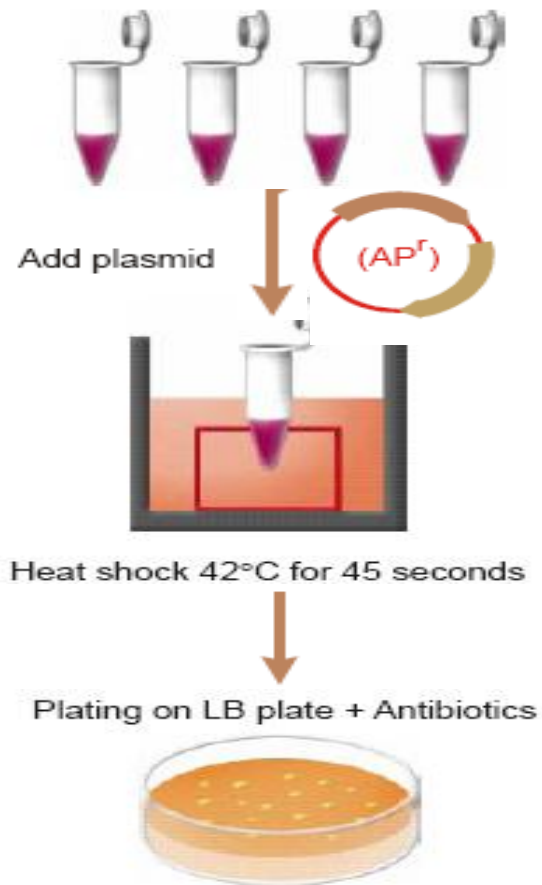


## LR Clonase Reaction



### 3. Postklonovací kroky

Chemically Competent *E. coli*



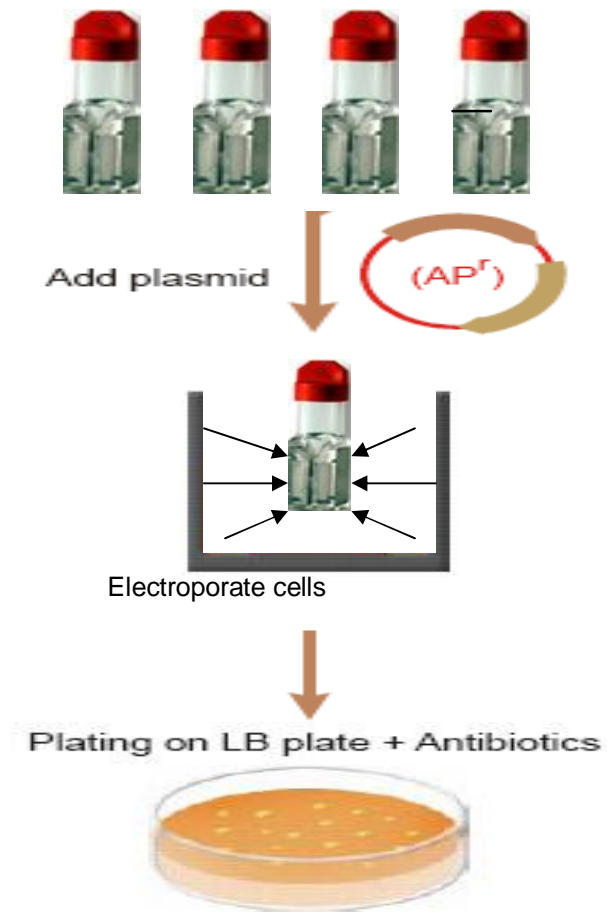
#### Chemical Transformation

1. Add DNA to pre-aliquoted cells
2. Heat shock 30 seconds @ 42C
3. Add recovery medium
4. Incubate cells and media 1hr
5. Spread onto plates
6. Incubate plates overnight

- No electroporator required
- One Shot® format for ultimate convenience
- Full range of transformation efficiencies
- StripWell format for high throughput cloning projects

### 3. Postklonovací kroky

#### Electroporation Competent *E. coli*



#### Electroporation

1. Add DNA to pre-aliquoted cells
2. Electroporate
3. Add recovery medium
4. Recover, in cuvette
5. Spread onto plates
6. Incubate plates overnight

- Shorter procedure than with chemical transformations
- Electroporator required
- Easier procedure with pre-aliquoted E-Shot™ Electrocomps™
- Expect higher transformation efficiencies with electroporation

# 3. Postklonovací kroky

Agarozová elektroforéza E-Gel

Insert gel in E-Gel®  
PowerBase™ v.4. or E-Base™



Pre-run for 2 minutes  
( E-Gel® PowerBase™ only )



Load Samples



Run the desired E-Gel  
Program (12-30 min.)



View Gel on UV box  
or Safe Imager™  
(SYBR Safe gels)

