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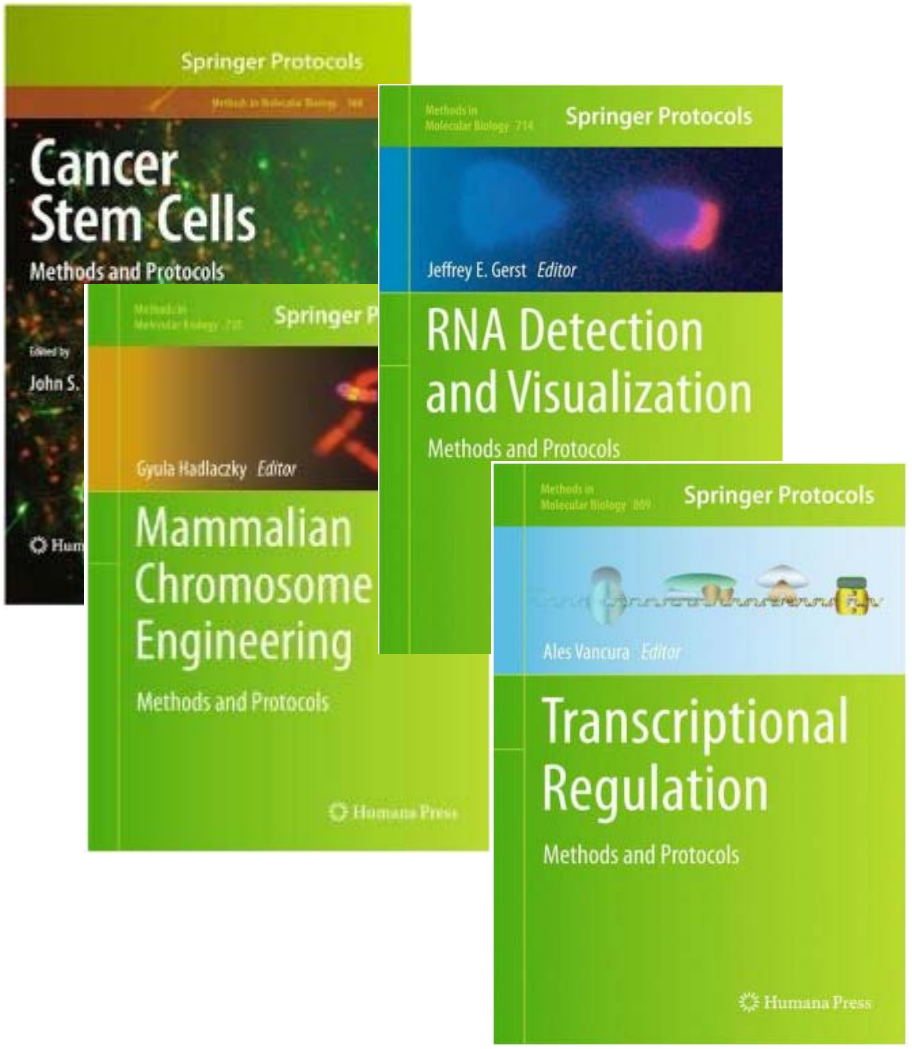

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Author(s): Martin H. Groschup, Frauke Junghans, Martin Eiden, Thorsten Kuczius
Pub. Date: July-13-2001; **DOI:**10.1385/1-59259-134-5:71
Summary: A number of different antibodies diluted in PBS-Tween at appropriate concentrations, can be used for the Immunoblot **detection** of **PrPSc**. For labeling murine **PrPSc** we use a polyclonal anti-peptide...
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Summary: . These protocols are mainly used for the **detection** of **PrPSc** in brain tissue of clinically suspected cases of human prion disease. The protocols may also be applied to animal prion disease such as BSE, chronic...
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Author(s): Victoria A. Lawson
Pub. Date: June-04-2008; **DOI:**10.1007/978-1-59745-234-2_7
Summary: labelling of PrPC enables the **detection** of the de novo-generated PrPres without **detection** of the input **PrPSc** seed. The proportionally large amount of **PrPSc** seed required to drive the CFC assay (**PrPSc**:PrPC...
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Author(s): Katarina Bedecs
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Summary: , avoiding clonal differences. In addition, GT1 cells are the only CNS-derived neuronal cells susceptible to prion infection today. 2. **Detection** of **PrPSc** in Infected Cells: Definition of Prion Infection...
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Author(s): Hanna Gyllberg, Kajsa Löfgren

Pub. Date: June-04-2008; **DOI:**10.1007/978-1-59745-234-2_3

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3. Immunodetection of PrP^{Sc} Using Western and Slot Blotting Techniques

By: Hanna Gyllberg[1], Kajsa Löfgren[1]

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Prion infectivity is often linked to presence of the protease-resistant isoform of prion protein (PrP), PrP^{res}; therefore, it is of highest interest to have convenient methods for rapid detection of PrP^{res} in the research laboratory. For detection of PrP^{res} in model systems to confirm infectivity, there are several methods that can be applied. This chapter focuses on detection of PrP^{res} by proteinase K digestion followed by Western blot, which is the only method that is both quantitative and qualitative. For large-scale screening of PrP^{res} content in samples, the dot blot method offers a great advantage for detecting PrP^{res}, and this method is also thoroughly described in this chapter.

Affiliation(s): (1) Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden

Book Title: Prion Protein Protocols

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Subject: Protein Science

Key Words: Dot blot - guanidinium thiocyanate - immunoprecipitation - nitrocellulose membrane - proteinase K digestion - PrP antibodies - PVDF membrane - reprobing - Western blot

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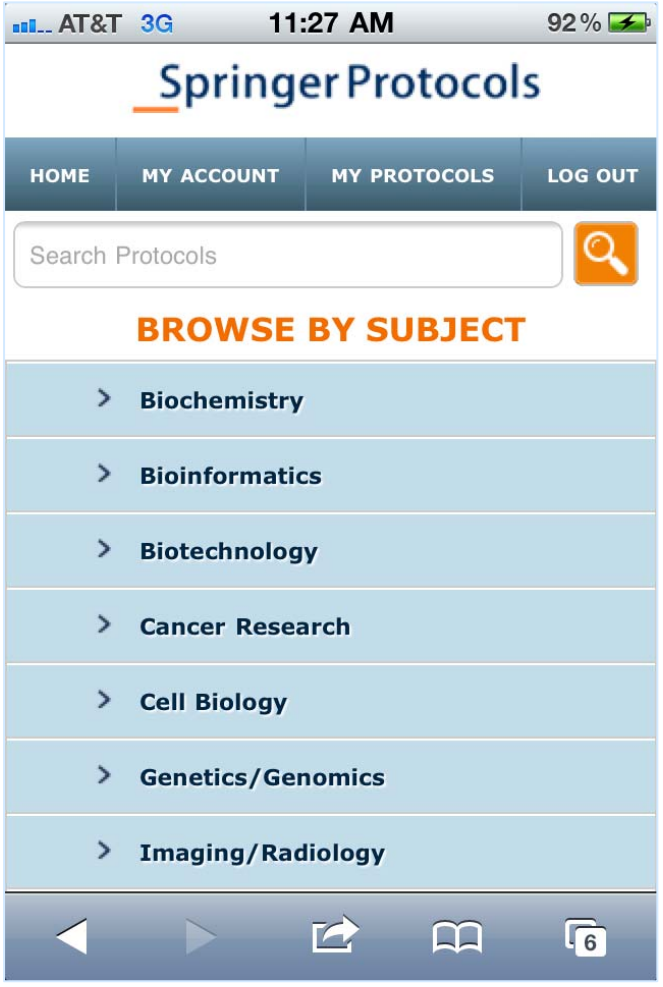

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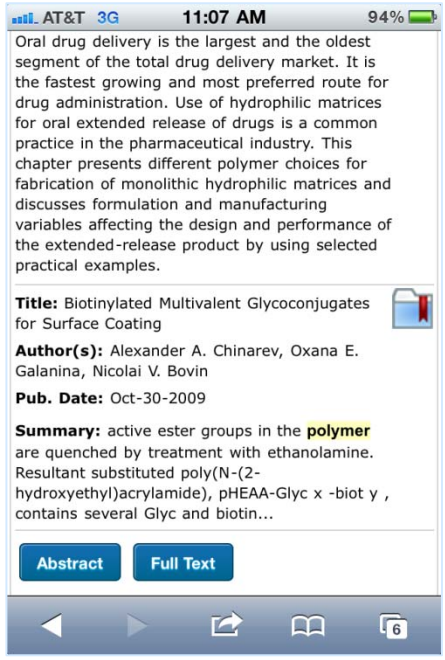

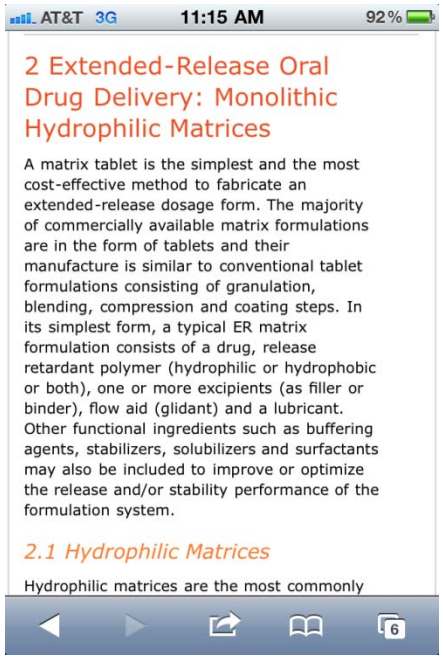

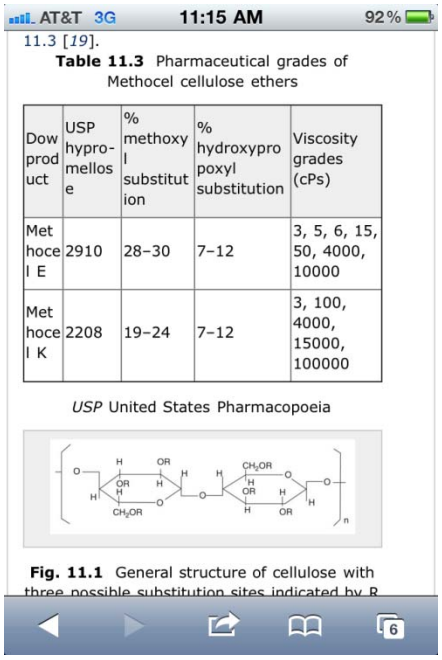

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案例 FFPE tissue analysis

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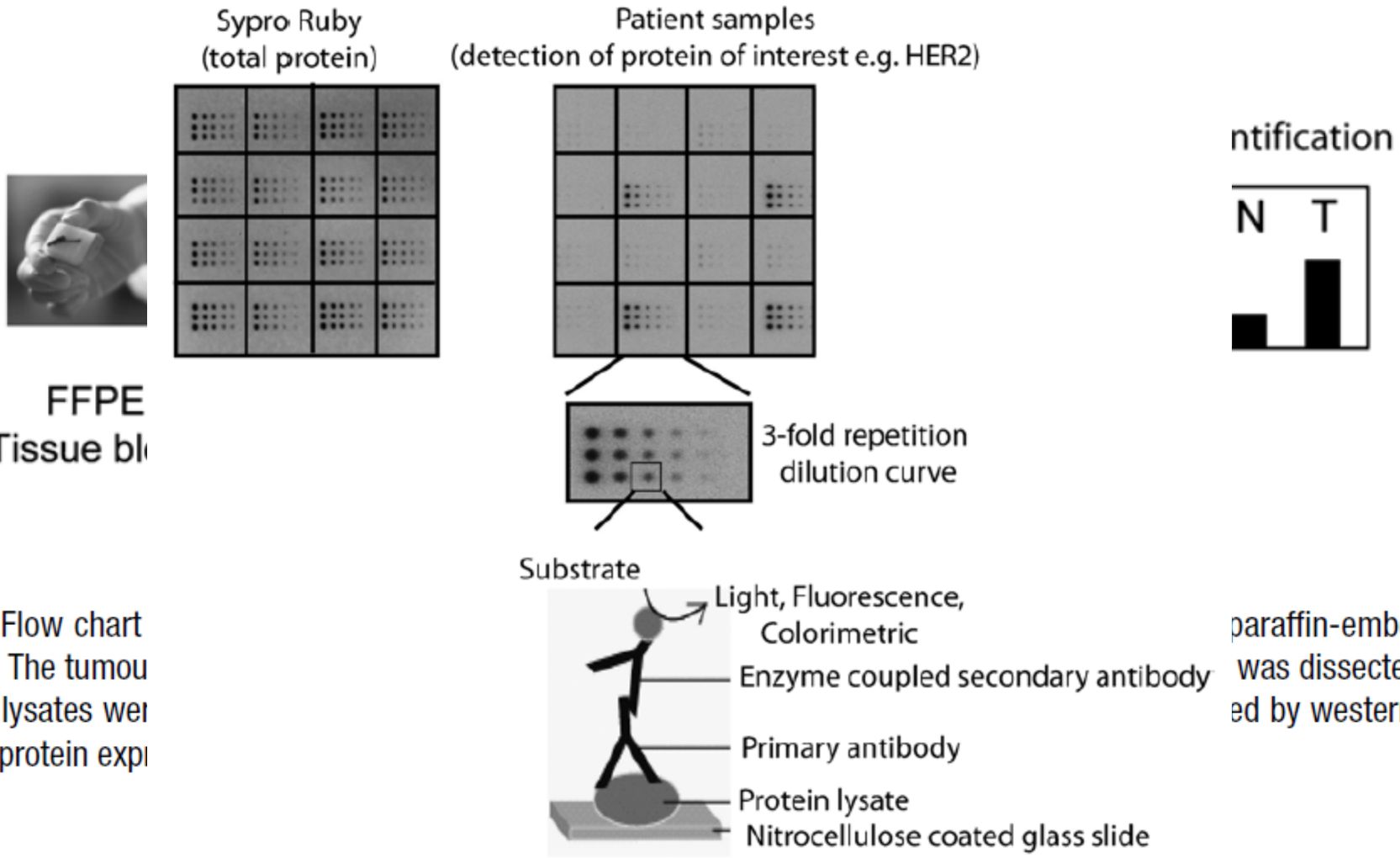

Fig. 1. Flow chart
tissues. The tumou
protein lysates wer
Finally, protein expr

paraffin-embedded
was dissected and
ed by western blot.

4. Notes

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3. Mangham, D.C., Williams, A., McMullan, D.J., et al. (2006) Ewing's sarcoma of bone: the detection of specific transcripts in a large, consecutive series of formalin-fixed, decalcified, paraffin-embedded tissue samples using the reverse transcriptase-polymerase chain reaction. *Histopathology* **48**, 363–376.
4. Sorg, I., and Metzler, A. (1995) Detection of Borna disease virus RNA in formalin-fixed, paraffin-embedded brain tissues by nested PCR. *J. Clin. Microbiol.* **33**(4), 821–823.
5. Mini-Protean®. 3 Cell Instruction Manual. Bio-Rad Laboratories, Inc.
6. QIAGEN®. OneStep RT-PCR Kit Handbook, February 2008.

fer-soaked filter paper. When each tray is full, it is covered with one long strip of heavy-duty plastic to prevent drying out. Trays are stacked and placed in a domestic vegetable steamer. Different cases are not mixed in the trays, and each case usually requires two trays.

Table 1

Table 2
Quick reference guide to troubleshooting FFPE FISH

Issue	Possible cause	Solution
No or weak FISH signals	Inappropriate tissue fixation	Ensure that only neutral-buffered FFPE tissue sections have been used
	Insufficient tissue digestion	Ensure that the appropriate digest temperature has been used and that the proteinase K has not passed its expiry date. Further digest, assess, and re-probe same slide
	Inadequate denaturation conditions	Check that the co-denaturation temperature used was at least 80°C for 10 min. Repeat the assay with an increased co-denaturation temperature. Temperatures as high as 95°C are necessary for certain tissue types
	Incorrect hybridization conditions	Ensure that hybridization occurred at 37°C for at least 14 h. Repeat with appropriate temperature and time
	Drying-out of probe during hybridization	Ensure that hybridization chamber is set up correctly, i.e., with 2× SSC to allow for sufficient humidity. Ensure that Fixogum is applied generously to completely seal the probe under the coverslip
	Excessive stringency of posthybridization wash conditions	Ensure that the recommended wash solutions, temperatures, and times are used. If necessary, decrease the time in, or even omit the 2× SSC/0.1% NP-40 wash
	Microscope not set up correctly	Check that an appropriate filter set is in use, a suitable mercury lamp is being used and is not beyond its expected life, the collector lens is not dirty or cracked, and that an appropriate fluorescence microscopy oil is in use
	Signals have faded	Minimize exposure to strong light sources and check probe stock
Region with no or patchy signals	Insufficient probe added	Ensure that the probe volume was sufficient to cover the entire area under the coverslip without any air pockets

unsuitable for FISH.

Optimal digestion	• Well-defined, intact cell borders and effective uptake of DAPI	Proceed to step 10 of Subheading 3.3

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- 玉米被广泛种植供人类食用，化学合成和乙醇生产。常规育种方法可能在质量和数量上达到了一定的改善，取得了较好的收成。然而，多种因素，如人口增长，环境压力和可再生能源的需求已经导致日益增加的需求，在数量和质量以及新的属性需要再进一步改善。基因工程在这方面提供了新的途径，并已成为一个在任何农作物改善计划中最重要的分子工具。找出如何以遗传工程改造玉米，到**SpringerProtocols**，在查询框中搜索“**maize**”和“**transformation**”。

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T Enhancer Trapping in Plants

Author(s): Sivanandan Chudalayandi

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Summary: Gene Gun Koprek et al. 2000 (77) University of California, Berkeley, CA Barley Gene trap Ds-Ubi Bar Ac from maize (Agrobacterial transformation) Zhao et al. 2006 (78) MPI for Plant Breeding Research...

Abstract | Full Text | PDF (1635K)

T Design of Gene Constructs for Transgenic Maize

Author(s): Dong Liu

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Summary: Design of Gene Constructs for Transgenic Maize The first step of any maize transformation project is to select gene expression elements that will make

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案例2 – 改造玉米

Table 1

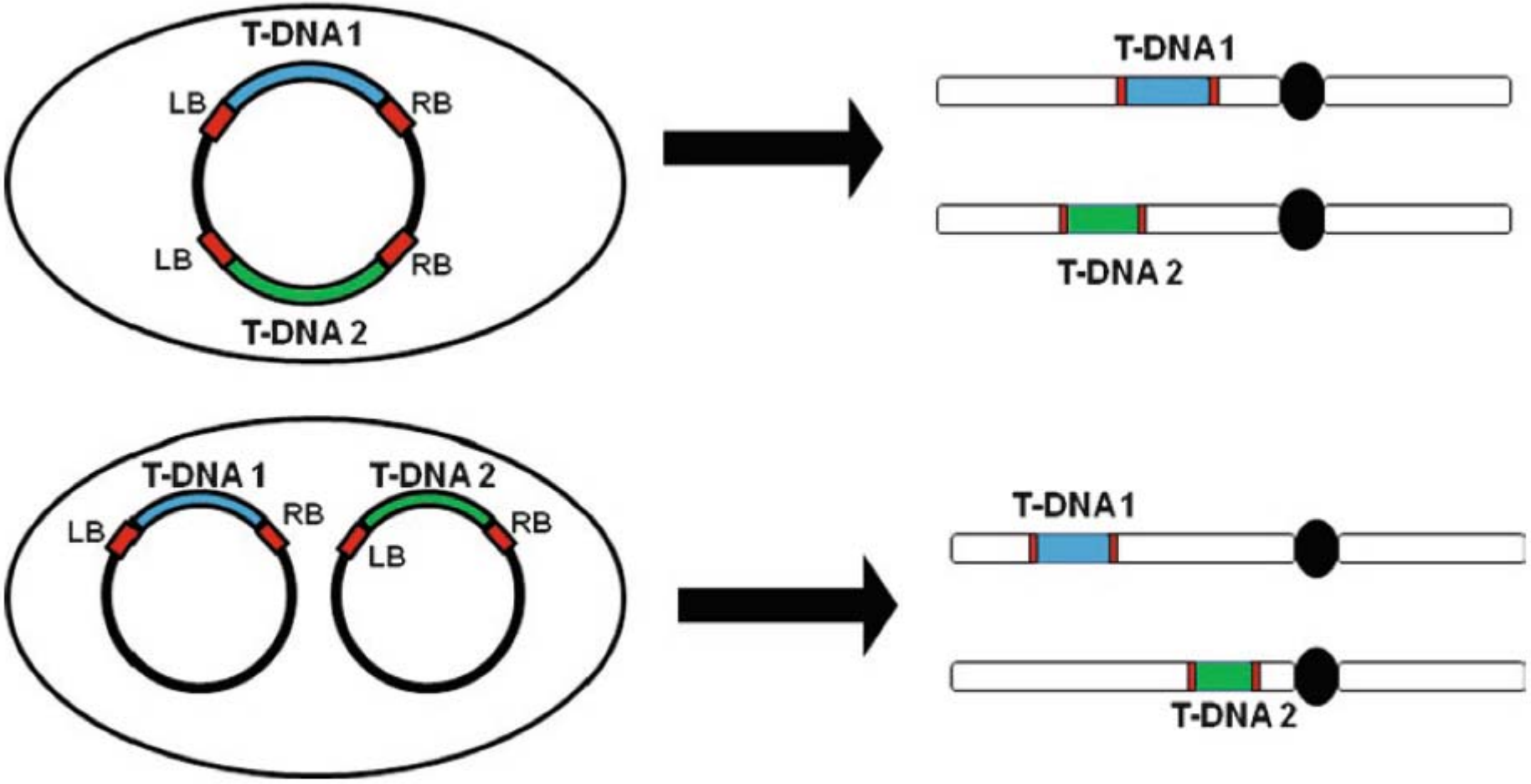


Fig. 1. Schematic representation of two T-DNA on single or two separate vectors in *Agrobacterium*. LB and RB: T-DNA left and right borders, respectively. After transformation, the cotransformed T-DNA integrates, along with its flanking sequences (the LB and RB), into the plant genome at different loci.

案例2 – 改造玉米

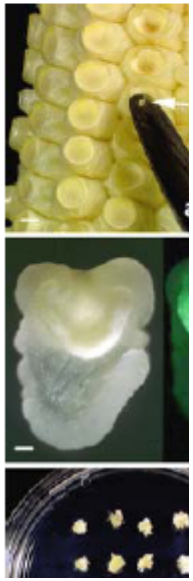
2. Materials

3. Methods

3.1. Transformation of Immature Embryos

3.1.2. Isolation of IE

1. Carefully remove the husks and silk from ears which are harvested 10–13 days post-pollination. Insert a blunt tip holder (can be forceps) at the basal end of ear (*see* **Note 6**).
2. Surface-sterilize ears for 20 min with a 50% dilution (v:v) of a 6.15% (active ingredient) solution of sodium hypochlorite and 10 µl of detergent (Tween 20). Occasionally swirl an ear during sterilization and rinse three times with sterile distilled water. As an alternative, ears collected from greenhouse grown plants can be simply sterilized with a 2 min rinse of 70% ethanol.
3. Grasp the holder in the ear base and transfer the ear to a large sterile plate or other sterile surface. With a fine scalpel remove the upper part of the kernels of an entire ear (remove a flap of pericarp).
4. With a blunt spatula pick up an embryo which lies at the basal edge of the endosperm of the immature caryopsis (**Fig. 1a**).



3.1.3. Inoculation and Co-culture

1. Isolated IE (1.5–2.0 mm) are collected for 15 min in an *Agrobacterium* cell suspension in 1.5-ml microcentrifuge tubes. After 15 min of embryo isolation the microfuge tube is set aside for 5 min.
2. Remove the *Agrobacterium* suspension using a pipette with fine tip. Transfer the embryos to standard ½ MS co-culture medium. Flip the embryos so the scutellum is facing up (*see* **Note 7**, **Fig. 1b**). Keep the coculture plates in a growth chamber set at 24°C and dark for approximately 24 h. Transient expression of GFP in IE after co-culture with *Agrobacterium* can be seen on **Fig. 1c, d**.

案例2 – 改造玉米

4. Notes

1. Sigma, St. Louis, M
nology Labs, Shawn
Sparks, MD.

Agrobacter

2. Antibiotics and acet
–20°C freezer in sm
up to 6 months as a
3. LB plates should b
at 4°C until ready t
1 month.
4. Keep glycerols on
5. After the initial 48
must be stored at 4
older than this sho
iments due to pot
6. Avoid ears with an

References

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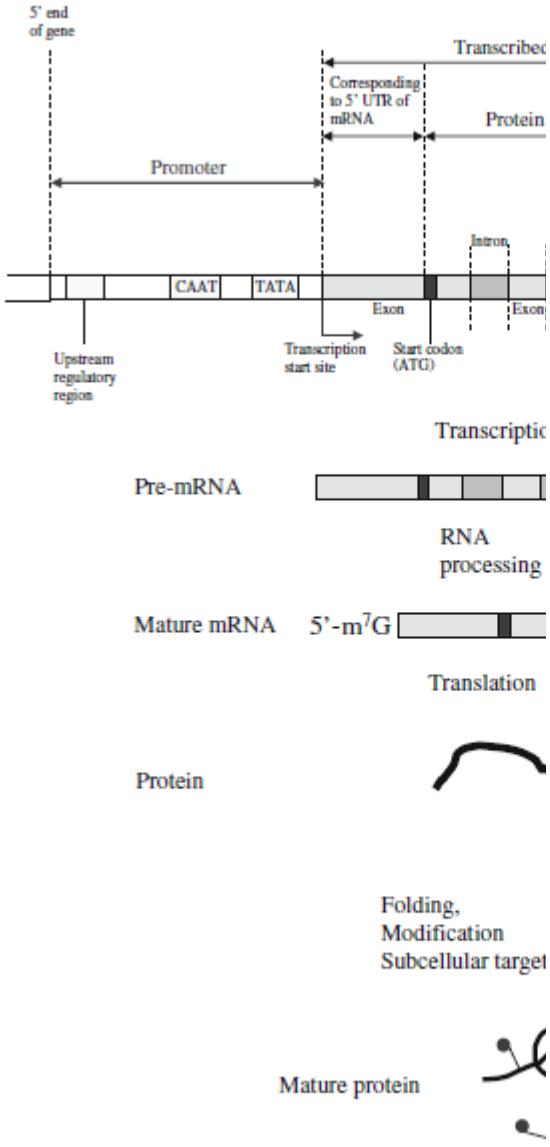

Fig. 1. An overview of the process of plant gene expression.

Table 1
Codon usage of 2,280 coding sequences of maize genes

Amino acid	Codon	Freq	Amino acid	Codon	Freq	Amino acid	Codon	Freq	Amino acid	Codon	Freq
Ala	GCU	21.1	Gln	CAA	13.3	Leu	TTA	5.7	Ser	TCG	10.5
Ala	GCC	31.2	Gln	CAG	23.5	Leu	CTG	25.8	Ser	TCA	11.2
Ala	GCA	16.7	Glu	GAG	40.9	Leu	CTA	7.3	Thr	ACC	16.5
Ala	GCG	23.1	Glu	GAA	20.0	Lys	AAG	39.6	Thr	ACT	10.8
Arg	AGG	14.8	Gly	GGT	14.3	Lys	AAA	15.0	Thr	ACA	10.5
Arg	CGC	14.3	Gly	GGC	30.2	Met	ATG	24.1	Thr	ACG	10.8
Arg	AGA	8.8	Gly	GGA	13.3	Phe	TTC	25.1	Trp	TGG	12.9
Arg	CGT	6.1	Gly	GGG	15.3	Phe	TTT	12.6	Tyr	TAC	19.3
Arg	CGG	9.4	His	CAC	14.8	Pro	CCA	13.9	Tyr	TAT	9.4
Arg	CGA	4.3	His	CAT	10.1	Pro	CCT	12.6	Val	GTC	21.1
Asn	AAC	22.2	Ile	ATC	23.0	Pro	CCC	13.5	Val	GTG	25.6
Asn	AAT	13.5	Ile	ATT	14.0	Pro	CCG	15.4	Val	GTT	15.8
Asp	GAC	32.2	Ile	ATA	8.4	Ser	TCC	16.2	Val	GTA	6.3
Asp	GAT	23.0	Leu	CTC	25.5	Ser	TCT	12.1	Ter	TGA	1.1
Cys	TGC	12.1	Leu	TTG	13.2	Ser	AGC	16.1	Ter	TAA	0.5
Cys	TGT	5.6	Leu	CTT	15.9	Ser	AGT	7.8	Ter	TAG	0.7

This table is adopted from the following website with some modifications: http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species – Zea + mays + [gbpln] *Freq*: occurred frequency per thousand codons