

Construction and Application of Epitope- and Green Fluorescent Protein-Tagging Integration Vectors for *Bacillus subtilis*

Marcus Kaltwasser et al. (2002)

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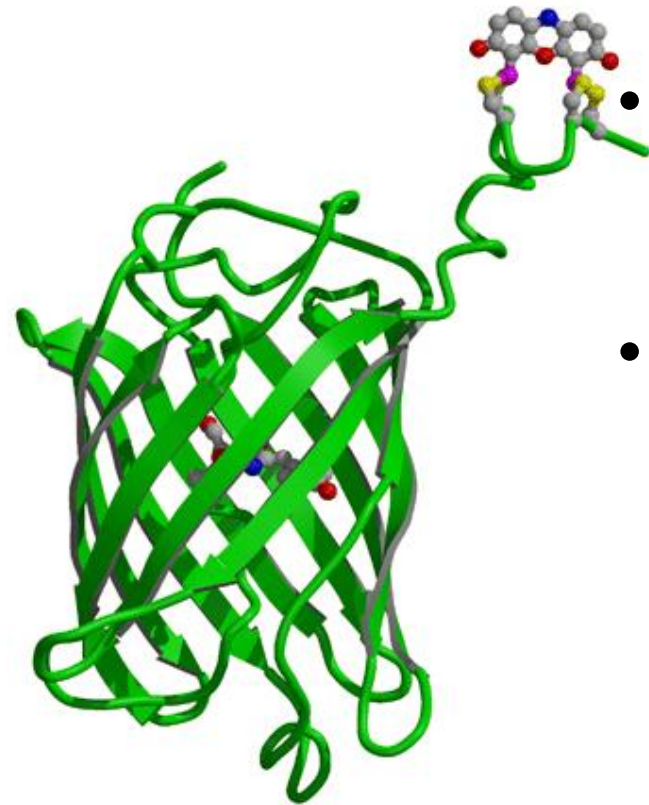
Outline

- 1. Introduction**
- 2. Objective**
- 3. Results**
- 4. Summary**
- 5. Take-Home-Lesson**
- 6. Discussion and current questions**

Epitope Tags

- **epitope = antigenic determinant; part of a protein which is able to react with antibodies**
- **FLAG: 8 amino acids, artificial peptide**
- **HA (= hemagglutinin): 9 amino acids, from influenza virus**
- **c-Myc: 10 amino acids, derived from the human Myc-gene**

Fluorescent Tags



- **GFP⁺ (green fluorescent protein):**
GFP-variant with higher fluorescence
- **CFP (cyan fluorescent protein) &**
YFP (yellow fluorescent protein):
variants of GFP with one amino acid
exchange

<http://www.tsienlab.ucsd.edu/Images/General/IMAGE%20-%20Molecule%20-%20GFP%20+%20FIAsH%20-%202002.gif>

Aufgerufen: 14.11.2011, 18:30 Uhr

pMUTIN-Ter

- **vector to originally integrate mutations & thereby knock out genes**
- **pMUTIN-Ter is derived from pDE01**
- **double STOP-codon to get more efficient translation termination**
- **possibility to activate downstream genes by an IPTG-controllable promoter**

Fusion Proteins

- **HtpG, a heat shock protein, is located in the cytoplasm**
- **FtsH is a ATP- & Zn²⁺-dependent protease and part of the inner membrane**

Bacillus subtilis

- **gram-positive**
- **~4100 genes**
- **in 2002: function of ~1600 genes was unknown**
 - systematical inactivation of these genes by usage of pMUTIN to find out their functions**
- **able to integrate the vector into its chromosome by homologous recombination**

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Is it possible to detect and localize gene products by usage of the six analyzed tagging-vectors?

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0) Usage of antibodies to identify gene products

- **classical procedure: purification of protein, immunization of an animal (normally: rabbit), extraction & purification of the antibody**
 - **expensive, time-devouring, differing quality**
 - **approach to the problem: epitope-tagging-vectors (1) or GFP-fusion-vectors (2)**

0) Usage of antibodies to identify gene products

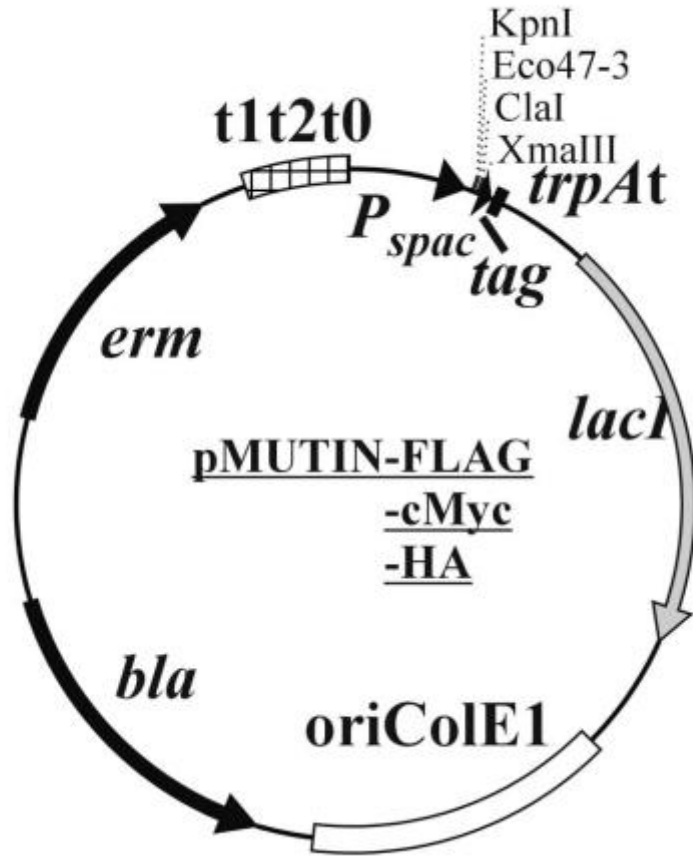
- **resulting problems in *B. subtilis*:**
 - (1) not commercially available for *B. subtilis***
 - (2) not applicable for chromosomal genes**
- **solutions: fusion of (1) HA-, FLAG- or c-Myc-tag resp. (2) GFP⁺, CFP or YFP to the 3'-end of desired gene by usage of a modified pMUTIN**

1) Fusion of the tagging-sequences to HtpG or FtsH

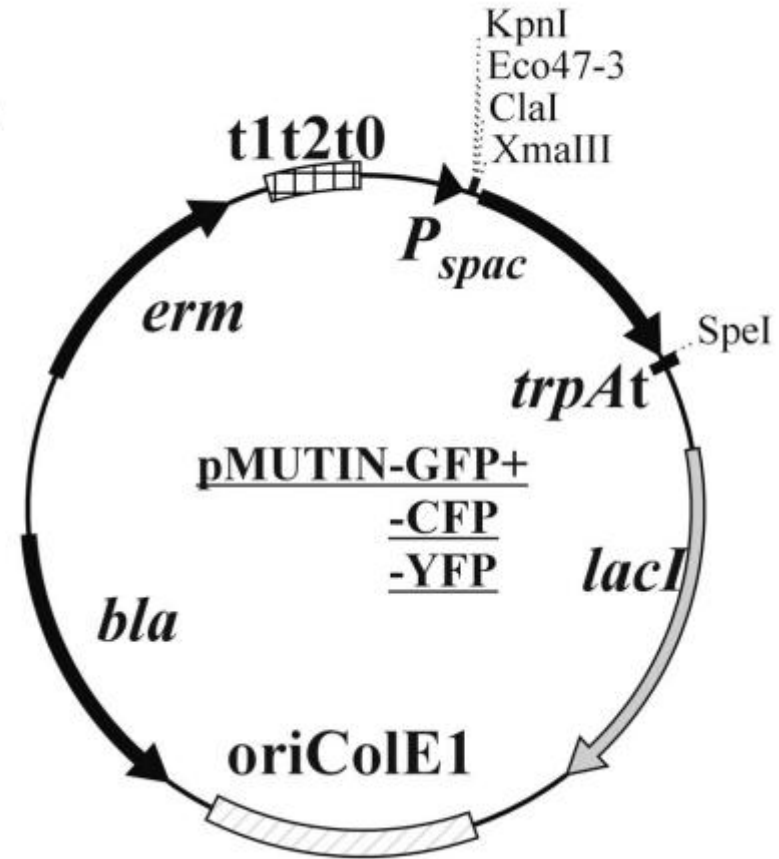
- **~300bp (derived from 3'-end of desired gene) without the STOP-codon are produced by PCR**
- **PCR-product & tagging-vectors are sliced with KpnI & XMaIII & assembled: 12 recombinant plasmides**
- **insertion into competent *B. subtilis***
- **plating & selection on LB plates (with erythromycin) and extra analyze by PCR**

- resulting vectors

A



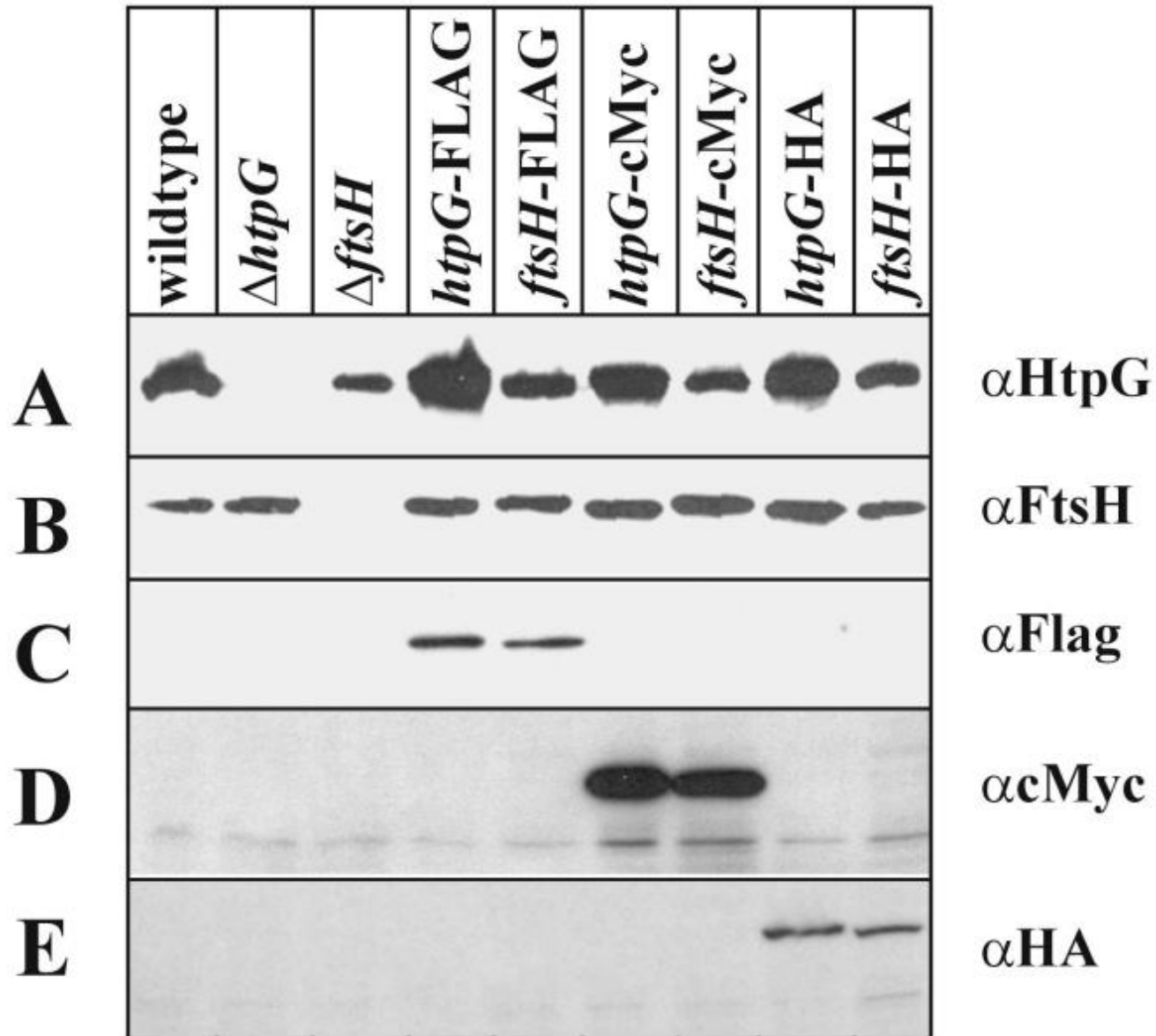
B



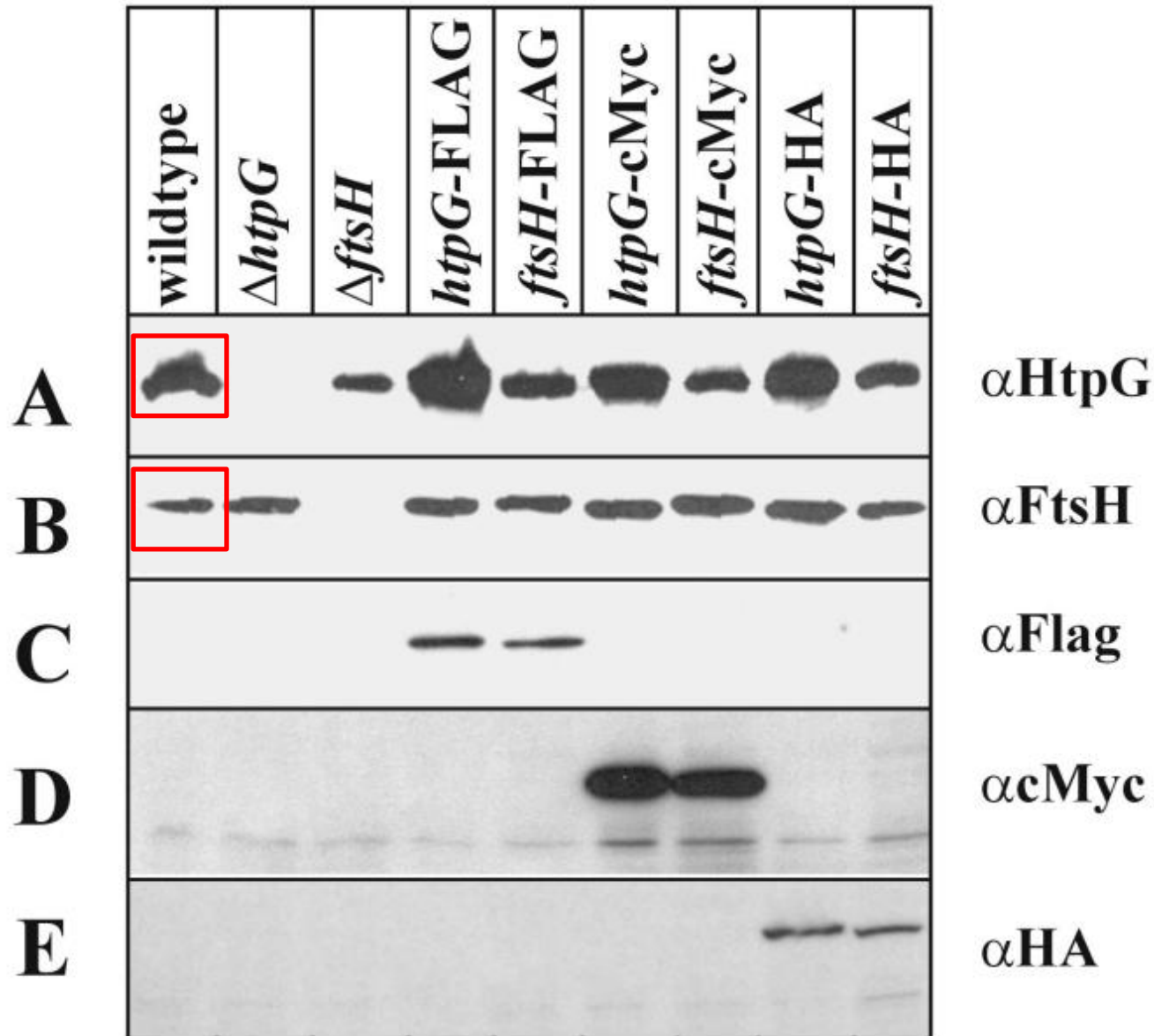
2) Detection of fusion proteins with epitope-tags

- **specific antibodies available**

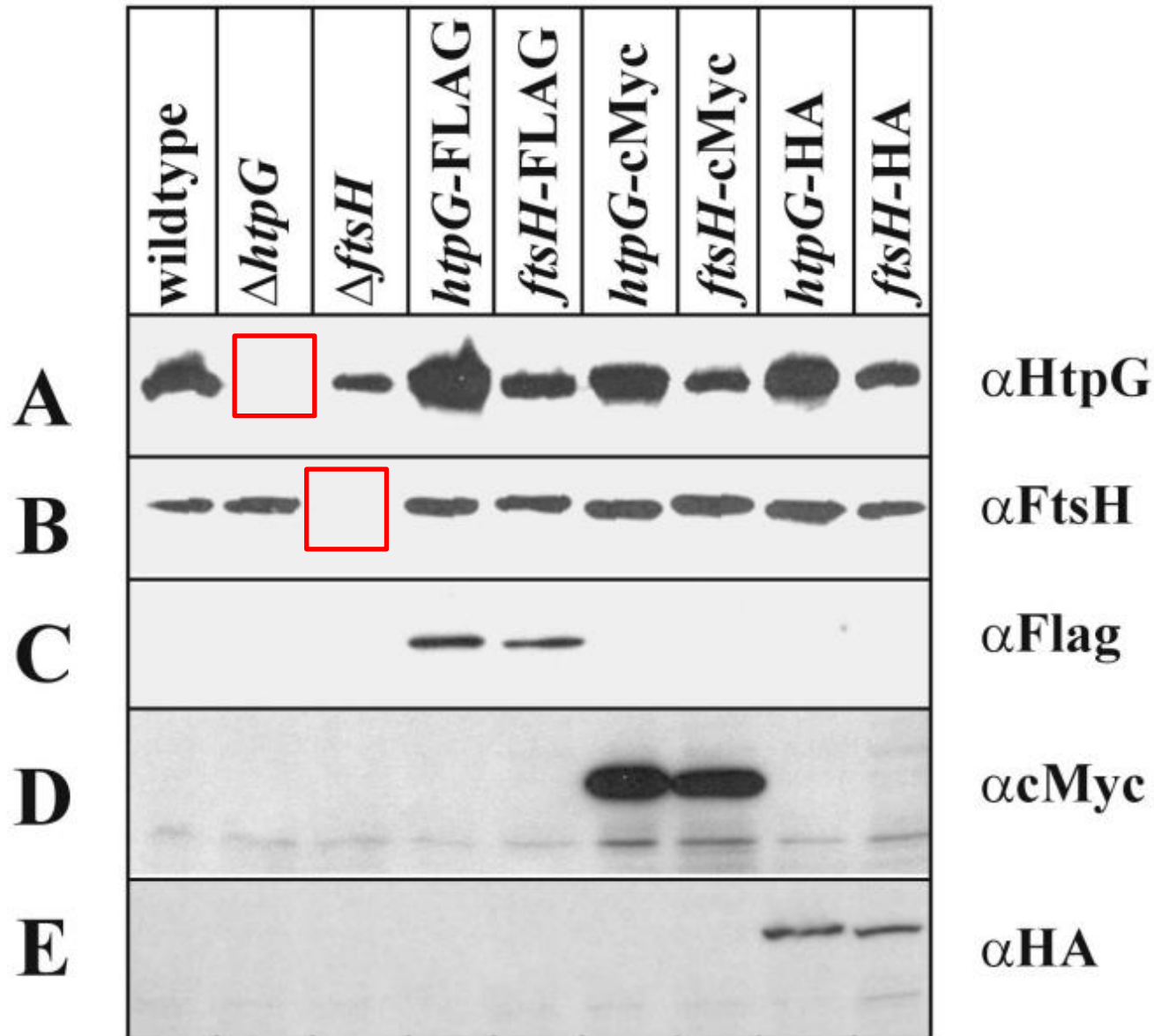
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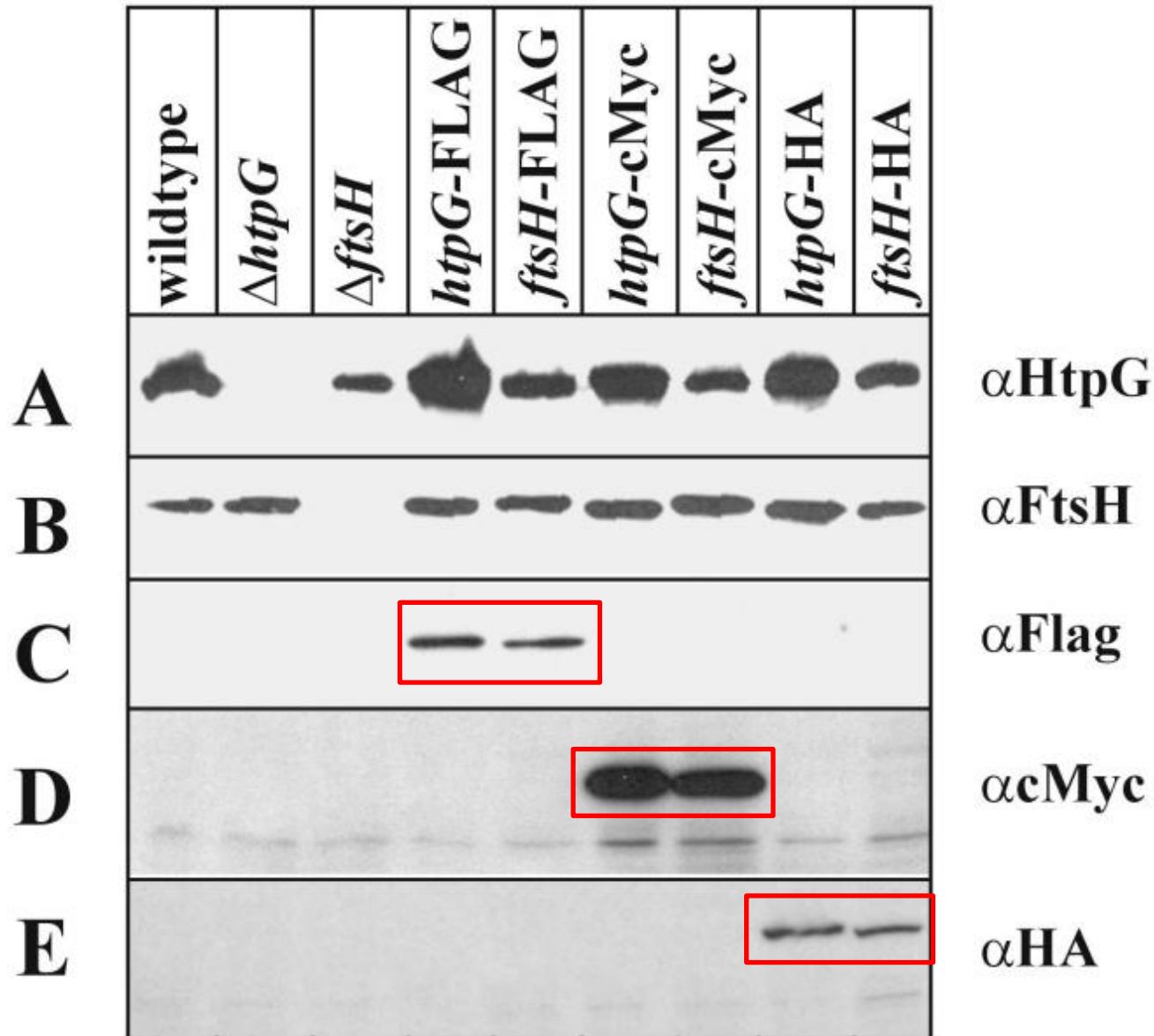
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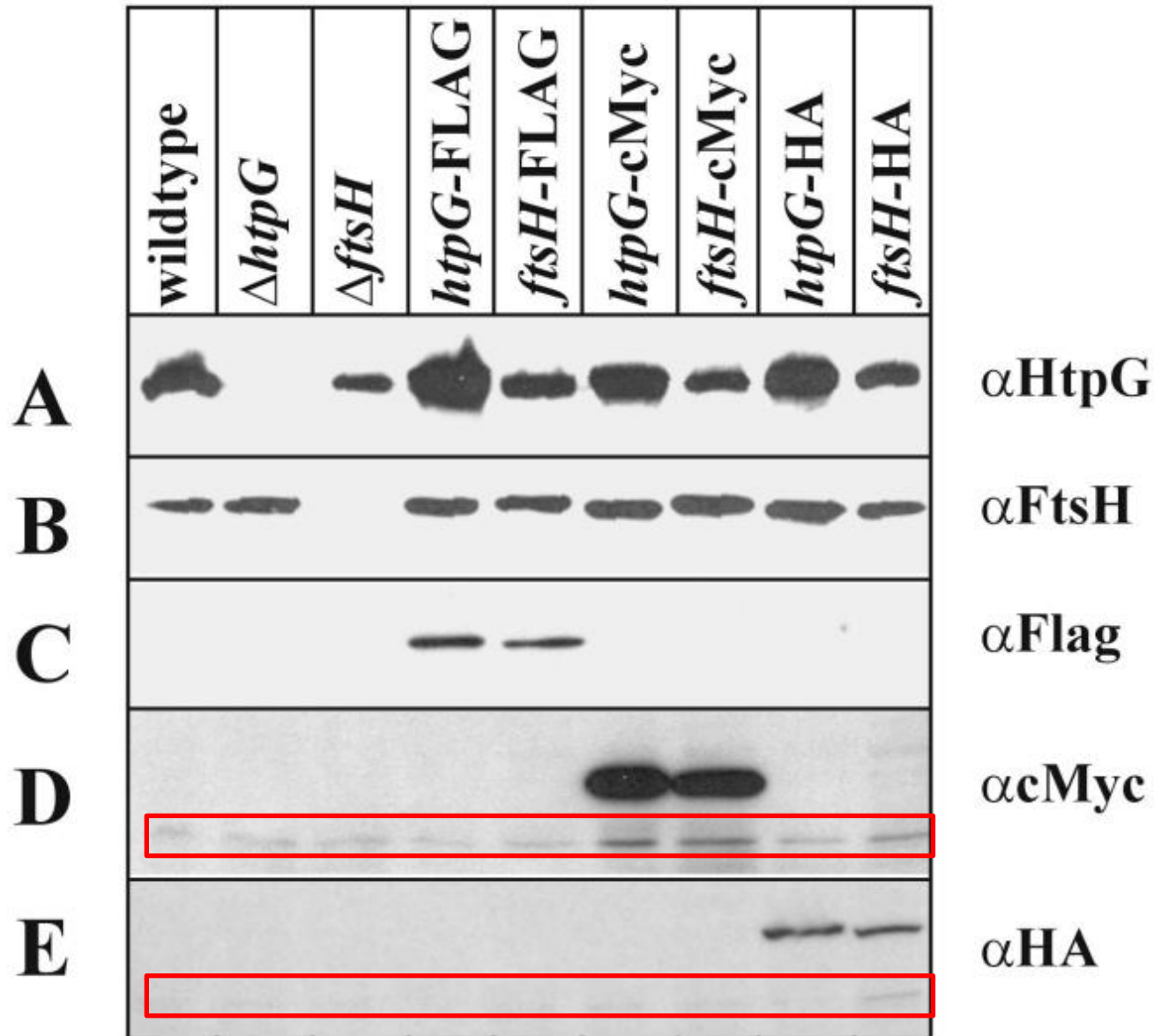
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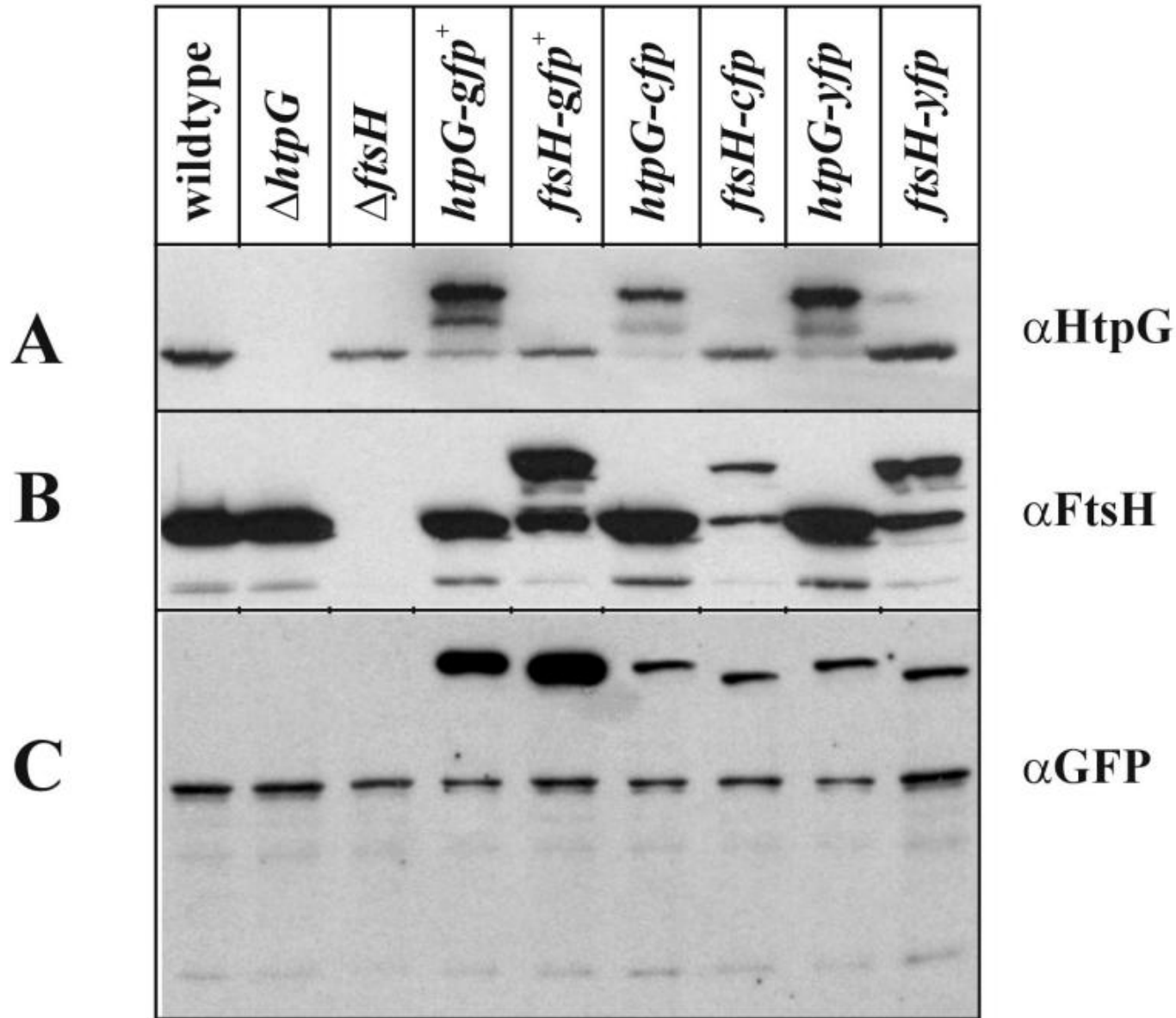


3) Detection of fusion proteins with fluorescent-tags

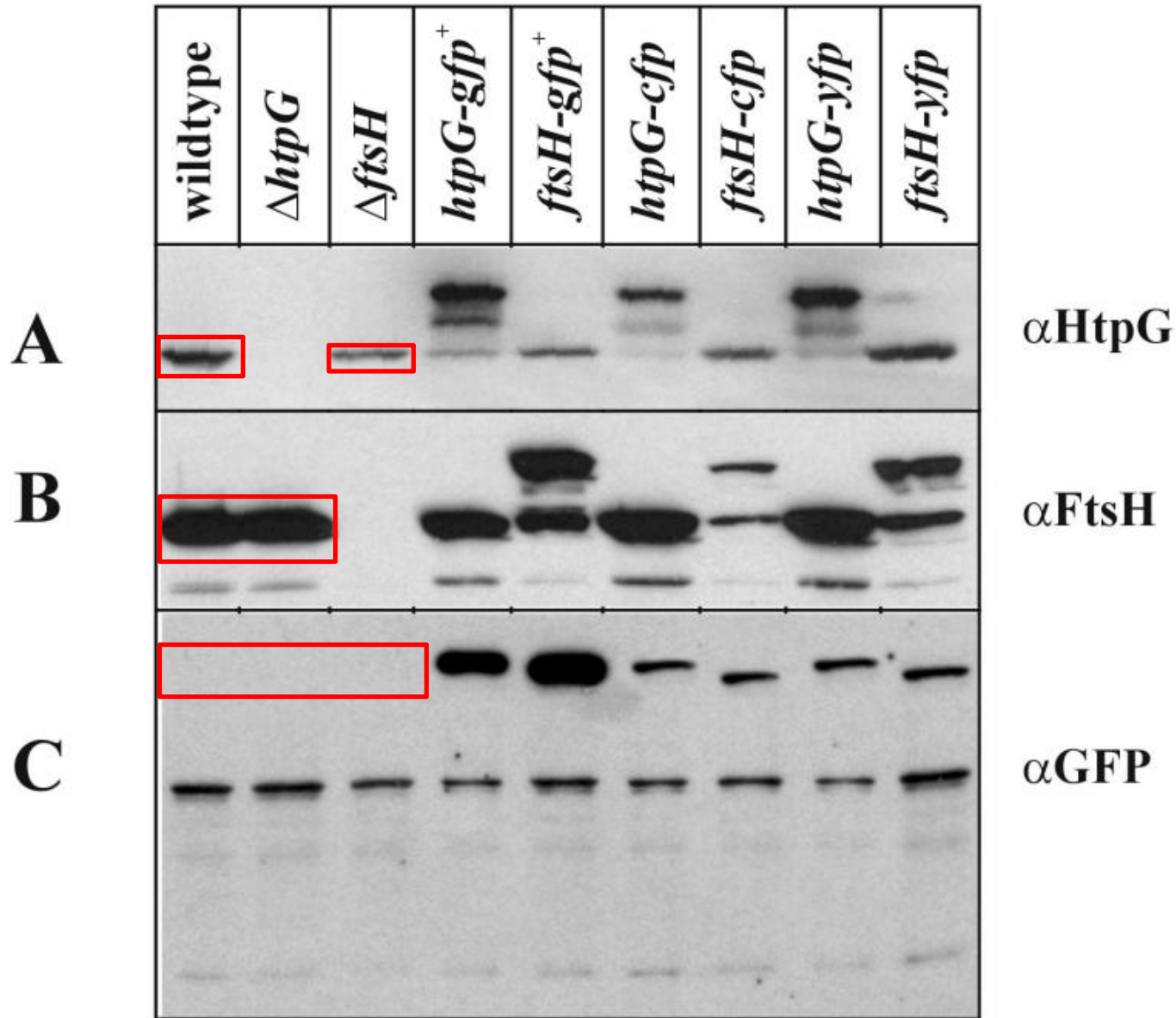
a) Detection by antibodies

- antibody: α GFP (works also for YFP and CFP)**

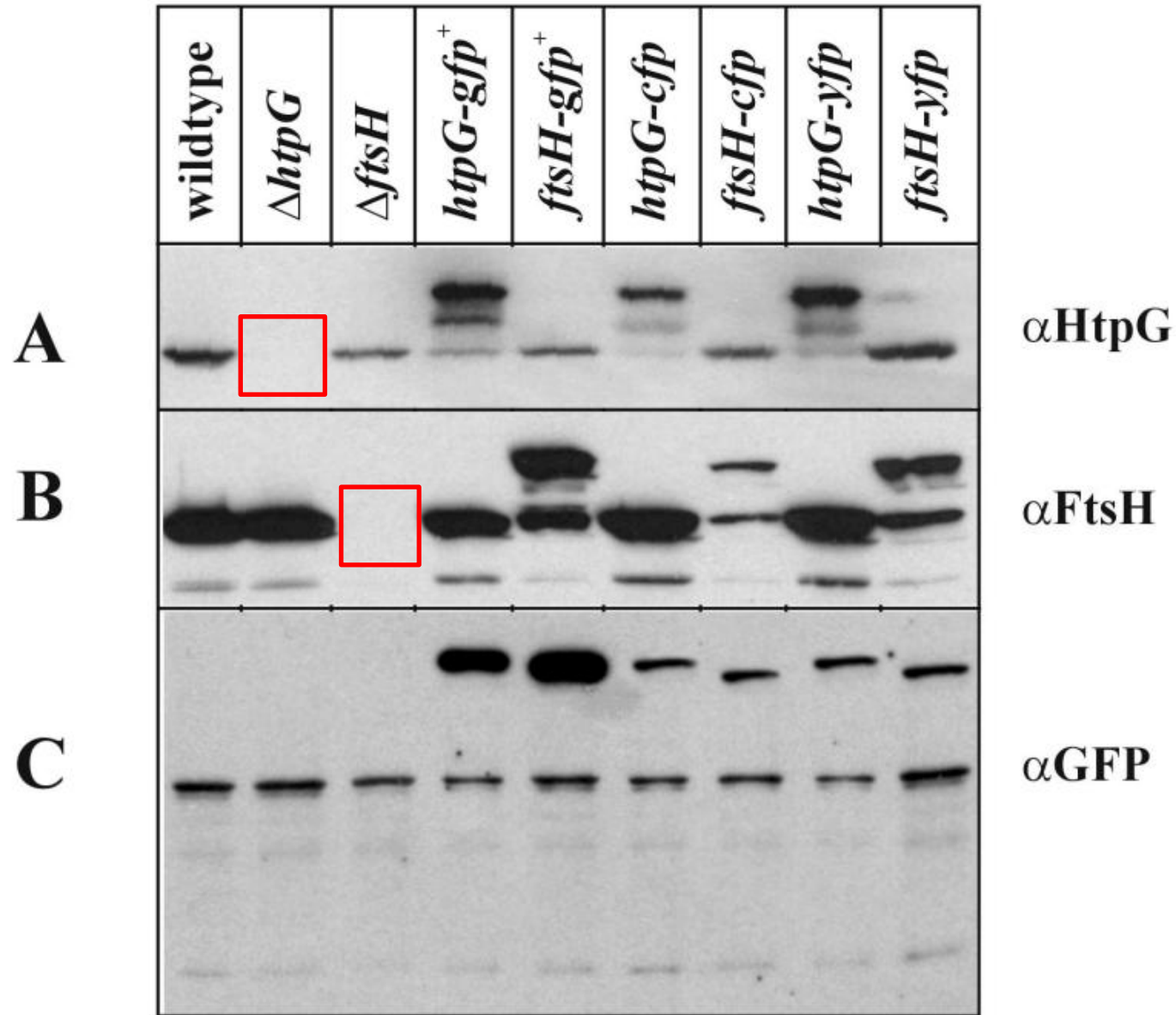
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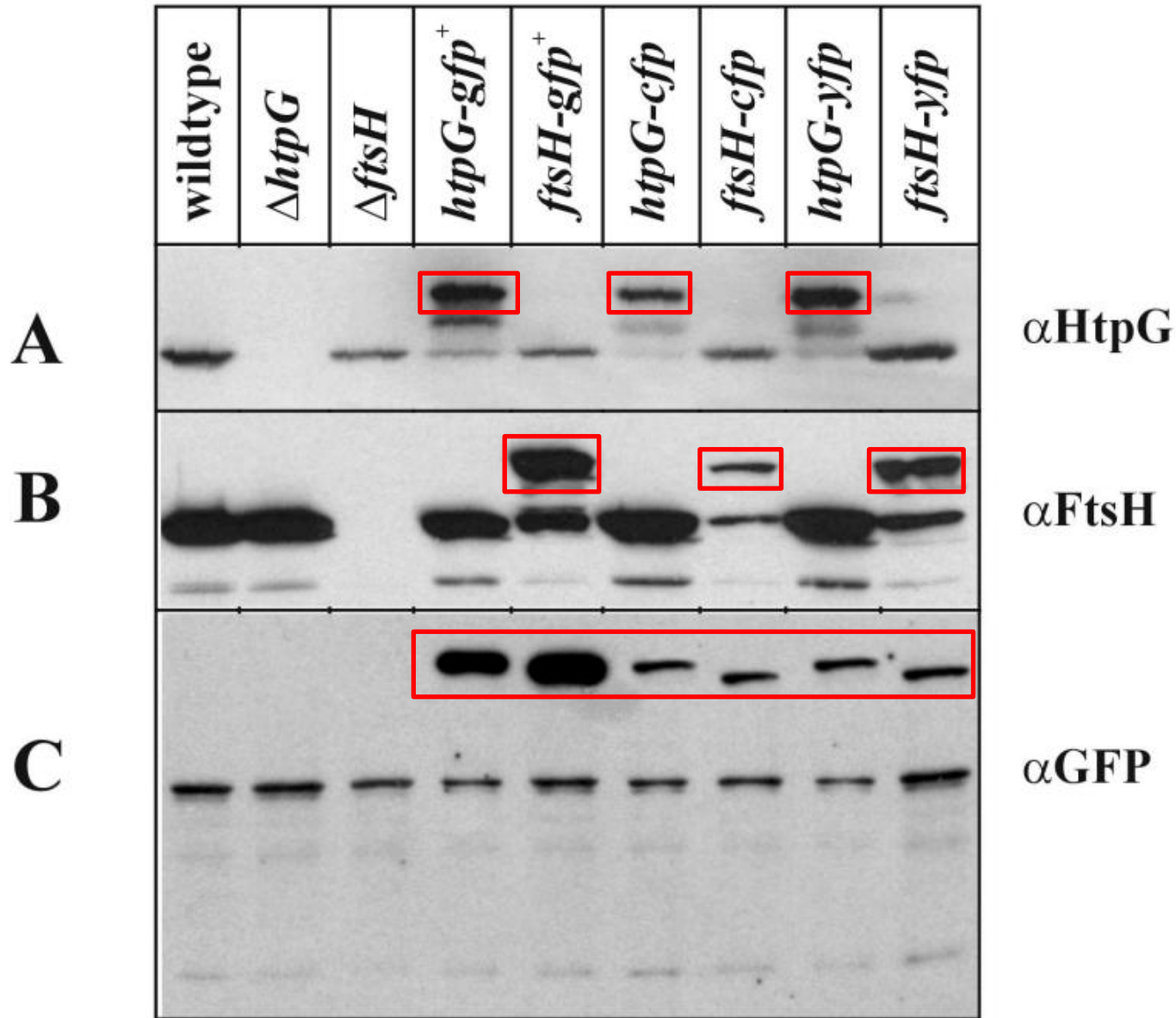
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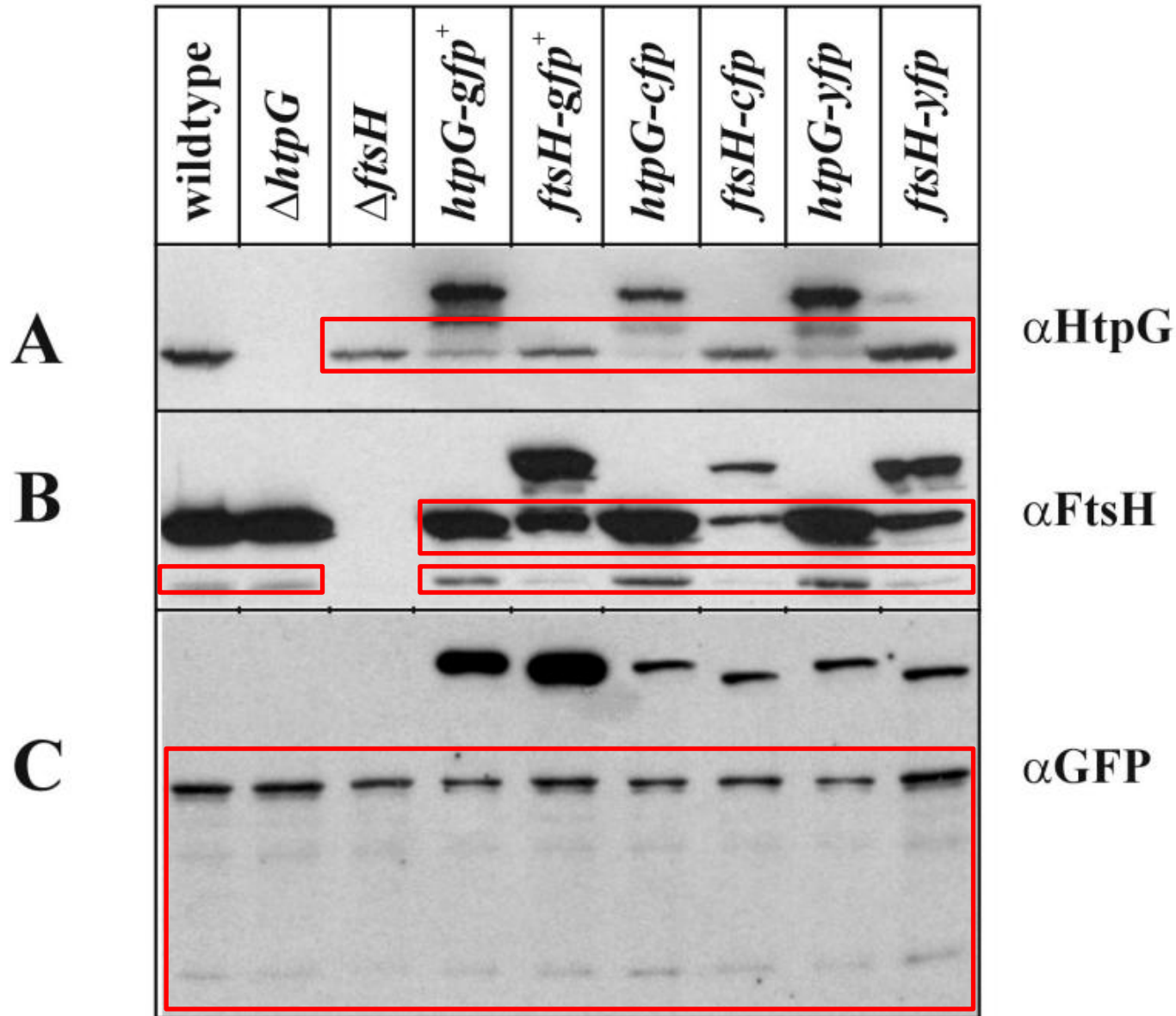
3) Detection of fusion proteins with fluorescent-tags



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3) Detection of fusion proteins with fluorescent-tags

b) Detection by fluorescence microscopy

- **problem: only filters for GFP are purchasable**
 - only GFP is detectable**
- **for FtsH-GFP: data already available from other research groups**

3) Detection of fusion proteins with fluorescent-tags

c) Detection by fluorometer

- all three fluorescent proteins detectable
- usage of HtpG-plasmids: inducible by heat shock

- **results:**

Fusion protein	Excitation at (nm):	Emission at (nm):	Relative fluorescence after heat shock	
			0 min	30 min
HtpG-CFP	433	475	0	5
HtpG-YFP	513	527	0	8
HtpG-GFP ⁺	488	510	10	25

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- **conclusion: all 3 fluorescent-plasmids work for cytoplasmic proteins (HtpG) as well as for membrane-bound (FtsH)**

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- **epitopes allow not only the detection of the tagged proteins (antibodies necessary) but furthermore their purification by affinity chromatography**
- **the localization of proteins by fluorescence-tagging is possible**
- **the validation for functionality of the six tagging-vectors was positive**
- **downstream-genes of a polycistronic operon are under the control of an IPTG-regulated promoter**
- **the plasmids can be used in every bacterium that is not able to replicate pBr322-based plasmids**

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The six tagging-vectors offer the possibility to fuse tagging sequences to any gene of *B. subtilis* or other bacteria.

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- 1. Was versteht man unter einem Epitop-Tag?**
- 2. Welchen Vorteil bietet die Fusion eines Epitop-Tags an ein Protein?**

Was versteht man unter einem Epitop-Tag?

Epitop = Teil eines Proteins (spezifische Gruppe/AS-Sequenz), das von einem Antikörper erkannt wird

Tag = zusätzliche AS an einem rekombinantem

Protein, die die Aufreinigung erleichtern oder z.B. der Lokalisation dienen

→ Epitop-Tag = zusätzliche AS am rekombinanten

Protein, die durch Antikörper erkannt werden

können

Welchen Vorteil bietet die Fusion eines Epitop-Tags an ein Protein?

- 1. Vorteil = schnelle, billige Methode, da Antikörper für die Epitope gekauft werden können; „normal“ hergestellte Antikörper sind teuer, zeitaufwändig und qualitativ schwankend (Protein reinigen, Immunisierung eines Tieres, Extraktion und Reinigung des Antikörpers)**
- 2. Vorteil = Epitop-Tag ist sehr klein und stört die Proteineigenschaften nicht/nur minimal**