

## BB3cH\_pGAP\_23\*\_pPFK300\_Cas9

(Plasmid #104912)

### Purpose

hCas9 under control of pPFK300 for direct cloning of HH-sgRNA-HDV PCR products for episomal expression in *P. pastoris* and selection on Hyg

### Depositing Lab

[Brigitte Gasser](#)

### Publication

[Gassler et al Methods Mol Biol. 2019;1923:211-225. doi: 10.1007/978-1-4939-9024-5\\_9.](#)

([How to cite](#) ↓)

### Sequence Information

[Sequences \(1\)](#)

## Ordering

This material is available to academics and nonprofits only.

Item	Catalog #	Description	Quantity	Price (USD)	
Plasmid	104912	Standard format: Plasmid sent in bacteria as agar stab	1	\$85	<a href="#">Add to Cart</a>

### Backbone

**Vector backbone:** Episomal ARS/CEN Backbone

**Vector type:** Yeast Expression, CRISPR

**Selectable markers:** Hygromycin

### Growth in Bacteria

**Bacterial Resistance(s):** Hygromycin, 200 µg/mL

**Growth Temperature:** 37°C

**Growth Strain(s):** DH5alpha

**Copy number:** High Copy

### Gene/Insert 1

**Gene/Insert name:** HH - FS23 linker - HDV

### Cloning Information for Gene/Insert 1

**Cloning method:** Restriction Enzyme

**5' cloning site:** BbsI (not destroyed)

**3' cloning site:** BbsI (not destroyed)

**5' sequencing primer:** pGAP fw

([Common Sequencing Primers](#))

### Gene/Insert 2

**Gene/Insert name:** human codon-optimized hcas9 sequence fused to a nuclear localization signal (NLS)

**Alt name:** hcas9

**Species:** *H. sapiens* (human); *Streptococcus pyogenes*, *Pichia pastoris*

**Insert Size (bp):** 4140

### Resource Information

**Supplemental Documents:**

- [BB3\\_pGAP\\_Link\\_BbsI\(intern\)\\_RPS25Att\\_pPFK300\\_Cas9\\_cycT\\_T\\_Hyg.gbk](#)

**A portion of this plasmid was derived from a plasmid made by:** The original Cas9 sequence was taken from from DiCarlo et al., (Addgene ID 43802). Reference: DiCarlo JE, Norville JE, Mali P, Rios X, Aach J, Church GM. 2013. Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. *Nucleic Acids Res* 41:4336-43.

## Terms and Licenses

### Academic/Nonprofit Terms:

- [UBMTA](#)

### Industry Terms:

- Not Available to Industry

### Trademarks:

- Zeocin® is an InvivoGen trademark.

## Depositor Comments

Additional Publication: Prielhofer, R. et al (2017) BMC Syst Biol. 11,123 (PMID:29221460)

### How to cite this plasmid

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These plasmids were created by your colleagues. Please acknowledge the Principal Investigator, cite the article in which the plasmids were described, and include Addgene in the Materials and Methods of your future publications.

For your **Materials & Methods** section:

BB3cH\_pGAP\_23\*\_pPFK300\_Cas9 was a gift from Brigitte Gasser (Addgene plasmid # 104912 ; <http://n2t.net/addgene:104912> ; RRID:Addgene\_104912)

For your **References** section:

**CRISPR/Cas9-Mediated Homology-Directed Genome Editing in *Pichia pastoris*.** Gassler T, Heisteringer L, Mattanovich D, Gasser B, Prielhofer R. *Methods Mol Biol.* 2019;1923:211-225. doi: 10.1007/978-1-4939-9024-5\_9. 10.1007/978-1-4939-9024-5\_9 [PubMed 30737742](#)

