

Resource Summary Report

Generated by [RRID](#) on Aug 18, 2026

pKM512

RRID:Addgene_140192

Type: Plasmid

Proper Citation

RRID:Addgene_140192

Plasmid Information

URL: <http://www.addgene.org/140192>

Proper Citation: RRID:Addgene_140192

Insert Name: zeocin resistance gene

Bacterial Resistance: Bleocin (Zeocin)

Defining Citation: [PMID:30538179](#)

Vector Backbone Description: Backbone Marker:K. Murphy; Backbone Size:816; Vector Backbone:pKM461; Vector Types:Bacterial Expression; Bacterial Resistance:Bleocin (Zeocin)

Comments: This plasmid can be used to removed ORBIT integrated plasmids leaving behind an Bxb1 attP site. After deletion of a target gene using ORBIT, the integrated plasmid (e.g., pKM464 - hygR) is excised by expressing phage Bxb1 Integrase and gp47 (directionality factor) from pKM512. A strain containing an ORBIT-generated deletion is transformed with pKM512 selecting for zeocin resistance. (This step cures the cell of pKM461 (kanR) used to make the deletion.) Select a zeoR colony containing pKM512 and grow to saturation in 7H10-zeocin. Dilute the culture 100-fold and regrow the strain in 7H10 without zeocin, but include 500 ng/ml anhydrotetracycline, (which induces expression of Integrase and gp47). Grow to saturation again, plate for single colonies, stab colonies on 7H10 plates containing 10% sucrose (Smeg) or 3% sucrose (Mtb) looking for hygromycin-sensitive colonies. Colonies should also be sensitive to kanamycin and zeocin. Once performed, the strain cannot be used for deletion of a second gene using ORBIT, as an attP site is now present at the site of the initial deletion.

Plasmid Name: pKM512

Relevant Mutation: Kanamycin resistance gene in pKM461 replaced with zeocin resistance gene by recombineering

Record Creation Time: 20240105T080217+0000

Record Last Update: 20260815T082830+0000

Ratings and Alerts

No rating or validation information has been found for pKM512.

No alerts have been found for pKM512.

Data and Source Information

Source: [Addgene](#)

Usage and Citation Metrics

We have not found any literature mentions for this resource.