Bird of Prey™ QUICKSTART: Mono-Effector Monogene

Bird of Prey genes in the form of CloneCards or CloneTabs can be stored at room temperature (15-25ºC) for up to 24 months.

For more information, please refer to the Bird of Prey Handbook, which can be found at www.ospreybio.com. For technical assistance, please contact us at clonecard@ospreybio.com

Notes before starting

- It is recommended to perform a plasmid prep for each gene involved in your vector design to ensure enough DNA is available for subcloning.
- We recommend 1µl of enzyme in restriction digests regardless of units/µl to ensure consistency, efficiency, and proper enzyme activity.

Materials Needed:

- BoP Vectors: Effector & Controller
- Restriction enzymes: PvuI & KasI
- T4 DNA Ligase and buffer
- Quick CIP
- Gel electrophoresis equipment
- DNA fragment purification kit
- Competent E. coli cells

Step-by-Step Procedure:

1. Design
   - Choose appropriate BoP Controller and Insert vectors.
2. Digest
   - Set up two separate digestion reactions:
     a) Controller Vector: PvuI + KasI
     b) Insert Vector: PvuI + KasI
   - Incubate for 2 hours at 37°C
   - Add CIP and incubate at 37°C for 1 hour

Sample ID	Buffer	Vector Acceptor (pDNA Backbone)	Insert Donor (DNA)	PvuI	KasI	CIP	H_20
x	4	20	-	1	1	1	13
xx	4	-	20	1	1	-	14

3. Gel Electrophoresis
   - Run digested samples on a 1% agarose gel using the above table volumes (in µl).
   - Visualize bands under UV light.

4. Fragment Purification
   - Excise desired bands from gel (try using OspreyBio's gel band cutters for faster and more accurate results).
   - Purify DNA using gel extraction kit (we recommend Zymo's "Zymoclean Kit" – Cat #D400 series).

5. Ligation
   - Mix purified Controller and Insert fragments in individual and labeled tubes.

- Add T4 DNA Ligase and buffer (see below table for values).
- Incubate at room temperature overnight.
- After 12-24 hours, heat shock at 37ºC to stop reaction

Sample ID	Buffer	Vector Acceptor (pDNA Backbone)	Insert	Ligase	H_20
TU1 (control)	10	5	-	1	84
TU1 + E1	10	5	10	1	74
TU1 + E2	10	5	10	1	74

6. Transformation
   - Transform ligation mixture into competent E. coli
   - Plate on Kanamycin antibiotic-containing agar
   - Incubate overnight at 37°C

7. Pick Colonies & perform miniprep
   - Pick two colonies from incubated plates
   - Add each to a culture tube of 3ml kanamycin LB broth
   - Place in a shaker incubator at 37°C overnight
   - Perform miniprep to isolate plasmid DNA
   - Verify construct via DNA sequencing
   - Proceed with downstream applications (e.g., protein expression)

Remember to always refer to the full Bird of Prey handbook for detailed protocols and troubleshooting advice. This QuickStart guide is intended as a concise reference for experienced users.