EIGC Genome Editing Workflow Objectives

Planning

- Design and plan gRNAs (up to 3 gRNAs if desired and available)
- Design genomic cleavage primers
- Design diagnostic primers and probes
- Order cell line, if needed

Testing the Guides

- Test gRNA in CRISPRMAX vs Neon Transfection Reagent

Transfection and Generation of Pool

- Perform transfection with gRNA using CRISPRMAX or Neon

Pool Analysis

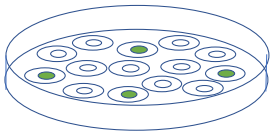
- Detect mutation using diagnostic test
  - ddPCR
  - genomic cleavage assay
  - RFLP
  - PCR with agarose gel; Sanger and Inference of CRISPR Edits (ICE)

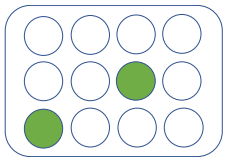
Clonal Isolation

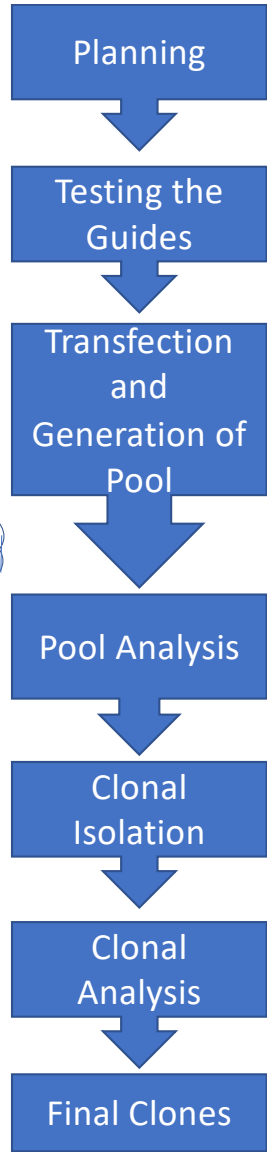
- Grow single cell clones

Clonal Analysis

- Determine positive clones by ddPCR, PCR with agarose gel, Sanger sequencing
- Expand clones and validate edits

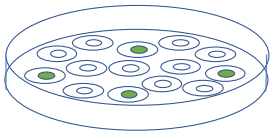


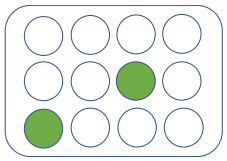


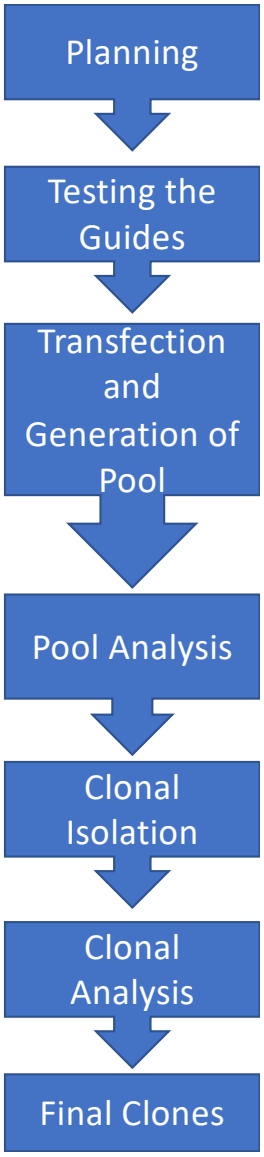


Genome Editing Options- Price Comparison

	EIGC Cost	Synthego Cost	Total Cost
Knock-in- Regular Cell Line			
Transfected Pool	$4,500	$9,250-$11,250	
Clones	$10,200	$18,500-$20,500	
Synthego/EIGC Partnership Clones	$5,750 + $9,250-$11,250		$15,000-$17,000
Knock-out- Regular Cell Line			
Transfected Pool	$2,800	$2,000-$4,000	
Clones	$5,800	$11,400-$13,400	
Synthego/EIGC Partnership Clones	$3,000 + $2,000-$4,000		$5,000-$7,000







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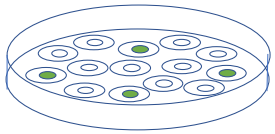
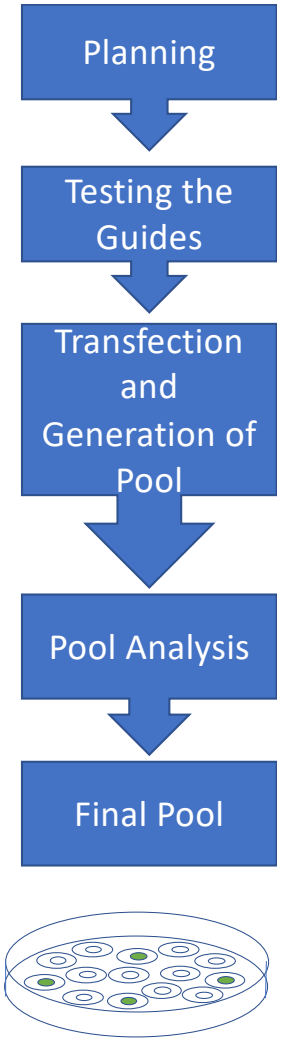
Knock-in: Transfected Pool



- Cost: $4,500
- What you get
  - gRNA design and file containing the sequence of any gRNA and primer utilized for editing and analysis
  - cell line will be validated before and after gene editing
  - transfection of cell line using CRISPRMAX or Neon Transfection System
  - 2 vials of a transfection pool
  - report of % of cells with positive edits
    - ddPCR for <10 bp knock-in
    - Sanger sequencing and ICE analysis for <200 bp knock-in
    - PCR at junctions and Sanger for >200 bp knock-in



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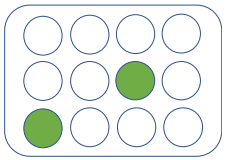
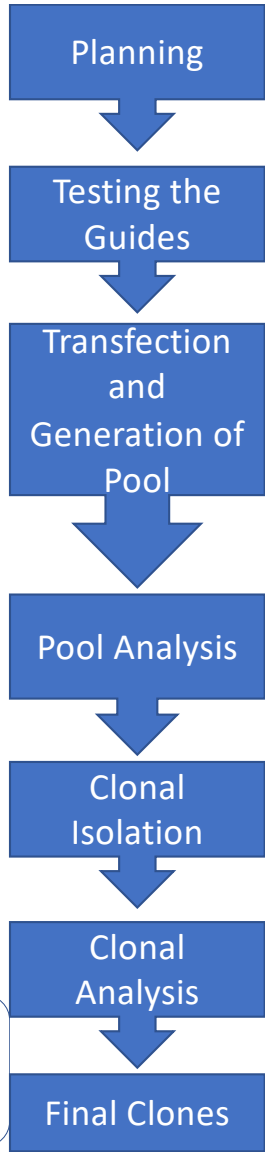
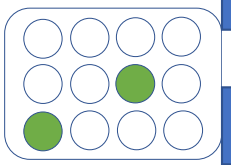
Knock-in: Clones



- Cost: $10,200
- What you get
  - gRNA design and file containing the sequence of any gRNA and primer utilized for editing and analysis
  - cell line will be validated before and after gene editing
  - transfection of cell line using CRISPRMAX or Neon Transfection System
  - 2 vials of a transfection pool
  - report of % of cells with positive edits
  - isolation of clones (homozygous and heterozygous when needed), 2 vials of each clone
  - report for each clone





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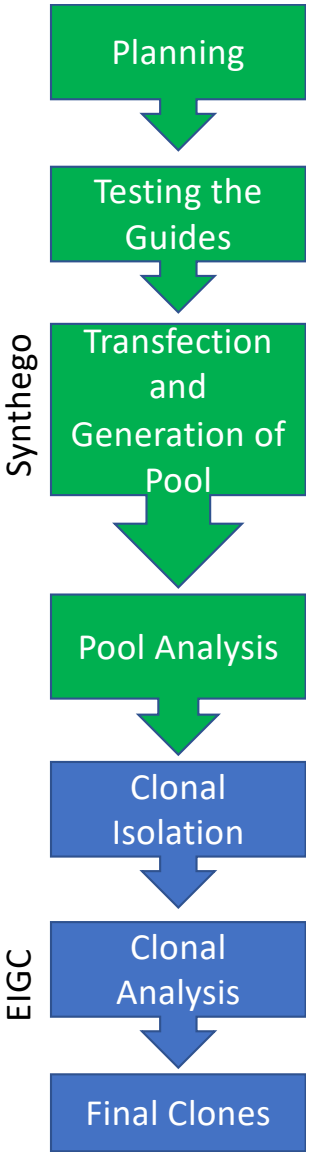
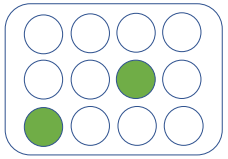
Knock-in: Synthego/EIGC Partnership Clones

- Total Cost: $15,000-$17,000
- Synthego Cost:
  - $9,250 (if Synthego has cells)
  - $11,250 (if Synthego needs to purchase the cells or you have to supply the cells)
- EIGC Cost: $5,750
- What you get:
  - Synthego:
    - 2 Vials of KI Pool (1M cells/vial), guaranteed or you're not invoiced
    - 2 Vials of Wild-Type Cells (1M cells/vial)
    - Milestone reports sent periodically; Final project report
    - Certificate of Analysis
    - Sequence of guides used; Primer sequences for PCR/sequencing
    - ICE Report (pre/post-thaw)
  - EIGC:
    - isolation of clones (homozygous and heterozygous when needed), 2 vials of each clone
    - report for each clone





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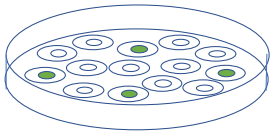
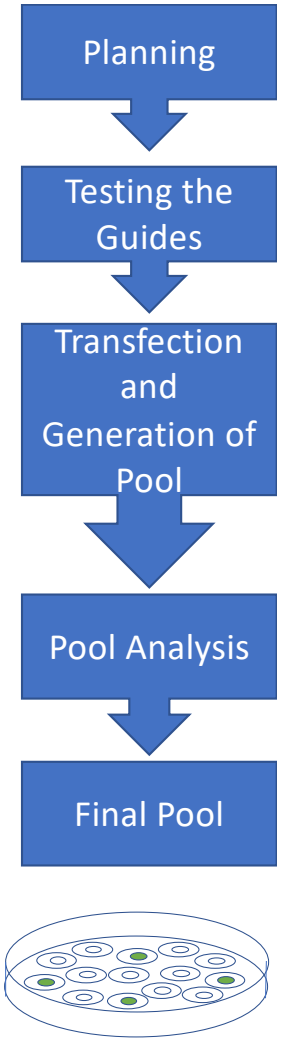
Knock-out: Transfected Pool



- Cost: $2,800
- What you get
  - gRNA design and file containing the sequence of any gRNA and primer utilized for editing and analysis
  - cell line will be validated before and after gene editing
  - transfection of cell line using CRISPRMAX or Neon Transfection System
  - 2 vials of a transfection pool
  - report of % of cells with positive edits
    - PCR and agarose gel
    - Sanger sequencing and ICE analysis



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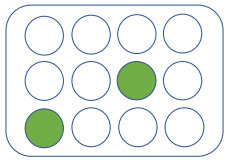
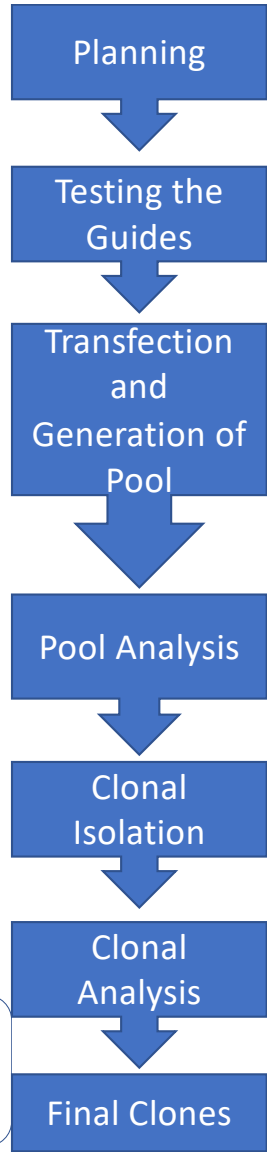
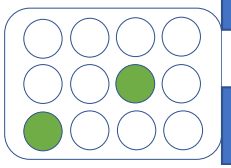
Knock-out: Clones



- Cost: $5,800
- What you get
  - gRNA design and file containing the sequence of any gRNA and primer utilized for editing and analysis
  - cell line will be validated before and after gene editing
  - transfection of cell line using CRISPRMAX or Neon Transfection System
  - 2 vials of a transfection pool
  - report of % of cells with positive edits
  - isolation of clones (homozygous and heterozygous when needed), 2 vials of each clone
  - report for each clone





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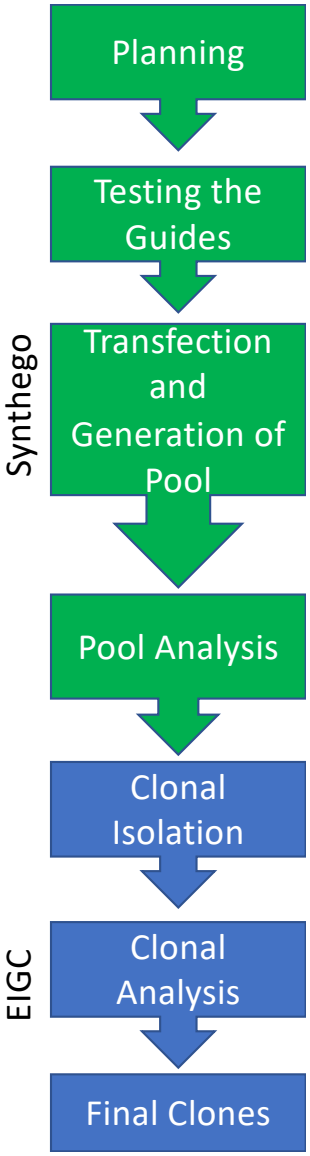
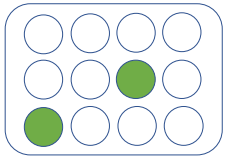
Knock-out: Synthego/EIGC Partnership Clones

- Total Cost: $5,000-$7,000
- Synthego Cost:
  - $2,000 (if Synthego has cells)
  - $4,000 (if Synthego needs to purchase the cells or you have to supply the cells)
- EIGC Cost: $3,000
- What you get:
  - Synthego:
    - 2 Vials of KO Pool (1M cells/vial), guaranteed or you're not invoiced
    - 2 Vials of Wild-Type Cells (1M cells/vial)
    - Milestone reports sent periodically; Final project report
    - Certificate of Analysis
    - Sequence of guides used; Primer sequences for PCR/sequencing
    - ICE Report (pre/post-thaw)
  - EIGC:
    - isolation of clones (homozygous and heterozygous when needed), 2 vials of each clone
    - report for each clone





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Summary

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Transfected Pool	$4,500	$9,250-$11,250	
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eigc@emory.edu