IND-enabling Small-Scale Guide RNA Production Under GMP for CRISPR Based Cell Therapies

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Disclosure

- I am an employee and shareholder of Editas Medicine

IND-enabling Small-Scale Guide RNA Production Under GMP for CRISPR Based Cell Therapies

This presentation will focus on the appropriate scale, final purity release specifications and GMP compliance for internal small-scale guide RNA production necessary to support our pre-clinical programs. It will also highlight the quality management system we have created and the guide RNA production clean rooms we have implemented at the Editas Boulder location.

Editas Medicine Research Pipeline

Cas12a advantage and how it relates to guide RNA production

Large scale manufacturing advantage of ~40/60mers vs 100mers

Strategy to create internal guide RNA manufacturing at the appropriate scale for our programs

Quality Systems to support GMP manufacturing

Internal GMP manufacturing will accelerate our pre-clinical programs

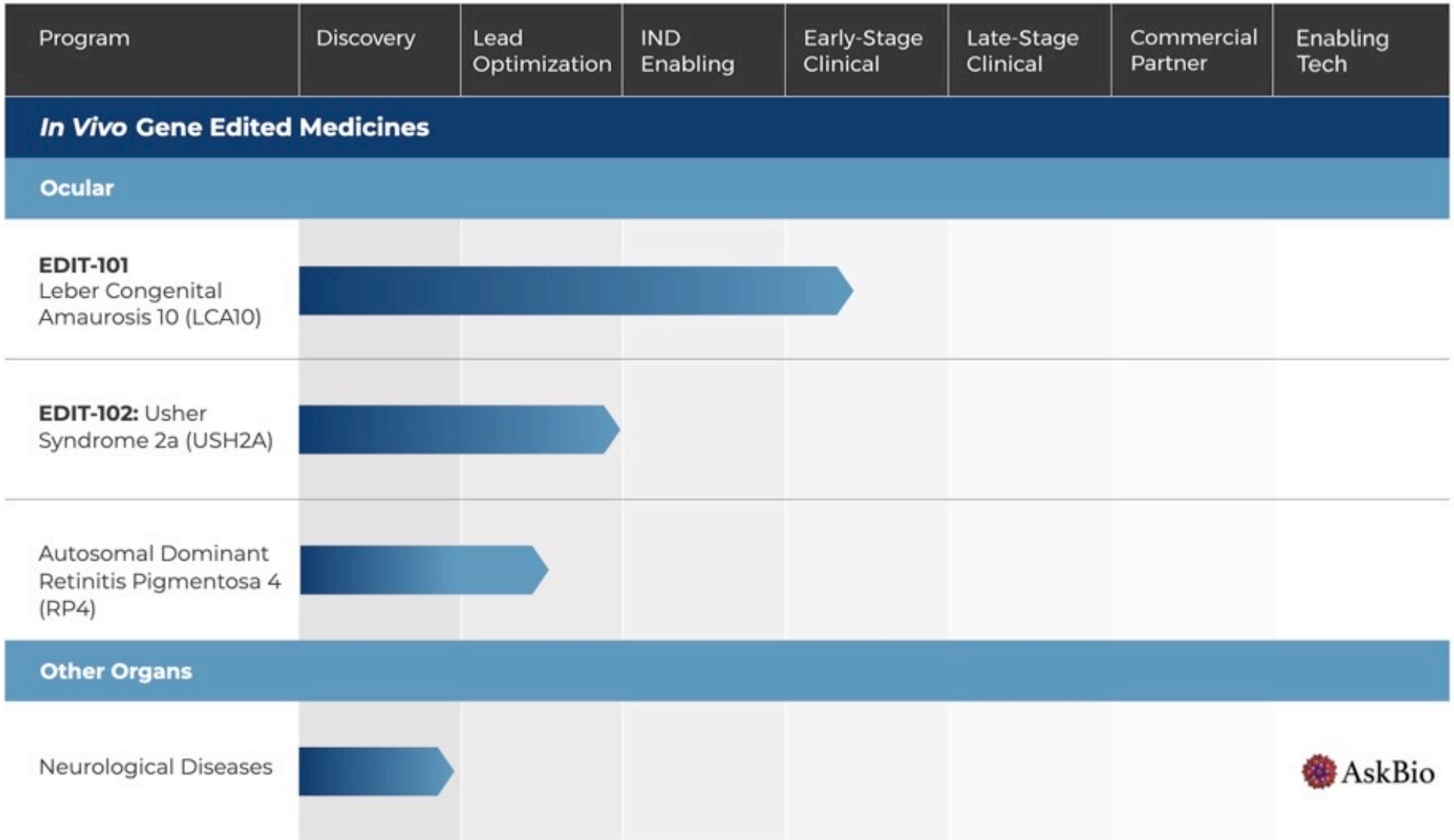
Editas Research and Pipeline

***In Vivo* Gene Edited Medicines**

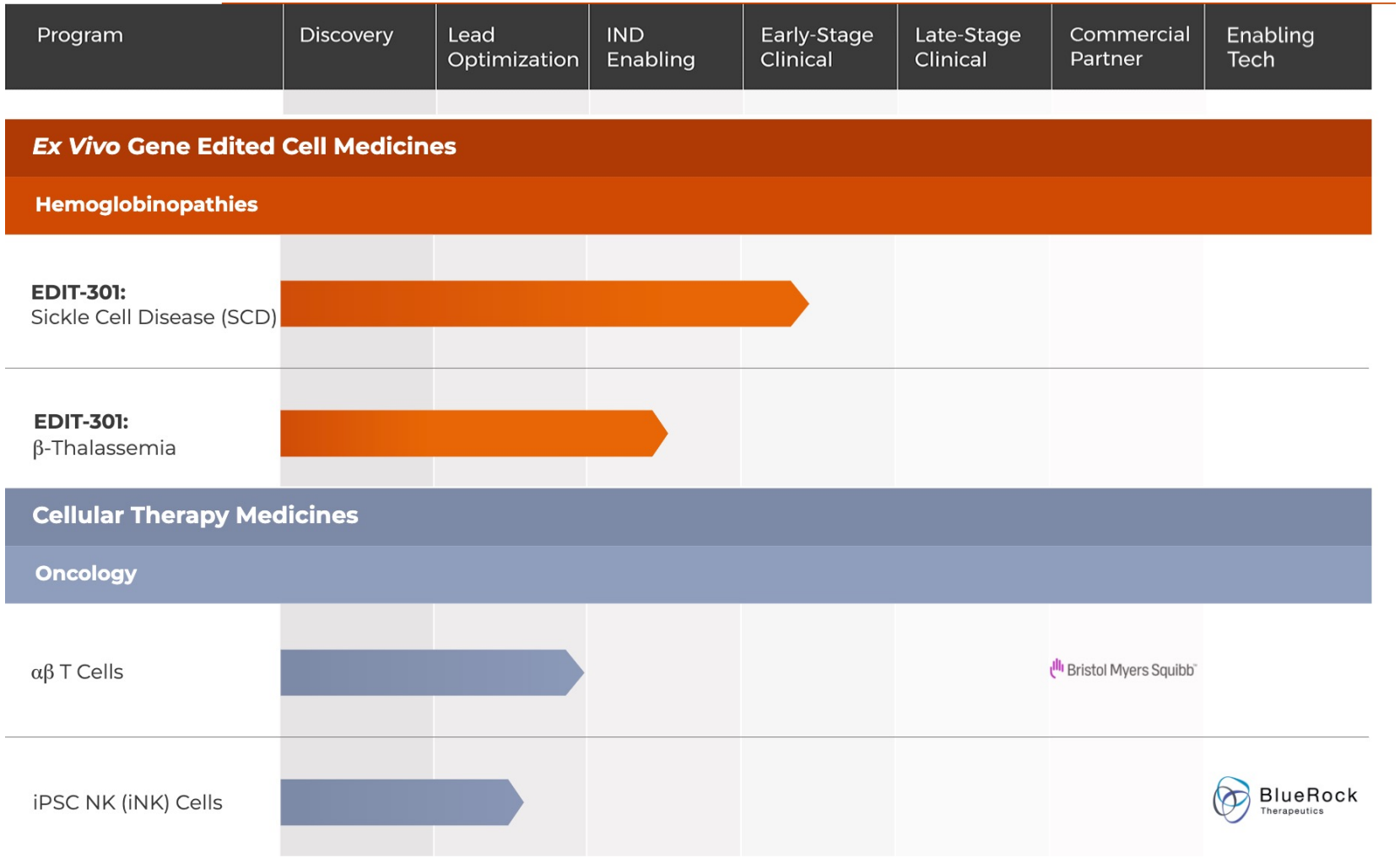
***Ex Vivo* Gene Edited Cell Medicines**

Cellular Therapy Medicines

In Vivo Research Pipeline



Ex Vivo Research Pipeline



Ex Vivo and Cell Therapy Medicines

EX VIVO	▪ Remove an individual's cells, edit them, then reintroduce them to the patient.
CELL THERAPY	▪ Create a universal cell population that can be edited and then given to any patient who needs it, without needing the patient to donate cells first.

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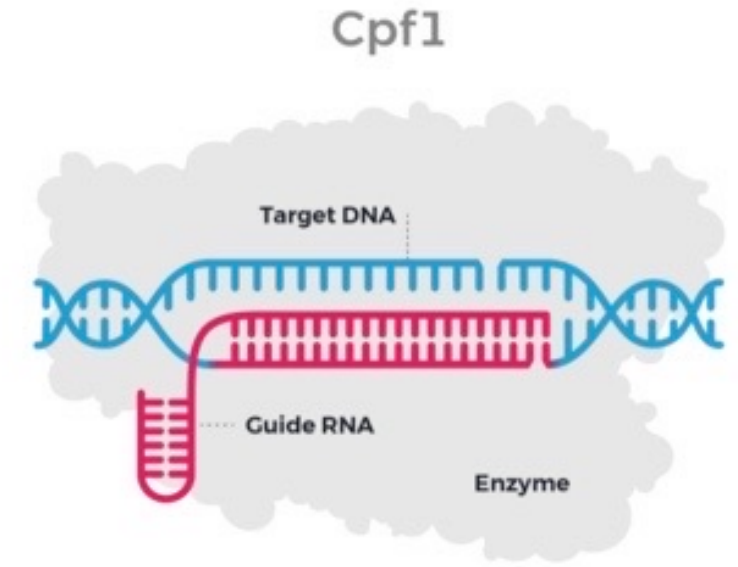
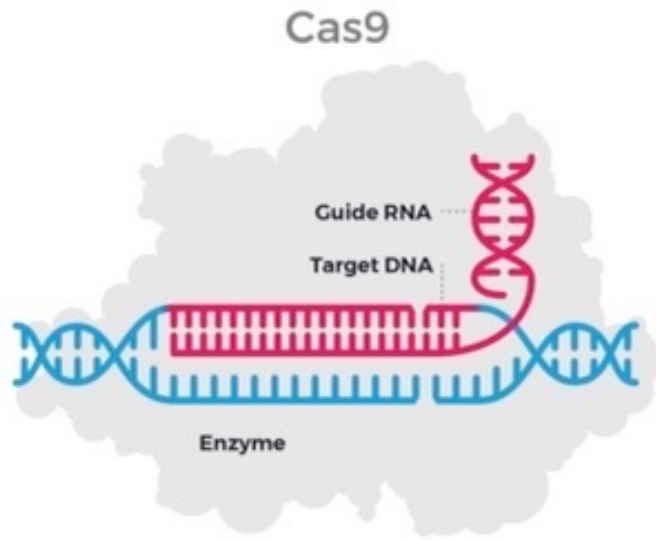
Cas12a/Cpf1 vs Cas9





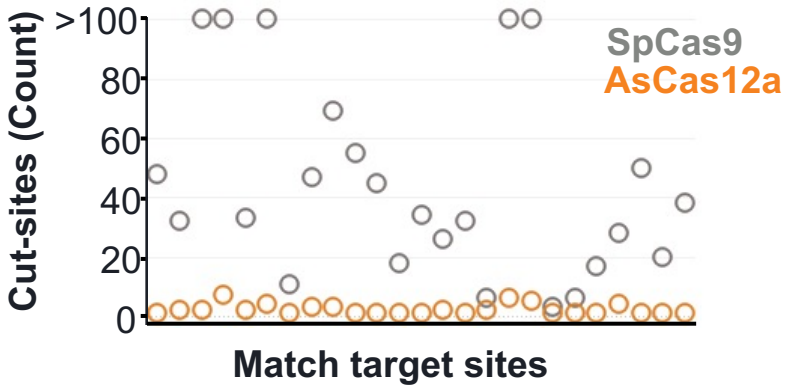
- gRNA has differences in protospacer adjacent motifs
- Staggered DNA cuts increase efficiency and accuracy for gene repair

- Programmable protein that specifically locates, binds to and edits the DNA of targeted genes
- Pairs with a guide RNA molecule that recognizes and initiates a double stranded break at target DNA sequence

The AsCas12a Nuclease has Higher Intrinsic Specificity and Higher Sequence Fidelity in the Shorter Chemically Synthesized Guide RNA

Specificity:

Matched Target Site (20-Ns):
TTTVNNNNNNNNNNNNNNNNNNNNNGG



Gotta et al. CSHL 2019

Takeaway:

AsCas12a is more specific across matched sites in the genome in contrast to SpCas9

Guide RNA synthesis:

Chemical synthesis of gRNAs occurs in the 3' → 5' direction and purity and yield of the entire gRNA sequence drops with increasing length

Cas12a gRNAs (~40mer) are much shorter than SpCas9 gRNAs (~100mer)

Cas9 guide is **most sensitive** to mismatches at 5' end which is the location of **lowest sequence fidelity** as this is where synthesis ends

Cas12a guide is **most sensitive** to mismatches at 3' end which is the location of **highest sequence fidelity** as this is where synthesis starts

Lack of sequence fidelity will lead to unanticipated off-target editing due to errors in RNA sequence targeting the protospacer region

Takeaway:

AsCas12a synthetic gRNAs have reduced risk of off-target editing that results from synthesis errors

References on Cas12a specificity:
Kim et al. Nat Biotech 2016, Kleinstiver et al. Nat Biotech 2016, Strohkendl et al. Mol Cell 2018, Swarts et al. Biochem Soc Trans 2019

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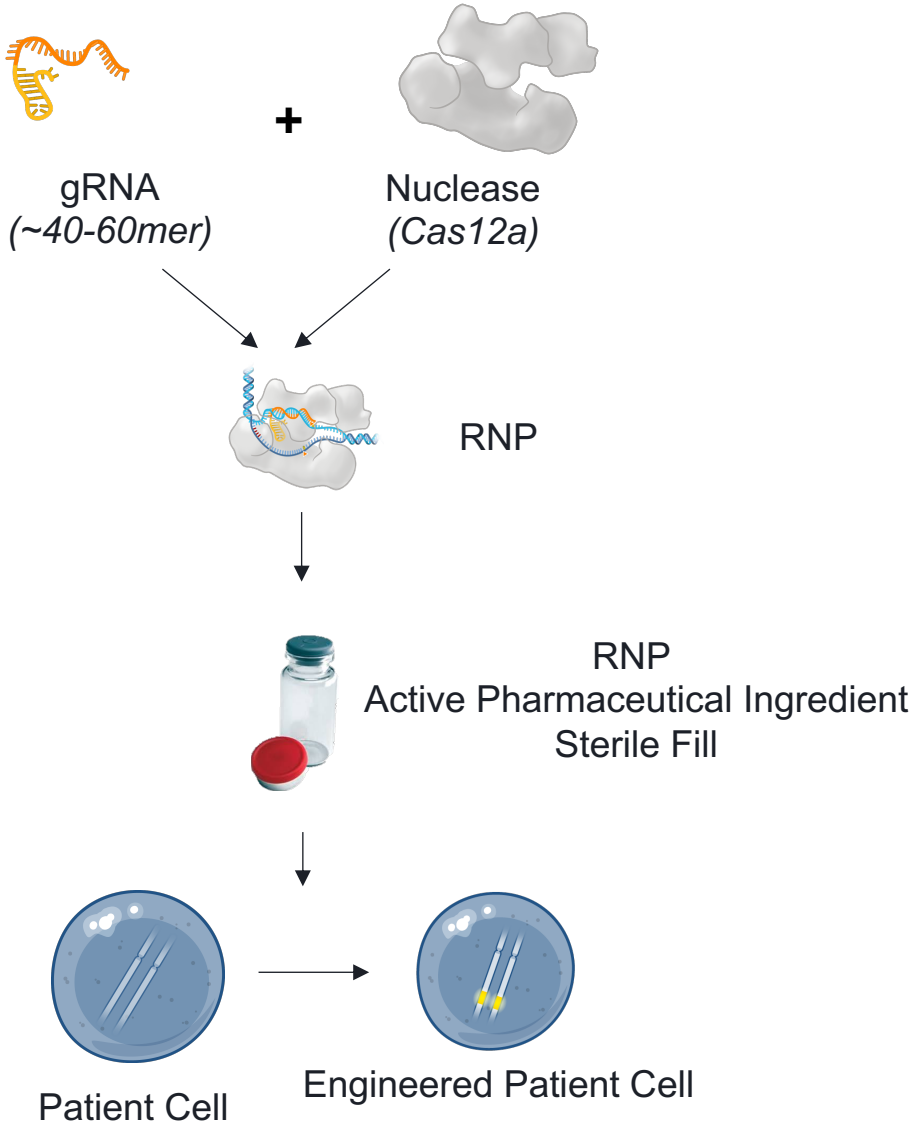
Ex Vivo Gene Edited Cell Medicine



Chemistry of guide RNA

RNA targeting region

DNA

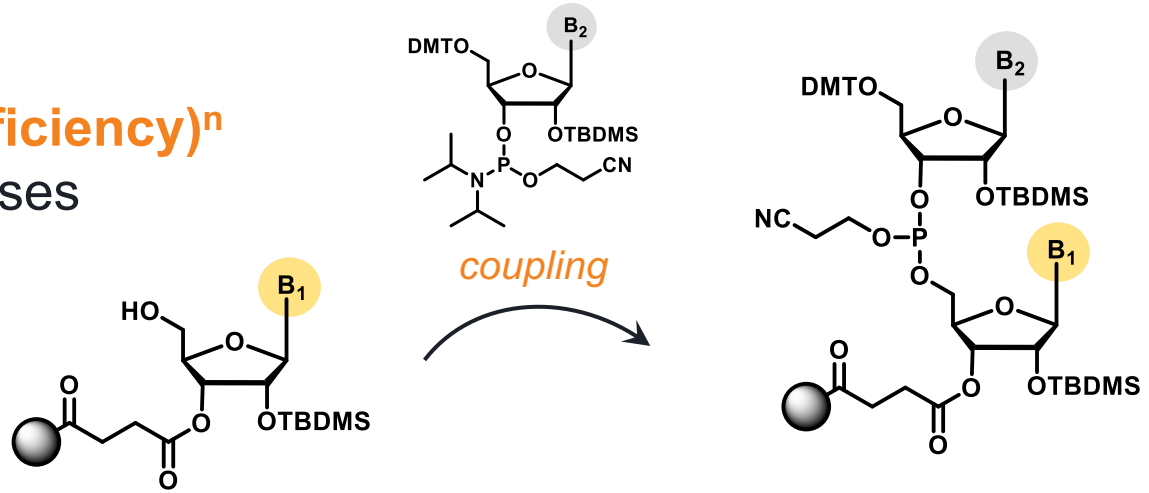
2’Omethyl modifications

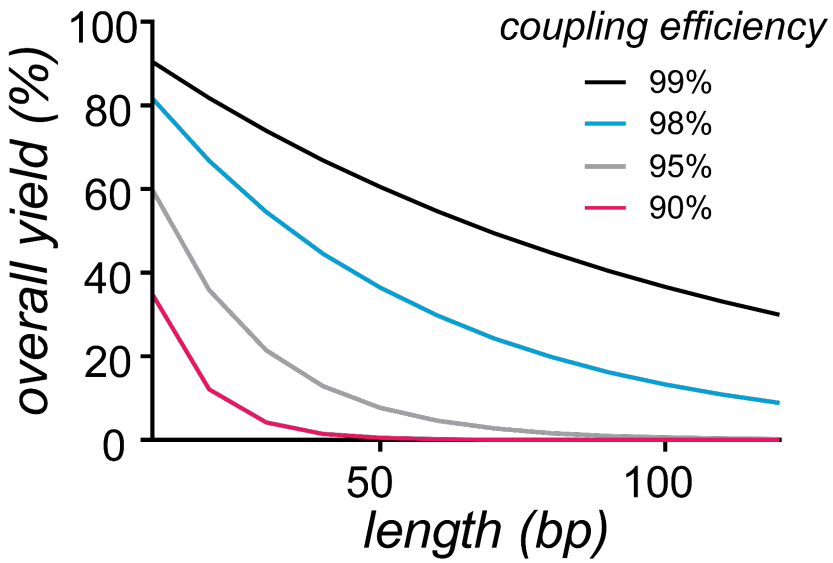
PS/PO

~40 to 60mers

Effect of coupling efficiency on yield

yield ~ (% coupling efficiency)^n
where n = # of bases



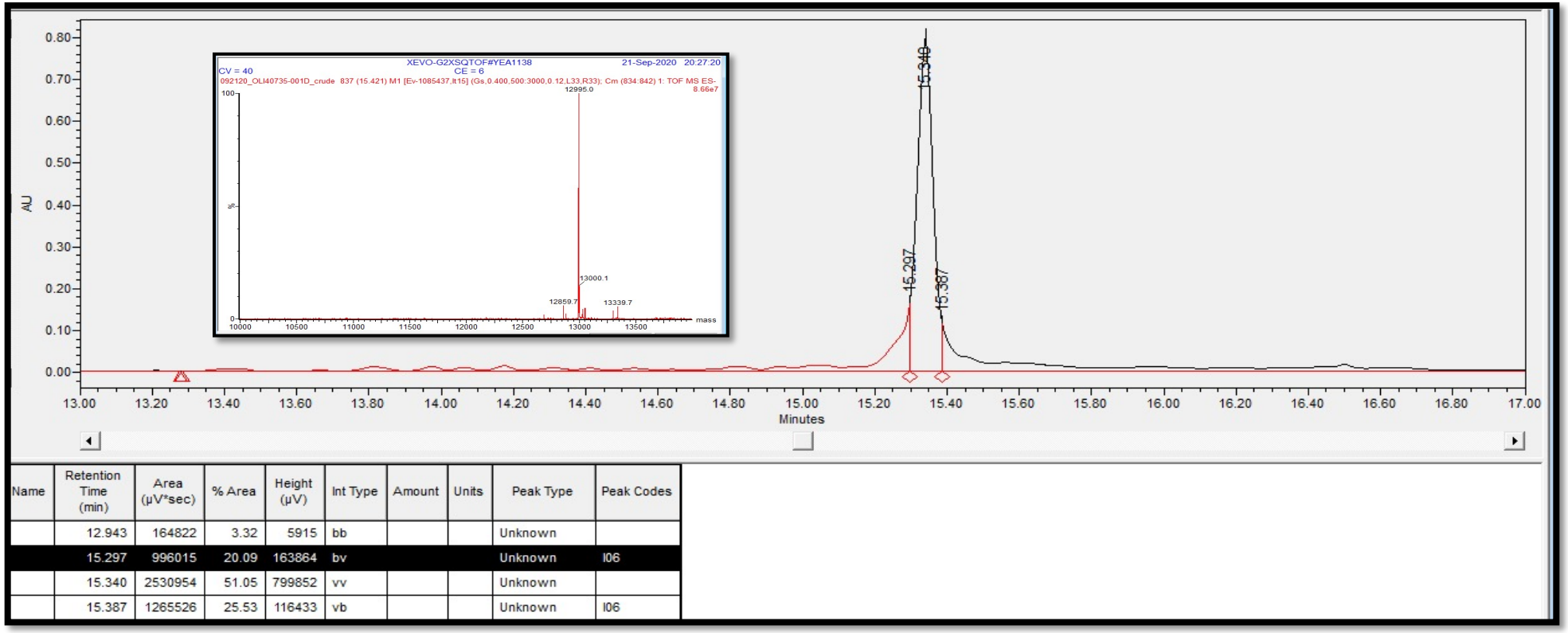


length	90%	95%	99%
34	2.8%	17%	71%
66	0.1%	3.4%	51%
100	<0.01%	0.6%	36%

shorter sequences:

- ❑ superior fidelity
- ❑ higher maximum yield

Typical Guide RNA Crude

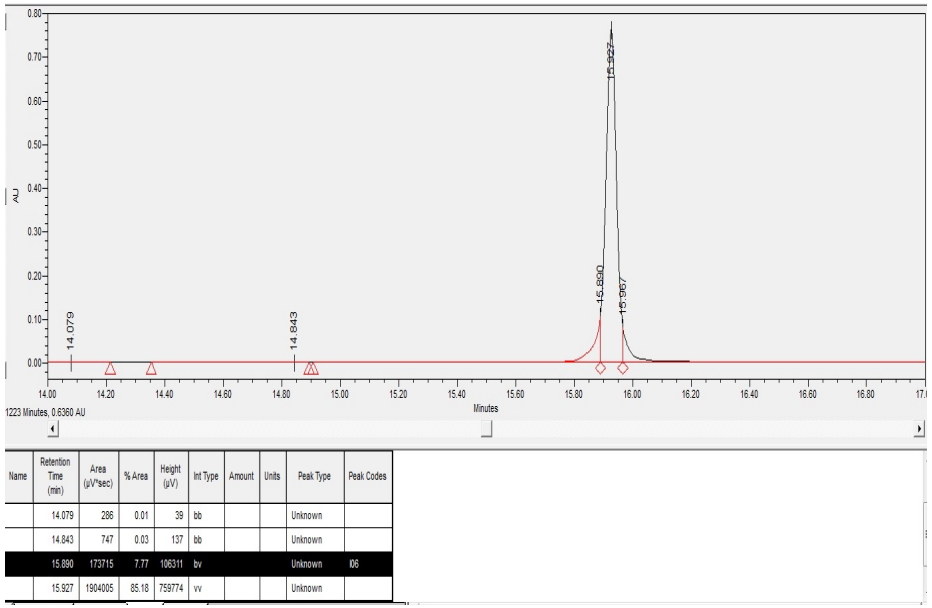


Name	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)	Int Type	Amount	Units	Peak Type	Peak Codes
	12.943	164822	3.32	5915	bb			Unknown	
	15.297	996015	20.09	163864	bv			Unknown	I06
	15.340	2530954	51.05	799852	vv			Unknown	
	15.387	1265526	25.53	116433	vb			Unknown	I06

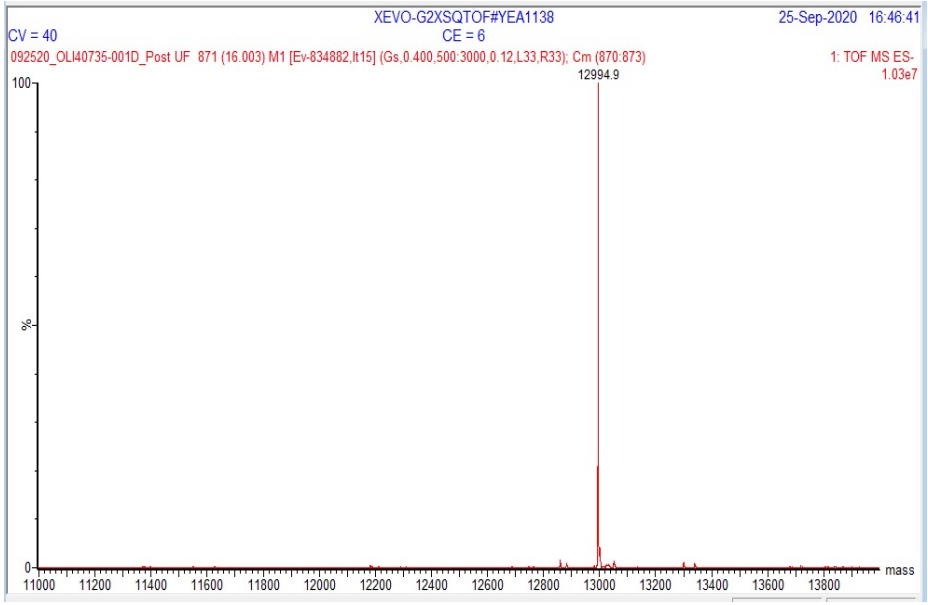
- 631 µmol scale synthesis
- Crude gross yield: 81700 ODU
- Crude full-length purity by UPLC: 51%
- Adjusted crude yield: 41667 ODU / 1287.02 mg
- Full length mass confirmed

Typical Guide RNA Final

OLI #	Batch#	Length	(A260-blank)	Pthlngth	Dil'n Fctr	Extinction Coefficient	moles/liter	uM	uL	pmol	nmol	mg
40735	D	41	0.649	1	200	420700	0.00030853	308.53	98000	30236272.88	30236.27	392.91



Name	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)	Int Type	Amount	Units	Peak Type	Peak Codes
	14.079	286	0.01	39	bb			Unknown	
	14.843	747	0.03	137	bb			Unknown	
	15.890	173715	7.77	106311	bv			Unknown	I06
	15.927	1904005	85.18	759774	vv			Unknown	



- 631 µmol scale synthesis
- Final yield: ~393 mg
- Final purity by UPLC: ~85%
- Full length mass confirmed

Overview

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Strategy to create internal guide RNA manufacturing at the appropriate scale for our programs

Quality Systems to support GMP manufacturing

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New Boulder Facility

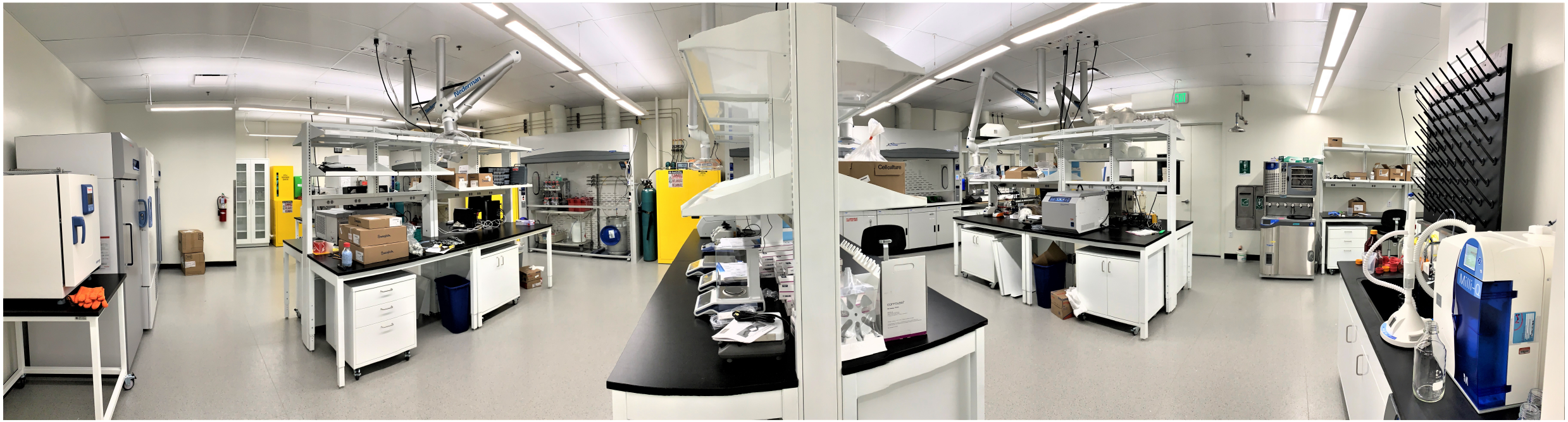
7,669 sf

Phase 1
Office Space

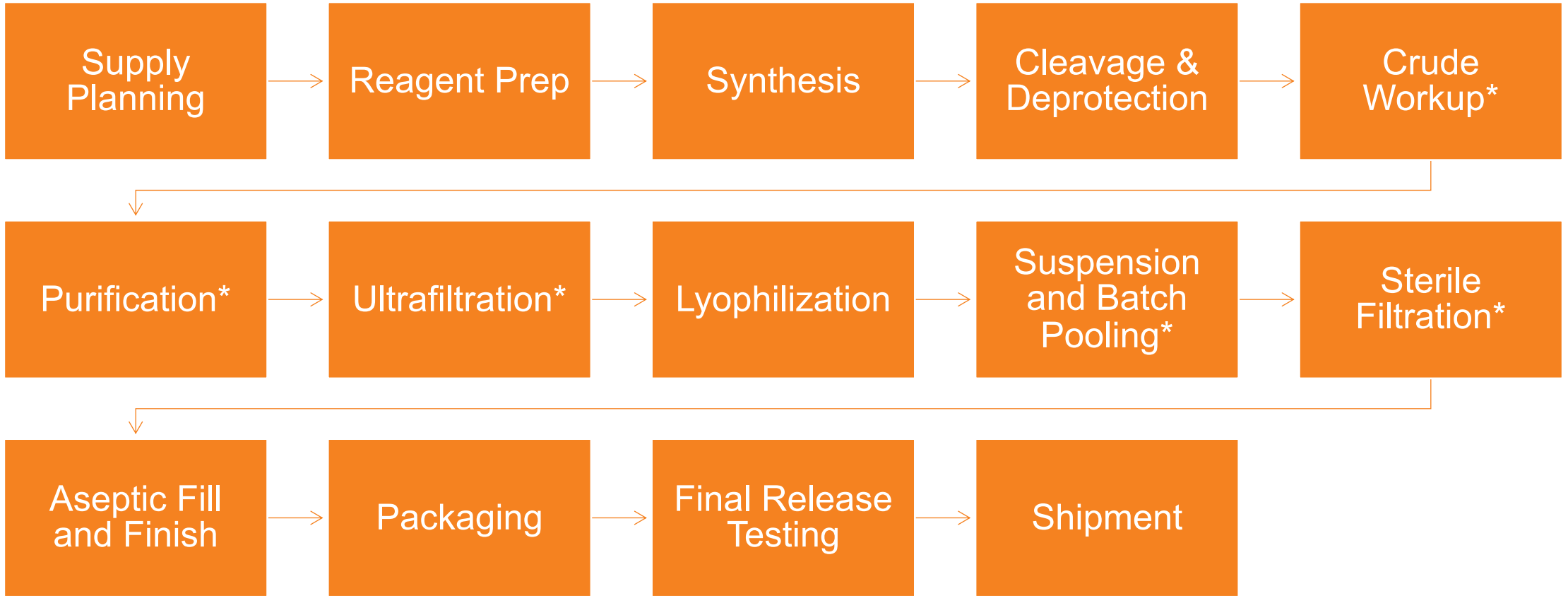
Phase 2
PD/AD Lab

Phase 3
GMP Manufacturing

New PD/AD Lab



Production Overview



* denotes an in-process testing point

gRNA GMP Manufacturing

Synthesis

- Akta™ Oligopilot™ 100 synthesizer
- Cleavage and Deprotection within enclosed hood space
- Located in controlled PD lab space

Purification

- Gilson Reverse Phase Prep System
- Ultrafiltration
- Located in Clean Room

Aseptic Fill and Finish

- Formulation
- Sterile Filtration
- Lyophilization
- Located in Clean Room

Boulder GMP Cleanroom

PD Lab Process Steps:
- Synthesis
- Cleavage / Deprotection
- Crude Ultrafiltration

Cleanroom Process Steps:
- Purification
- Final Ultrafiltration
- Lyophilization
- Final Fill & Finishing

In-Process QC analysis will be completed at Editas.

Final release testing will be completed at an external contract lab.

First GMP batch initiation expected in Q1 2022

Dual Duty: Lead Guide PD/AD and Synthesis Upstream Processing

PROCESS DEVELOPMENT/ANALYTICAL DEVELOPMENT

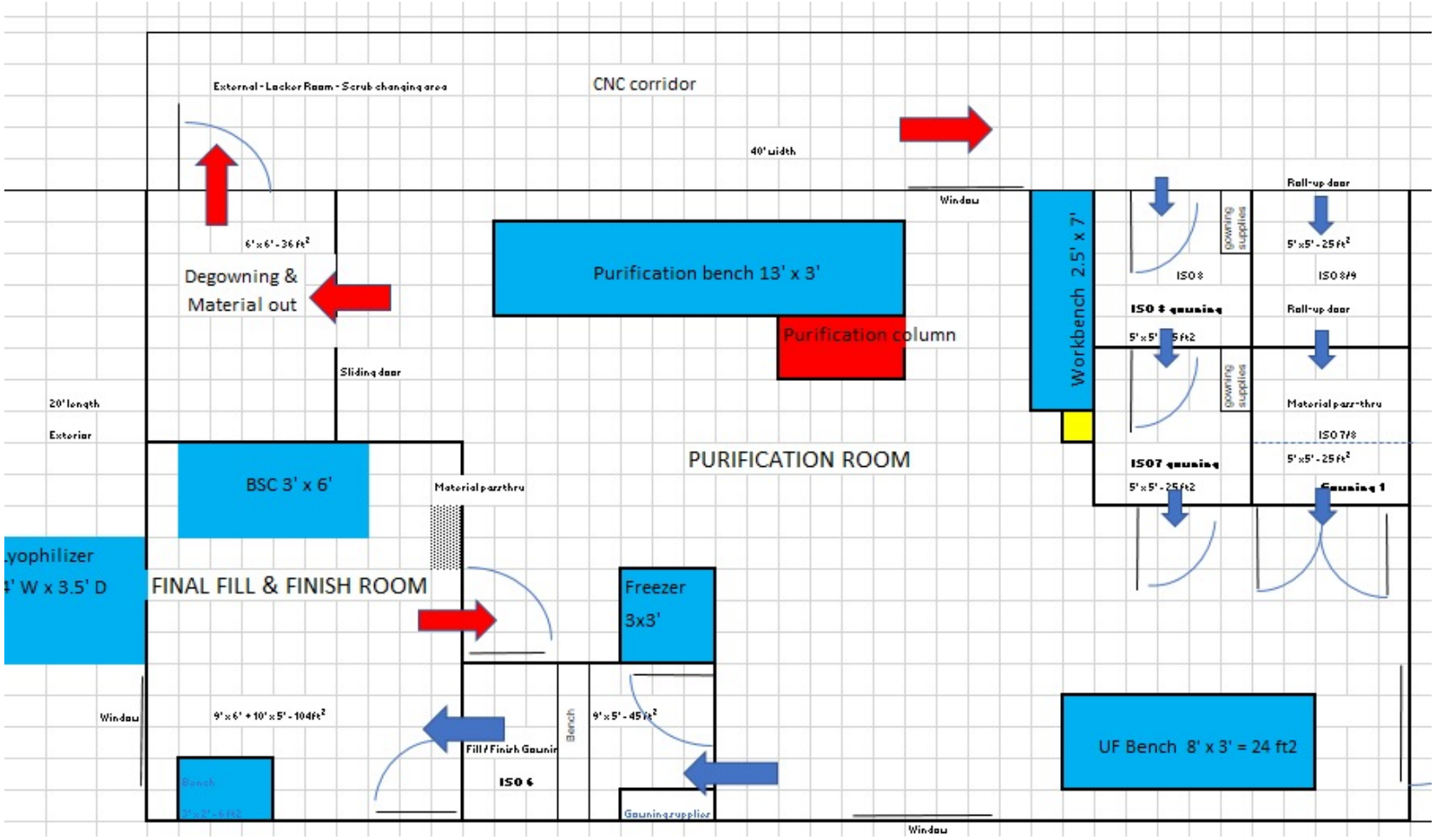
- Guide process optimization once a lead is declared by research
- Phosphoramidite and Reagent Prep
- Synthesis
- Cleavage and Deprotection
- Crude Ultrafiltration
- Purification
- Fraction Collection
- Final Ultrafiltration
- Lyophilization

SYNTHESIS UPSTREAM PROCESSING

- Phosphoramidite and Reagent Prep
- Synthesis
- Cleavage and Deprotection
- Crude Ultrafiltration

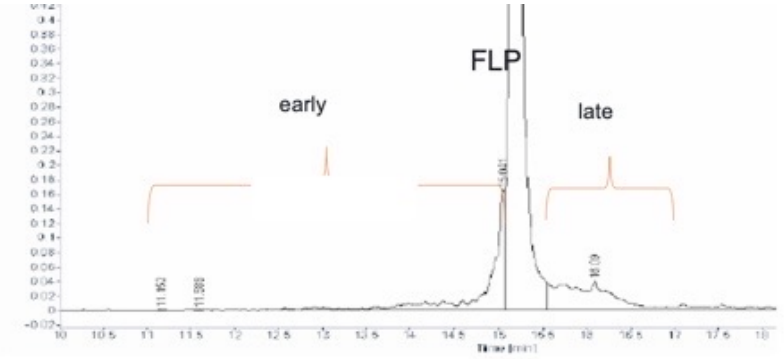
Boulder GMP Cleanroom

- CNC area (ISO 9)
- First airlock (ISO 8)
- Purification room (ISO 7)
- Fill / Finish Room (ISO 7)
- BSC (ISO 5)



Current gRNA Internal Analyses

Category	Attribute	Method	Current Acceptance Criteria
Identity	Molecular Weight	LCMS (TM-0001)	Consistent w/ Theor. Mass ±0.2%
Strength	Assay % w/w (anhydrous) by UV Assay	A_{260} UV/Vis (TM-0002)	Report Results
Purity	Purity	IP-RP-UHPLC (TM-0001 – final) (TM-0003 – faster fraction method)	Final product: ≥ typically 80.0% Crude and fractions: report results
	Total Impurities (Product Related)	IP-RP-UHPLC (TM-0001)	Report to 1 decimal: a) Early Eluting Cluster b) Late Eluting Cluster c) other peaks ≥ 0.1 %
	Impurity Quant/ID	IP-RP-UHPLC (TM-0001) (custom slow gradient methods)	Report Results



GMP guide RNA Testing (at CRO)

Category	Attribute	Method	Current Acceptance Criteria
Safety	Bioburden	USP <61>	≤100 CFU/100 mg TAMC/ TYMC
	Endotoxin	USP <85>	≤ 0.25 EU/mg (to two decimal places)
General	Appearance	Agilent QC-GNM-6005	White to off-white solid, free of visible particulates
	pH	USP <791>	Report Results
Identity	Molecular Weight	LCMS	Consistent with Theoretical Mass ±5 Da
	Sequence	Sanger	>99% accuracy for each base call
Strength	Assay % w/w (anhydrous) by UV Assay	A_{260} UV/Vis	Report Results
	Moisture Content	Karl Fisher	≤10% w/w
Purity	Purity	IP-RP-UHPLC	≥ 80.0% Main Peak
	Impurities (Product Related)	IP-RP-UHPLC	Report to 1 decimal each of the following: a) Early Eluting Cluster b) Late Eluting Cluster c) peaks ≥ 0.1 % area not included in other peaks
Impurities (Process Related)	Sodium	Flame AA Spectroscopy	Report Results
	Residual Solvents	GC-FID	2,6-lutidine: Report Acetonitrile: ≤ 410 ppm Ethanol: ≤ 5000 ppm Isopropanol: ≤ 5000 ppm Pyridine: ≤ 200 ppm Toluene: ≤ 890 ppm
	Elemental Impurities (Metals)	ICP-MS	As: ≤ 19 Cd: ≤ 03 Cr: ≤ 1383 Cu: ≤ 377 Fe: Report Hg: ≤4 Mn: Report Mo: ≤ 1886 Ni: ≤ 25 Pb: ≤ 6 Ti: Report
	Triethylamine	GC-MS	Report Results (note: first two GMP lots of OLI21036: 4, 6 ppm)
	N,N-Dimethyl Formamide	GC-FID	Report Results (note: first two GMP lots of OLI21036: <170 ppm)
	Dimethyl Sulfoxide	GC-FID	Report Results (note: first two GMP lots OLI21036: <0.004, <50 ppm*)

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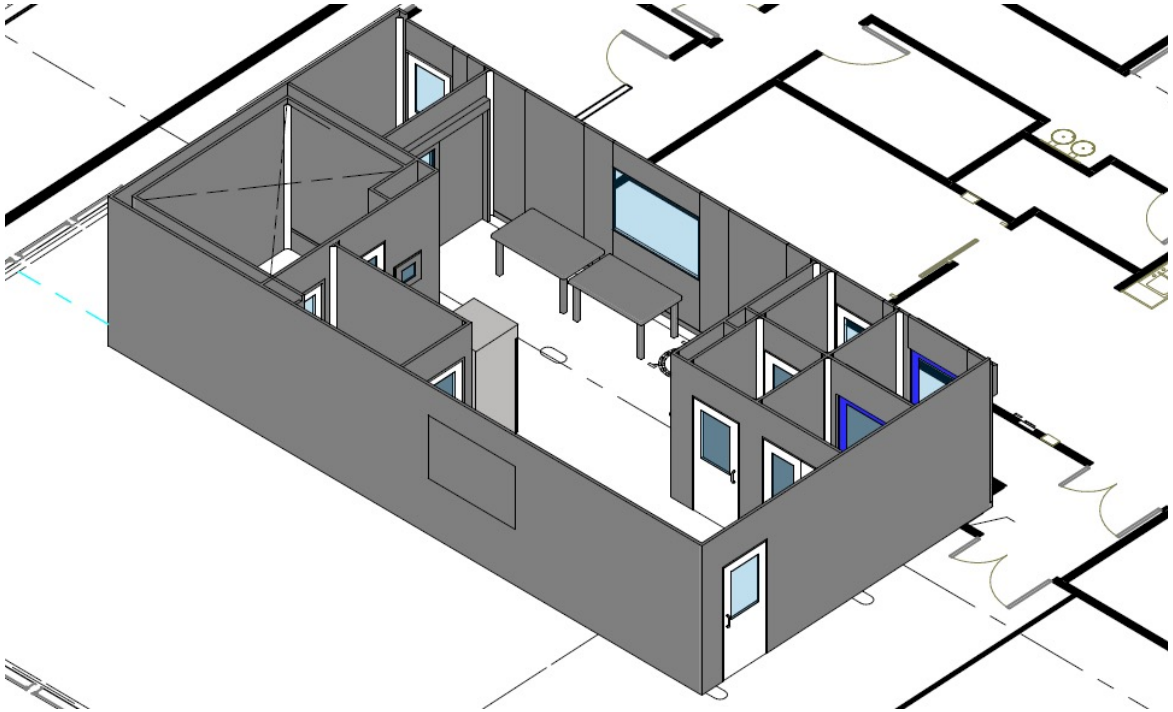
GMP Systems Implementation for gRNA Production



Materials Management System

- Procure appropriate grade of materials for gRNA production
- Create Internal Material Specifications
- Procedures for receipt and disposition of incoming raw materials
- Vendor Qualification for suppliers/manufacturers

Production Records

- Master Batch Records

Cleaning Program

- Evaluation of cleaning agents
- Development of cleaning procedures
- Utilization of contract cleaning company

Gowning Program and Gowning Qualification

GMP Systems Implementation for gRNA Production

Environmental Monitoring Program and Qualification

- HVAC operation
- Monitoring of particulates and air sampling
- Aseptic conditions

Equipment

- Operation, Calibration and Maintenance procedures
- IQ/OQs

Data Integrity

- Data backup

Validation

- CSV for GMP equipment
- Server and network

QC

- In Process Testing performed in-house
- Method Development and Qualification
- Release testing and stability testing to occur at a contract test facility

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Large scale manufacturing advantage of ~40/60mers vs 100mers in terms of cost, purity and time

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Internal GMP Manufacturing Accelerates our Pre-Clinical Programs

- Appropriate scale customized to actual need
- 6 to 12 month lead time required to hold a manufacturing slot
- Ability to prioritize internal and collaborative programs based on evolving research timelines
- All IP retained internally (every gRNA is unique so process development efforts can turn into important IP)

Process Chemistry Team Acknowledgement

Medicinal Chemistry

- Vy Le (Scientist)
- Austin McFarlin (Research Associate)

Research Scale Guide RNA Production

- McKenzie Weiss (Research Associate)
- Mark Jones (Research Associate)
- Shelby Beer (Research Associate)
- Research Associate

Process Development

- Stephen Pietrasiewicz (Scientific Technical Leader)
- Jill Fletcher (Research Associate)
- Research Associate

GMP Manufacturing

- Tim Corre (Manufacturing Lead)
- Manufacturing Associate
- Manufacturing Associate