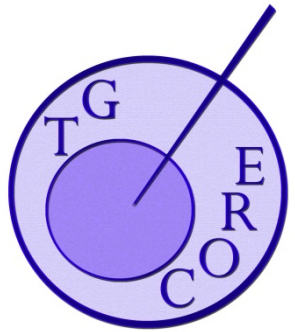


Germ line transmission with IKMC ES cells by aggregation with outbred host embryos

Marina Gertsenstein

Lauryl Nutter

EMMA cryopreservation workshop

Madrid, May 7-8, 2012

Outline

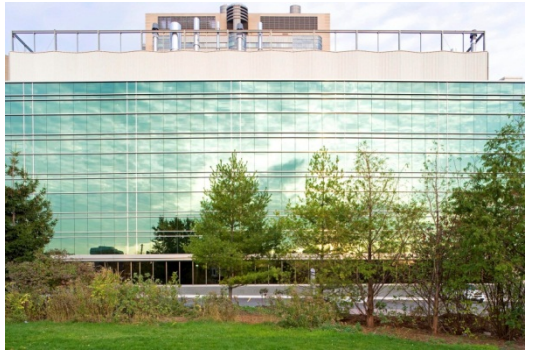
- TCP Transgenic Core
- Methods of chimeras' generation
- C57BL/6 ES cells
- IKMC ESC GLT chimeras

Toronto Centre for Phenogenomics
Joseph and Wolf Lebovic Centre

MOUNT SINAI HOSPITAL
Joseph and Wolf Lebovic Health Complex
Samuel Lunenfeld Research Institute

SickKids®

TCP



- Centralized mouse facility (~36,000 cages)
- Mouse Imaging Centre (MICe)
- CMHD Physiology Core
- CMHD Neurobiology and Behaviour Cores
- CMHD Pathology Core
- Canadian Mouse Mutant Repository (CMMR)
- Transgenic Core
    - *Embryo, sperm cryopreservation (CMMR)*
    - *Generation of chimeras*

Two projects - part of IMPC
Genome Canada NorCOMM2
NIH KOMP2-DTCC

Generation of chimeras

BALB/c, C57BL/6-Tyr^{c} (FVB/N) ↔ B6 ESC ↔ SW (outbred); C57BL/6-Tyr^{c}

Morula vs Blastocyst host (129 ESC)

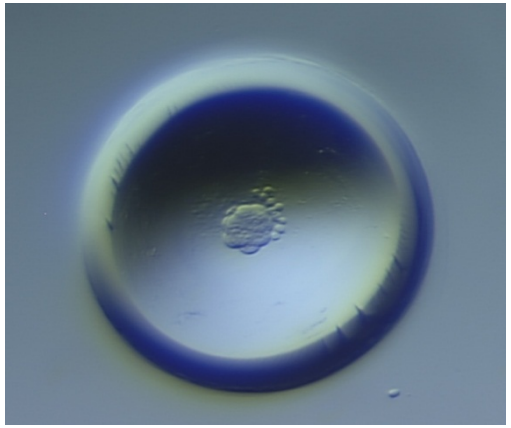
- Easier to inject
- Less pups at term
- More chimeras
- Higher ESC contribution
- More efficient GLT

Methods

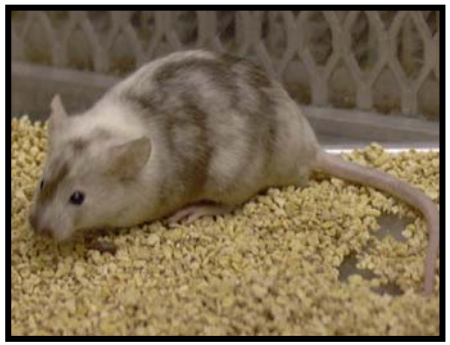
- Conventional
- Ca/Mg (-)
- Zona slit
- Piezo
- Laser

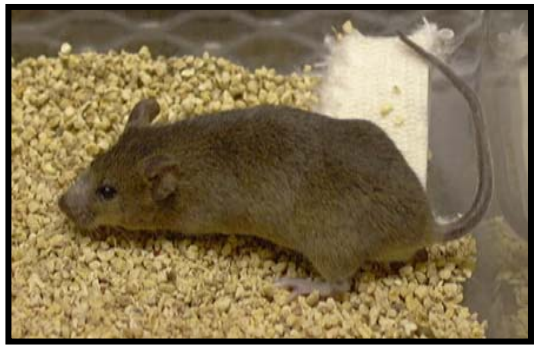
Morula aggregation

R1 ESC:129X1 x 129S3



ICR(CD-1): host





GLT

C57BL/6 ES cells

- Large scale KO mouse projects – IKMC
- Best characterized strain, genome sequence
- B6 ESC since 1990s (but ~100 KO before IKMC)
- Difficult to culture, less efficient GLT chimeras
- Instability and deterioration in culture not due to the high passage number
- Gene expression shifted to more differentiated state compared to 129 ESC

C57BL/6 ESC culture conditions

- B6 strain is often considered refractory for ESC derivation in standard FBS conditions
- KnockOut™ Serum Replacement (KOSR)
- Medium conditioned by a rabbit fibroblasts transduced with rabbit LIF (RESGRO™)
- VGB6 (Regeneron): KO-DMEM-SR and medium conditioned by L-cells (Wnt3a)
- Inhibitors of Erk and GSK3 pathways (2i)
- KO-DMEM-SR + supplements + LIF + 2i

PLoS One. 2010; 5(6): e11260
http://www.ncbi.nlm.nih.gov/pubmed/20582321

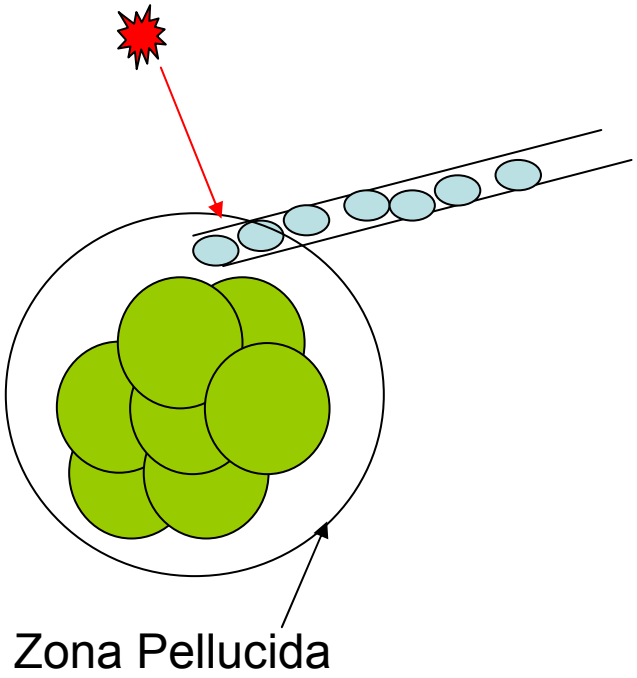
8-cell injection vs aggregation

Method	Host embryos	Number of clones		
		Total	♂ chimeras >50% coat colour (%)	F0 animals (%)
Aggregation (TCP)	ICR	10	6 (60%)	5 (50%)
8c Injection (Regeneron)	Albino B6 and SW	10	8 (80%)	6 (60%)

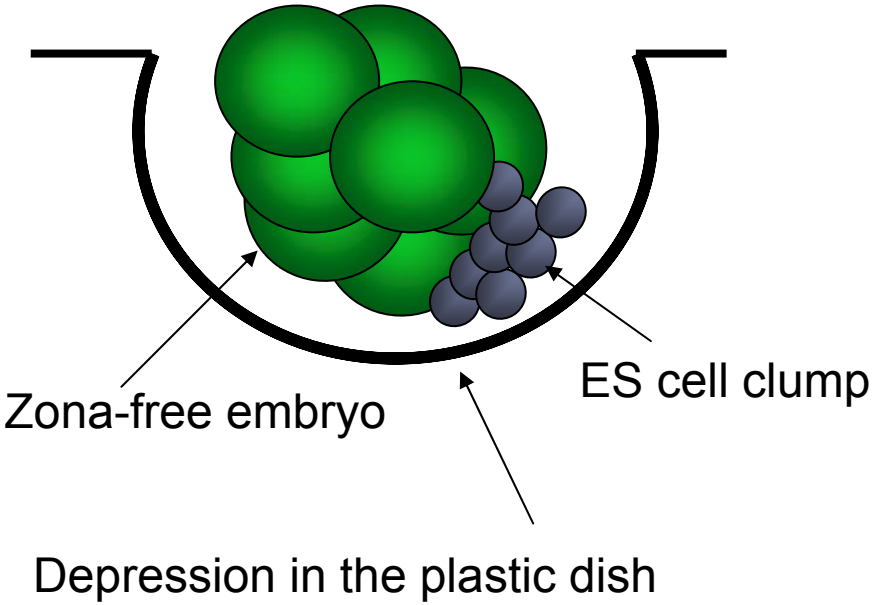
- The same 10 VGB6 clones were aggregated at TCP and injected at Regeneron (laser assisted)
- ESC were cultured in the same VGB6 medium during targeting & before aggregation/injection

8-cell injection vs aggregation

Conventional,
Laser, piezo



- ES cells quality
- Embryonic stage
- Host embryo strain



GLT chimeras with targeted C2 - C57BL/6NTac ESC

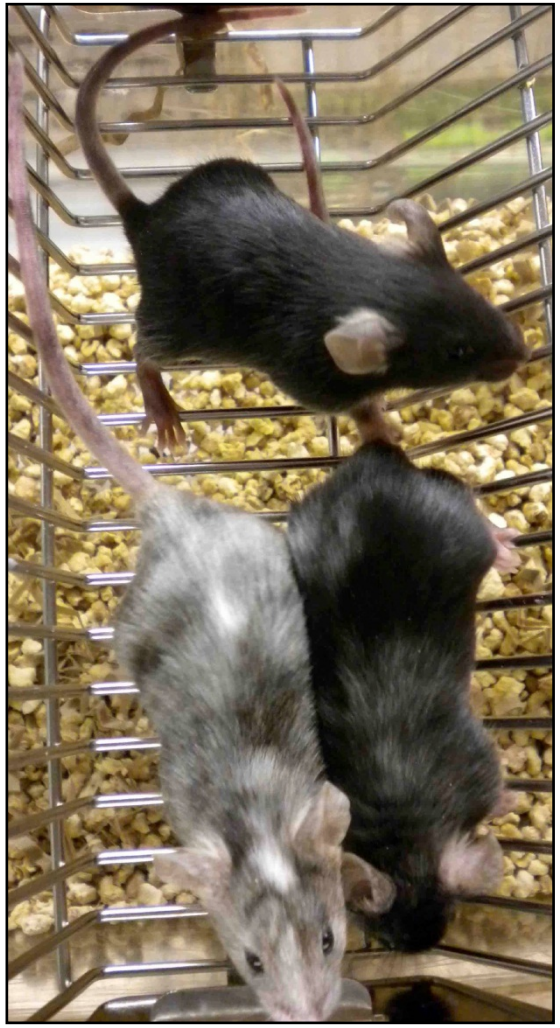
KO-SR + 2i vs VGB6

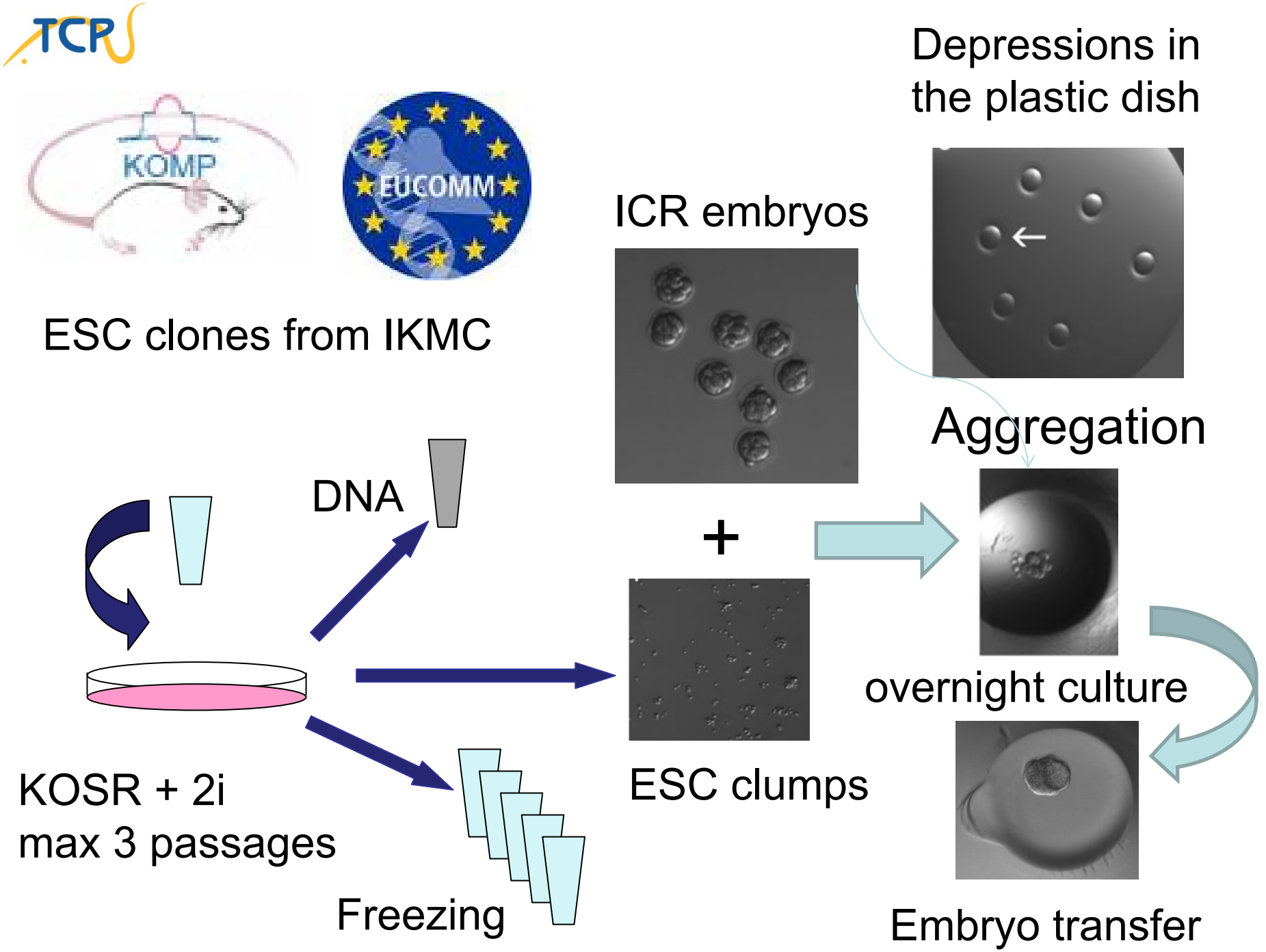
- VGB6: higher # & proportion of chimeras
- KOSR+2i: better survival of chimeras to weaning
- Some statistical but no functional differences
- Same GLT rate

Overall: 26 GLT from 31 clones -> 84% GLT rate

PLoS One. 2010; 5(6): e11260
http://www.ncbi.nlm.nih.gov/pubmed/20582321

Aggregation chimeras
B6 ESC↔ICR morula




KOMP
EUCOMM
ESC clones from IKMC
Depressions in the plastic dish
ICR embryos
Aggregation
DNA
+
overnight culture
KOSR + 2i
max 3 passages
ESC clumps
Freezing
Embryo transfer

IKMC B6 ESC chimeras

Parental ESC	Total # clones	# clones with >50% ♂ chimeras	% clones with >50% ♂ chimeras	# clones in progress	# GLT	% GLT $\frac{\text{\# GLT}}{\text{(\# aggr - \# in progress)}}$
JM8	3	3	100%	0	3	100%
JM8.F6	11	9	82%	0	5	50%
JM8.N4	15	14	93%	1	13	93%
JM8A3.N1	32	29	91%	6	12	46%
JM8A1.N3	7	7	100%	3	3	75%
VGB6	12	12	100%	1	9	82%
TIGM B6	4	3	75%	0	2	50%
C2	34	33	97%	0	29	85%
TOTAL	**117**	**110**	**93%**	**11**	**76**	**72%**

Overall % GLT from all attempted B6 ESC clones: 65 %

Tech Dev

- Early vs late ICR morula
- Aggregation vs injection ICR morula
  - 8 clones tested (4 GLT breeding in progress)
  - Aggregation: No difference in GLT by stage
  - Injection: 4 GLT 8c stage, no GLT from late
- Optimization of ESC culture conditions

Funding

- NorCOMM2

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Janet Rossant

Lee Adamson

Genome Canada

The Ontario Genomics Institute (OGI-051)

- KOMP2

In collaboration with Kent Lloyd

NIH Knockout Mouse Production and Cryopreservation (U42) RFA-RM-10-013

References

- Early B6 ESC: Ledermann and Burki, 1991; Kontgen et al. 1993; Kawase et al. 1994
- Difficulties with B6 ESC: Brook and Gardner, 1997; Auerbach et al. 2000; Ware et al. 2003; Seong et al. 2004; Hansen et al. 2008; Hughes et al. 2007, Sharova et al. 2007
- Culture conditions: Cheng et al 2004, Schoonjans et al. 2003, Poueymirou et al. 2007
- Morula vs blastocyst host: Lallemand and Brullet,1990; Stewart 1993

Tokunaga et al.1992; Yagi et al.1993 (ZP slit); Poueymirou et al. 2007 (laser); Huang et al. 2008 (piezo)

- First mouse aggregation chimeras: Tarkowski 1961, Mintz 1962; Aggregation EC cells: Stewart 1980, 1982; Nagy et al. 1990: R1 ESC
- 2i: Ying et al. 2008; Nichols et al. 2009; Buehr et al. 2008; Li et al. 2009